MATING BEHAVIOUR, EPIBIOTIC GROWTH, AND THE EFFECT OF SALINITY ON GROOMING ACTIVITY IN THE HERMAPHRODITIC SHRIMP *LYSMATA WURDEMANNI*

A Dissertation

by

TUHIN GIRI

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

August 2003

Major Subject: Zoology

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Approved as to style and content by:

Mary K. Wicksten (Chair of Committee)	Jane M. Packard (Member)
Merrill H. Sweet (Member)	S. Bradleigh Vinson (Member)
	V. M. Cassone (Head of Department)

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ABSTRACT

Mating Behaviour, Epibiotic Growth and the Effect of Salinity on Grooming Activity in the Hermaphroditic Shrimp *Lysmata wurdemanni*. (August 2003)

Tuhin Giri, B.Sc., University of Toronto;

M.Sc., University of Toronto

Chair of Advisory Committee: Dr. Mary K. Wicksten

Many species of caridean shrimp are protandrous hermaphrodites, maturing initially as males but developing into females as they age and grow. A unique sexual system was recently discovered in the peppermint shrimp, *Lysmata wurdemanni*. In this species, individuals are initially male, but become simultaneous functional hermaphrodites over time. As in most caridean shrimp, *L. wurdemanni* can mate as a male during the intermoult period, but can reproduce through female function for only a short period after moulting. Ecdysis does not occur *en masse* in this species, and thus the operational sex ratio found in populations of *L. wurdemanni* is extremely male-biased. Sexual selection theory suggests that these conditions will result in increased competition for access to mates. Evolutionary pressures should therefore have selected for mechanisms that permit individuals to quickly identify and locate potential mating partners.

L. wurdemanni were exposed to chemical stimuli collected from recently moulted conspecifics of varying reproductive condition. Test animals were able to distinguish among the different conditions, and physically manipulated only the plastic nozzle used

to pump solutions collected from shrimp with ovaries filled with vitellogenic oocytes. It was subsequently hypothesized that methyl farnesoate, a hormone associated with ovarian maturation in crustaceans, might be a key component of sex pheromones used by *L. wurdemanni*. However, a series of methyl farnesoate concentrations did not elicit responses, indicating this shrimp species does not use this hormone alone when determining reproductive condition.

Reproductive behaviour in *L. wurdemanni* was observed to differ both before and after copulation, as well as with increasing population density. Intermoult individuals were more likely to approach, follow and remain in the vicinity of a near-moult shrimp before mating could occur, and under high density conditions. The near-moult shrimp approached conspecifics only under low density conditions, and performed rapid escape behaviours only after copulation had occurred.

The unusual occurrence of epibiota upon *L. wurdemanni* was described, and the location, size and age of barnacles quantified. The effect of salinity upon grooming activities was tested. Results indicated that carapace grooming was depressed at low salinities, and could account for the considerable epibiota found in this region.

DEDICATION

This dissertation is dedicated to Dr. David Dunham and Dr. Janet Halperin for taking a 2nd year undergraduate under their collective wing and showing him how exciting behavioural research (and crustaceans) can be.

ACKNOWLEDGMENTS

Numerous people have helped me over the course of this degree. I am most grateful to my advisor, Dr. Mary Wicksten, for treating me as an independent scientist, free to create and test my own hypotheses regarding the reproductive biology of crustaceans. Dr. Jane Packard provided statistical advice, and was always available for discussions regarding concepts and methodologies. The experiments described within this dissertation (as well as studies planned for the future) were greatly improved through interactions with the other two members of my advisory committee, Drs. Merrill Sweet and Brad Vinson.

Special thanks are due to Dr. Larry Griffing for letting me use his microscopes, digital cameras, computers and video capture equipment; for teaching me the skills needed for image capture and analysis; and also for the opportunity to help teach his courses on Image Analysis and Electron Microscopy. Dr. Duncan MacKenzie helped develop the methodology used for testing responses to crustacean hormone in Chapter III.

Gratitude is also extended to the various academic advisors who have occupied the Graduate Advising Office. In particular, I would like to thank Michelle Allen and Dr. Kay Goldman for letting me know when I was about to encounter bureaucratic troubles, and then for subsequently rescuing me when I failed to heed their advice in a timely fashion. Drs. Mark Zoran and Duncan MacKenzie also provided much helpful advice during their respective terms as the departmental graduate advisor.

Several friends I made while in Texas have helped me survive the stresses of graduate school. Sarah Bernhardt, Veronica Martinez, and Jeannie Skalsky (as well as Obi, Jasmine, and Carbon) have commiserated and celebrated both my failures and successes over the past few years. They have been joined by others who have become really good friends, as well as excellent shrimp collectors—Asha Samuel, Trent Apple, Kirsten Williams and Chris Ramey (as well as Cabot, Cleo, Jasmine II, Josie, Vinnie, Maddie and Sierra) —can each consider themselves the recipient of a golden dipnet award.

Several undergraduates helped me collect specimens, performed animal care and analysed some data. This dissertation would have taken considerably longer if it were not for Jesse Carrasco, Christa Connell, Jason Graves, Liz King, Kim Sparks, and Chris Woodruff. Other graduate students within the Wicksten lab broadened my horizons, and Butler Hall was certainly enriched by the presence of Archie Ammons, David Hess, and Scott Harrison.

Thanks are also due to the numerous Biology personnel who filled out paperwork, constructed tanks, and did any number of tasks on my behalf. I am particularly appreciative of the efforts made by Ann Harris and David Reed. Rhonda Patterson and the BioAquatics staff deserve every accolade and award they have received.

I would also like to thank all my friends north of the border (some of whom were temporarily displaced south of the 49th) for numerous e-mails and packages of "Canadian content." I really appreciate all the support and votes of confidence I

received from Erika Crosse, Radu Guiasu, Kate Jackson, Ronald Kan, José Perez, Nasreen Rahman, Dr. David Dunham, as well as my parents.

Research funding for this dissertation was provided in the form of mini-grants from the Department of Biology. Personal support was provided by Texas A&M University, the Department of Biology, and the family of Dr. Lawrence S. Dillon. Although I never had the privilege of meeting Dr. Dillon, he was by all accounts a *virtuoso*—able to discuss anything with anyone. I hope this dissertation lives up to both his reputation and memory.

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CHAPTER I

GENERAL INTRODUCTION

Studies of sexual selection, mating systems and reproductive behaviour have concentrated on species that are gonochoristic and have separate sexes—males and females. This is not surprising, as it is in these systems that the occurrence and intensity of sexual selection is most easily observed and quantified (e.g. review by Andersson (1994). Males are often larger, more conspicuously coloured, and may possess exaggerated traits that females lack. In addition, males may perform distinctive behaviours in order to attract potential mates.

Although under-represented in the literature on sexual biology, hermaphrodites are quite common. Hermaphroditism is the norm amongst plants (Morgan 1994), and 20 of 28 animal phyla have hermaphroditic representatives (Michiels 1998). Some of these phyla, such as the Porifera (sponges), Ectoprocta (bryozoans), Platyhelminthes (parasitic and free-living flatworms), Chaetognatha (arrow worms), Ctenophora (comb jellies) and Urochordata (sea squirts) are almost exclusively hermaphroditic. This mode of reproduction thus appears to be plesiomorphic, but may have also evolved several times in many different lineages.

In species classified as hermaphroditic, individuals possess both functional male and female reproductive systems over at least a portion of their lifetime. A species is classified as *simultaneously hermaphroditic* if male and female genitalia are present over

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This dissertation follows the style and format of *Animal Behaviour*.

much of an individual's lifetime, and if reproduction occurs through both male and female sex allocation (Michiels 1998). *Sequential hermaphrodites* also use both sex allocations for reproduction, but not simultaneously. Instead, these organisms 'change sex' over the course of their lifetime (Warner 1975), and the order of gender-expression is used to further categorise sequential hermaphroditism. *Protandrous* hermaphrodites are initially male, but lose masculine reproductive function and become females (Ghiselin 1969). In contrast, *protogynous* hermaphrodites are initially female, but eventually become functional males. Again, there is a loss of reproductive function through one sex but a gain in the other (Ghiselin 1969).

Hermaphroditism is favoured if the overall reproductive success obtained by a hermaphrodite is greater than that achieved by pure males or pure females (Charnov et al. 1976; Heath 1979; Charnov 1982). Ghiselin (1969) outlined three conditions under which this selection could occur. He postulated that simultaneous hermaphroditism would be favoured when (1) population densities or motility are low, or (2) if the availability of optimal mates is low. At low population density or motility (the "low density model"), the probability of encountering conspecifics of the opposite sex is reduced. Selection should therefore favour simultaneous hermaphrodites under this scenario. Similarly, a risk of inbreeding or a bias in sex ratio could decrease the flow of genes, and the availability of optimal mates ("gene dispersal" model). Simultaneous hermaphrodites would also experience greater reproductive success under this condition.

The "size advantage" model suggests that sequential hermaphroditism will be selected when one gender gains more matings at a particular size than does the other sex.

Individuals which assumed the gender advantageous to a particular size would increase their reproductive potential. Protandrous species may delay reproduction through female function if there is a strong correlation between size and fecundity (e.g. Anger & Moreira 1998), while protogynous species may require territorial defence or parental care as a component of male function (e.g. Knowlton & Keller 1982; St. Mary 1993; DeWitt 1996; Tallamy 2000). Although sequentially hermaphroditic individuals may accrue a serious cost (decreased mating opportunities or success) during the restructuring of gonadal tissue, there is likely a large increase in reproductive success once this process is complete (Hoffman et al. 1985).

Most crustaceans are gonochoristic, but hermaphroditism does occur in a number of phylogenetically distant groups. Juchault (1999) hypothesized that the ancestral crustacean sexual system was one of simultaneous hermaphroditism, a condition that can still be observed in two of the most primitive crustacean classes, the Remipedia and Cephalocarida. Hermaphroditism is also common amongst the barnacle sub-Class Cirripedia (Furman & Yule 1990). It has been suggested that the evolution of separate sexes in Crustacea occurred following the integration of parasitic DNA which inhibited the expression of 'male genes' in hermaphrodites (Juchault 1999). Current evidence for this proposed model can be found in the isopod *Armadillidium vulgare*, where cytoplasmic factors in parasitic Wolbachia bacteria have been observed to repress the gene coding for differentiation of the androgenic gland. Repression of this gene results in embryonic gonadal tissue differentiating into an ovary, rather than an androgenic gland (Juchault 1999).

Most decapod crustaceans (such as lobsters, crabs and shrimp) are gonochoristic. However, there also exist several descriptions of sequential hermaphroditism (e.g. Perez Farfante (1992)), especially amongst the caridean shrimp. Of the 37 species of decapod crustaceans observed to exhibit protandry, 31 belong to the Infraorder Caridea (reviewed in Bauer (2000)). Protandric hermaphroditism found in these shrimp is believed to have evolved from a gonochoristic ancestor, but the mechanism by which this occurred has yet to be resolved. Hoffman (1972) suggested that this form of sequential hermaphroditism could have occurred through selection acting upon female fecundity, as there is a relationship between body size and fecundity in many caridean shrimp (Anger & Moreira 1998). Charniaux-Cotton & Payen (1985), however, proposed that protandry could have evolved through the early degeneration of androgenic glands in genetic males. The subsequent reduction in male hormones would have resulted in these individuals maturing as females.

Although there is a general pattern of male to female transition in these species, there are also a number of variations present (Fig. 1.1). Simple protandry can be observed in *Pandalus platyceros, P. hypsinotus,* and *Pandalopsis dispar* (Butler 1964). In these populations, individuals are initially male, but later develop into females (Fig. 1.1A).

Boddeke et al. (1991) describe a different form of protandry in the shrimp *Crangon crangon* (Fig. 1.1B). Most of the individuals sampled from populations of this species were observed to transition from male to female reproductive function. In a portion of the population, however, juveniles mature directly as females ("primary females"), and

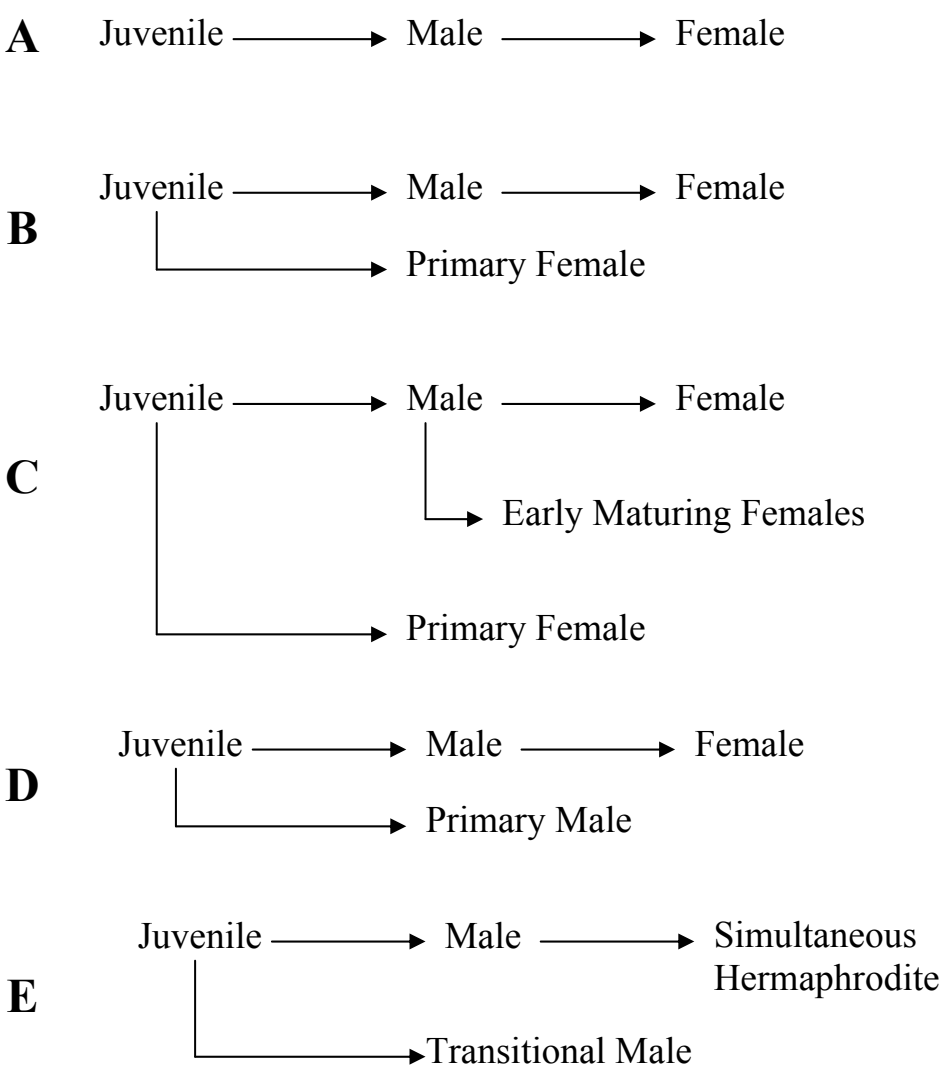


Figure 1.1. Variations of protandric hermaphroditism found in caridean shrimp. (A) Simple protandry; (B) Protandry with development of primary females in portion of population; (C) Protandry with primary females, as well as individuals that experience a very short male phase; (D) Protandry with primary males; (E) Protandric simultaneous hermaphroditism. (After Bauer (2000))

skip male function altogether. This system has also been observed in *Processa edulis* (Noel 1976).

Pandalus borealis exhibits the pattern of protandry illustrated in Fig. 1.1C (Bergstrom 1997). As in *C. crangon*, the majority of individuals initially mature as males, but become females with increasing age and size. A small subset of the population, however, matures directly as primary females (5-9% of samples collected), while 4-12% of individuals pass through a brief male-phase before maturing into females ("early maturing females"). Chiba et al. (2000) describe a similar occurrence in *Pandalus latirostris*.

Bauer (1986) describes the presence of primary males in *Thor manningi*. In this form of protandry, almost half of the population passes through a male phase before final maturation as females (Fig. 1.1D). Another 50% mature as "primary males", and breed only through this reproductive function. Primary females make up only 1% of the population. Interestingly, two congenerics, *T. dobkini* and *T. floridanus*, are completely gonochoristic and do not exhibit any form of protandry (Bauer & VanHoy 1996). Bauer (1986) hypothesized that primary males persisted in high numbers in *T. manningi* because they were able to compete more effectively for mates than their smaller-sized protandric male competitors. Gherardi & Calloni (1993) describe a similar hermaphroditic condition in the alpheid shrimp *Athanas indicus*.

Until recently, shrimp of the genus *Lysmata* were considered protandrous hermaphrodites. Morphological studies using *L. seticaudata* by Dohrn (1950) and Berreur-Bonnenfant & Charniaux-Cotton (1965) found the presence of male

characteristics in smaller individuals, while female characteristics predominated in larger animals. Despite observations that the testicular portion of the ovotestis did not disappear completely in larger shrimp, these studies concluded that *L. seticaudata* was a protandrous hermaphrodite. Couturier-Bhaud (1974) also examined *L. seticaudata* and concluded that this species is a protandric hermaphrodite, with female vitellogenesis dependent upon coastal water temperatures.

Kagwade (1981) reported the occurrence of simultaneous hermaphroditism in *Exhippolysmata ensirostris* (as *Hippolysmata ensirostris*), a sister group to *Lysmata* (Christoffersen 1987). Sperm was found within the ovotestis of all the shrimp examined, regardless of body size. Larger specimens were also found to possess 'claspers' on the distal ends of the pleopods, a characteristic not usually found in females. Sukumaran (1981), in a different study using the same species, stated that *E. ensirostris* is a protandric hermaphrodite. Morphological examinations in this study suggested that the testicular portion of the ovotestis degenerated with increased body size. Unfortunately, neither study performed mating experiments to determine which form of hermaphroditism exists in this species.

There have also been many anecdotes in the aquarium hobbyist literature describing *Lysmata* as hermaphroditic, although these reports are not consistent as to the nature of this hermaphroditism. Delbeek (1987) and Wilkerson (1994), for example, describe simultaneous hermaphroditism in *Lysmata* (with an emphasis on *L. amboinensis*), whereas Debelius (1984) describes protandric hermaphroditism in *L. seticaudata*.

Several studies recently indicated that *Lysmata* spp. are simultaneously hermaphroditic for at least a portion of their lifetime. Fieldler (1998) observed that female-phase *L. amboinensis* produced viable embryos only when paired. When pairs were separated, individual shrimp continued to spawn, but these clutches of eggs were inviable and soon discarded. Histological examinations of the ovotestis indicated that both oocytes and sperm were present. These results together suggest that *L. amboinensis* is an outcrossing simultaneous hermaphrodite. Simultaneous hermaphroditism has also been described in *L. debelius* (Fletcher et al. 1995; Rufino & Jones 2001), *L. grabhami* (Wirtz 1997) and *L. seticaudata* (C. D. d'Acoz, pers. comm)

Bauer & Holt (1998) found morphological and behavioural evidence of simultaneous hermaphroditism in large *L. wurdemanni*, but also discovered that small individuals were exclusively male. In this sexual system (defined as "protandric simultaneous hermaphroditism" by Bauer (2000); Fig. 1.1E), smaller (presumably younger) individuals reproduce only as males, but become simultaneous functional hermaphrodites as they age and grow. In addition to characteristics typical of caridean males (e.g. male gonopores, ejaculatory ducts, cincinnuli (hooks) on the first pair of pleopods and appendices masculinae on the second), small individuals possess an ovotestis with an undeveloped ovarian portion. Larger, hermaphroditic individuals retained the male gonopores and ejaculatory ducts, but showed a reduction or absence of pleopod characters. In addition, the ovarian portion of the ovotestis was fully developed. Mating experiments demonstrated that copulations between hermaphrodites resulted in the production of viable embryos. Interestingly, a small portion of the population

sampled was found to possess a vitellogenic ovotestis while also retaining all the characteristics typical of males. Bauer & Holt (1998) suggested that these "transitional" forms were males that have not completed the transition to a hermaphroditic phase, or are males that never made this transition. An examination of small *L. grabhami* yielded a similar result—smaller individuals possessed well-developed male characteristics, while a complete ovotestis could be observed within larger individuals (Wirtz 1997). Protandric simultaneous hermaphroditism may thus be widespread among the *Lysmata*, and may also occur in related taxa.

Lysmata are found in both warm temperate and tropical waters, and also at various population densities (Table 1.1). They are generally known as 'cleaner shrimp' (view videoclip Cleaning.avi), and are often observed removing debris, diseased tissue and parasites from fish (Criales 1979; Fletcher et al. 1995; Zhang et al. 1998; Wicksten 2000). Dreyer (1994) compared the cleaning abilities of a temperate species, *L. wurdemanni*, with that of *L. grabhami*, a species found in the tropics. The results of this study suggested that tropical species are obligate cleaners, and depend upon hosts as a food source. They are usually conspicuously coloured and individuals will typically be found in one location for long periods of time. Temperate species, in contrast, are facultative cleaners, and obtain only a portion of their nutritional needs from cleaning activities. Individuals of these species usually wander, and are typically not as conspicuously coloured as their tropical relatives.

It is not clear how a sexual system of protandric simultaneous hermaphroditism could have evolved, given the wide range of environmental and social conditions in

Table 1.1 Ecological and behavioural patterns found in those *Lysmata* spp. where reproductive biology has been described

Species	Population Density	Biogeography	Cleaning Ability
L. amboinensis	Low, found in pairs	Tropical Pacific	Obligate?
L. debelius	Low?	Tropical, Deep	Obligate?
L. grabhami	Low, found in pairs.	Tropical Atlantic, Caribbean	Obligate
L. californica	High	Warm temperate	Facultative
L. seticaudata	High	Warm temperate	Facultative
L. wurdemanni	High, found in groups	Tropical to warm temperate	Facultative

which *Lysmata* are found. It is clear that this unique sexual system is ancestral within the genus, as the majority of species appear to exhibit it. Bauer (2000) hypothesized that protandric simultaneous hermaphroditism may have evolved first in a protandric tropical ancestor that was found in low densities. If few mating partners were available, the "low density" model suggests a selection for simultaneous hermaphroditism would occur (Ghiselin 1969). Colonization of temperate regions and subsequent speciation could result in the pattern of distributions observed today. This model would suggest, however, that the general cleaning behaviours found in temperate species would have evolved from a specialized tropical cleaner. Further research is therefore needed to determine the phylogenetic relationships among the various species within this genus.

Darwin (1871) suggested that sexual selection in hermaphrodites would be low because both genders are united within a single organism. Subsequent research has refuted this hypothesis, and indicated that hermaphrodites can compete for a limited number of ova (e.g. Bateman 1948; Morgan 1994; Michiels 1998). The competition for mates could be fairly strong in several *Lysmata* species, especially amongst those that live in high densities.

The peppermint shrimp, *L. wurdemanni*, can be found along the Atlantic and Caribbean coasts of North and South America from New Jersey to Brazil (Williams 1984). This species is usually associated with hard substrates such as jetties and rocky outcroppings, and can also be found in relatively high densities (pers. obs., but see Debelius (1984)). As in most caridean shrimp, mating in *L. wurdemanni* occurs after an individual has moulted. Reproduction through male function can occur at any point

during the intermoult period, but breeding as a female only occurs for a short time immediately after moulting. Copulation behaviours (Bauer & Holt 1998) are similar to those described for gonochoristic caridean species (e.g. Bauer 1976; Correa et al. 2000), and culminate with a spermatophore being placed on the external abdominal surface of the recently moulted partner.

Emlen & Oring (1977) defined "operational sex ratios" as the ratio of fertilizable females to sexually active males. In *L. wurdemanni*, the operational sex ratio is very male biased. While every small *L. wurdemanni* and every intermoult hermaphrodite can potentially act as a male partner, the number of 'females' is determined by the number of recently moulted individuals. Since *L. wurdemanni* do not appear to moult *en masse* (pers. obs.), the number of 'females' encountered in a population at any point in time is very low. In addition, not all recently moulted individuals will be able to produce viable embryos—moulting can occur without vitellogenic oocytes being present in the ovaries. Under these conditions, there should be much intra-sexual competition for access to potential mates.

Individual *L. wurdemanni* may use several behavioural mechanisms to obtain mating opportunities. Andersson (1994) reviews several categories of pre-copulatory actions that organisms typically use during competitions:

(1) Scrambles—individuals quickly search and locate potential mates. Scrambles may explain why certain organisms have well-developed sensory and locomotory organs. Since *L. wurdemanni* is typically found in high population densities, the quick identification and localization of potential mates would maximize reproductive

success. Since crustaceans are remarkably chemosensitive (Ache & Derby 1985), chemical cues may be used by *L. wurdemanni* to determine the reproductive condition of conspecifics.

(2) Endurance Rivalry—individuals remain reproductively active during a large part of the season. This mechanism appears to be active in *L. wurdemanni*, as individuals can mate as males during intermoult stages. Since there is no terminal moult in this species, individuals can also reproduce several times using female function.

(3) Contests—fights over mates select for strength, through such characteristics as large size and weapons. Unlike some other other caridean shrimp (e.g. snapping shrimp), *L. wurdemanni* do not possess heavy chelae. Larger individuals may be more successful at obtaining mates, however.

(4) Mate Choice—in many species, individuals possess traits or perform behaviours that attract and stimulate mates. Individuals may also offer a resource that a mate needs. This does not appear to occur in *L. wurdemanni*, although it does occur in other caridean shrimp (e.g. Mathews (2002a) observed heterosexual sharing of burrows in the snapping shrimp *Alpheus angulatus*.)

(5) Sperm competition—Pre- and post-copulatory actions may also be performed if there is a risk that a rival may fertilize a mate. Common tactics used by male crustaceans include mate guarding (e.g. Waddy & Aiken 1991; Correa et al. 2000), and the use of a mating plug (e.g. Bauer & Min 1993). The high population densities associated with *L. wurdemanni* suggest mate guarding may occur. Since spermatophore

placement in caridean shrimp is external (but see Boddeke et al. (1991) for a description of internal fertilization), sperm plugs are not found.

Chapters II-IV examine reproductive behaviour in *L. wurdemanni*. Chapter II describes how *L. wurdemanni* is able to distinguish among different reproductive conditions using chemical cues. An experiment to determine whether methyl farnesoate, a crustacean hormone used in ovarian maturation, is a major component of *Lysmata* sex pheromone is detailed in Chapter III. Pre- and post-copulatory mating tactics performed by *L. wurdemanni* are examined in Chapter IV.

During a collecting trip to obtain specimens for use in the above studies, several *L. wurdemanni* with considerable epibiota attached were found. The presence of an estuarine barnacle species, epistylidid ciliates and bryozoans upon the external surfaces of *L. wurdemanni* was unusual, as shrimp spend a considerable amount of time engaged in grooming behaviours (Bauer 1975). The pattern of barnacle settlement upon these shrimp, and the environmental conditions surrounding shrimp fouling events (both current and historical) is described in Chapter V.

The *L. wurdemanni* used in these studies were collected in Galveston, Texas, a coastal area that is influenced by freshwater inflow from the Trinity and San Jacinto river basins. As a result, organisms living around and within the collecting area can be exposed to dramatic changes in salinity. Fluctuating salinity levels were also common in other areas where shrimp fouling has been observed. Chapter VI tests the hypothesis that a decrease in salinity affects the performance of grooming activities in *L.*

wurdemanni, and correlates a change in the pattern of grooming behaviours with the observed pattern of barnacle settlement found in Chapter V.

CHAPTER II

DISCRIMINATION OF REPRODUCTIVE CONDITION BY THE HERMAPHRODITIC SHRIMP *LYSMATA WURDEMANNI*

INTRODUCTION

Decapod crustaceans use chemical information in numerous ecological and social contexts. Several studies have implicated the role of chemical cues in the initiation and maintenance of feeding behaviours, habitat selection, aggression, and mating behaviour. Hirtle and Mann (1978) found that american lobsters, *Homarus americanus*, are able to discriminate among prey items using waterborne odors, while McLeese (1970) observed that *H. americanus* would respond to both extracts of prey foods as well as several amino acids and organic compounds. Rebach (1996) reported that the stone crab (*Cancer irroratus*) will manipulate artificial prey when food odours are present, but ignore identical objects in the absence of chemical cues. Chemosensory responses have been used to determine appropriate bait for commercially important species (Zhou & Shirley 1997).

The hermit crab *Clibinarius vittatus* uses both chemical and visual cues while searching for shells to inhabit (Diaz et al. 1994). Punzalan et al. (2001) observed that juveniles of the burrowing crayfish *Fallicambarus fodiens* displayed a preference for mud saturated with water that had previously contained conspecifics, and were able to distinguish conspecific-built chimneys from controls through chemical cues. Similarly,

(Zimmerfaust et al. 1985) observed that spiny lobsters (*Panulirus interruptus*) are attracted to shelters by chemical substances released by conspecifics.

Karavanich and Atema (1991) found that chemical communication also plays an important role during agonistic interactions in *H. americanus*. Chemical stimuli contained within urine excreted by combatants during these interactions appears to be used in the establishment of stable dominance relationships (Breithaupt & Atema 1993; Karavanich & Atema 1998). Similar results were found for the crayfish *Orconectes rusticus* (Schneider et al. 2001). Chemical cues also appear to be used for individual recognition in a number of crustacean species (Johnson 1966; Caldwell 1985), and are likely used for species recognition (Tierney & Dunham 1982).

The performance of aggressive or reproductive behaviours is likely influenced by specific sex pheromones. An experiment by Ameyaw-Akumfi and Hazlett (1975) demonstrated that male crayfish (*Procambarus clarkii*) could discriminate between genders in the absence of visual or tactile information. Atema and Cowan (1986) observed a similar result in *H. americanus*, where males responded strongly to urine collected from other males and intermolt females. Courtship responses were initiated in male blue crabs (*Callinectes sapidus*) following the presentation of urine collected from pubertal and recently-moulted females (Gleeson 1980), while Stebbing (2003) observed that male signal crayfish (*Pacifastacus leniusculus*) would seize, mount and deposit a spermatophore upon an air stone through which was injected water conditioned by reproductively-ready females.

The unique sexual system of the caridean shrimp *Lysmata wurdemanni* was described by Bauer and Holt (1998). Individuals are initially exclusively male, possessing a testis and morphological characteristics typical of male caridean shrimp. As individuals age and grow, however, an ovary begins to develop and each shrimp becomes a simultaneous functional hermaphrodite. Studies on this species, as well as on *Lysmata amboinensis* indicate that self-fertilization does not occur (Bauer & Holt 1998; Fiedler 1998).

In caridean shrimp, females are reproductively receptive for only a short period immediately after moulting. This pattern is also true of *L. wurdemanni*. Hermaphroditic individuals are able to mate as females immediately after moulting, but play a male mating role during the intermoult period. Since there are relatively few recently moulted individuals at any single point in time, the operational sex ratio (Emlen & Oring 1977) found in a population of *L. wurdemanni* is decidedly biased towards males. As a result, there should be increased competition among inter-molt hermaphrodites and younger males for access to receptive individuals. The performance of behaviours that may increase the probability of successful matings (e.g. mate guarding) depends upon individuals identifying conspecifics that are soon to enter a reproductively receptive stage. This study examines if *L. wurdemanni* use chemical cues released by recently moulted conspecifics to determine reproductive condition.

METHODS

Lysmata wurdemanni were collected along a rocky groin in Galveston, Texas (29°16'N 94°49'W) (Fig. 2.1) during nocturnal low tides and subsequently transported to Texas A&M University (College Station, Texas). Shrimp were initially housed in community aquaria measuring 21 (w) x 41 (l) x 25 (h) cm at densities of 12-15 animals per aquarium, and under a 12h:12h light:dark cycle. Seawater was maintained at 33-35‰ and at a temperature of 26-27°C. Corner filters were used for fitration and aeration. All animals were fed frozen brine shrimp every other day until use.

Stimulus Solutions

Stimulus solutions were created using *L. wurdemanni* at three different reproductive stages (Fig. 2.2). A subset of the shrimp collected in Galveston were anaesthetized in a clove oil solution and were then examined under a dissecting microscope for distinctive morphological characteristics. Shrimp that were defined as 'males' (M; N=5) had hook-like cincinnuli present on the first pair of pleopods, and well-developed appendices masculinae on the second pair. Individuals were classified as hermaphrodites if these two traits were reduced in size or absent (c.f. Bauer & Holt 1998). Hermaphrodites were further subdivided into two groups, based on the stage of ovarian development visible through the shrimp carapace (Bauer 1986). *L. wurdemanni* at stage 4 of ovarian development had green vitellogenic oocytes clearly filling the ovary (H4; N =7; Fig. 2.2). Individuals with no green oocytes visible were classified as being at stage 1 of ovarian development (H1; N =8). Only stage 4 and stage 1 hermaphrodites were used to

Figure 2.1. Collecting site in Galveston, Texas. *L. wurdemanni* are associated with hard substrates, such as rocky groins (A). Dipnets were used to collect shrimp from areas between rocks (B & C). Note the turbidity of water in (C)

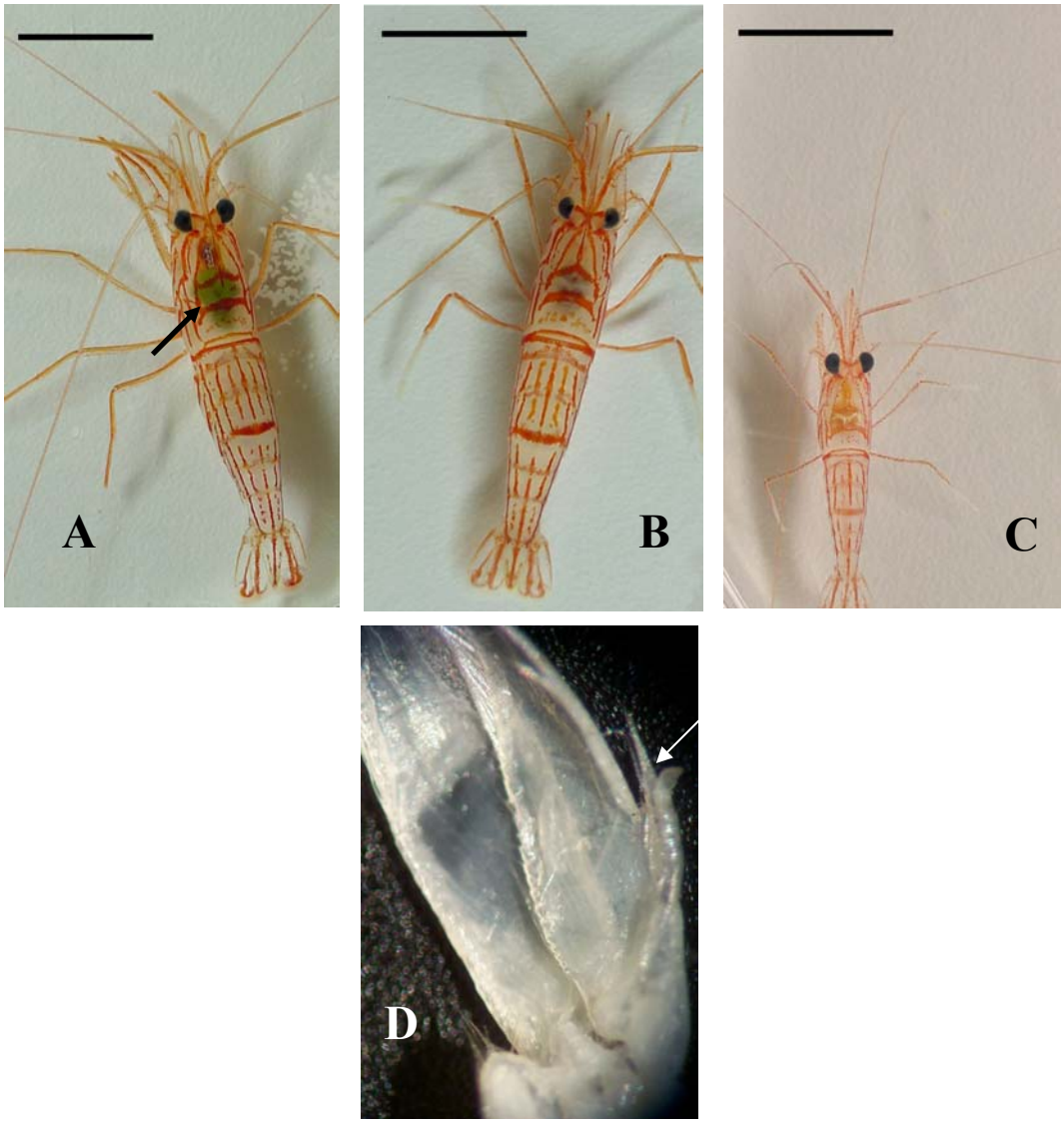


Figure 2.2. Reproductive conditions found in *L. wurdemanni*. (A) Stage 4 hermaphrodite (H4) with green vitellogenic oocytes visible in the ovary (indicated by arrow); (B) Stage 1 hermaphrodite (H1) with no oocytes in ovary; (C) Non-hermaphroditic *L. wurdemanni*. Small individuals possess male characteristics, such as an appendix masculina on the second pleopod (indicated by arrow on D). Scale bar indicates 1 cm.

create stimulus solutions; shrimp that were at stages of ovarian development between these two extremes were excluded.

A Panasonic video camera (Model GPKR222) attached to a dissecting microscope and connected to a PowerMacintosh 7200 was used to capture digital images of the dorsal surface of each shrimp. The software package ImageJ (version 1.22) was used to measure the carapace, from a point mid-way between the eyes to the junction with the abdomen. Shrimp were then housed in 2L glass jars, with the volume of water standardized at a ratio of 50 mL of seawater for each millimetre of carapace length (range: 250-555 mL). Water conditions were as described above, but were aerated only. The volume of water in each jar was replaced daily with freshly-mixed stock.

Shrimp were checked every 12 hours. If a shed exoskeleton was observed, the freshly-moulted shrimp was transferred by dipnet into an aquarium and the conditioned water within the housing jar was collected in a beaker. The moult water was then divided into 10 ± 0.1 mL aliquots. Each aliquot was stored within a 14 mL Falcon® tube and frozen at -20^{o}C until use.

Three stimulus solutions (M, H1, H4) were created from three 10 mL aliquots collected from three different shrimp of the same reproductive condition. Nine tubes (three for each reproductive condition) were initially thawed within the testing tank until conditioned water solutions were isothermic with the testing environment. Once thawed, the solutions for each reproductive condition (30 mL total) were mixed together in 50 mL beakers. Each of the three stimulus solutions were created immediately before

testing occurred. Water from the testing tank was collected in a separate beaker and used as a control solution.

Testing

The responses of *L. wurdemanni* at stage 4 of ovarian development (N=14) were examined. Each shrimp was housed in a 2L glass jar (water conditions described above) for 24 hours prior to testing. Each shrimp was tested only once, and was returned to a community aquarium after use.

A large plastic tank measuring 50 (l) x 38 (w) x 21 (h) cm was used for testing responses to the stimulus and control solutions (Fig. 2.3). Plexiglas dividers were used to create a 30 x 30 cm testing arena within the larger tank. This arena was lined with coral gravel to a depth of 1 cm, and the entire tank was filled to a depth of 10 cm. All testing occurred during the dark portion of the light cycle, and was videotaped under infrared illumination using a Sony TR-517 camcorder positioned above the arena. The camcorder was connected to a TV and VCR located in another room.

A four-chambered HaakeBuchler polystaltic pump was used to pump the control and stimulus solutions into the testing arena at a controlled rate of 1.0± 0.2 mL/min. Four equal lengths of Tygon tubing extended from the pump to the testing arena, and were threaded through holes drilled in the widest section of four identical moulded aquarium rocks. Tubing was anchored in place with plastic nozzles, and one rock was placed in each corner of the testing arena.

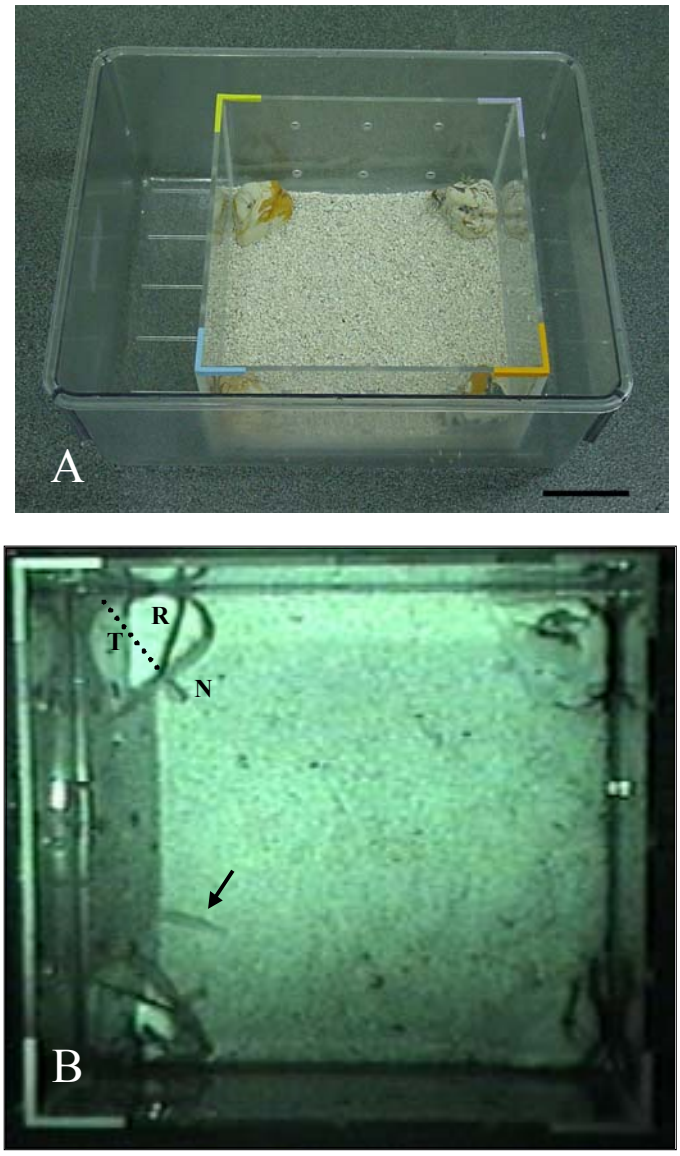


Figure 2.3. Arena used for testing responses. (A) Empty testing tank with colour coded corners. Scale bar represents 10 cm. (B) Video capture of the testing arena used for filming. All filming was performed under infrared light. Moulded decorative rocks (R) were placed in each corner. Tygon tubing (T) was threaded through a hole drilled through each rock indicated by dashed line, and anchored in place by a plastic nozzle (N). Arrow points to *L. wurdemanni* being tested. Arena measures 30 cm x 30 cm.

The opposite ends of the Tygon tubing were threaded through four hollow tubes extending through a circular piece of plastic. This plastic disc was wide enough to rest upon four beakers arranged in a 2x2 formation, with each tube extending into a single beaker. This apparatus allowed all four tubes to be moved at once, and also allowed solutions to be drawn simultaneously into the four lengths of tubing. Colour-coding the beakers, tubing, the four quarters of the plastic disc and the upper corners of the testing arena with pieces of coloured tape ensured that individual solutions were pumped to the appropriate rocks. The corners from which each stimulus and control solution entered the testing arena was randomized, but balanced over the course of the experiment.

The plastic disc and tubing were initially placed over four beakers containing control solutions. These solutions were pumped into all four corners of the testing arena. A test animal was then gently placed in the centre of the testing tank and allowed to explore for 20 minutes. The plastic plate was then lifted, and placed over the four beakers containing the three stimulus and one control solution. Since a small amount of air was pumped into the tubing during transfer, the appearance of bubbles indicated when the stimulus solutions had begun to enter the testing arena. Solutions were pumped into the arena for 30 minutes.

The Plexiglas dividers and the larger tank were rinsed thoroughly following testing. The Tygon tubing was flushed several times with distilled water and air was then pumped through each length. Coral gravel was rinsed and autoclaved between uses.

Data Collection

The first 10 minutes following entry of stimulus solutions into the testing tank were used for analysis. Two activities performed by test animals were examined. The duration of time shrimp spent within a designated zone around each stimulus entry point was recorded, as was the number of times individuals physically manipulated the plastic nozzles anchoring the Tygon tubing into the rocks.

A quarter-circle region in each corner was defined as a 'stimulus zone' and included within it both the rock and plastic nozzle (Fig. 2.4). Zone boundaries were based upon an estimate of the diffusion pattern of stimulus solutions within the testing arena. The arena and contents (excluding test animal) were set up as normal, and a mixture of blue food dye and seawater was then pumped in and videotaped (Fig. 2.5). The distance that the dye-seawater solution was able to travel from each of the plastic nozzles was measured from videotape images with Image J. The area of greatest intensity was measured and added to measurements made of the rocks and nozzles. The mean was used to determine the radius for each zone (10.9 ± 0.27 cm SE).

In *L. wurdemanni*, mating typically involves the grasping of a freshly moulted individual by the intermoult mating partner. Physical manipulations of the plastic nozzle were thus considered to be demonstrations of mating behaviour.

Statistics

Statistical advice was sought from the Statistical Consulting Service at Texas A&M University. Multiple comparison Wilcoxon signed-ranks tests were used to compare the

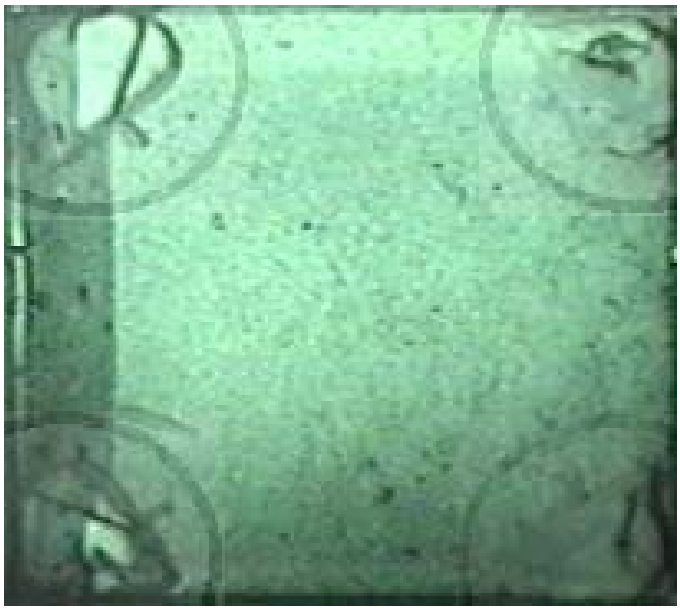


Figure 2.4. "Stimulus zones" used in this study. Zones were designated around each rock and were based on areas of highest dye concentration after 10 minutes of solution entry.

Figure 2.5. Dye test used to determine stimulus diffusion within testing arena. Images were captured before solution entry (0), and at (5) and (10) minutes after solution dispersal.

durations of time that test animals spent within each zone. This procedure was used because the simultaneous presentation of control and stimulus solutions had the potential to affect the responses of test animals. An increased response to one stimulus solution reduced the total time each shrimp could respond to any of the other solutions (J. Jeng and D.W. Kwok, Statistics Dept., Texas A&M University, pers. comm). An initial α-level of 0.10 was also recommended because of the high variability in observed responses. An increased α-level is typical of many agricultural studies (J. Jeng and D.W. Kwok, Statistics Dept., Texas A&M University, pers. comm). This α-level was then corrected for multiple comparisons, such that the final level used was α=0.017.

Poisson tests were used to compare the manipulation responses directed towards the plastic nozzles. This test was suggested in light of the high number of non-responses to M, H1 and control solutions. Mean responses to each stimulus solution were examined separately to determine if they lay within a Poisson distribution; those that lay outside this distribution were considered to be significantly different from those that lay within the distribution.

RESULTS

The duration of time spent by the 14 *L. wurdemanni* within each stimulus zone is illustrated in Fig. 2.6. Shrimp spent a significantly greater amount of time within the H4 stimulus zone than in the H1 zone (multiple Wilcoxon signed-ranks test, N=14, P=0.001) or M zone (multiple Wilcoxon signed-ranks test, N=14, P=0.004). A comparison with the control zone approached significance for the corrected α-level

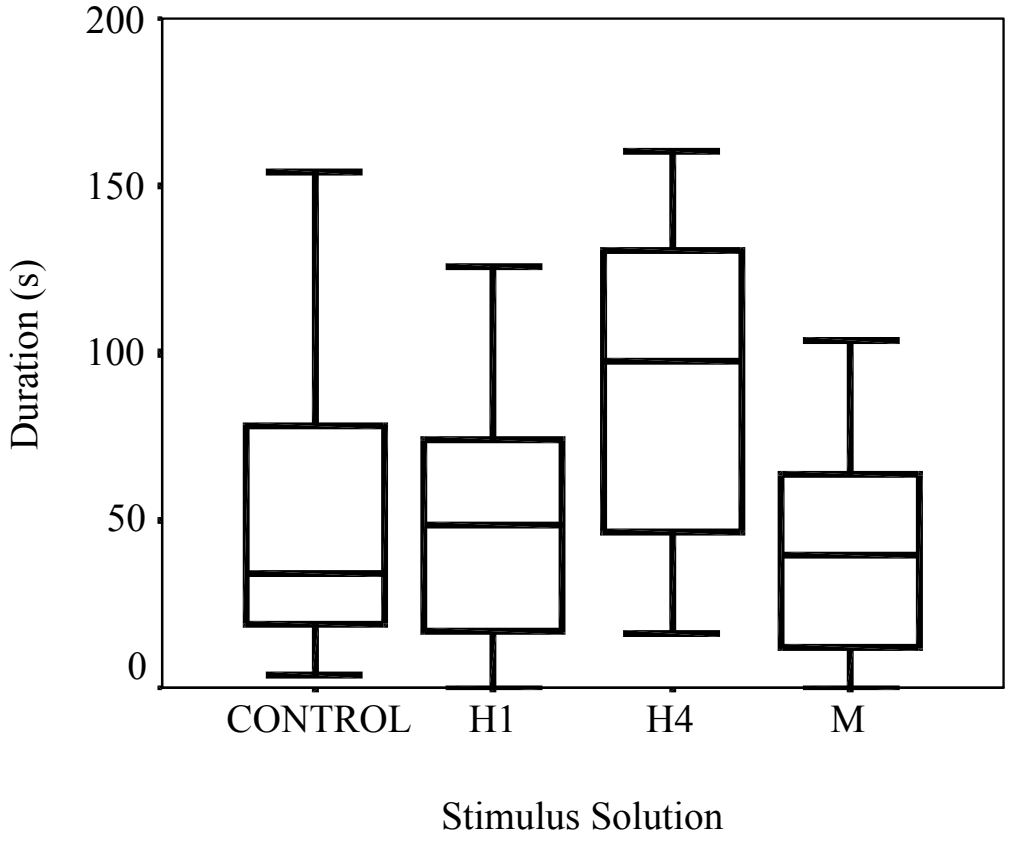


Figure 2.6. Median duration spent by *L. wurdemanni* within each stimulus zone. Bars represent 25th, 50th (median) and 75th percentiles. Whiskers represent upper and lower extremes. Stimulus solutions were collected from males (M), and hermaphrodites with vitellogenic oocytes within the ovary (H4) and without oocytes (H1).

(multiple Wilcoxon signed-ranks test, N=14, P=0.035). All other comparisons were very non-significant (P>0.40 in each case).

Eleven of the 14 shrimp physically manipulated the plastic nozzles used for solution entry, with the vast majority of manipulations directed towards the H4 stimulus (Fig. 2.7). Poisson tests indicated that the responses performed towards the control, M and H1 stimuli all fell within the same distribution (P>0.60 for all three cases). Responses performed towards the H4 stimulus, however, lay outside a Poisson distribution and were therefore significantly different (Poisson test, P=0.0189)

DISCUSSION

The results of this study demonstrate that *L. wurdemanni* can use chemical information to discriminate among different reproductive condition. Individuals tested in this experiment spent a greater amount of time in the vicinity of the H4 stimulus than in either the M or H1 zones. In addition, almost all of the physical manipulations performed by individuals were directed towards the H4 nozzle. Since mating behaviour in *L. wurdemanni* involves the manipulation of a freshly moulted individual by the intermoult mating partner, the physical interactions with the nozzle suggest that test individuals chemically viewed this structure as a potential mate.

Other species of decapod crustaceans have long been known to use chemical cues to distinguish the gender of conspecifics. Ameyaw-Akumfi and Hazlett (1975) observed that male *P. clarkii* could recognize sex in the absence of visual or physical contact. In that study, the behaviour of test animals were observed following the presentation of

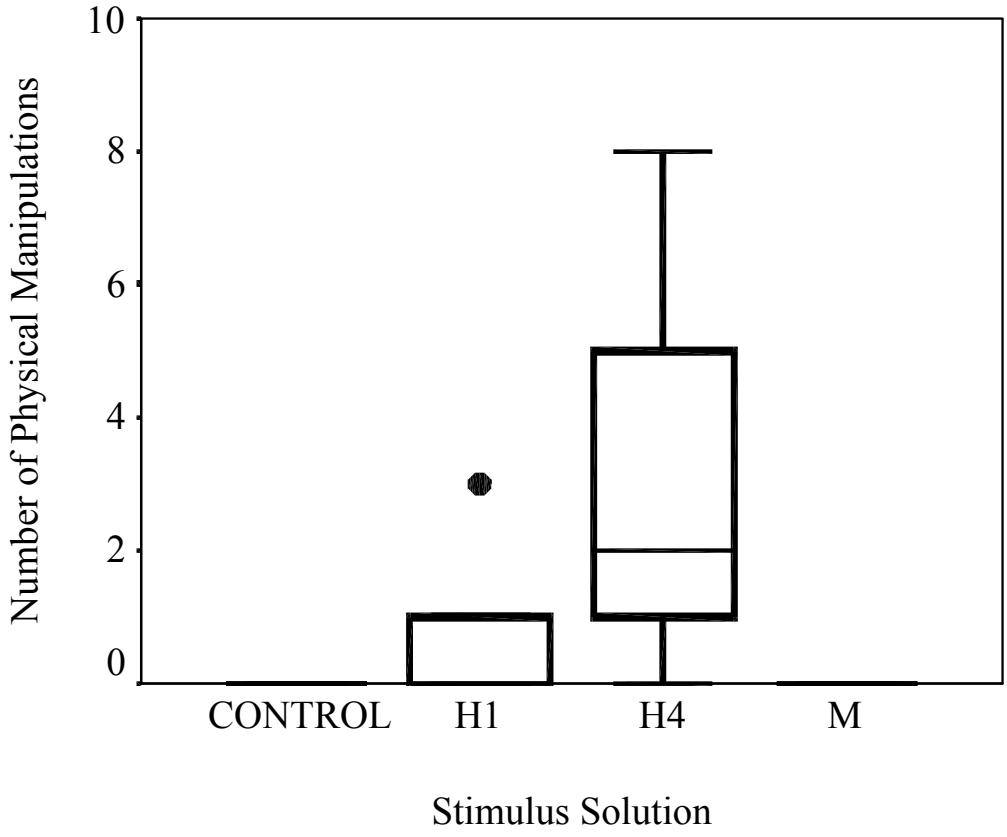


Figure 2.7. Median number of physical manipulations performed by *L. wurdemanni* towards plastic nozzles. Bars represent 25th, 50th (median) and 75th percentiles. Whiskers represent upper and lower adjacent values. Black circle indicates outlier. Stimulus solutions were collected from males (M), and hermaphrodites with vitellogenic oocytes within the ovary (H4) and without oocytes (H1).

stimulus animals located within opaque perforated plastic containers. When males were in the stimulus container, test individuals maintained postures typical of agonistic behaviours. The presentation of female stimulus animals, however, elicited submissive actions.

Hazlett (1985) found that male crayfish (*Orconectes virilis*) reacted differently to water conditioned by males vs. females. Observed animals generally raised their chelipeds and cephalothorax when presented with conditioned water, but maintained this posture for a longer duration when the stimulus solutions were conditioned by males. Interestingly, female *O. virilis* did not appear to distinguish between male and female stimuli.

Differential responses to male and female stimuli were also observed in the snapping shrimp *Alpheus heterochaelis* (Hughes 1996). No responses were detected when only chemical stimuli were presented. However, the presentation of shrimp-conditioned water in association with a visual stimulus (a model of an open chela), resulted in the performance of open chela displays by test animals. Male *A. heterochaelis* performed significantly fewer displays when the chela model was paired with female stimulus solutions than when stimulus solutions were collected from males. No significant difference was reported for females.

Similarly, Sneddon et al. (1999) found that agonistic interactions between paired males of shore crabs (*Carcinus maenus*), were longer in duration when female-conditioned water was concurrently pumped into the testing arena, than when male- or control solutions were used.

The differential responses observed in the studies described above are likely the result of sex pheromones released by female crustaceans. In addition to sex recognition, these pheromones appear to suppress male aggression and initiate the performance of courtship and mating behaviours. Water collected from tanks of recently-moulted female *H. americanus*, for example, induced courtship displays by males, whereas moulted-male conditioned water triggered both aggressive and feeding patterns (Atema & Engstrom 1971). The same pattern of behaviours was observed when animals were allowed to interact physically. Atema and Engstrom (1971) further demonstrated the importance of chemical cues in the reproductive behaviour of *H. americanus* by siphoning conditioned water into tanks containing intermoult females. Introduced males were initially aggressive, but soon began to court the non-receptive females.

Bamber and Naylor (1997) observed that mate guarding behaviours were initiated by male shore crabs (*C. maenus*) following exposure to female-conditioned seawater. Similarly, Gleeson (1980) found that urine from pubertal-females elicited courtship behaviours by male *C. sapidus*. Visual information was found not to be necessary for mating in this species, as the male located and cradle-carried females even in complete darkness.

Results similar to those observed in the current study were reported by Stebbing et al. (2003). Signal crayfish (*Pacifastacus leniusculus*) were exposed to one of three solutions injected through an airstone—water conditioned by mature females or immature females, or a freshwater control. Male crayfish presented with the mature

female stimulus were more active and also seized and mounted the airstone. Males exposed to control or immature female solutions did not perform these activities.

The results of this study suggest that receptive *L. wurdemanni* release a sex pheromone that initiates reproductive behaviours in intermoult individuals. Several studies using other decapod crustaceans have indicated that this pheromone is released in urine (e.g.McLeese et al. 1977; Christofferson 1978; Gleeson 1980; Atema & Cowan 1986), but the source of this pheromone is still uncertain. Although Ryan (1966) and Christofferson (1974) showed that blockage of the antennal gland of pre-moult female *Portunus sanguinolentus* crabs reduced both the flow of urine and subsequent attractiveness to males, other studies (e.g.McLeese et al. 1977; Bamber & Naylor 1997; Bushmann 1999) indicate that sources other than urine may also elicit sexual responses and successful pairings.

The chemical nature of any sex pheromone has also to be established. Kitteredge et al. (1971) reported that crustecdysone, the crustacean moulting hormone, induced precopulatory behaviour. Rudd and Warren (1976) examined responses of rock lobsters *Jasus lalandii* to crustecdysone, and observed the occurrence of behaviours similar to that performed towards conditioned stimulus solutions. The methodologies used in these two studies were criticized by Dunham (1978), and subsequent experiments by Gagosian and Atema (1973), Gleeson et al. (1984) and Seiphert (1982) were unable to demonstrate any sexual response to crustecdysone or structurally related compounds.

The responses of intermoult *L. wurdemanni* in this study indicate that crustecdysone does not serve as a sex pheromone in this species, and that the site of production for a

pheromone may be the developing ovary. If crustecdysone were used as a sex pheromone, the individual shrimp tested would have responded equally to each of the solutions conditioned by freshly moulted shrimp. Instead, shrimp responded almost exclusively to the solutions collected from shrimp with vitellogenic oocytes completely filling the ovary. McLeese et al. (1977) examined the behavioural responses of *H. americanus* to tissue extracts and concluded that the sex pheromone in lobsters originates in the ovary. Similarly, Jones and Hartnoll (1997) found a correlation between ripe ovaries and attractiveness in the spider crab *Inachus dorsettensis*. Those results, in addition to the responses of *L. wurdemanni* in this study, indicate that future research on the source of crustacean sex pheromone should focus on biochemical products produced or stored by the developing ovary.

CHAPTER III

THE POTENTIAL ROLE OF METHYL FARNESOATE IN THE PERFORMANCE OF REPRODUCTIVE BEHAVIOUR BY *L. WURDEMANNI*

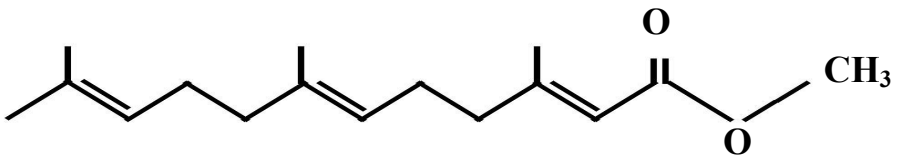
INTRODUCTION

Chemical stimuli are known to be important in the mating behaviour of many crustacean species (Dunham 1978). The shrimp *Lysmata wurdemanni* was observed to discriminate among reproductive conditions using chemical cues, and performed mating behaviours towards stimulus sources collected from female-phase hermaphrodites with vitellogenic oocytes present in the ovary (Chapter II). This finding, along with similar observations in other decapod species (e.g. McLeese et al. 1977; Jones & Hartnoll 1997), suggested that sex pheromones are likely produced or stored within the ovary, and excreted in urine.

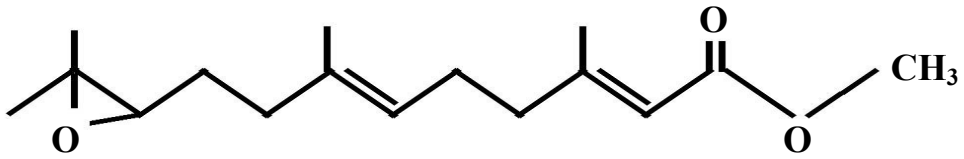
Previous studies have examined the use of crustecdysone in crustacean reproductive behaviour. This hormone (also known as β-ecdysone, 10-hydroxyecdysone, ecdysterone, and isoinokosterone) is known to accumulate in ovaries during ovarian development (Chaix & De Reggi 1982; Borst et al. 1987; Spindler et al. 1987; Wilder et al. 1990; Wilder et al. 1991), and was initially identified as a sex pheromone (Kittredge et al. 1971; Rudd & Warren 1976). Subsequent studies, however, were unable to demonstrate any sexual response to this hormone (Gagosian & Atema 1973; Seifert 1982; Gleeson et al. 1984).

Another potential candidate is methyl farnesoate (MF), the unepoxidated form of insect juvenile hormone III (JH III) (Fig. 3.1). MF was first identified in the haemolymph of the spider crab, *Libinia emarginata* (Laufer et al. 1987), but has since been identified in 33 other crustacean species (reviewed in Laufer & Biggers (Laufer & Biggers 2001), Olmstead & LeBlanc (2002)). MF is synthesized by the mandibular organ (MO) (Laufer et al. 1987; Landau et al. 1989; Tsukimura & Borst 1992), and negative regulation of MF production by MOs is performed by mandibular organ inhibitory peptides (MOIH) (Borst et al. 2001) This neuropeptide hormone is itself produced by the X-organ sinus gland complex found in the eyestalk.

Several functions have been attributed to MF, including regulation of metamorphosis, reproduction and behaviour. These functions are similar to those of JH III in insects (Laufer & Borst 1983). Abdu et al. (1998) found that MF altered larval development in the shrimp *Macrobrachium rosenbergii*. Larvae fed MF-enriched *Artemia* developed a smaller carapace than controls, had slower development, and had significantly altered the pattern of larval metamorphosis (i.e. production of "intermediate" forms). In male crustaceans, MF has been found to stimulate testicular growth (Kalavathy et al. 1999), and appears to be involved in male morphogenesis of *L. emarginata*. Males of this species can be classified into 6 morphotypes based on chela size, carapace size and epicuticle condition (Laufer & Ahl 1995). Only 3 of these morphotypes have been observed to mate when presented with receptive females (Sagi et al. 1994); these three forms also have the highest levels of circulating MF in their



Methyl Farnesoate



Juvenile Hormone III

Figure 3.1. Molecular structures of methyl farnesoate and juvenile hormone III

haemolymph (Laufer & Ahl 1995). MF thus appears to affect both male morphology and behaviour in this species.

MF has also been implicated in the ovarian maturation of the crayfish *Procambarus clarkii* (Laufer et al. 1998; Rodriguez et al. 2002b), the crabs *Cancer pagurus* (Wainwright et al. 1996), *Oziotelphusa senex senex* (Reddy & Ramamurthi 1998), and *L. emarginata* (Jo et al. 1999). Trials with the shrimp *Penaeus vannamei* indicated that MF could be administered to enhance egg production and almost double larval production (Laufer et al. 1997). Natural oscillations in MF levels were also observed to correlate with vitellogenic cycles in the deep-sea Norway lobster, *Nephrops norvegicus* (Rotllant et al. 2001).

Recently, MF was also implicated in sex determination by *Daphnia magna* (Olmstead & LeBlanc 2002). Exposure to MF caused adult daphnids to switch from parthenogenetically producing female broods to the production of males.

This study was designed to examine the reproductive responses of *L. wurdemanni* to various concentrations of MF. Since the presence and circulating concentration of this hormone has yet to be identified in this shrimp species, test individuals were exposed to a variety of MF concentrations that covered the range of circulating levels reported in the literature thus far (Table 3.1).

GENERAL METHODS

The shrimp used in this experiment were originally collected for use in other studies on the reproductive behaviour of *L. wurdemanni*. The location and methods used for

Table 3.1 Methyl farnesoate levels found in the haemolymph of decapod crustaceans

Species	Common Name	ng MF/mL haemolymph	Reference
Macrobrachium rosenbergii	Freshwater shrimp	17.3 - 24.0	Sagi et al. (1991)
Macrobrachium rosenbergii	Freshwater shrimp	4.3 - 8.0	Wilder et al. (1995)
Scylla serrata	Mud crab	2.10	Tobe et al. (1989)
Oziotelphusa senex senex	Freshwater crab	2.0	Kalavathy et al. (1999)
Cherax quadricarinatus	Crayfish	5.1	Abdu et al. (2001)
Procambarus clarkii	Red swamp crayfish	0.60 – 1.28	Laufer et al. (1998)
Homarus americanus	American lobster	0.4 – 0.8	Tsukimura & Borst (1992)
Nephrops norvegicus	Norway lobster	0.604 - 0.856	Rotllant et al. (2001)
Libinia emarginata	Spider crab	11-25	Borst et al. (1987)
Libinia emarginata	Spider crab	<0.04 - 104	Borst et al. (1987)
Libinia emarginata	Spider crab	15.8 – 40.3	Laufer & Ahl (1995)
Libinia emarginata	Spider crab	0.31 – 1.15	Jo et al. (1999)
Libinia emarginata	Spider crab	0.17 - 2.23	Rotllant et al. (2000)

collection, as well as subsequent housing conditions, were described in Chapter II. Shrimp were maintained in community tanks under similar temperature, salinity, density and feeding regimes until their use in this experiment.

Since methyl farnesoate (MF) is insoluble in water, this hormone is first dissolved in methanol before use in aqueous solutions (Duncan MacKenzie, pers. comm). Two experiments were conducted in this study. The first examined responses of *L. wurdemanni* to various concentrations of methanol and was used to determine the point at which individual shrimp would respond to methanol. The second experiment examined the performance of reproductive behaviours following the presentation of methanol-MF test solutions. Since normal distributions were found for all the results obtained, parametric statistics were used for analyses.

EXPERIMENT 1: RESPONSES TO METHANOL

Methods

Five methanol solutions (10%, 1%, 0.1%, 0.01% and 0.001%) were created by mixing 1 mL, 0.1 mL, 0.01 mL, 0.001 mL, or 0.0001 mL of methanol with 10 mL of freshly mixed seawater (SW). The same reservoir of 33‰ SW was used to create all solutions used in this study, and was also used in all testing tanks. Salinity was checked with a refractometer that had been calibrated with reverse-osmosis water.

The apparatus used in this experiment was modified from Chapter II. A small aquarium measuring 30 (l) x 20 (w) cm in area, lined with 1 cm of coral gravel and filled to a water depth of 15 cm was used to test shrimp (Fig. 3.2). There was no filtration or

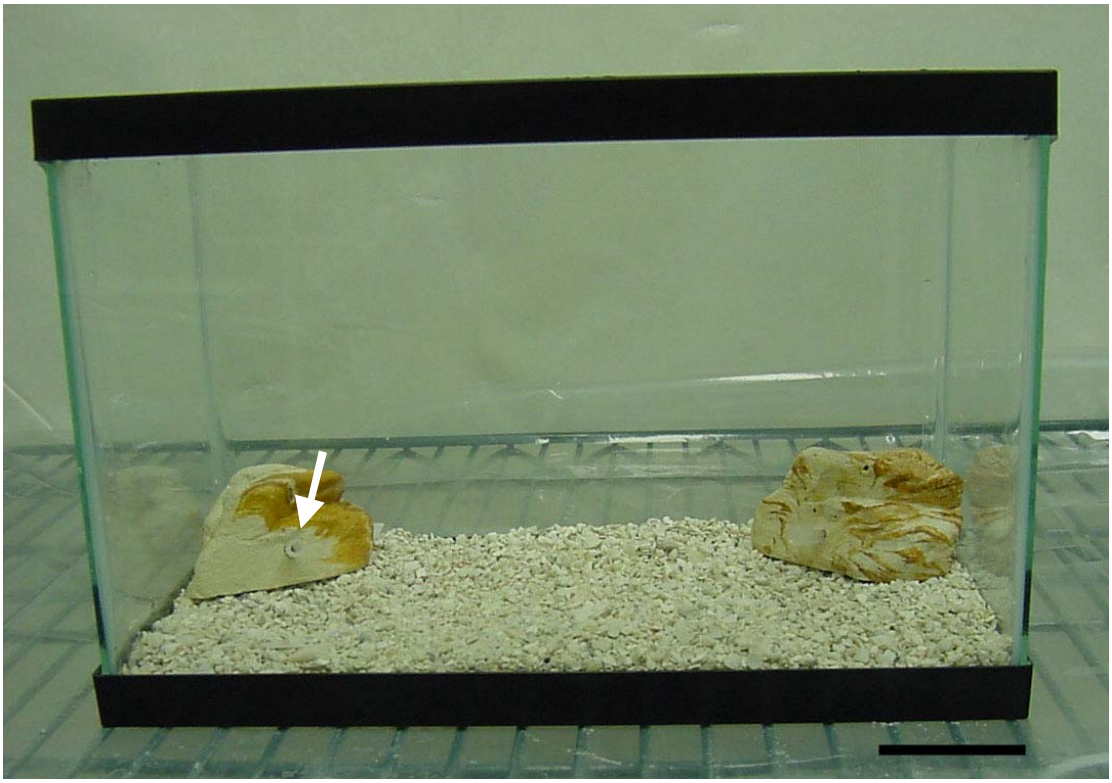


Figure 3.2. Testing tank used to examine responses to methanol solutions. Solutions were pumped into the aquarium through Tygon tubing (not shown) that was anchored within each rock by a plastic nozzle (indicated with arrow). Scale bar represents 5 cm.

aeration of the testing tank. A moulded aquarium rock was placed in each corner at the rear of the testing tank. Each rock had a hole drilled through the widest section. Equal lengths of Tygon tubing were threaded through the holes, and were anchored in place with plastic nozzles. Tubing extended out of the tank to a Haake-Buchler polystaltic pump that was used to pump solutions for 4 min at a known rate of 2.0± 0.2 mL/min. Solutions were pumped through only one of the rocks for each replicate, and this was balanced over the course of the experiment.

The responses of five intermoult, non-gravid shrimp were examined. Each shrimp was placed individually in the aquarium and allowed to habituate for 1h before testing. Methanol solutions were presented from lowest to highest concentration, and were both preceded and followed by SW control solutions (i.e. SW control, 0.001%, 0.01%, 0.1%, 1%, 10%, SW control). After testing, each shrimp was returned to a community aquarium. Individuals were used only once. The aquarium and rocks were rinsed thoroughly following testing. The Tygon tubing was flushed several times with distilled water and air was then pumped through each length. Coral gravel was rinsed and autoclaved between uses.

The frequency of four behavioural responses performed following the presentation of each stimulus and control solution was examined:

Antennal waving: the long bifurcated antennules and/or single-branched antennae are slowly "waved" upwards and down. The distal half of the antennule or antenna may be flexed. Schmitt (1979) found that the rapid movements of antennules by the lobster

Panulirus argus separated chemosensory aesthetasc hairs and enhanced the response of olfactory receptors.

Touching Nozzle: physical contact occurs between the nozzle and any part of the test shrimp.

Nozzle Manipulation: *L. wurdemanni* physically manipulates the plastic nozzle. The first two pairs of pereiopods were typically used to grasp and pick at the nozzle. Pereiopods were sometimes inserted into the hollow centre of the nozzle. Manipulation of the nozzle was used as an indicator of reproductive behaviour in Chapter II.

Scramble: rapid "grabbing" action performed by the first pair of pereiopods, and directed towards the solution stream being pumped into the tank. There was no contact with the plastic nozzle.

Results

No significant overall difference was observed in the frequency of Antennal Waving (Fig. 3.3; Friedman's test, P=0.763). Multiple comparison tests found no significant pairwise comparisons among the different methanol concentrations.

Touching Nozzle activity was also non-significant (Fig. 3.4; Friedman's test, P=0.231). No significant pairwise differences were detected.

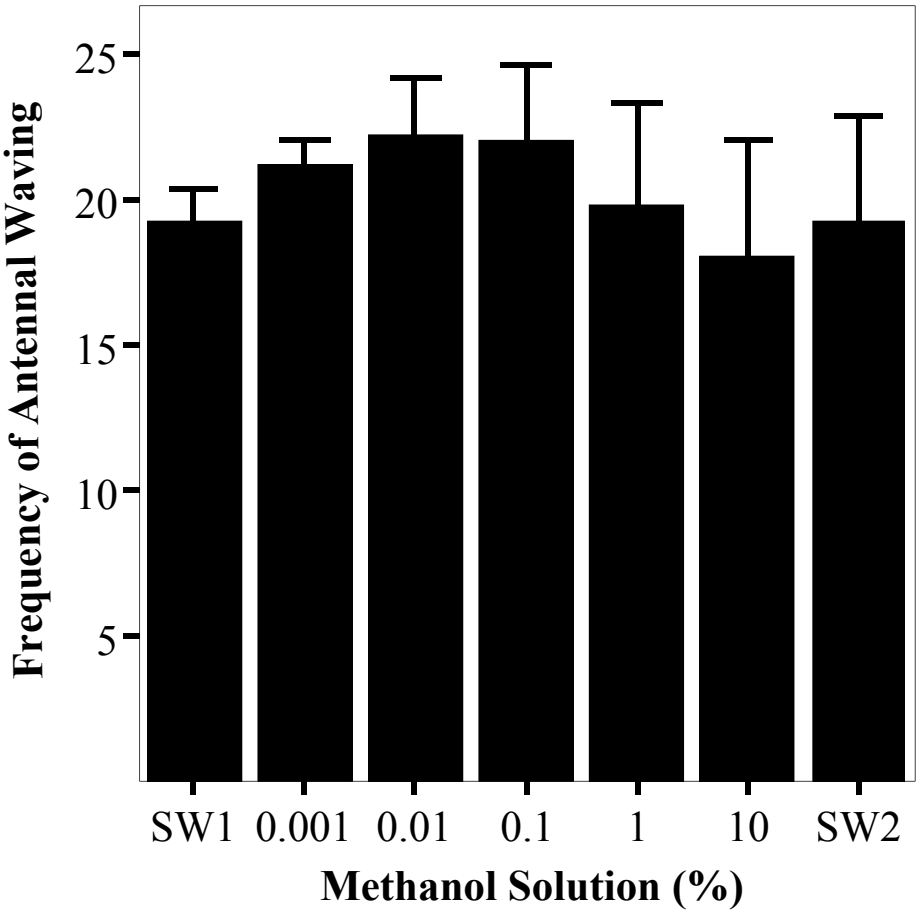


Figure 3.3. Frequency of Antennal Waving (mean ± SE) performed to various methanol solutions. Seawater controls (SW1 and SW2) were presented both before and after a graded series of methanol-seawater solutions.

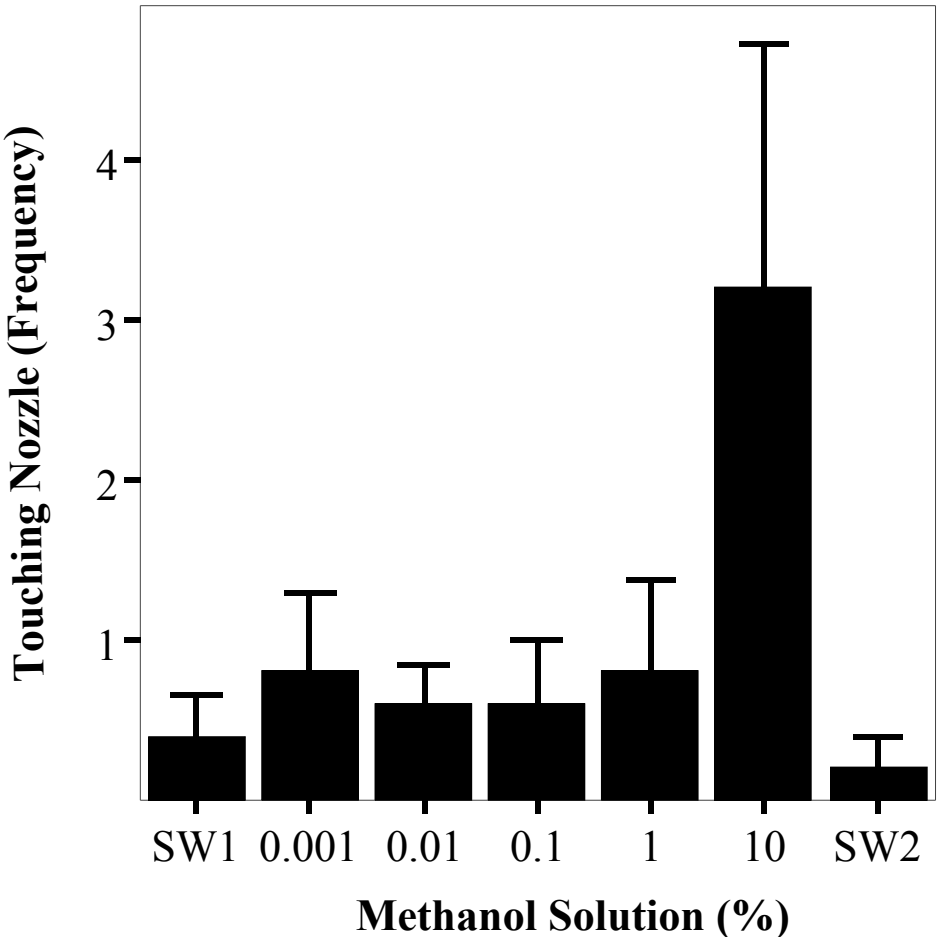


Figure 3.4. Frequency of Touching Nozzle (mean ± SE) performed to various methanol solutions. Seawater controls (SW1 and SW2) were presented both before and after a graded series of methanol-seawater solutions.

A significant overall difference was found in the performance of Nozzle Manipulation (Fig. 3.5; Friedman's test, P=0.033). However, no significant differences were observed in any of the pairwise comparisons examined. The mean and standard error was identical for both the 0.001% and 0.01% test solutions, suggesting that the 0.01% solution may be the point at which shrimp increase their responses to methanol presentations.

An extremely significant overall difference was observed in the frequency of Scramble behaviour (Fig. 3.6; Friedman's test, P<0.0001). This was not surprising, as Scrambling only occurred after presentation of the 10% methanol solution. As a result, Dunn's multiple comparisons tests found highly significant differences between the 10% solution and all other methanol or control solutions (P<0.01 in all cases).

EXPERIMENT 2: RESPONSES TO METHYL FARNESOATE

Methods

Methyl farnesoate was purchased from Echelon Biosciences Inc. (Salt Lake City, Utah). A stock solution was created by dissolving the hormone in methanol at a ratio of 1mg MF to 1 methanol. A serial dilution was used to create the MF test solutions used in this study. 20 μL of the stock solution was added to 180 mL of freshly mixed 33‰ seawater, creating a test solution with a MF concentration of 1000 ng/mL. A tenfold dilution—adding 20 mL of this dilute MF solution to 180 mL of SW—was used to make a 100 ng/mL MF test solution. This procedure was repeated to formulate 10 ng/mL,

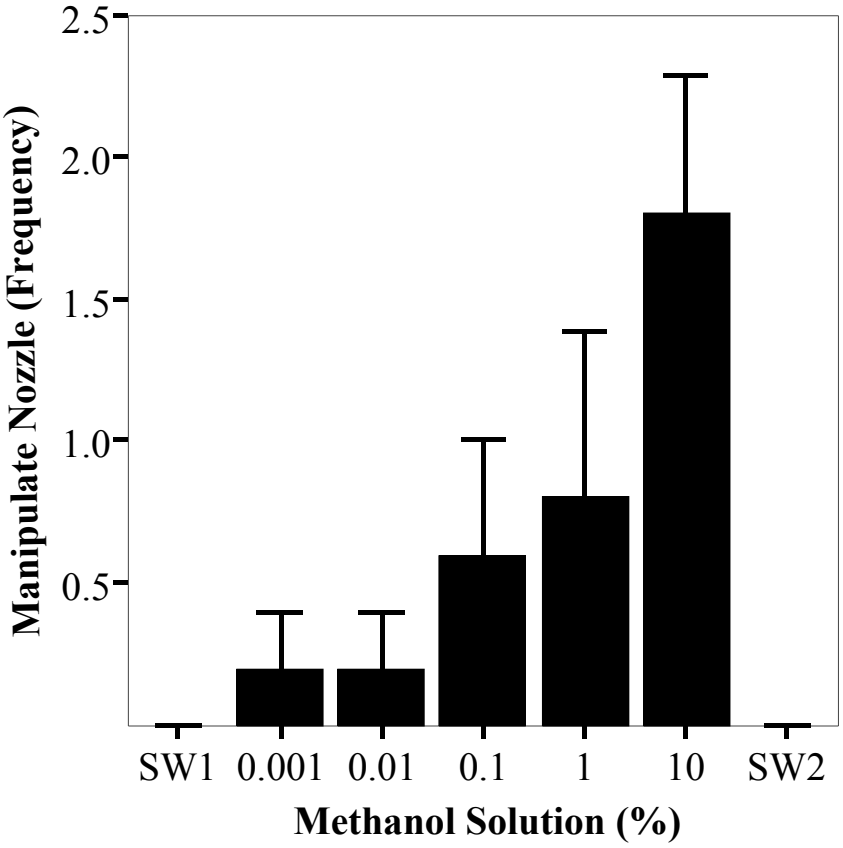


Figure 3.5. Frequency of Manipulating Nozzle (mean ± SE) performed to various methanol solutions. Seawater controls (SW1 and SW2) were presented both before and after a graded series of methanol-seawater solutions.

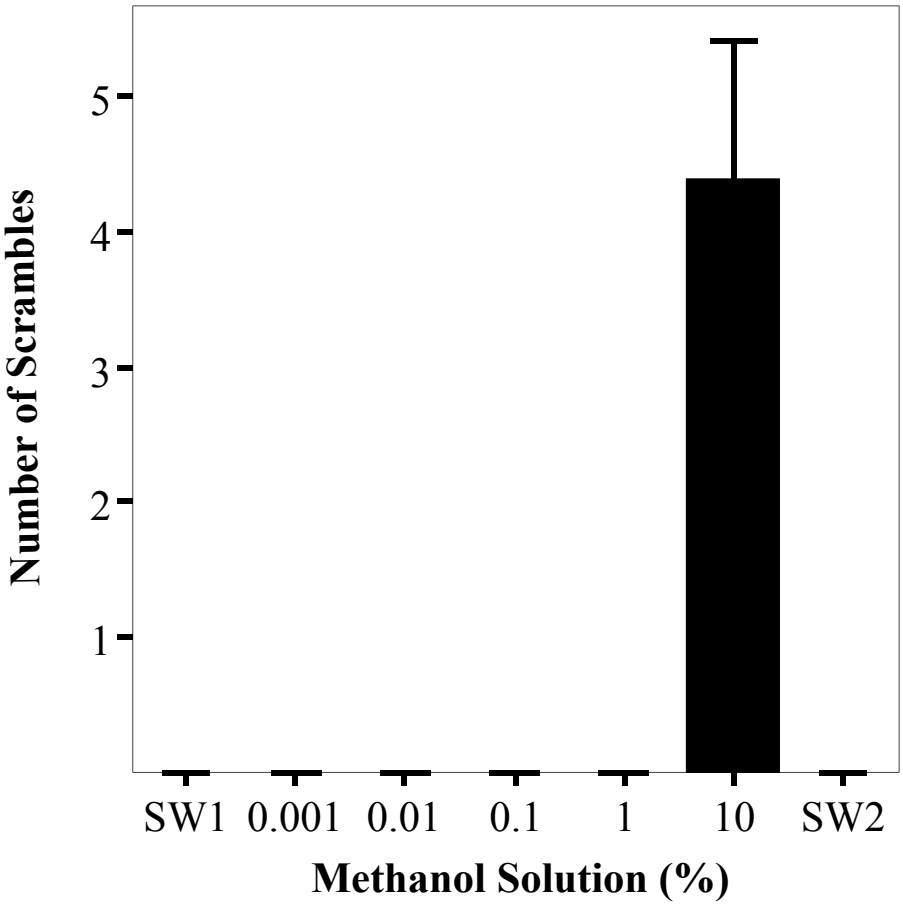


Figure 3.6. Frequency of Scrambles (mean ± SE) performed to various methanol solutions. Seawater controls (SW1 and SW2) were presented both before and after a graded series of methanol-seawater solutions.

1 ng/mL and 0.1 ng/mL test solutions. The same process, without the addition of MF, was used to create methanol control solutions. Each solution was stored in 14 mL Falcon® tubes and frozen at –80°C until use.

Shrimp responses were examined using an aquarium setup identical to that used in Experiment 1, with methanol and MF solutions pumped into the testing tank simultaneously. The corners from which the stimulus and control solutions entered the tank were randomized, but balanced over the course of the experiment.

A single intermoult non-gravid shrimp was placed in the aquarium and allowed to habituate for 1h. A SW control solution was initially pumped through both lengths of tubing. This was followed by control and MF test solutions from lowest to highest concentration (i.e. 0.1 ng/mL, 1.0 ng/mL, 10 ng/mL, 100 ng/mL, 1000 ng/mL). A second SW control solution was then pumped into each tank. Each solution was pumped into the testing aquarium for 4 minutes. After testing, each shrimp was returned to a community aquarium. Individuals were used only once. The aquarium and rocks were rinsed thoroughly following testing. The Tygon tubing was flushed several times with distilled water and air was then pumped through each length. Coral gravel was rinsed and autoclaved between uses.

The results obtained in Experiment 1 indicated that Nozzle Manipulation and Scramble actions were the most indicative behaviours. These two activities were thus examined in this experiment.

Results

Table 3.2 lists the results obtained for Experiment 2. Neither Nozzle Manipulation nor Scramble behaviours were performed to any of the methanol-MF, or SW control solutions.

DISCUSSION

This study was designed to determine if methyl farnesoate, a crustacean hormone implicated in ovarian maturation, influences the performance of reproductive behaviour in *L. wurdemanni*. The results of Experiment 2 suggest that this shrimp species does not use MF as a chemical cue when determining the reproductive state of conspecifics.

There are several possible explanations for the observed result. It is possible that the concentration of MF may have been too low for *L. wurdemanni* to detect. This seems improbable, however, as the different concentrations used in this study cover the wide range of MF concentrations that have been naturally found in decapod haemolymph thus far (Table 3.1). Although not yet proven, it is also likely that MF circulates within the haemolymph of *L. wurdemanni*, since it has been discovered in every decapod species in which this hormone's presence has been examined (Laufer, pers. comm). A collaborative effort currently underway with Hans Laufer (University of Connecticut) should determine the presence or absence of MF in *L. wurdemanni*, as well as potential changes associated with ovarian development.

It is also unlikely that methanol would have interfered with the detection of MF. In Experiment 1, high methanol concentrations elicited large responses of Nozzle

Table 3.2. Frequency of Nozzle Manipulation and Scramble behaviours performed by *L. wurdemanni* following presentation of seawater (SW) control and methanol-methyl farnesoate (MF) solutions

Solution (ng MF/mL SW)	**Frequency of Nozzle Manipulation**	**Frequency of Scramble**
SW Control	0	0
0.1	0	0
1.0	0	0
10	0	0
100	0	0
1000	0	0
SW Control	0	0

Manipulation and Scramble behaviours, and Nozzle Manipulations were also performed at lower concentrations. It is unclear why behavioural responses were performed towards the higher levels of methanol. The lower concentrations used in the methanol solutions (i.e. 0.001 mL methanol/10 ml SW and 0.0001 mL methanol/10 ml SW), however, are among the higher concentrations used to create the methanol-MF solutions used in Experiment 2. Any responses performed to these test solutions would therefore have been a response to the addition of the MF hormone.

Although MF has been found in at least 33 crustacean species thus far (Laufer & Biggers 2001; Olmstead & LeBlanc 2002) this hormone may play different roles in the reproductive behaviour of different taxa. Laufer et al. (1998) and Rodriguez et al. (2002a; 2002b) both observed that the administration of MF to the crayfish *P. clarkii* stimulated ovarian growth and resulted in the maturation of a greater number of oocytes. In contrast, Abdu et al (2001) observed that the injection of MF into mature female *Cherax quadricarinatus* crayfish did not enhance spawning, but instead accelerated moulting. Similarly, MF levels in the lobster, *Homarus americanus*, were found to be lowest when ovarian maturation was in progress, suggesting that MF plays no role in ovarian maturation in this species (Tsukimura & Borst 1992). It was hypothesized that these differing observations were the result of species-specific physiological differences (Abdu et al. 2001).

The experimental design used in this study used solutions composed of relatively pure MF. Since natural solutions used by *L. wurdemanni* and other decapods are a combination of stimuli, the solutions used here and in other studies (e.g. Gleeson et al.

1984) are extreme oversimplications. Previous studies examining chemical cues suggested that crustecdysone was used as a sex pheromone (Kittredge et al. 1971; Rudd & Warren 1976), although subsequent experiments failed to find any sexual response to this hormone or related compounds (Gagosian & Atema 1973; Seifert 1982). Gleeson et al. (1984) presented blue crabs, *Callinectes sapidus*, with crustecdysone and urine stimuli, and found that the test animals responded to conspecific urine, but did not perform courtship responses when crustecdysone alone was presented.

No study as yet has tested crustacean responses to a combination of MF and crustecdysone. Ecdysteroids, the steroidal group to which crustecdysone belongs, are known to control moulting in a large number of insect and crustacean species (reviewed in Skinner (1985)). Fluctuations in crustecdysone levels are associated with moult stages, with the highest concentrations observed shortly before moulting. Although crustecdysone is accumulated in the ovaries during development (Chaix & De Reggi 1982), it is not clear that they are necessarily involved in ovarian development. The ablation of the Y-organ (where ecdysteroid synthesis occurs) prevented vitellogenesis in the isopods *Porcellio dilatatus* and *Armadillidium vulgare* (Besse & Maissiat 1971; Meusy et al. 1977; Suzuki 1986). Similarly, oocyte maturation in the shrimp *Palaemon serratus* was found to be correlated with the accumulation of ecdysteroids in oocytes during the premoult stages (Lanot & Cledon 1989). However, the administration of crustecdysone had no positive effect on vitellogenesis in the amphipod *Orchestia gammarella* (Blanchet et al. 1975), and actually inhibited the onset of vitellogenesis in *Lysmata seticaudata* (Touir & Charniau 1974). Recently, Okumura & Katsumi (2000)

found that ecdysteroid levels fluctuated with moult cycle stages in *M. rosenbergii*, but did not observe any differences between reproductive and non-reproductive moult cycles. This suggests that ecdysteroids are involved in moulting, but not in ovarian development.

A crustecdysone-MF combination, if used as a reproductive pheromone, would provide individual *L. wurdemanni* with a great deal of information about potential mating partners. An evaluation of crustecdysone levels could reveal how close a conspecific was to moulting, and MF concentrations could reveal the state of ovarian development. High levels of crustecdysone coupled with low levels of MF would suggest that a conspecific was soon to enter ecdysis, but would not be an appropriate mating partner, as oocytes are likely not present in the ovary. High levels of both MF and crustecdysone would indicate an individual with many oocytes was soon to moult. This identification could initiate the subsequent performance of reproductive tactics that increase the chances of successful mating by intermoult individuals. Further study is needed to examine this possibility.

CHAPTER IV

PRE- AND POST-COPULATORY MATING TACTICS PERFORMED BY *L. WURDEMANNI* UNDER DIFFERENT DENSITY CONDITIONS

INTRODUCTION

Sexual selection is the result of the competition that occurs among individuals for access to mates (Andersson 1994; Moller 1998). Since reproductive success is dependent upon the number of successful fertilizations obtained over a lifetime, individuals should perform behaviours that maximize mating opportunities. A classic study by Bateman (1948) demonstrated that males and females have differential investments in reproduction. While female fertility (number of offspring) is limited by the ability to produce eggs, a male's reproductive success is limited by the number of females inseminated nd eggs fertilized. Reproductive behaviours have been influenced by this intersexual conflict—males perform actions that increase the number of successful matings, and females preferentially select among potential mates.

Andersson (1994) outlined several mechanisms that are used in the competition over mates (outlined in Chapter I), including Scrambles and Sperm-Competition. In species which use a mating system involving scrambles, individuals quickly search for and locate potential mates. Wickler & Seibt (1981) proposed that this system ("pure searching") would be selected if males are able to encounter at least 2 females per day (Fig. 1 in Wickler & Seibt (1981)). Scrambling is common in many invertebrate species, such as the fiddler crab *Uca paradussumieri* (Murai et al. 2002), and is also found in a

number of vertebrates as well (e.g. Belding's ground squirrel *Spermophilus beldingi*) (Sherman 1989). In both of these species, males resume searching once copulation is completed.

Sperm competition occurs when there is a risk that a rival male may fertilize a female's eggs. To reduce the possibility of this occuring, individuals may perform mate guarding behaviours, as a male that guards a female before ovulation (pre-copulatory mate guarding) will increase greatly his chances of successful insemination and subsequent fertilization. This is particularly true of species in which females can mate with several males, but where a first-male advantage (i.e. the first mating partner fertilizes the greatest number of eggs) is not observed. In species where male mating partners can remove from females the ejaculates of prior mates, post-copulatory guarding is expected to occur. Grafen & Ridley (1983) proposed that strong mate guarding or permanent monogamy evolves when sex ratios are either equal or male biased, and when female receptivity is short, predictable, and identifiable. Similarly, Wickler & Seibt (1981) suggested that precopulatory mate guarding would be selected when female interspawn duration is greater than 3-4 days, and when males encounter up to 2 females per day.

Observations of pre- and post-copulatory mate guarding are common, and this phenomenon has been studied in a number of taxa, especially birds (reviewed by Andersson 1994; Black 1996; Radwan & Siva-Jothy 1996; Currie et al. 1998; Dunn 1998; Bateman & MacFadyen 1999; Tadler et al. 1999; Wada et al. 1999; Holdsworth & Morse 2000). Amongst crustaceans, mate guarding has been reported in the isopod

Idotea baltica (Wada et al. 1999), american lobster *Homarus americanus* (Atema 1986), blue crab C*allinectes sapidus* (Gleeson 1980; Jivoff & Anson 1998), snow crab *Chionoecetes opilio* (Rondeau & Sainte-Marie 2001), fiddler crab *Uca tetragonon* (Goshima et al. 1996), and the hermit crabs *Pagurus middendorfii* (Wada et al. 1999) and *P. filholi* (Minouchi & Goshima 1998). Mate guarding has also been observed in several caridean shrimp, such as the rock shrimp, *Rhyncocinetes typus* (Correa et al. 2000), painted shrimp *Hymenocera picta* (Wickler & Seibt 1981) and the snapping shrimp, *Alpheus angulatus* (Mathews 2002b).

The unique sexual system, reproductive cycle and morphological characteristics of the shrimp *Lysmata wurdemanni* provide support for both the occurrence and absence of mate guarding in this species. In crustaceans with mate guarding behaviours, males are typically larger than females, possess large chelipeds, and engage in aggressive interactions with conspecifics (e.g. Kittredge et al. 1971; Gleeson 1980; Atema & Cowan 1986; Jormalainen et al. 2000). In *L. wurdemanni*, larger individuals are hermaphroditic (Bauer & Holt 1998), and can mate as either gender (but see below). The chelae found in *L. wurdemanni* are not enlarged, and aggressive interactions are rarely observed. This morphological data suggests that mate guarding is not expected to occur.

However, the sexual system of *L. wurdemanni* suggests there should be a strong selective pressure for the performance of mate guarding behaviours. Like many decapod crustaceans, mating in *L. wurdemanni* is associated with moulting—individuals mate as "males" during their intermoult phase, while reproduction as a "female" occurs only

during a short period after moulting. Since *L. wurdemanni* does not exhibit synchronous moulting (pers. obs.), the number of "females" available at any point in time is fairly low. However, *L. wurdemanni* is capable of distinguishing reproductive condition using chemical cues (Chapter II), and can quickly identify individuals that are near ecdysis.

In addition, populations of *L. wurdemanni* also include small non-hermaphroditic individuals that possess functional testes. These shrimp, although incapable of mating as a female while at small size, are potential mating partners/competitors. The operational sex ratio (Emlen & Oring 1977) of this species is thus severely male-biased, as every intermoult individual and every small non-hermaphroditic *L. wurdemanni* is capable of mating with a recently moulted conspecific.

These observations meets criteria laid out for the evolution of mate guarding by both the Grafen & Ridley (1983) and Wickler & Seibt (1981) models. The extreme mating competition that potentially exists in this species suggests that mate guarding should occur, although this activity may not be as obvious as that observed in those species where mate guarding has already been well-documented (Grafen & Ridley 1983).

This experiment examines mating tactics performed by *L. wurdemanni* both immediately before and after a mating event. The occurrence of mate guarding or searching behaviours was also examined at two different population densities, to determine if the performance of these activities was dependent upon the number of competitors present.

METHODS

The collection and maintenance of *L. wurdemanni* used in this study is described in Chapter II. Shrimp were maintained under similar environmental and density conditions until use in this experiment. The same light cycle (12h L:12h D) was also used during testing.

Testing Tank

A plastic tank measuring 50 (l) x 38 (w) x 21 (h) cm was used to hold a testing enclosure constructed of white plastic mesh (Fig. 4.1). Three sides of the enclosure were perpendicular to the bottom. The fourth side was angled outwards at 45^{o} to prevent the formation of shadows. In a previous experiment (Chapter II), directed infrared light illumination was observed to create shadows extending the length of a testing tank. The base of the enclosure measured 25 (l) x 32 (w) cm, and increased such that area at the water surface was 32.5 (l) x 32 (w) cm..

Coral gravel lined the base of the enclosure to a depth of 1 cm. The outer plastic tank was filled with 33‰ seawater to a depth of 10.5 cm, and a water temperature of 28^{o}C was maintained through the use of an aquarium heater. Filtration and aeration were performed by an AquaClear power filter attached to the side of the plastic tank. Both the heater and filter overhang (outflow) were placed between the angled enclosure wall and the plastic tank. A plastic baffle placed in front of the filter outflow directed water downwards, and prevented disruptions of the water surface.

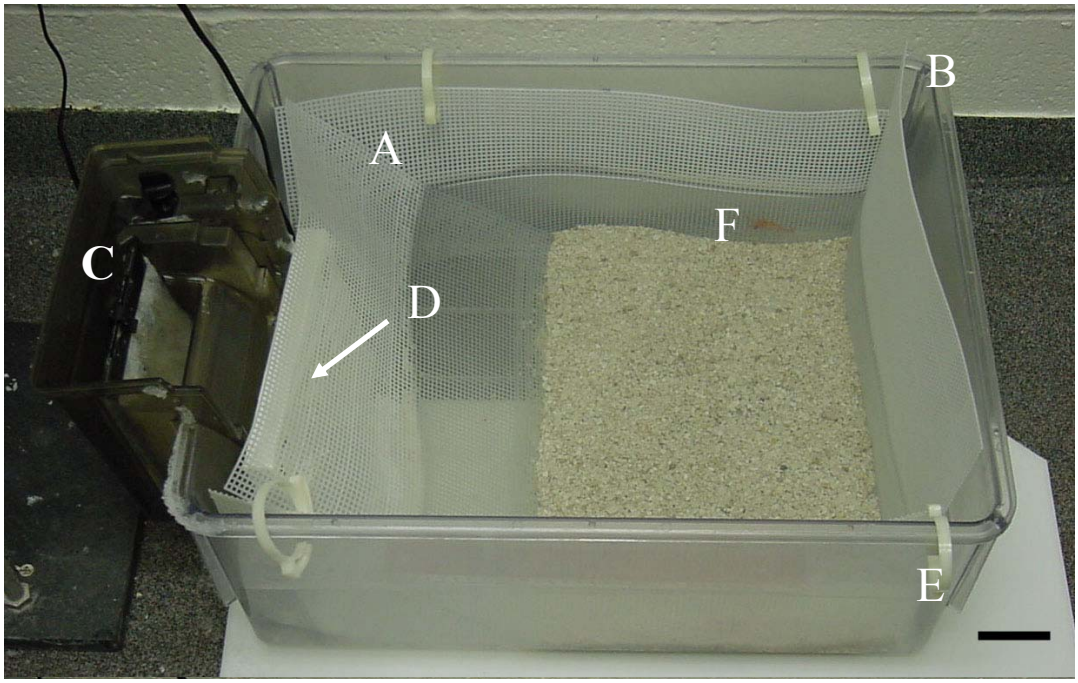


Figure 4.1. Testing tank used to examine mate guarding. A plastic enclosure (A) was placed within a larger plastic tank (B). A power filter (C) was used for filtration and aeration, and water outflow was directed downwards by a baffle (D). Plastic clips (E) were used to prevent upper enclosure walls from curling inwards. One *L. wurdemanni* is visible (F). Scale bar represents 5 cm.

Density & Identification

L. wurdemanni used in this experiment were selected from community tanks. A non-gravid hermaphroditic shrimp with green vitellogenic oocytes filling the ovary (H4, see Chapter II) was first collected and placed in a large glass dish filled with 33‰ seawater isothermic with testing tanks. A variable number of hermaphroditic non-vitellogenic conspecifics (H1, see Chapter II) were also collected and subsequently placed with the H4 shrimp. Each shrimp was then individually removed and placed upon a sheet of paper towel that had been soaked in seawater immediately before animal collection. Digital calipers were used to measure the carapace of each shrimp, from a point mid-way between the eyes to the junction with the abdomen. These measurements were used to size-match each H4 shrimp with either 1 or 5 H1 conspecifics (the two densities used in this study). Within any replicate, the H1 individuals used were within 6% of the carapace length measured for the H4 shrimp.

To aid in identification, a piece of reflective foil was attached to every H4 test individual (view videoclip Reflection.avi). While placed on the wet paper towel, the dorsal surface of the carapace was gently blotted dry and a piece of reflective foil attached with a drop of epoxy glue. After 10s of air drying, the shrimp was returned to the glass dish.

Immediately after measuring, the shrimp were gently placed within the testing enclosure. Over the course of the experiment, reverse-osmosis water was added to compensate for the effects of evaporation. Shrimp feeding schedule was identical to that used during housing. Individuals were used only once in this experiment.

Video Recording & Testing

Two video camcorders located above the tank and aligned with the angled wall of the testing enclosure, were used to film interactions among *L. wurdemanni* (Fig. 4.2). A Yashica KD-H170 camera was used to film during the "daylight" portion of the light cycle, while an infrared-sensitive Sony TR517 camcorder operated during the "night" An infrared LED (light emitting diode) array was positioned under the cameras and provided additional illumination of the testing area. Each camera was connected (using a splitter) to two VCRs located in another room. These four VCRs (Toshiba W604, W603; JVC HR-VP78U; Samsung 30405) were set to continuously record onto a 6h videotape during different portions of the light cycle. Two VCRs were thus used to record a video feed collected from the daytime camcorder, while the other VCRs recorded video feed from the night camera.

The presence of eggs was used to indicate that a shrimp had moulted and mated at some point during the previous 24h. Shrimp remained in the enclosure and were continuously videotaped until eggs were observed under the abdomen of the H4 individual. Video recording was then stopped, and all test individuals transferred to an aquarium. Gravid shrimp were checked over the next 48h, to see if eggs were discarded. The removal of egg masses was not observed in this study, suggesting that successful fertilization had occurred.

The tank, plastic enclosure, filter and heater were thoroughly rinsed following testing. Coral gravel was rinsed and autoclaved between uses.

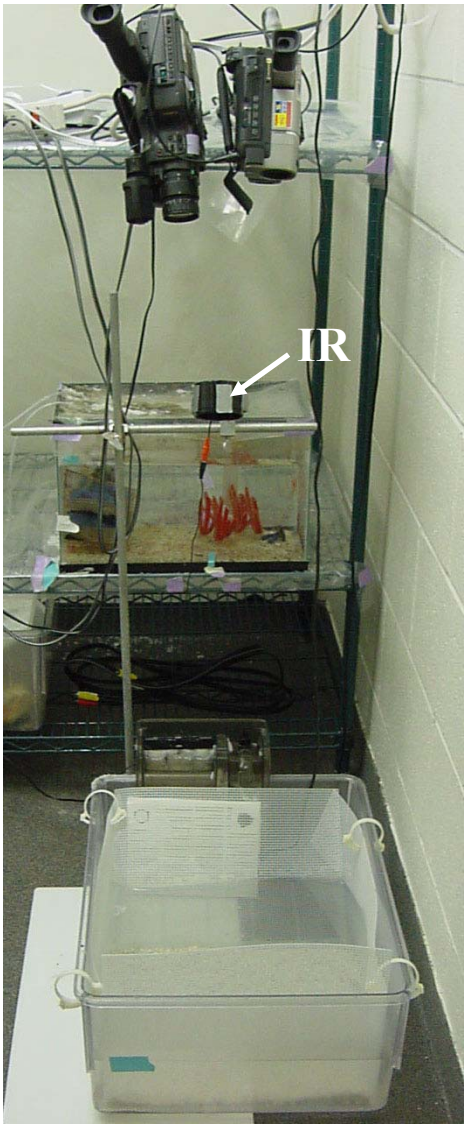


Figure 4.2. Placement of video equipment. Two camcorders were positioned above the testing tank. One camera was used for day recording, the other for night. An infrared LED array (IR) provided infrared illumination.

Data Collection

A PowerMacintosh G3 computer connected to a VCR (Toshiba W604 or a Panasonic AG1960) and running the Apple Video Player software was used to scan the last 24h of video recording for moulting and mating events. The latency from moulting to copulation was recorded, as was copulation duration (view videoclips MateLow.avi and MateHigh.avi). The software program JWatcher (version 0.9) operated concurrently on the computer was used to record specific behaviours for the 10 minute period immediately before mating ("precopulatory"), as well as for the 10 minutes subsequent to mating ("postcopulatory"):

H1 Approaches H4—H1 individuals face H4 shrimp and move towards them. This behaviour was recorded if approaching H1 shrimp moved to within 1 body length of the H4 individual.

H4 Approaches H1—H4 shrimp faces H1 individuals and moves towards them. This approach is characterised by slower locomotion, and may have brief periods of inactivity (stopping).

Following—H4 shrimp is closely followed by H1 individual(s) no more than 1 body length behind. H1 individuals will follow the exact path taken by H4 individuals, even when locomotion is rapid. The number of H1 individuals involved in a bout of

Following was also recorded (view videoclip Follow.avi). This behaviour has been observed in other shrimp species (e.g. Bauer 1996b).

Vicinity to H4—H1 shrimp are positioned within 1 body length of H4, and are either facing, or are lateral to the H4 individual. Shrimp may be moving slowly , or not at all. Excluded from this category are instances where shrimp pass each other within 1 body length of distance. The number of H1 individuals in close vicinity to the H4 was also recorded (view videoclip Vicinity.avi)

H1-H4 Contact—Physical contact between H1 and H4 individuals. Since *L. wurdemanni* have long antennae and antennules, individuals that had a quarter body-length or less between them were considered to be in physical contact.

H1-H1 Contact—Aggressive physical contact between H1 individuals. Since it was impossible to observe fine movements on the recorded video, contact was assumed to have occurred if one individual performed tailflips or other rapid avoidance actions while within 1 body length of another H1 conspecific. Rapid movement towards an H1 conspecific ("charging") was also included within this category.

H4 Escape—Rapid movement away from H1 conspecifics. This action can be accomplished through tailflips or through rapid swimming, and was typically observed following approaches or contact by H1 individuals (view videoclip H4Escape.avi).

RESULTS

Of the 10 replicates filmed for each density treatment, only 6 of each were ultimately analyzed. This reflects the time and effort required to locate a brief event (moulting and subsequent mating) in 24 hours of recording.

Copulations in *L. wurdemanni* occur shortly after moulting. A significant difference was observed in the timing of moulting, as 10 of 12 H4 shrimp performed ecdysis during the "night" portion of the light:dark cycle (χ^2 test, P=0.021).

No difference was observed in the latency to mating (Fig. 4.3; Mann-Whitney U test, P=0.914). In both the low and high density conditions, H1 individuals copulated with the H4 shrimp within 40s of moulting. In six replicates (evenly divided amongst high and low densities), mating occurred while the H4 individual was in the process of moulting, or occurred immediately afterward.

Copulation duration significantly differed under low and high density conditions (Fig. 4.4, Mann-Whitney U test, P=0.010). Copulation duration was much shorter when there were a greater number of H1 competitors present.

Pre- & Post-Copulatory Behaviours

No difference was found in the frequency of approaches performed by H1 shrimp towards the H4 in the 10 min before and after copulation under low densities (Fig. 4.5; Mann-Whitney U test, P=0.091). A very significant difference was observed in the high density condition, however, with a greater number of approaches performed in the post-copulatory period (Mann-Whitney U test, P=0.008).

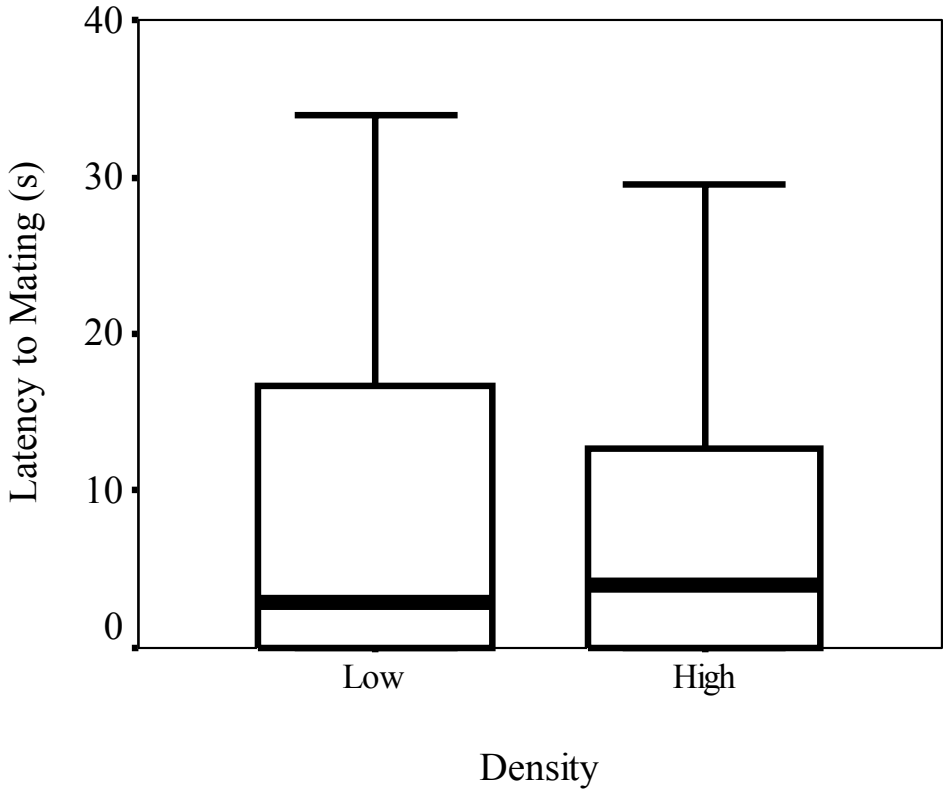


Figure 4.3. Latency to mating. Horizontal bars represent 25th, 50th (median) and 75th percentiles. Whiskers indicate range.

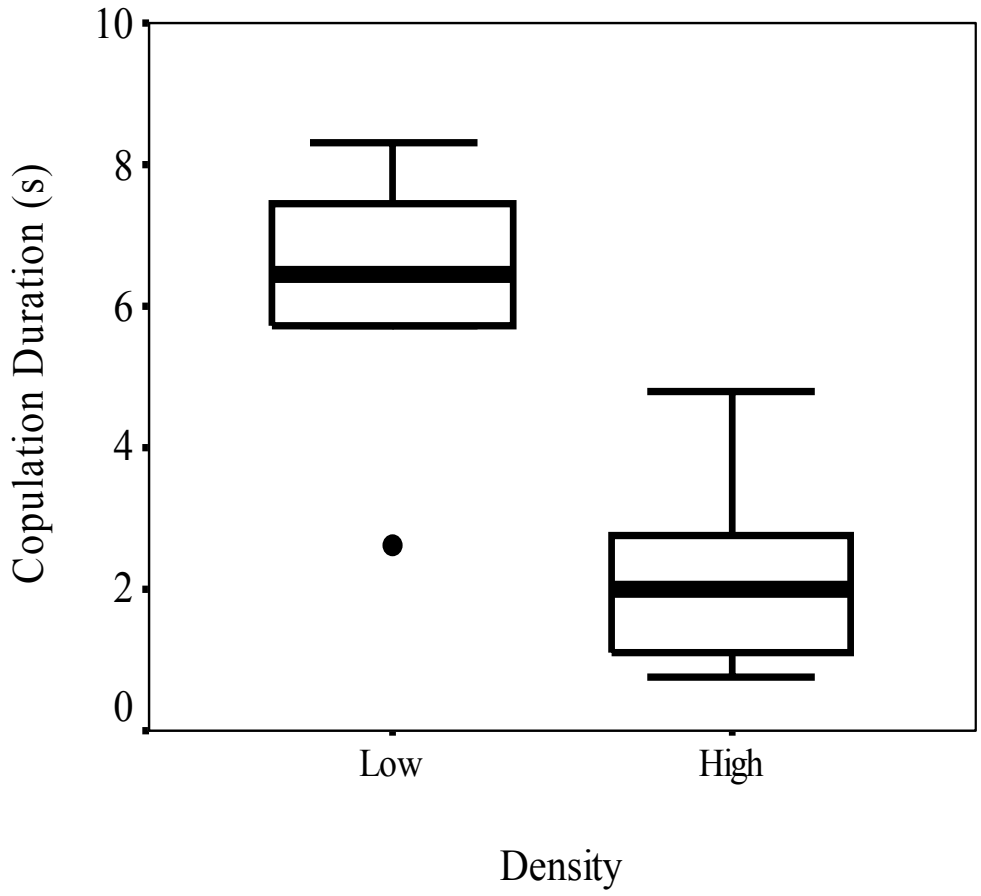


Figure 4.4. Copulation duration at different densities. Horizontal bars represent 25th, 50th (median) and 75th percentiles. Whiskers indicate range. Circle represents outlier.

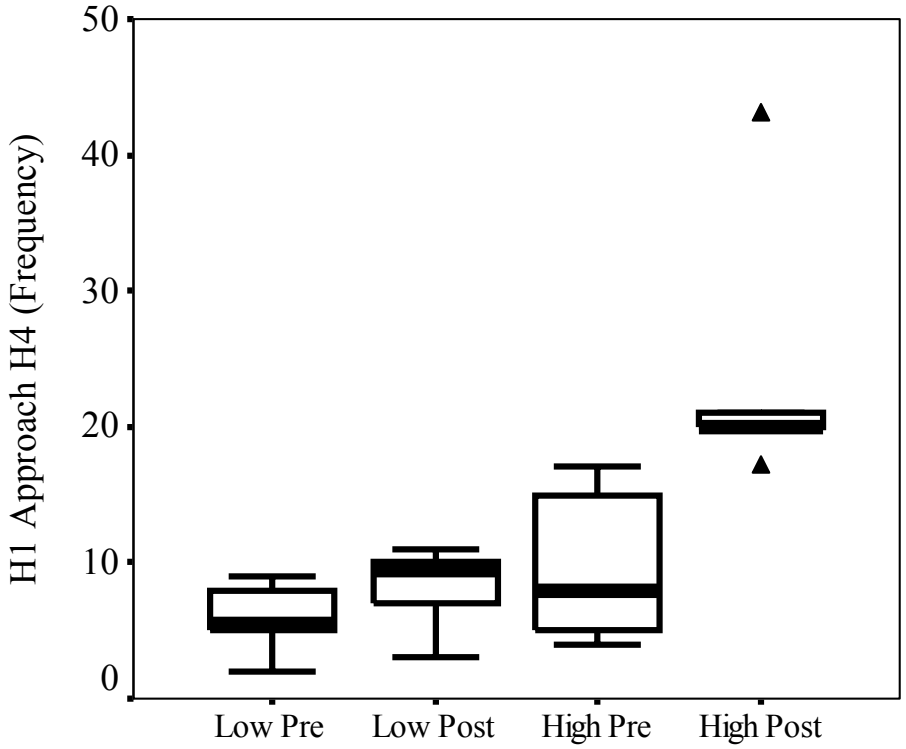


Figure 4.5. Frequency of pre- and post-copulatory "H1 approaching H4 shrimp" behaviours at different densities. Low = One H1 shrimp; High = Five H1 shrimp. Horizontal bars represent 25th, 50th (median) and 75th percentiles. Whiskers indicate range. Triangles represent extremes.

A similar pattern was observed in the duration of Following behaviour (Fig. 4.6). In the low density condition, no significant difference was observed in the amount of following behaviour (Mann-Whitney U test, P=0.1922). In the high density condition, however, H1 shrimp followed the H4 individual for greater durations during the 10 min pre-mating period than in the post-copulatory 10 minutes (Mann-Whitney U test, P=0.010).

Fig. 4.7 illustrates the duration of time H1 shrimp spent within 1 body length of the H4 individual. A wide range of responses was observed, with H1 shrimp spending up to 326 seconds in the vicinity of the H4 shrimp. Although no significant difference was observed between pre- and post-copulatory periods under the low density condition (Mann-Whitney U test, P=0.l319), a highly significant difference was observed under increased mating competition (Mann-Whitney U test, P=0.006). H1 shrimp remained in the vicinity of the H4 individual longer periods of time prior to copulation than afterwards.

There were more physical contacts between H1 and H4 shrimp prior to copulation than during the post-copulatory period (Fig. 4.8). The observed differences were significant in both the low density (Mann-Whitney U test, P=0.019) and high density conditions (Mann-Whitney U test, P=0.0057).

Physical contacts between H1 competitors were almost non-existent. A total of 2 H1-H1 contacts were observed over the 120 minutes of high-density recordings that were analyzed (Fig. 4.9).

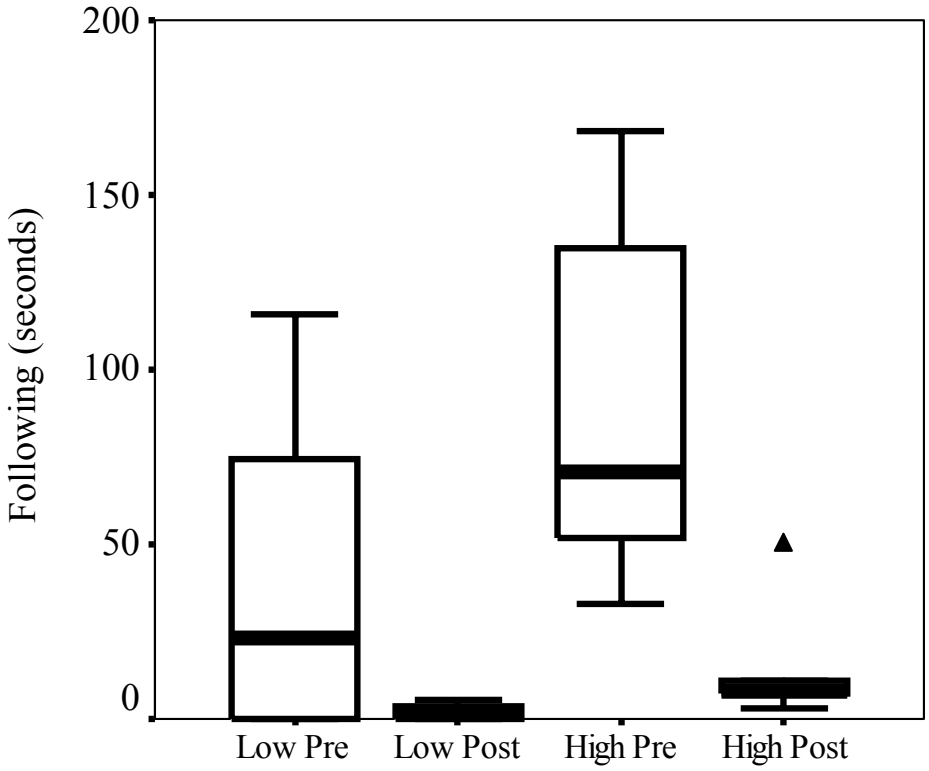


Figure 4.6. Duration of pre- and post-copulatory following behaviour at different densities. Low = One H1 shrimp; High = Five H1 shrimp. Horizontal bars represent 25th, 50th (median) and 75th percentiles. Whiskers indicate range. Triangle represents extreme value.

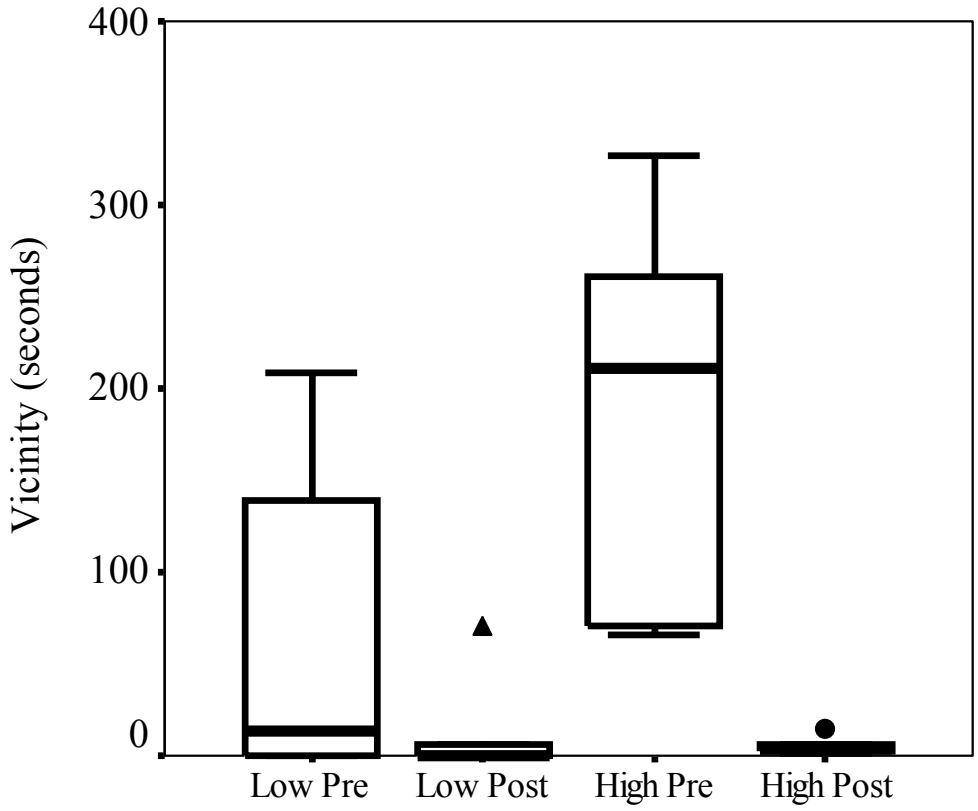


Figure 4.7. Duration of time H1 shrimp spent within 1 body length of H4 individuals during pre- and post-copulatory periods at different densities. Low = One H1 shrimp; High = Five H1 shrimp. Horizontal bars represent 25th, 50th (median) and 75th percentiles. Whiskers indicate range. Circle represents outlier, triangle represents extreme.

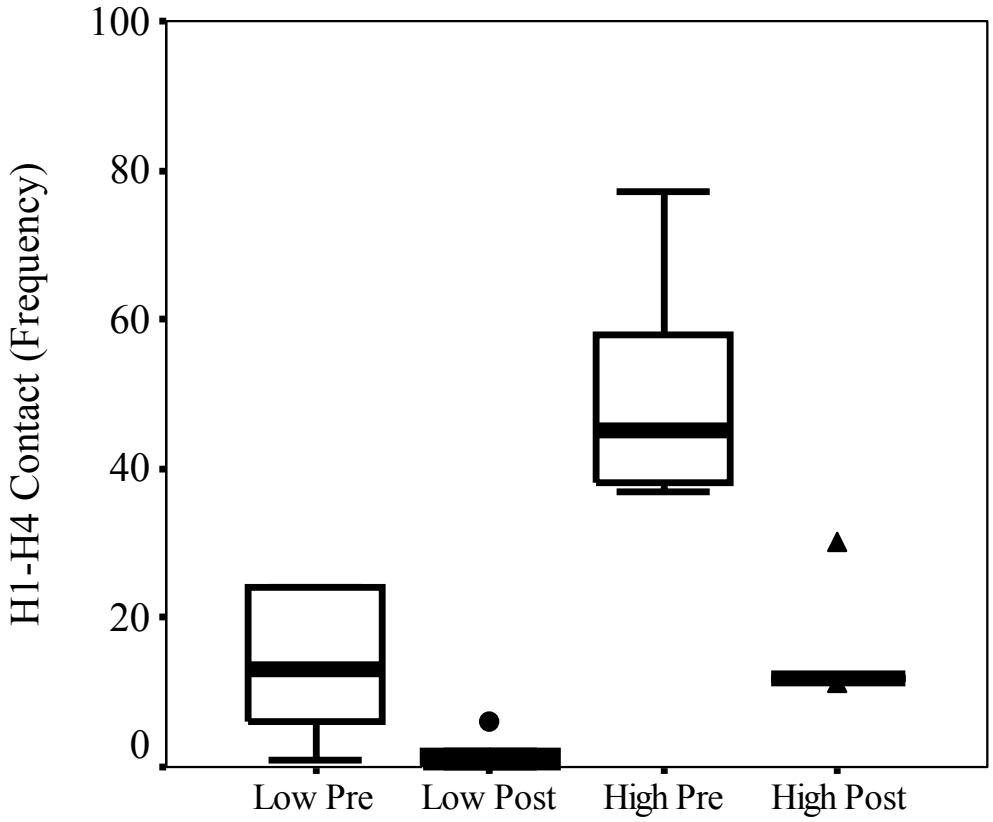


Figure 4.8. Frequency of pre- and post-copulatory contacts between H1 and H4 shrimp at different densities. Low = One H1 shrimp; High = Five H1 shrimp. Horizontal bars represent 25th, 50th (median) and 75th percentiles. Whiskers indicate range. Circle represents outlier, triangle represents extreme.

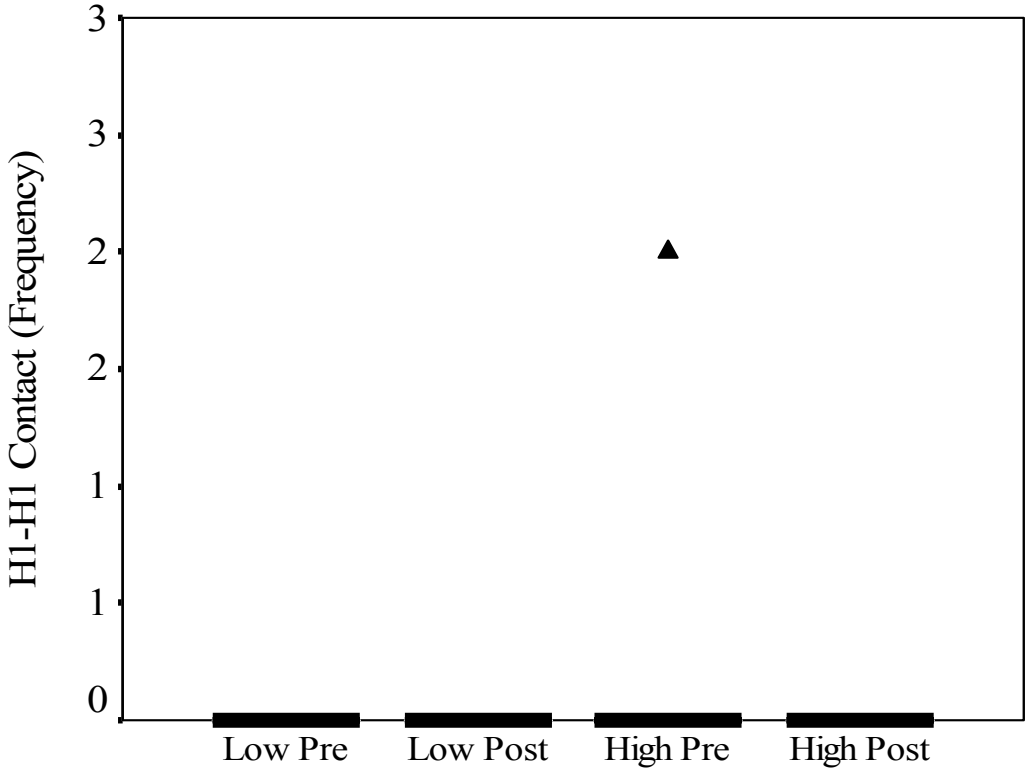


Figure 4.9. Frequency of pre- and post-copulatory contacts between H1 competitors at different densities. Low = One H1 shrimp; High = Five H1 shrimp. Horizontal bars represent 25th, 50th (median) and 75th percentiles. Whiskers indicate range. Triangle represents extreme.

Significant differences were also found in several of the responses of H4 shrimp. At low densities, H4 individuals approached the H1 shrimp before mating significantly more often than after (Fig. 4.10, Mann-Whitney U test, P=0.002). No significant difference was observed in the number of approaches performed by H4 shrimp when 5 H1 conspecifics were present (Mann-Whitney U test, P=0.361).

Escape reponses were more frequent during the post-copulatory period. H4 shrimp performed a greater number of tailflips and other avoidance behaviours after mating in both the low density (Fig. 4.11, Mann-Whitney U test, P=0.006) and high density conditions (Mann-Whitney U test, P=0.006).

Density-Dependent Effects

The effect of density conditions upon reproductive behaviours was determined by examining pre-copulatory and post-copulatory behaviours separately. Difficulties were encountered, however, when dealing with the results obtained for the high density conditions. The large testing area filmed, the relatively small size of the shrimp used and the rapidity of actions occurring immediately before and after both moulting and mating made it impossible to follow individual H1 shrimp. Although the total frequency or duration of behaviours performed by H1s were recorded, the absence of data for specific individuals meant that a traditional or weighted average could not be calculated. It could not be determined, for example, whether the same H1 individuals performed the majority of following behaviours, or if this activity was spread evenly amongst the 5 conspecifics. Density effects were therefore examined indirectly, by comparing the

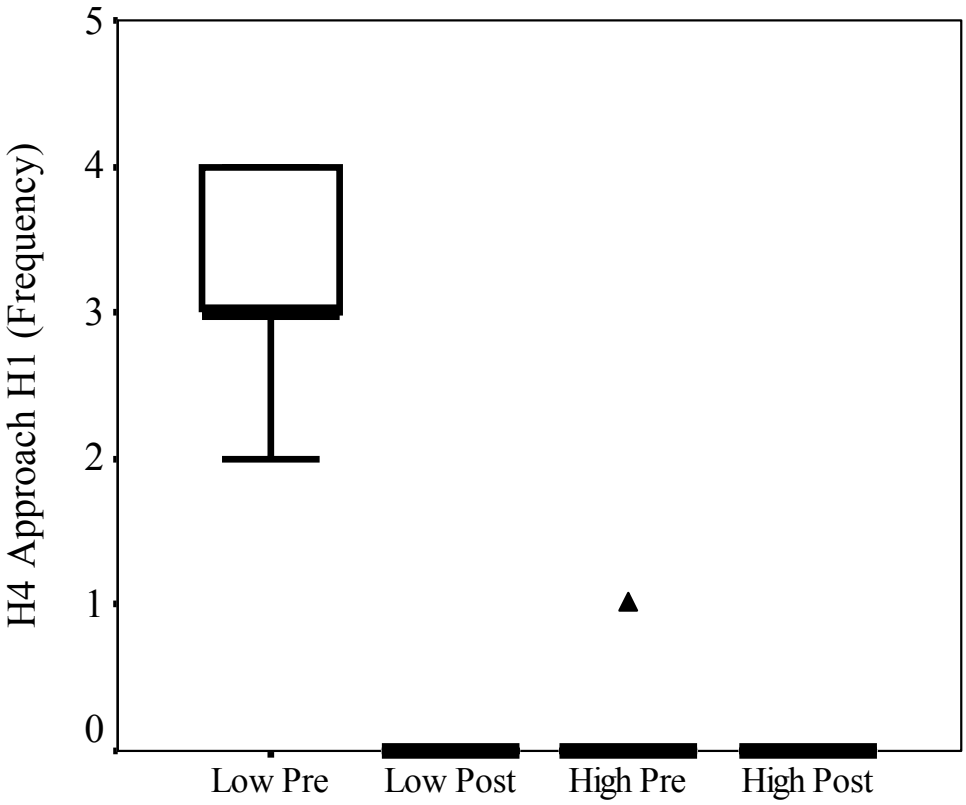


Figure 4.10. Frequency of pre- and post-copulatory "H4 approaching H1 shrimp" behaviours at different densities. Low = One H1 shrimp; High = Five H1 shrimp. Horizontal bars represent 25th, 50th (median) and 75th percentiles. Triangle represents extreme.

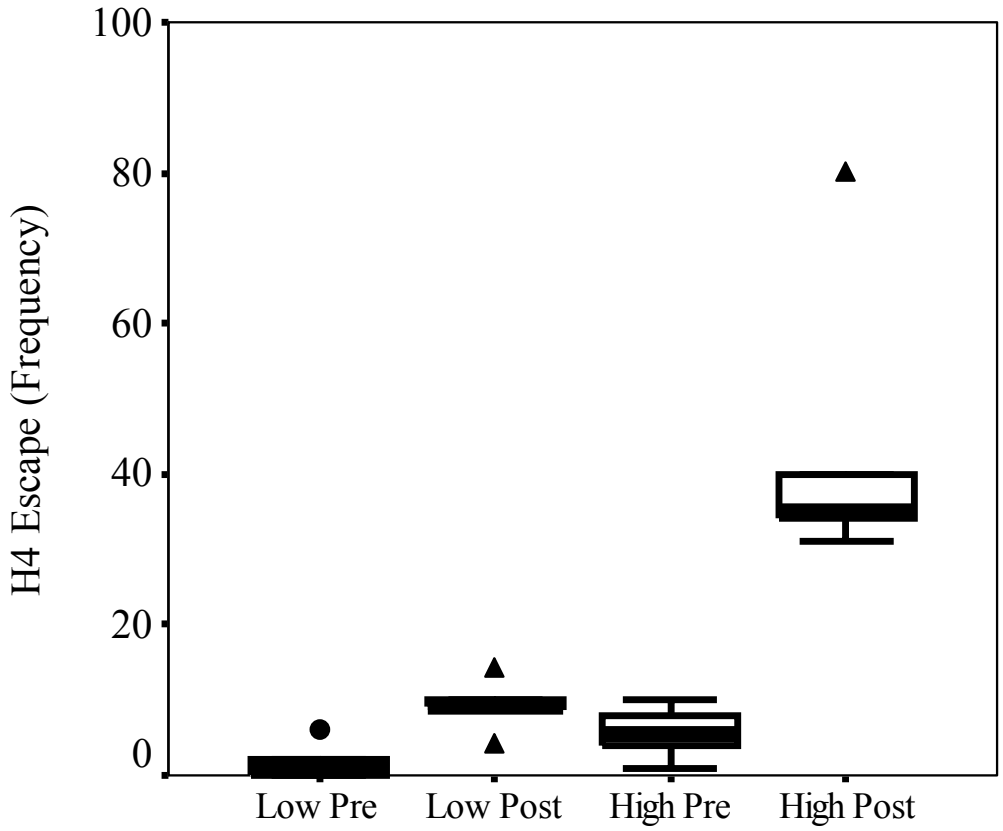


Figure 4.11. Frequency of pre- and post-copulatory escape behaviours performed by H4 shrimp. Low = One H1 shrimp; High = Five H1 shrimp. Horizontal bars represent 25th, 50th (median) and 75th percentiles. Whiskers indicate range. Circle represents outlier, triangles represents extremes.

number of responses directed towards the H4 shrimp by all of the H1 shrimp in the high and low densities.

Surprisingly, during the pre-copulatory period, no significant difference was observed between the low and high density conditions in the number of approaches made towards the H4 shrimp (Mann-Whitney U test, P=0.2937). Recently moulted H4 individuals, however, were approached significantly more often when there were numerous H1 shrimp present (Mann-Whitney U test, P=0.006).

No significant difference was observed between density conditions in the duration of following behaviour experienced by the pre-moult H4 (Mann-Whitney U test, P=0.109). A significant difference was found, however, once the H4 had moulted and been mated. In the 10 minutes following copulation, this shrimp was followed significantly longer (Mann-Whitney U test, P=0.018).

The H4 shrimp had an H1 conspecific within 1 body length for a greater period of time in high density conditions than when only 1 H1 was present (Mann-Whitney U test, P=0.037). There was no significant difference once mating had occurred (Mann-Whitney U test, P=0.269).

H4 shrimp experienced a greater number of physical contacts when 5 H1 shrimp were present. This difference was very significant prior to copulation (Mann-Whitney U test, P=0.004), but only marginally non-significant post-mating (Mann-Whitney U test, P=0.055).

The responses of H4 shrimp were also compared at the two densities. Prior to mating, H4 individuals approached H1 conspecifics far more often under low density

conditions than high (Mann-Whitney U test, P=0.003). No approaches were observed after mating, in either density condition.

Significant differences were observed in the number of H4 escape behaviours both before and after mating. Escape and avoidance behaviours were significantly more frequent under the high density condition than the low, for both the pre-copulatory period (Mann-Whitney U test, P=0.0431), as well as the post-copulatory observation session (Mann-Whitney U test, P=0.0057).

DISCUSSION

This experiment was performed to determine if mate guarding is a component of the reproductive behavioural repertoire in *L. wurdemanni*. Although this species does not display overt aggressive behaviour or possess enlarged chelae, the male-biased operational sex ratio and short period of "female" receptivity suggested that mate guarding could occur.

The results of this study do not support the predictions outlined by Grafen & Ridley (Grafen & Ridley 1983). Despite a "male" biased sex ratio, and a "female" receptivity that is identifiable, short and predictable, individual *L. wurdemanni* did not spend a considerable amount of time in the vicinity of the H4 shrimp. In fact, there was a considerable range in the response of H1 shrimp, as some individuals did not spend any extended periods of time within 1 body length of the H4, while one individual spent over 300 seconds in the vicinity of this soon-to-moult shrimp. In addition, following behaviour by H1 shrimp was also relatively low, with the maximum duration less than

200 seconds. If mate guarding was occurring in this species, individual shrimp would have spent considerably longer periods of time either following, or remaining in the vicinity of the H4, especially during the 10 minutes immediately prior to moulting. Mate guarding occurs for extended periods of time in other crustacean species. Mathews (2002b), for example, reported that male snapping shrimp, *A. angulatus,* will reside in a burrow with a female and defend the mating partner and territory for 13 to 15 days. Wada et al. (1999) found that mate guarding behaviours in the hermit crab *Pagurus middendorffii* averaged roughly 200 hours when male-female encounter rates were low. At high encounter rates, males performed approximately 30 hours of guarding behaviours. In the isopods *Thermosphaera thermophilum* and *T. milleri*, mate guarding occurs for 4 to 9 days.

Further evidence that mate guarding does not occur in *L. wurdemanni* can be found in the absence of agonistic behaviours among H1 competitors. Although numerous H1 shrimp could be in close contact with each other (e.g. when several *L. wurdemanni* would engage in following behaviour), there were only 2 instances of aggressive interactions observed. Since the very term 'mate guarding' implies the defence of a resource by one individual from conspecifics, the absence of aggression amongst size-matched individuals indicates that guarding does not occur.

L. wurdemanni appear to have a mating system of Scrambles, where individuals perform "pure searching" to locate potential mates (Wickler & Seibt 1981; Andersson & McGregor 1999). This mating system has been observed in the crab *Macrophtalmus hirtipes* (Jennings et al. 2000), the penaeid shrimp *Sicyonia dorsalis* (Bauer 1996a) and

the caridean shrimp *Palaemonetes pugio* (Bauer & Abdalla 2001). In both high and low densities, physical contact between H1 and H4 *L. wurdemanni* occurred in greater frequency prior to copulation. This observation suggests that H1 individuals are determining the reproductive state of conspecifics they encounter. A previous study demonstrated that *L. wurdemanni* can chemically distinguish among different reproductive conditions (Chapter II); tactile responses and cues may provide intermoult individuals with more information about potential mating partners.

Under the model created by Wickler & Seibt (1981), pure searching is expected to evolve if males are able to encounter at least 2 receptive females per day. It is presently unknown how often individual *L. wurdemanni* encounter receptive conspecifics. In both Galveston and Port Aransas, Texas, *L. wurdemanni* are found living amongst the rocks and crevices of rocky groins and jetties. While recently-moulted shrimp are rarely collected (pers. obs.), it is possible that these individuals move to more sheltered areas where the risk of predation and injury (and thus collection) is lower. A detailed census of *L. wurdemanni* in these areas should clarify the true operational sex ratios.

Copulation duration was observed to decrease with competitor density. Interruption by conspecifics occurred in three of the six high density replicates, and likely explains the brief copulations observed. However, the remaining (uninterrupted) matings were still considerably shorter than the copulation durations recorded in the low density replicates. This suggests that competitor density had an effect on copulation duration. Brief matings should be selected if they are energetically expensive, increase the risk of predation or reduce the time available for other activities, such as searching for other

mates (reviewed in Eberhard 1996). However, longer copulations may evolve to avoid sperm competition—by preventing a partner from remating, a male may increase his chances of successful fertilizations, while also reducing the risk of competition with future conspecific ejaculates (reviewed in Andres & Rivera 2000). The risk of sperm competition in *L. wurdemanni* has yet to be determined. Future studies need to examine whether egg broods display patterns of multiple paternity, or if post-copulatory H4 behaviour limits this possibility (see below).

An examination of the spermatophore contents ejaculated by intermoult *L. wurdemanni* also needs to be performed. It is possible that *L. wurdemanni* increases sperm production as a result of increases in mating competition, or when there is an increased chance of obtaining copulations. A shift in sex allocation towards male function has been observed under these conditions in other simultanoues hermaphrodites, such as the parasitic cestode *Schistocephalus solidus* (Scharer & Wedekind 2001) and the land snail *Arianta arbustorum* (Locher & Baur 2000). If there is an increase in sperm production, *L. wurdemanni* may be able to provide recently moulted conspecifics with large amounts of sperm in a shorter period of time.

Specific reproductive behaviours also appear to have been affected by competitor density. Although it had been expected that comparisons of raw data between low and high densities would yield consistently significant results in favour of high density responses, a number of behaviours were found to not differ. The frequency of pre-copulatory approaches performed by H1 shrimp towards H4 individuals was not significantly different between the two density conditions. This suggests that H1 shrimp

in the high density replicates reduced the number of approaches made. Similarly, there was no difference observed between the two densities in the duration of pre-copulatory following behaviour. It is unclear why there may have been a reduction in these activities by the high density animals, especially considering the very significant differences that were observed once the H4 individual had moulted and been mated.

Approaches by H4 shrimp also appear to be density-dependent. In the low density replicates, H4 shrimp were observed to make several pre-copulatory approaches towards H1 individuals. Approaches were not observed when densities were higher. Approaches may represent some form of mate-choice on the part of hermaphroditic *L. wurdemanni* which are near ecdysis. Since inter-sexual conflict suggests that females should attempt to select preferentially from among potential mates (Andersson 1994), the H4 approaches may be a method by which "female" hermaphrodites can evaluate potential mates. Approaches would not be necessary in the high density condition, as the increase in searching by the numerous H1 shrimp would result in the H4 being frequently contacted by the intermoult individuals. Mate evaluation could occur passively, without the H4 shrimp actively searching for conspecifics.

Differences were observed in the performance of pre- and post-copulatory behaviours. In general, behaviours performed by H1 shrimp occurred with greater frequency or duration prior to copulation. In the low density conditions, significant decreases in post-copulatory approaches and contacts may be the result of sperm depletion in the H1 mating partner. Lin & Zhang (2001) observed that intermoult *L. wurdemanni* were unable to successfully mate through male function if mating as a male

had occurred in the previous 24h. If H1 shrimp are unable to mate for an extended period following copulation, individuals may be less likely to participate in reproductive activities that may be energetically expensive.

In the high density condition, H1 individuals approached H4 shrimp more often during the post-mating period. This increase is likely caused by increased activity in H1 shrimp resulting from the detection of a freshly moulted individual. Search activities may be most intense in the few minutes immediately before and after moulting.

The significant reduction observed in H1 post-copulatory following and vicinity behaviours was influenced by H4 actions. Recently moulted H4 shrimp were observed to perform more escape and avoidance behaviours after mating. The higher response at the higher density reflects the greater number of interactions with H1 conspecifics. H4 shrimp appeared to tailflip or swim rapidly away whenever contact was made with an H1 individual. In addition, H4 shrimp would also perform escape reactions as conspecifics approached. The rapid nature of these escape movements would make it difficult for an H1 shrimp to remain within one body length of the H4, and would also prevent conspecifics from following effectively.

The increase in H4 escape response may be the result of an increased mortality risk. Shrimp which have recently undergone ecdysis are extremely soft, and thus highly susceptible to damage. One of the H4 shrimp used in this experiment was killed immediately after mating, as it was subsequently grabbed by three other H1 individuals, each of whom attempted copulations (view videoclip Death.avi). The frenzied physical manipulations of several conspecifics almost certainly caused severe damage to the H4

individual, resulting in its death. It is therefore plausible that selection pressures should favour the performance of avoidance activities immediately after mating.

Responses by the recently moulted mating partner may further explain the absence of mate guarding in this species. The performance of escape responses and the avoidance of further copulations by mated *L. wurdemanni* should reduce the risk of sperm competition. This decrease, in turn, would result in sexual selection pressures being weaker during the post-copulatory period, and may not favour the establishment of mate guarding behaviours. If encounter rates with receptive individuals is found to be greater than 2 per day (Wickler & Seibt 1981), then a pre-copulatory scramble by H1 individuals followed by H4 escape responses may yield the greatest reproductive success.

CHAPTER V

FOULING OF THE CARIDEAN SHRIMP, *L. WURDEMANNI* BY THE BARNACLE, *BALANUS IMPROVISUS* AND OTHER EPIBIONTS*

INTRODUCTION

Exposed substrata in marine environments are quickly colonized (fouled) by sessile organisms, such as protozoans, hydroids, bryozoans, barnacles, and a variety of benthic plants. Since the availability of hard substrate is a limiting resource (Jackson & Buss 1975), these epibiotic organisms will often attach to the external surfaces of benthic fauna and flora.

The calcified body surface of decapod crustaceans appears to be suitable habitat for epibionts (Connell & Keough 1985). Many of these encrusting species select settlement sites using a variety of cues, such as substrate texture (Crisp 1974; O'Connor & Richardson 1996; Berntsson et al. 2000). Epibionts found attached to decapod crustaceans include peritrich and suctorial ciliates (reviews by Fernandez-Leborans & Tato-Porto 2000b; Fernandez-Leborans & Tato-Porto 2000a), bryozoans (Dick et al. 1998; Key et al. 1999), hydroids (Gili et al. 1993), sponges (Maldonado & Uriz 1992), and barnacles (e.g. Dawson 1957; Eldred 1962).

The attachment of epibiota can be beneficial to the host organism. Some crabs, such

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*Reprinted with permission from "Fouling of the caridean shrimp, *Lysmata wurdemanni* (Gibbes, 1850) by the barnacle *Balanus improvisus* Darwin, 1854 and other epibionts" by T. Giri & M. K. Wicksten, 2001. *Crustaceana,* 74**,** 1305-1314.

as the Majidae and Dromidae, actively acquire sponges and other sessile organisms and use them for protective camouflage (Cutress et al. 1970; McLay 1983; Maldonado & Uriz 1992; Wicksten 1993). Epibiota can, however, impart many disadvantages, through the higher energetic costs required for movement (Xu & Burns 1991), the inhibition of moulting (Glynn 1970), and a potential increase in predation risk (Willey et al. 1990).

During a recent collecting trip to obtain specimens for another study, several individuals of the peppermint shrimp, *Lysmata wurdemanni* with considerable epibiota attached were collected. This paper reports the first occurrence of the barnacle *Balanus improvisus* and other epibiota upon this caridean shrimp species. The pattern of barnacle settlement on these shrimp is detailed, and the effect of salinity on epibiont attachment examined.

METHODS

Lysmata wurdemanni were collected at nocturnal low tides along a rocky groin in Galveston, Texas (29^{o} 16´N 94^{o} 49´W) on April 13 and 17, 2001. Shrimp were transported and housed under conditions similar to those described in Chapter II.

As some shrimp had moulted during transport or within a few days of collection, the identification of epibiota, and counting and measurement of barnacles were made using moults, live shrimp, and one animal which died 1 day after collection.

A dissecting microscope was used to identify epibiota and count the number of barnacles on the following regions of each shrimp: antennae and antennules; dorsolateral regions of the antennal scales, rostrum, carapace, abdomen, telson, and uropods; and the

ventral surfaces of the telson and uropods, the abdomen, walking legs, and antennal scales. Dorsolateral barnacles were further separated into dorsal (defined as a region between the eye orbits, extending the length of the animal), or lateral positions. Digital images of each barnacle and other epibiota were captured using a dissecting microscope with a Panasonic GP-KR222 video camera or a Kodak DC-290 digital camera connected to a personal computer running Microsoft Windows.

The carapace length of each shrimp and the basal area of each barnacle was measured from these digital images. A SummaSketch II pen and tablet was used to trace the carapace length of each shrimp, and the perimeter of each barnacle shell. The software package Image J subsequently was used to calculate area. Three area measurements were taken of each barnacle, and the average used for analysis.

A voucher specimen of a *L. wurdemanni* fouled by *B. improvisus* has been deposited in the collections of the United States National Museum of Natural History.

RESULTS

Of the 65 *Lysmata wurdemanni* collected over two nights, 10 (15.38%) had epibiotic growth (Fig. 5.1). The mean carapace length (± SE) of fouled shrimp was 9.20 ± 0.42 mm. Bryozoans (*Membranipora* sp.) were found on the carapace of one shrimp (Fig. 5.1B), while peritrich cilates (family Epistylididae) were found on two others (Fig. 5.2). On both of these shrimp, ciliate encrusting was highest along the pleopods and lateral body surfaces. Although ciliates were also observed on the pereiopods, they were absent from the most distal regions.

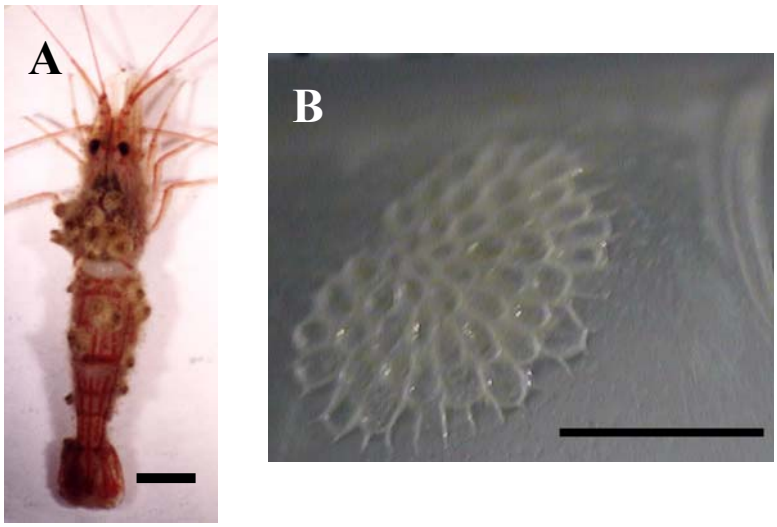

Figure 5.1. Epibiota found on *Lysmata wurdemanni*. (A) A recently-dead *L. wurdemanni* with considerable fouling by barnacles (*Balanus improvisus*) on dorsolateral surfaces of carapace and abdomen. Scale bar represents 1 cm. (B) A bryozoan (*Membranipora* sp.) found upon carapace moult. Scale bar represents 1mm.

Live barnacles were found on every shrimp with epistylidid colonies (Fig. 5.2). The number of barnacles attached was highly variable, ranging from 1 to 48 barnacles per individual. Table 5.1 lists the number of barnacles present on each of the different body regions examined. For analysis, all regions except the dorsolateral surface of the carapace and abdominal segments were combined, creating three regions of approximately equal surface area. A significant difference was found when comparing barnacle counts across these three regions (Friedman test, $P<0.002$), and subsequent pairwise tests indicated that there were more barnacles attached to the dorsolateral surfaces of the carapace than to the other areas.

A significant difference also was observed in the pattern of barnacle settlement, with far greater numbers attached to the dorsolateral body surfaces than to the ventral ones (Wilcoxon signed-ranks test, $P=0.002$). No difference was observed between the number of barnacles attached to dorsal versus lateral surfaces (Wilcoxon signed-ranks test, $P=0.718$).

The mean barnacle basal area was obtained for each region of each shrimp. Areas with no barnacles present on any individual were excluded from further analysis. Correlations among the remaining areas were analysed to determine if independence existed among these body regions. Since the majority of regions were only weakly positively correlated, independence between regions was assumed to exist (Z. Hu, Dept. of Statistics, Texas A&M University, pers. comm.). Barnacle measurements were compared across the dorsolateral regions of the carapace and abdomen and all other

Figure 5.2. Live barnacles and ciliates (family Epistylididae). Note dense ciliate growth along edge of carapace, but near absence of ciliates along distal portion of periopod; dark spot in lower right corner is eye. Scale bar represents 1mm.

Table 5.1. Total number of barnacles found attached to specific body regions of *L. wurdemanni*. (AS=antennal scale; RA=rostrum; CA=carapace; PL=abdomen; UR=uropods and telson; VAS=ventral surface of antennal scale; WL=pereiopods; VPL=ventral surface of abdominal region; VUR=ventral surface of telson and uropods; AN=antennules and antennae)

	Dorsolateral					Ventral				Other
	AS	RA	CA	PL	UR	VAS	WL	VPL	VUR	AN
Total number of barnacles found	4	0	61	22	0	0	0	1	2	1
Number of shrimp with barnacles attached	2	0	9	4	0	0	0	1	1	1

regions (Fig. 5.3). An ANOVA indicated that there was no significant difference in barnacle size across these three areas (ANOVA, P=0.839).

The three largest barnacles were measured using the method employed by (Costlow & Bookhout 1953). A comparison between the controls in that study (which were housed in Beaufort Harbour, North Carolina) and the measurements taken in the present one indicate that the largest barnacles were between 20-26 days old, assuming growth rates are similar in both Beaufort Harbour and the Gulf of Mexico.

DISCUSSION

Epistylidid ciliates, the bryozoan (*Membranipora* sp.), and the barnacle *Balanus improvisus* were found attached to the caridean shrimp, *Lysmata wurdemanni*. Each of these epibionts has also been found on a number of other crustacean species. In their review of protozoan epibionts, (Fernandez-Leborans & Tato-Porto 2000b) reported that among decapod crustacean hosts, members of the family Epistylidae have been found on *Astacus astacus*, *Astacus leptodactylus*, *Penaeus duorarum*, *Ploeticus robustus*, *Coenobita clypeatus*, *Geograpsus lividus*, *Pachygrapsus transversus*, and *Scylla serrata*. The bryozoan *Membranipora* sp. has been observed on the horseshoe crab *Tachypleus gigas* (Patil & Anil 2000), the tanner crab *Chionoecetes bairdi* (Dick et al. 1998) and the blue crab *Callinectes sapidus* (Key et al. 1999).

Barnacles have also been found attached to a number of living substrates. Silina & Ovsyannikova (2000) noted the occurrence of *Balanus rostratus* on the scallop

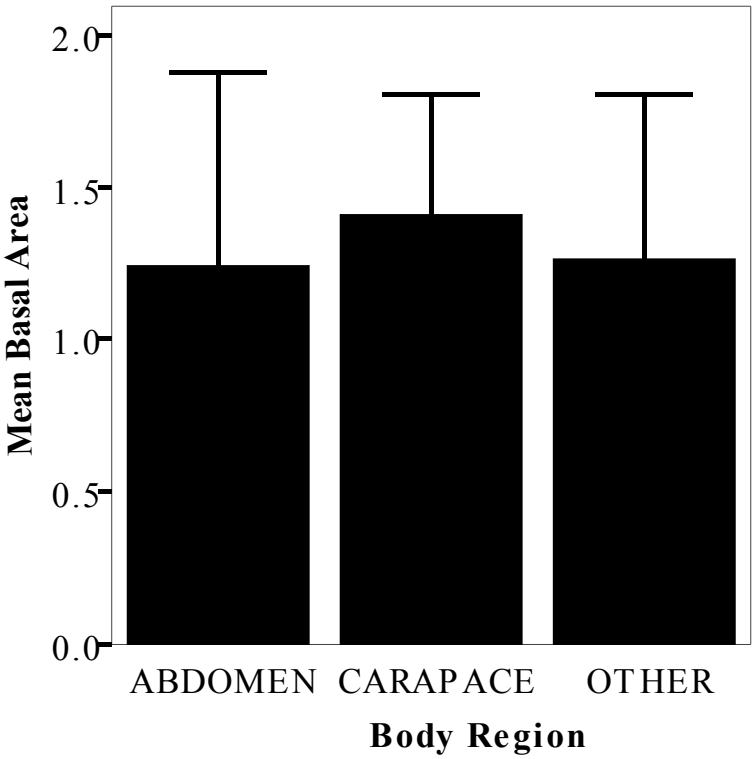

Figure 5.3. Mean basal area (± SE) of barnacles found attached to various areas of *L wurdemanni* . 'Abdomen' and 'Carapace' refer to the dorsolateral surfaces of these regions. 'Other' refers to all other body regions combined.

Patinopecten (*Mizuhopecten*) *yessoensis*. *Balanus variegatus* and an unidentified stalked barnacle species have been observed on two tropical marine crabs,*Metapograpsus quadridentatus* and *Hexapus anfractatus* (Becker & Wahl 1996), while *Balanus amphitrite* has been found attached to the horseshoe crab *Tachypleus gigas* (Patil & Anil 2000), as well as the shrimp *Penaeus setiferus* (Dawson 1957) and *Sicyonia dorsalis* (Eldred 1962). Eldred (1962) also reported that several barnacle species have been found on spiny lobsters *Panulirus argus*, stone crabs *Menippe mercenaria,* and the blue crab, *Callinectes sapidus*.

The barnacle identified in this study, *Balanus improvisus*, has been previously found on mussels (Furman & Yule 1990). An examination of specimens in the Marine Invertebrate Collection at Texas A&M University found *B. improvisus* also encrusting shells of *Pollineces duplicatus*, *Stramonita haemastoma floridana* and *Busycon perversum pulleyi*.

Shrimp spend considerable time performing grooming and cleaning activities (Bauer 1975). This behavioural repertoire may explain the pattern of barnacle encrustment upon these species. Dawson (1957) reported the presence of barnacles only along the mid-dorsal region of the abdominal segments of *Penaeus setiferus*, while Eldred (1962) found barnacles along the mid-dorsal line of both the carapace and abdomen on *Sicyonia dorsalis*, with the majority of barnacles found on the more anterior region. A similar result was found in this study, with the carapace acting as substrate for roughly 60% of the barnacles counted, and almost 22% attaching to abdominal segments. Very few

barnacles were found attached to the ventral surfaces in any of the shrimp species examined.

A combination of shrimp grooming behaviours and barnacle attachment strategies may explain the observed settlement pattern. Bauer (1975) reported that in the caridean shrimp *Pandalus danae*, the last pair of walking legs are used to brush and scrub the carapace and abdomen, removing epizoites and ectoparasites. Since the mid-dorsal line of the carapace and abdomen may be at the furthest reach of these appendages, this area may not be cleaned as effectively, allowing barnacle settlement to occur. In addition, shrimp groom the abdomen, telson, and uropods by flexing the abdomen beneath the body. This activity likely permits more efficient grooming, and may account for the reduced number of barnacles found on the abdominal segments of *L. wurdemanni* and *S. dorsalis*.

Berntsson et al. (2000) report that *Balanus improvisus* cyprids preferentially explored smooth surfaces over micro-textured ones. This finding supports the observations in this study, as barnacle cyprids should select the large and relatively flat carapace for settlement over the ventral surface and its numerous bases for pereiopods, pleopods and maxillipeds.

Grooming behaviour by itself, however, is unlikely to explain the few instances of barnacles encrusting upon shrimp. McGaw et al. (1999) found that decreases in salinity can alter the behavioural responses of crabs. Species classified as efficient osmoregulators increased the frequency of antennal, antennular and mouthpart cleaning at lower salinities, while weaker regulators typically became less active. It is not known

whether *Lysmata wurdemanni* is an effective osmoregulator, but a decrease in grooming, caused by an environmental factor such as salinity, could permit settlement by a greater number of cyprids.

Measurements taken at the site where fouled *L. wurdemanni* were collected indicated that salinity levels were around 18‰. This is considerably lower than average, and tests performed during subsequent trips to the same area (May 12 and 26, 2001) showed increases in salinity (26‰ and 31‰, respectively). Epibiotic growth was not found on any shrimp collected on these dates.

Salinity at the coastal collection site used in this study is influenced by the Trinity and San Jacinto River Basins. Fresh water outflow from these two rivers enters Galveston Bay, and from there travels to the Gulf of Mexico. A large influx of fresh water from these two rivers can thus severely decrease the salinity found along the local coastal region. The date of settlement was estimated using the age of the largest barnacles found. Precipitation data for Galveston County were then examined for the 26 day period between settling and collection, and also for the 90 days prior to settlement. During the initial 90 days, this region received 276.35 mm of rain, 135% of the normal amount. Between the dates of settlement and collection, 62.23 mm of precipitation was received, 121% greater than the norm for the area (Texas State Climatologist, pers. comm.). This increase in freshwater is likely the cause of decreased salinity measurements on the date of collection.

Similarly, salinities at the collection sites used by Dawson (1957) and Eldred (1962) (i.e., Ocean Springs Harbour (Biloxi Bay), Mississippi; the Edisto River System, South

Carolina; and the Tortugas, Florida) may be determined by fresh water outflow as well. Biloxi Bay lies between the Wolf and Pascagoula river systems. The outflow from these and other rivers affects the salinity of the more southern Mississippi Sound, which ranges from 2 to 22‰ (Eleuterius 1977). The Edisto River System is formed from the confluence of two other rivers and several swamp systems, and merges with the Dawho river before draining into the Atlantic. Salinity in this region can vary from 0 to 34‰ (F. Holland, pers. comm.). The Tortugas are a part of the Florida Keys, and lie downstream of the Kissimmee River-Lake Okeechobee-Everglades watershed. Extended precipitation along part of these river systems would increase the amount of freshwater outflow, and decrease salinity along the area of coastal drainage.

Salinity is known to affect settlement in a number of barnacle species (Dineen & Hines 1992; Dineen & Hines 1994a; Dineen & Hines 1994b). The two species observed to encrust upon shrimp (*Balanus amphitrite* and *B. improvisus*) are typically estuarine, and exhibit normal development at lower salinities (Dineen & Hines 1992; Qiu & Qian 1999). Bousfield (1955) found the cyprid mode of *B. improvisus* occurred at 17‰, while (Dineen & Hines 1992) observed peak settlement at 10 and 15‰. These values are similar to the salinity level found in Galveston during mid-April 2001. The large amount of precipitation, and the subsequent increase in freshwater outflow, could have increased the number of barnacle larvae and cyprids flushed into the Gulf of Mexico. The resulting decrease in salinity along the coast may have created conditions optimal for settlement of cyprids upon both non-living and living substrata, and a depression in grooming activities would preclude *Lysmata wurdemanni* from removing them.

In early June 2001, a tropical storm along the coast of southeast Texas deposited between 24.8 and 93.9 cm of rain on the Galveston and Houston areas (National Oceanic and Atmospheric Administration data). This massive outflow of freshwater resulted in reduction of salinity levels along the coast. Approximately two weeks after this storm, *L. wurdemanni* were collected at the same rocky groin to determine if fouling by barnacles would occur again. Salinity at this site was measured at 23‰. *L. wurdemanni* were not found in large numbers during sampling. One of the 14 shrimp collected had 7 barnacles attached to the dorsolateral surface of its carapace, with the largest of these barnacles estimated to be 8-10 days old (method outlined in Costlow & Bookhout 1953). Since no fouled *L. wurdemanni* were found on two previous collecting trips when coastal salinity was higher (May 12 and 26, 2001), the observation of fouling following a large outflow of freshwater provides some support for the hypothesis outlined above. A more intense collecting effort and controlled experiments are required to fully test this hypothesis.

Preliminary observations indicated that *L. wurdemanni* fouled by barnacles were less active than unfouled individuals. This is not unexpected, as the additional mass presumably increased the energetic costs required for movement, and should therefore have decreased general activity. Although the presence of epibiota would presumably make individual *L. wurdemanni* more visible to predators, a reduction in activity may instead make them inconspicuous. Bass & Weis (1999), for example, found that *Palaemonetes pugio* (Holthuis, 1952), if parasitized by the isopod *Probopyrus*

pandalicola (Packard, 1879), were less likely to be preyed upon, as they were less active than unparasitized conspecifics.

Crustaceans may find it very difficult to escape ectoparasites such as *P. pandalicola* (Cash & Bauer 1993). *L. wurdemanni*, however, were able to escape barnacles and other epibiota through moulting. Ciliates, bryozoans, and barnacles remained on the exuviae, while the freshly moulted shrimp did not appear to have any epibiotic growth (Fig. 5.4). Moulting may therefore serve as a natural antifouling mechanism in this species.

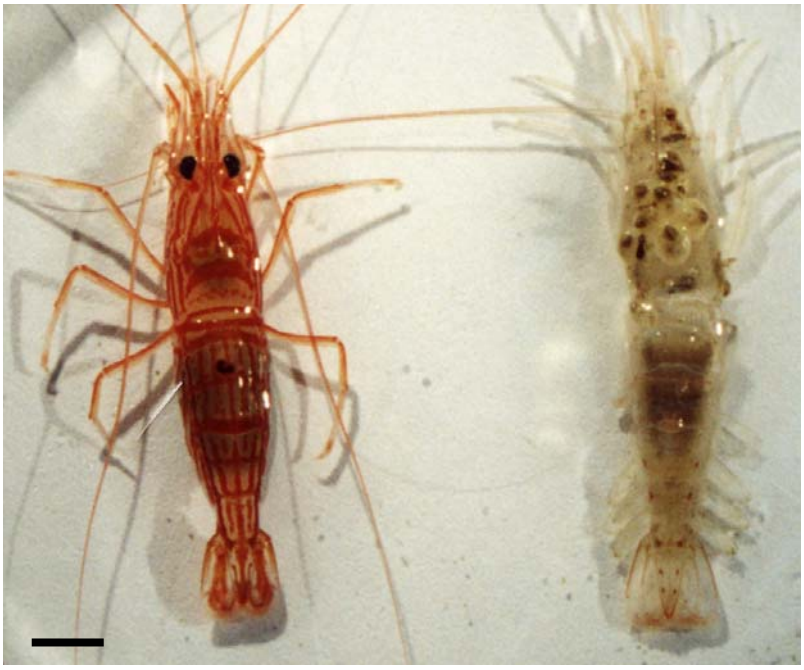

Figure 5.4. Recently moulted *L. wurdemanni* and shed exuvium. Epibiota were shed with old skeleton. Scale bar represents 1 cm.

CHAPTER VI

THE EFFECT OF SALINITY CHANGE ON GROOMING ACTIVITIES IN *L. WURDEMANNI*

INTRODUCTION

In marine environments, sessile organisms release larvae or spores into the water column. Colonization occurs when these juvenile forms locate and subsequently attach to hard surfaces. Since the availability of suitable substrate is a limited resource (Jackson & Buss 1975), many larvae will instead settle upon hard surfaced animals, such as decapod crustaceans. The attachment of epibiotic organisms such as bacteria, algae, diatoms, protozoans, fungi, bryozoans, tube-dwelling polychaetes, and barnacles (Bauer 1977; Wahl 1989; Buckley & Ebersole 1994; Davis & White 1994; Giri & Wicksten 2001) can be deleterious for the host, as they may interfere with locomotion (Xu & Burns 1991; Bass & Weis 1999) overgrow sensory or respiratory structures (Bauer 1999), inhibit moulting (Glynn 1970), and increase the risk of predation (Willey et al. 1990). Antifouling adaptations, such as grooming activities, are thus an important component of the behavioural repertoire of many decapod crustaceans.

Giri and Wicksten (2001) described the unusual occurrence of barnacles, bryozoans and epistylidid ciliates upon the shrimp *Lysmata wurdemanni.* This species can be exposed to dramatic changes in salinity in portions of its distribution. Salinity levels in Galveston, Texas, for example, are influenced by freshwater outflow from the Trinity and San Jacinto River Basins, and measurements of salinity have ranged from 18 to 33‰

(pers. obs.). The barnacles found attached to *L. wurdemanni* were identified as *Balanus improvisus*, a species typically found in estuarine environments. An analysis of settlement patterns indicated that the vast majority of barnacles had settled upon the carapace, followed by the abdominal region. It was hypothesized that an increase in precipitation flushed barnacle cyprids into coastal areas, and that the resultant decrease in salinity may have reduced grooming behaviour in *L. wurdemanni*. As a result, cyprids were likely able to settle successfully upon individual shrimp.

While it is possible that barnacle cyprids may be resistant to grooming efforts, the considerable number of ciliates also found on fouled shrimp suggests this explanation is less plausible. Ciliate colonies should be much easier for individuals to remove, but the large numbers of ciliates indicates that grooming did not occur, or was greatly reduced following a decrease in salinity.

Relatively few studies have examined behavioural responses to changes in salinity. The studies that have studied this relationship in crustaceans typically focus on salinity choice (Jury et al. 1994; Tankersley et al. 1998), transport and settlement of larvae (Dineen & Hines 1992; Dineen & Hines 1994a; Welch & Forward 2001), and habitat use by adults (Watson et al. 1999; Perry et al. 2000; McGaw 2001). McGaw et al (1999) examined physiological and behavioural responses to salinity change in four crab species. The responses observed appeared to correlate with each species' osmoregulatory ability. *Callinectes sapidus* and *C. maenas*, two efficient osmoregulators, were found to increase grooming of both the mouthparts and the antennae or antennules (the only grooming activities examined). Weak osmoregulators

and osmoconformers (*Cancer magister* and *Libinia emarginata*, respectively) typically became inactive at lower salinities.

This experiment examines how salinity affects the performance of specific grooming activities in *L. wurdemanni*. Two alternate hypotheses were examined: (i) a decrease in salinity reduces the general performance of grooming behaviours, or (ii) a decrease in salinity alters the pattern of grooming activities.

METHODS

The location and methods used to collect *L. wurdemanni*, as well as their subsequent housing, were described in Chapter II. Shrimp were collected in Fall 2001 and initially maintained in community tanks under similar temperature, salinity, density and feeding regimes until their use in April 2002.

Sixteen intermoult, non-gravid shrimp were selected for use in this experiment. Although individuals were selected in a quasi-random manner, an attempt was made to obtain a wide range of body sizes. Each shrimp was examined under a dissecting microscope for the presence of epibiota, such as barnacles, bryozoans and ciliates (Giri & Wicksten 2001). Fouling organisms were not found on any of the individuals selected.

The 16 shrimp were divided into four groups, such that each group had two large and two small individuals. Each group was housed in an aquarium measuring 20 (w) x 40 (l) cm in area, with a water depth of 15 cm, and a 1 cm layer of coral gravel spread along the bottom (Fig. 6.1). Salinity was maintained at 33 ± 0.5‰ and checked every 12 hours

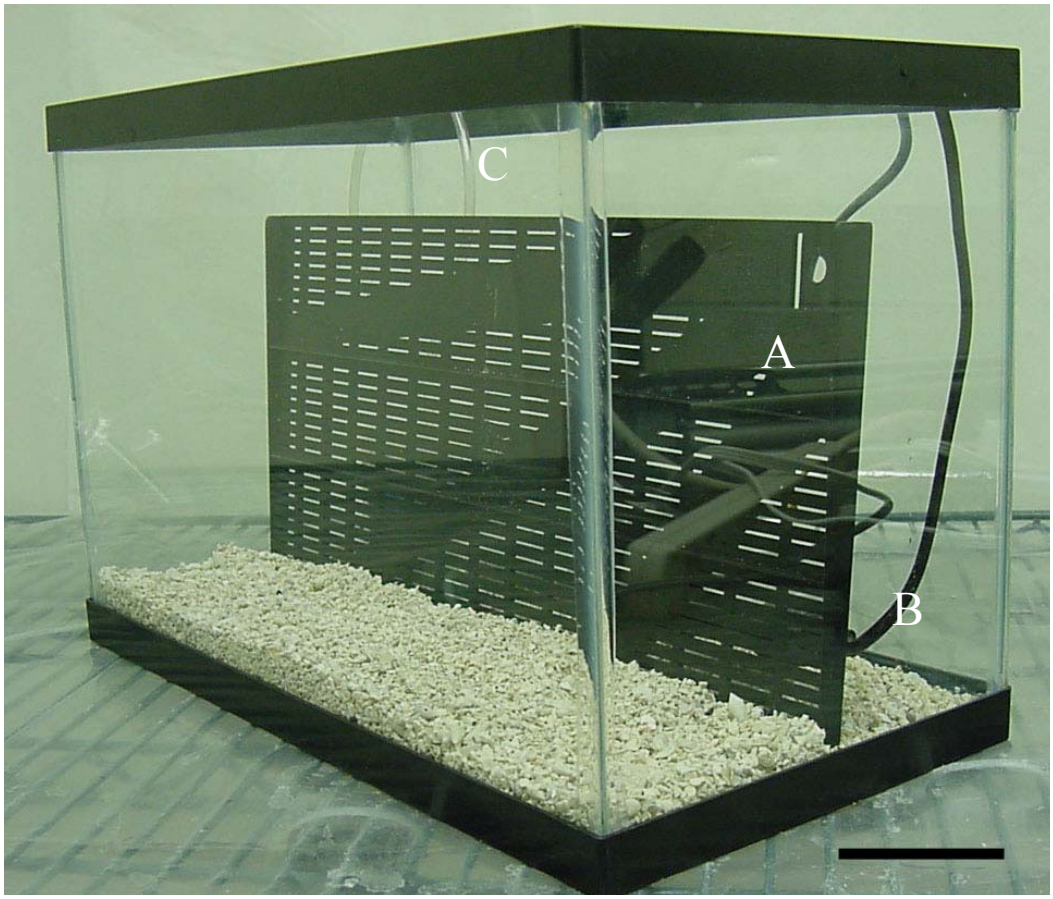

Figure 6.1. Testing tank used to examine grooming responses. A piece of black perforated plastic (A) was used to limit shrimp to the front half of the aquarium. An aquarium heater (B) and box filter (C, showing airline tubing) were located behind the perforated divider. Scale bar represents 5 cm.

using a refractometer that had been calibrated with a reverse-osmosis water standard. Reverse-osmosis water was added to aquaria as needed throughout this study to maintain specific salinity levels. Temperature was held at 28 ± 0.5°C through the use of aquarium heaters, and overhead fluorescent lights were used to create a 12h:12h day:night cycle. Box filters were used for both aeration and filtration. Shrimp were fed pieces of frozen brine shrimp daily, and any uneaten food was removed after an hour. Each group of shrimp was maintained under these conditions for one week before testing.

Rectangular pieces of black perforated plastic (38.5 x 19 cm in area) were inserted into each tank and used to limit the movements of *L. wurdemanni* to the front half of each aquarium (10 x 40 cm in area). Sections of plastic tubing were fitted around the ends of each insert, so that each piece would fit tightly into the testing tank when vertically inserted. Filters and heaters were kept behind the inserts, but the perforations permitted water circulation and filtration. Each plastic piece was inserted 24h prior to testing, and was used to ensure proper focus of shrimp during filming.

Shrimp were maintained at 33 ± 0.5‰ for 48h, and were filmed during the two night cycles. On the third day, reverse osmosis water was slowly added to each tank over an 8h period to reduce the salinity to 20 ± 0.5‰. Salinity was again checked with a calibrated refractometer. Once the appropriate salinity level was obtained, water was slowly drained from the tank until water depth again reached 15 cm. Individuals were again filmed each night for two nights.

Salinity was then increased through the addition of hypersaline water (and subsequent removal of water volume) over a 4h period until a salinity level of 27 ± 0.5‰

was reached, and each shrimp was filmed over another two nights. Salinity was subsequently increased to the initial 33 ± 0.5‰ and shrimp filmed for two additional nights.

Individual shrimp were videotaped under infrared light using a Sony TR517 camcorder and a Sony IR-HVC infrared light source (view videoclip Grooming.avi). Each shrimp was filmed for five minutes per night. Body size was used to distinguish individuals, and ensured that each was only filmed once per session.

A PowerMac G3 computer connected to a VCR and running the Apple Video Player software was used to subsequently watch videos of each shrimp. The software program JWatcher (version 0.9) operated concurrently on the computer and was used to record how often individuals performed bouts of specific grooming behaviours (modified from Bauer (1975; 1981)). All bouts were less than 10 seconds in duration. Each bout was defined as a period of uninterrupted activity, composed of one or more acts of the same behaviour (Fig. 6.2):

Antennular grooming—the medial or lateral branch of either antennule is flexed and scraped through the third pair of setose maxillipeds. Grooming of the non-bifurcated antennae could not be distinguished from antennular wiping, and is thus included in this category. This grooming behaviour has also been described in many other decapod species (e.g. Wasserthal & Seibt 1976; Bauer 1981; Martin & Felgenhauer 1986; Wroblewska et al. 2002).

Pereiopod grooming—walking legs are swung forward and groomed by the maxillipeds. The ventral bases of pereiopods may be groomed by other pereiopods.

Abdominal grooming—the 5th pereiopod is extended backwards, and is used to rapidly scratch and scour the dorsolateral surfaces of the abdomen. Bauer (2002) describes a similar behaviour in the crayfish *Procambarus clarkii*, as well as in *P. danae* (Bauer 1975). Read et al. (1991) report that this pereiopod is also used for abdominal grooming in the snapping shrimp *Alpheus heterochaelis*.

Uropod/Telson grooming—the abdomen is flexed and curled forward, bringing the tailfan underneath the body. The 5th pereiopod is used to scratch and scour the surface of the uropod and telson.

Pleopod grooming—the multiarticulated 2nd pereiopod is used to groom the fan-like pleopods. The entire body is raised, and the pereiopods are used to perform picking and scraping actions along the pleopods. Similar actions have been reported in the shrimp *Heptacarpus pictus* (Bauer 1979) as well as in the crab genus *Aegla* (Martin & Felgenhauer 1986).

Carapace grooming—anterior areas of the carapace, including dorsal spines, rostrum, and eyes are groomed by the chela of the multiarticulated 2nd pereiopod. Posterior regions are typically groomed by the 5th pereiopod. Similar activities have been reported for a number of other caridean shrimp (Bauer 1978; Read et al. 1991).

Maxilliped grooming—the rapid and repeated rubbing of setose maxillipeds against each. Debris acculumulates on the maxillipeds, as these body parts are used to groom the antennules and pereiopods. The rapid movements of maxilliped setae in opposite directions against each other ("autogrooming") moves debris to the tip of the limb, where it drops off. This activity has been reported in *Aegla* spp. crabs (Martin & Felgenhauer 1986), and in the stomatopod *Gonodactylus oerstedii* (Bauer 1987)

Although there are a number of methods by which decapod crustaceans prevent and remove gill fouling, this category of grooming behaviour was not examined in the present study. Bauer (1998; 1999) divides gill cleaning into "active" and "passive" components. In active grooming, chelipeds are inserted into the branchial chamber and subsequently brush, scrape and pick at gill filaments. In contrast, passive grooming involves the use of setae located among the gills (Bauer 1998; Suzuki & McLay 1998; Batang & Suzuki 1999; Bauer 1999). These setae are attached to thoracic appendages,

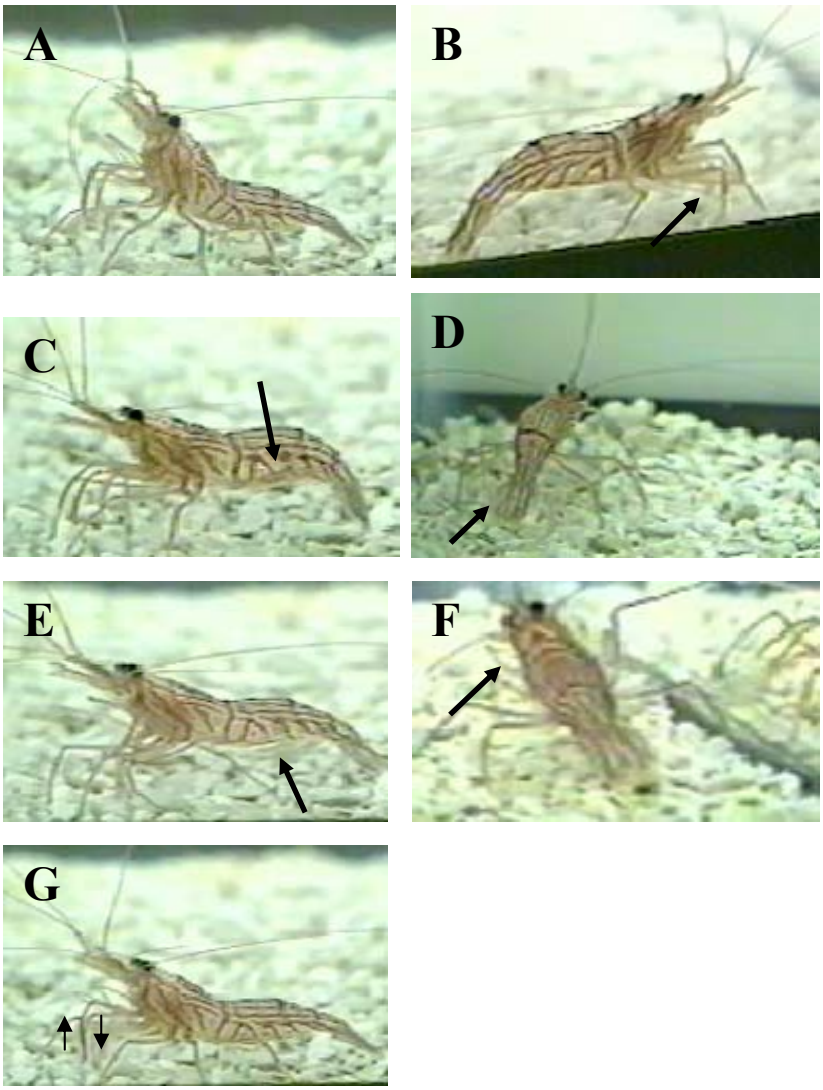

Figure 6.2. Grooming behaviours performed by *L. wurdemanni*. (A) Antennular grooming by maxillipeds; (B) Pereiopod grooming by other pereiopods; (C) Abdominal grooming by 5th pereiopod; (D) Uropod grooming by 5th pereiopod; (E) Pleopod grooming by 2nd pereiopods; (F) Carapace grooming by 2nd pereiopod; (G) Maxilliped "autogrooming". Arrows in (B)-(F) indicate appendage used for grooming activity; arrows on (G) indicate directional movements of maxillipeds.

and the movements of these limbs (e.g. during locomotion) moves the setae along gill filaments. Since passive gill cleaning could not be observed in this study, gill cleaning in general was excluded from analyses.

The identities of individual shrimp were determined using measurements of body length. Still images were captured from the video feed when a shrimp was positioned upon the black plastic insert. These images were imported into the software program ImageJ and the body length of each shrimp was measured. Perforations visible on the black plastic insert were used as an internal fiduciary standard.

RESULTS

The possibility of a 'day effect' was examined by comparing the grooming responses performed on each day of the salinity testing regime. Sign tests found no significant differences between Day 1 and Day 2 responses, at any salinity level (Siegel & Castellan Jr. 1988). Since no differences were observed between days, grooming behaviours were added together and these sums used for subsequent analyses.

Grooming behaviours were frequently performed during the total observation period (Fig. 6.3). A Friedman's test found an overall significant difference in the total number of grooming actions performed across the different salinities (P=0.028). Subsequent pairwise comparisons failed to detect any significant differences, but an analysis of rank sum differences found marginally non-significant differences between responses performed at 27‰ and 20‰, as well as between 27‰ and the final 33‰ level.

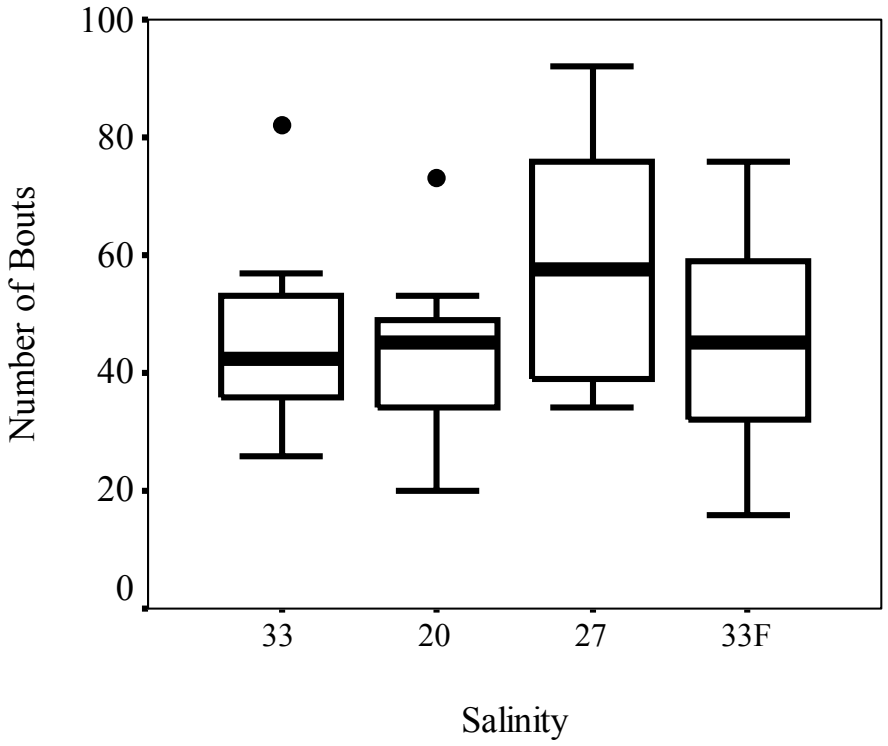

Figure 6.3. Total grooming activities performed by *L. wurdemanni* at salinities of 33‰, 20‰, 27‰ and a final 33‰ (33F). Horizontal bars represent 25th, 50th (median) and 75th percentiles. Whiskers indicate range. Circles represent outliers.

An overall significant difference was found in abdominal grooming (Fig. 6.4, Friedman's test, P=0.030), but subsequent pairwise analyses did not detect significant differences. An examination of rank sum differences found a marginally non-significant difference between abdominal grooming actions performed at 20‰ and 27‰.

An overall significant difference was also found in pleopod grooming (Fig. 6.5, Friedman's test, P<0.024). No significant difference was found in subsequent pairwise comparisons, but analyses of the rank sum differences indicated that responses at 27‰ were almost significantly different from those observed at 27‰ and the final 33‰.

A very significant overall difference was found in the performance of maxilliped auto-grooming (Fig. 6.6, Friedman's test, P=0.004). Pairwise comparisons found a significant difference between auto-grooming performed at 27‰ and the initial 33‰ (Dunn's test, P<0.05), as well as between 27‰ and the final 33‰ level (Dunn's test, P<0.01). All other comparisons were non-significant.

A extremely significant difference was observed in the number of bouts of carapace grooming (Fig. 6.7, Friedman's test, P<0.0001). Pairwise comparisons indicated that the large depression in carapace grooming at 20‰ was significantly different from actions performed at the initial 33‰ (Dunn's test, P<0.001), at 27‰ (Dunn's test, P<0.05) and at the final 33‰ (Dunn's test, P<0.01). Other comparisons were non-significant.

No significant differences were observed in pereiopod grooming (Fig. 6.8, Friedman's test, P=0.643), antennular grooming (Fig. 6.9, Friedman's test, P=0.157 or the uropods (Fig. 6.10, Friedman's test, P=0.158).

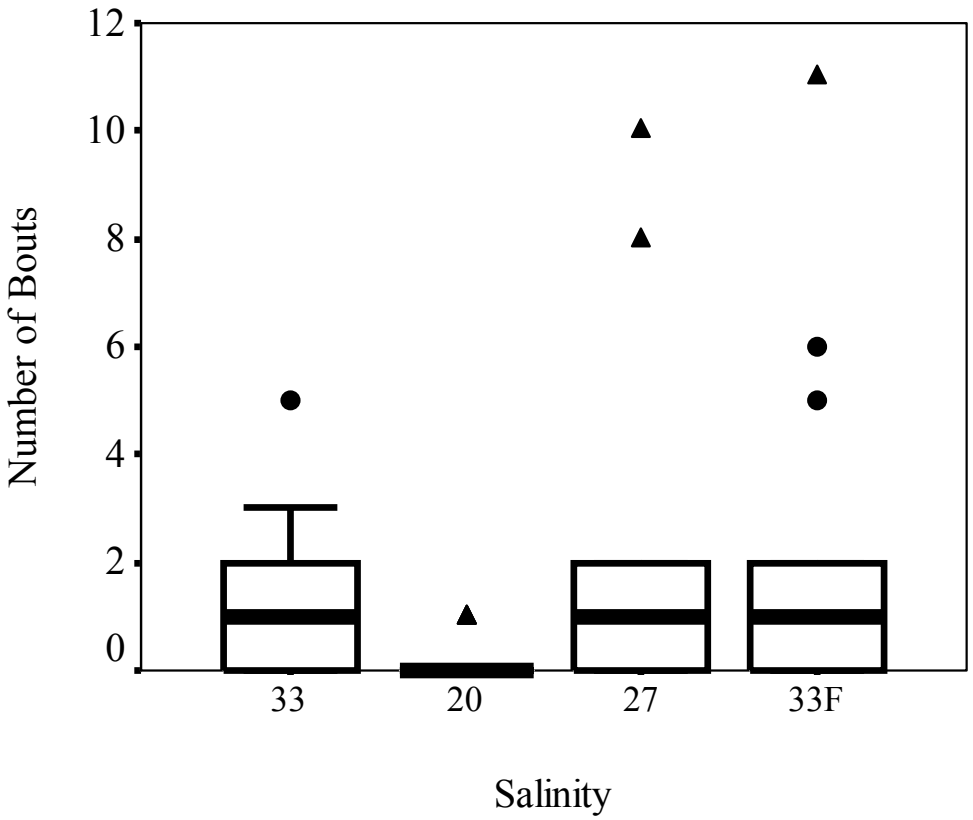

Figure 6.4. Abdominal grooming performed by *L. wurdemanni* at salinities of 33‰, 20‰, 27‰ and a final 33‰ (33F). Horizontal bars represent 25th, 50th (median) and 75th percentiles. Whiskers indicate range. Circles represent outliers, triangles are extreme values.

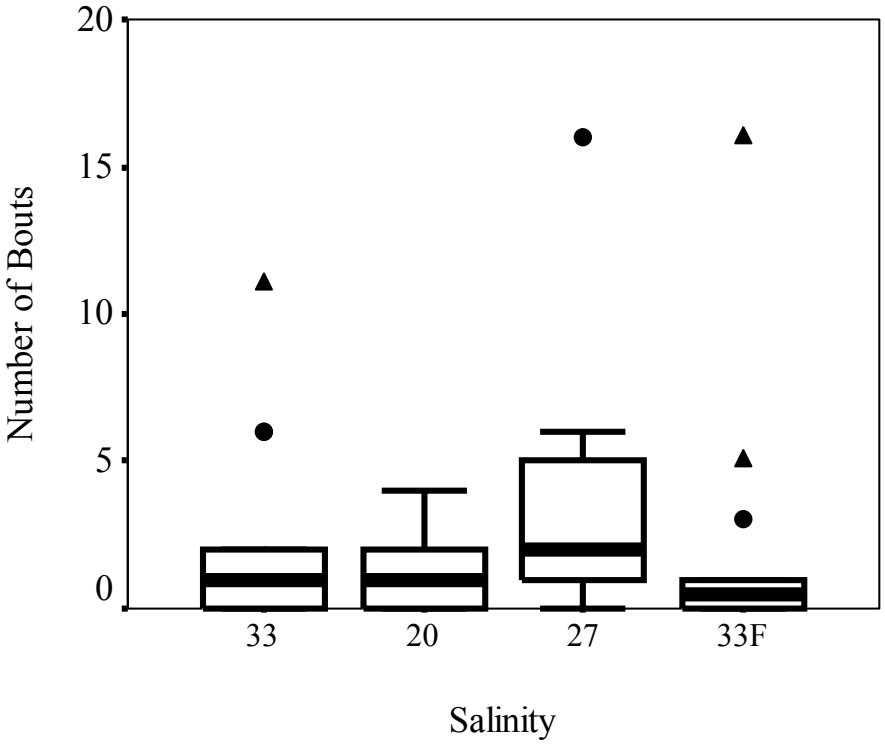

Figure 6.5. Grooming of pleopods as performed by *L. wurdemanni* at salinities of 33‰, 20‰, 27‰ and a final 33‰ (33F). Horizontal bars represent 25th, 50th (median) and 75th percentiles. Whiskers indicate range. Circles represent outliers, triangles are extreme values.

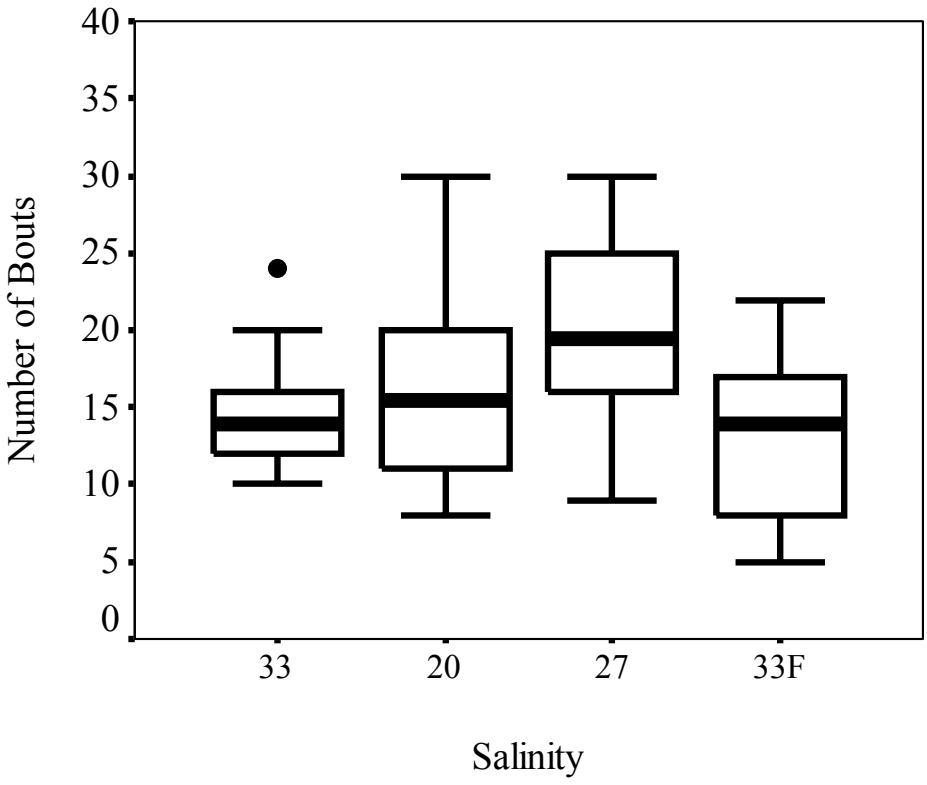

Figure 6.6. Maxilliped autogrooming performed by *L. wurdemanni* at salinities of 33‰, 20‰, 27‰ and a final 33‰ (33F). Horizontal bars represent 25th, 50th (median) and 75th percentiles. Whiskers indicate range. Circle represents outlier.

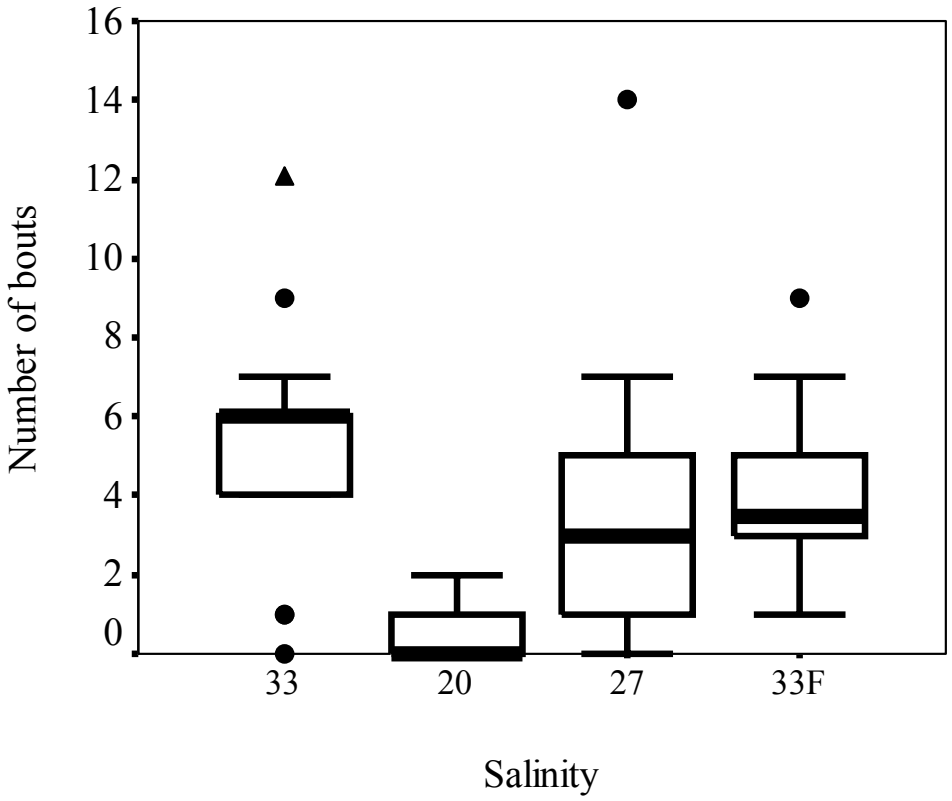

Figure 6.7. Grooming of the carapace by *L. wurdemanni* at salinities of 33‰, 20‰, 27‰ and a final 33‰ (33F). Horizontal bars represent 25th, 50th (median) and 75th percentiles. Whiskers indicate range. Circles represent outliers, triangle is an extreme value.

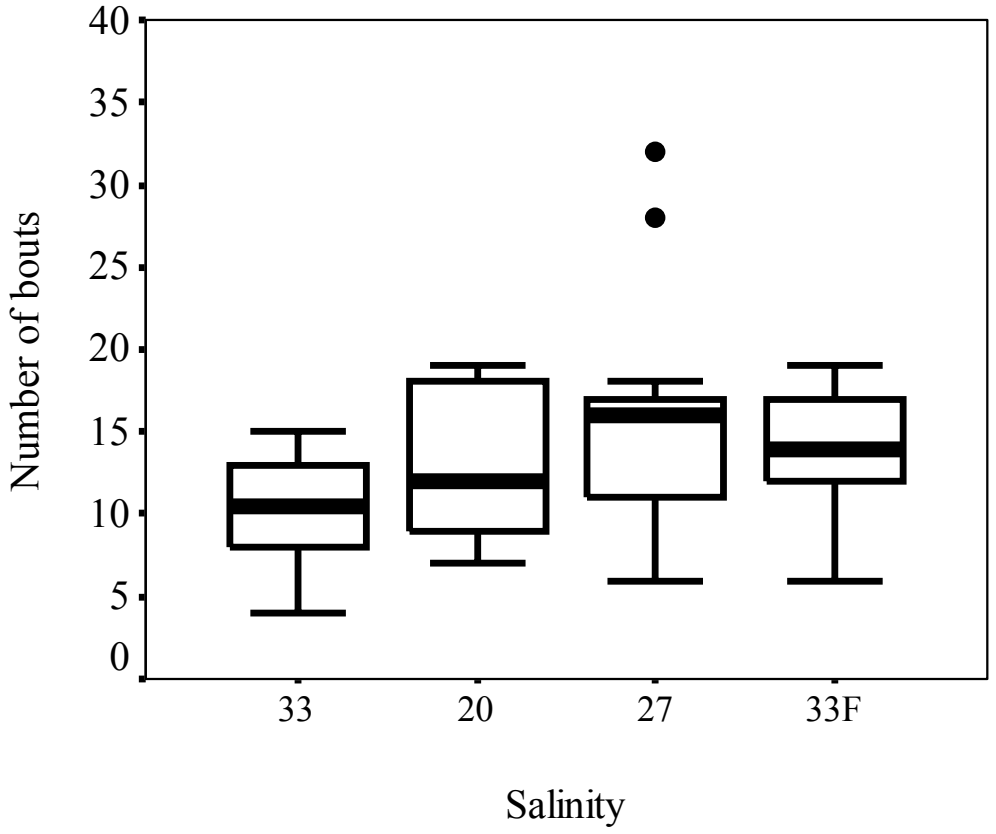

Figure 6.8. Grooming of the pereiopods by *L. wurdemanni* at salinities of 33‰, 20‰, 27‰ and a final 33‰ (33F). Horizontal bars represent 25th, 50th (median) and 75th percentiles. Whiskers indicate range. Circles represent outliers.

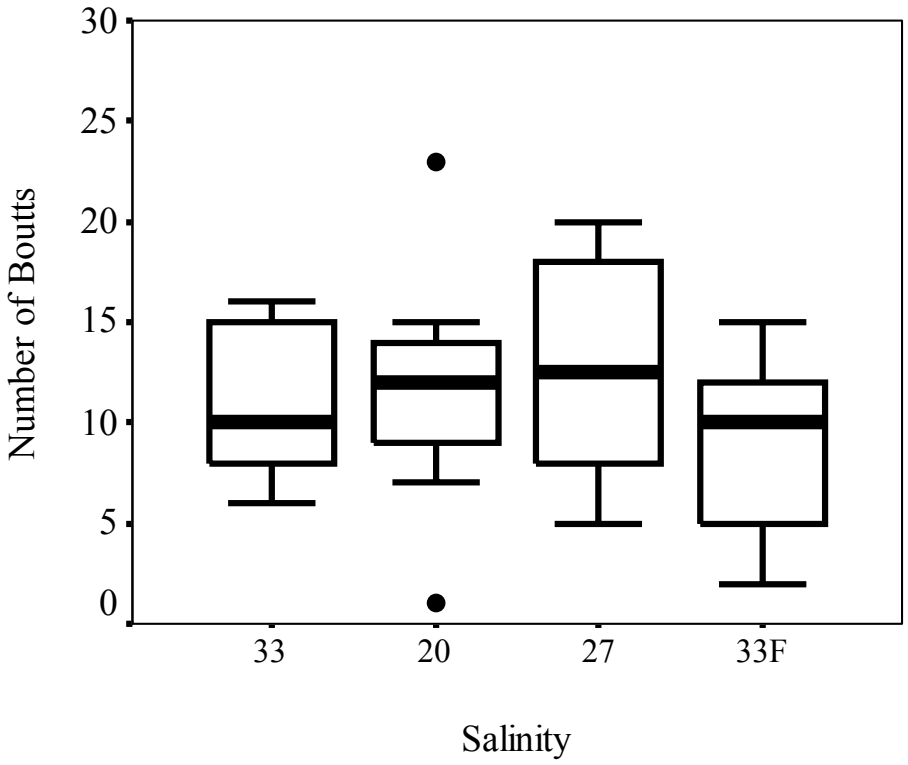

Figure 6.9. Grooming of the antennules and antennae by *L. wurdemanni* at salinities of 33‰, 20‰, 27‰ and a final 33‰ (33F). Horizontal bars represent 25th, 50th (median) and 75th percentiles. Whiskers indicate range. Circles represent outliers.

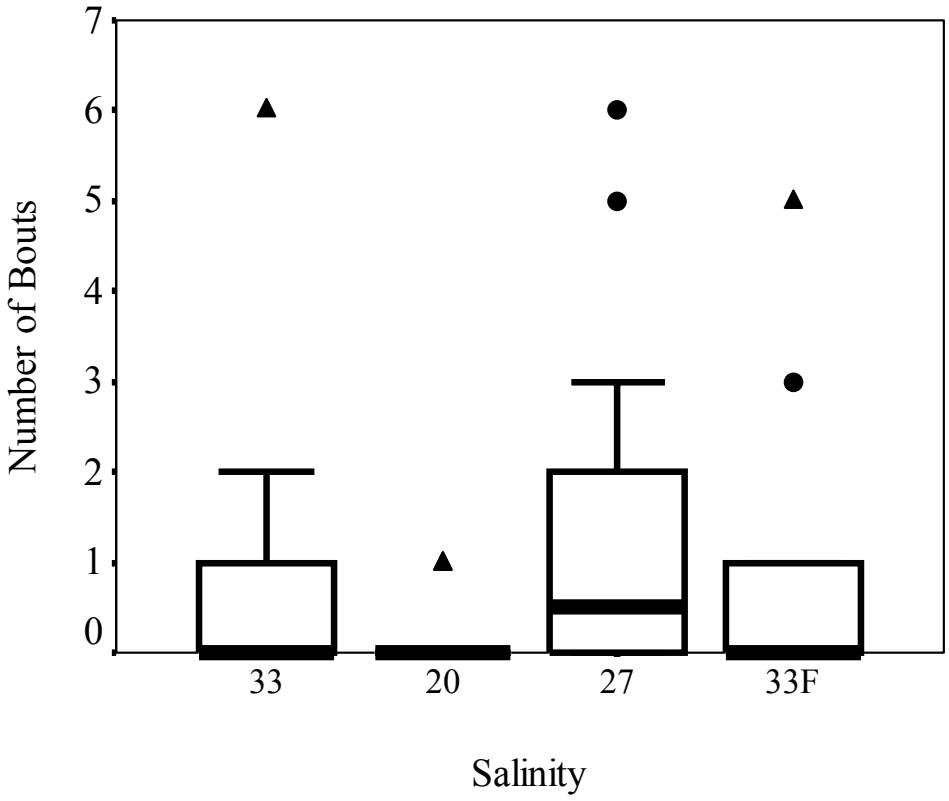

Figure 6.10. Grooming of the uropods and telson by *L. wurdemanni* at salinities of 33‰, 20‰, 27‰ and a final 33‰ (33F). Horizontal bars represent 25th, 50th (median) and 75th percentiles. Whiskers indicate range. Circles represent outliers, triangles are extreme values.

DISCUSSION

Grooming is an important part of the behavioural repertoire of crustaceans, and is used to remove epibiotic growth and sediment from body surfaces and structures. The sequence of events performed during grooming is quite similar across many taxa. Indeed, the mechanisms and structures employed in anti-fouling activities have been used to reconstruct possible phylogenetic relationships among decapod crustaceans (Bauer 1981; Bauer 1989; Suzuki & McLay 1998)

In this study, grooming of the pereiopods, maxillipeds and antennules were the most frequent grooming activities performed at the initial and final 33‰ salinity levels. A similar observation has been made in other crustaceans. Bauer (1989) reports that antennular grooming using the third maxillipeds is the most frequent grooming activity performed by decapods. Antennular grooming was also frequent in the stomatopod *G. oerstedii* (Bauer 1987). *L. wurdemanni* performed other grooming activities far less frequently, an observation that has also been reported for other decapods (Bauer 1989). Body grooming is rare in brachyuran crabs, but Becker & Wahl (1996) report that fouling of body surfaces may be avoided though burial within sediment, nocturnal activity and exposure to air (among intertidal species).

The results of this experiment indicate that a rapid decrease in salinity does not cause a general reduction in the performance of grooming behaviour in *L. wurdemanni*, but instead results in changes to the pattern of grooming activities. The carapace was groomed significantly less often when salinity was decreased to 20‰. This depressed response in carapace grooming correlates with the barnacle settlement pattern observed

in Giri & Wicksten (2001), where the vast majority of barnacles were found to have attached to the carapace of fouled *L. wurdemanni*. Individual shrimp collected in that study likely performed little carapace grooming, allowing barnacle cyprids to successfully colonize this large dorsolateral surface.

Giri & Wicksten (2001) also report that barnacles settled upon the dorsolateral surfaces of the abdomen. Fig. 6.4 suggests that this region also experienced a reduction in grooming, although no significant pairwise differences were found among salinity levels. However, abdominal grooming was not performed very often during the observation periods, and this general absence of grooming may partly explain the attachment of barnacles upon this region.

Pleopod and uropod grooming were also rarely performed. Giri & Wicksten (2001) found very few barnacles attached to these body surfaces. Cyprids may have avoided the pleopods in favour of larger flatter body regions (Berntsson et al. 2000). It is unclear, however, why barnacle cyprids did not settle upon the flat uropod and telson regions.

There were no significant differences observed in the grooming of pereiopods, maxillipeds, or antennules between the initial 33‰ and subsequent 20‰ conditions. Chemoreceptors are found on each of these body regions, and are used by decapod crustaceans during foraging, courtship and other social interactions (e.g.Case & Gwilliam 1961; Ache 1972; Shepheard 1974; Ameyaw-Akumfi 1977; Schmitt & Ache 1979; Derby & Atema 1982; Fontaine et al. 1982; Gleeson 1982; Tierney et al. 1984; Corotto et al. 1992; Voigt & Atema 1992; Bayha et al. 1993; Giri & Dunham 1999; Giri

& Dunham 2000). Under stressful environmental conditions, individuals may groom certain areas preferentially, and attempt to keep chemosensitive regions free of fouling.

Bauer (1977) used ablations to determine the effectiveness of antennular grooming in *H. pictus*. The antennules of shrimp which had maxillipeds ablated were quickly fouled by the filamentous bacterium *Leucothrix* sp., ciliates, and particulate matter. Fouled individuals were also observed to lose olfactory aesthetasc hairs from the antennules. The loss of aesthetascs and the potential clogging of other receptors would severely impact the ability of individuals to obtain information about the surrounding environment, and may have a detrimental effect on survival.

In addition, fouling of body surfaces is known to affect the locomotory ability of crustaceans. The occurrence of epibiota could increase drag and thus deleteriously affect swimming ability. Bauer (1981) describes a reduction in body grooming that is correlated with an evolutionary trend from swimming to walking. Grooming actions are more common amongst swimming shrimp-like crustaceans than among the crab-like walking forms (Brachyura). Interestingly, intense bouts of grooming can be observed in portunid crabs, where swimming has developed secondarily. This suggests that active grooming via body appendages is correlated with the maintenance of swimming efficiencies.

Fouling can potentially affect the ability of individual *L. wurdemanni* to obtain fertilization opportunities. A pure searching mating tactic requires intermoult individuals to be both mobile and able to chemically distinguish receptive individuals from other conspecifics. The attachment of epibiota to outer body surfaces and

chemosensory structures may preclude the successful performance of this tactic and dampen the number of successful matings obtained.

The distribution of *L. wurdemanni* along coastal regions ensures that individuals may be exposed to marked variations in salinity. The osmoregulatory abilites of *L. wurdemanni* are as yet unstudied, but the responses observed in this experiment suggest that this shrimp species may be a fairly effective osmoregulator. McGaw (1999) found that crab species which were efficient osmoregulators increased antennular cleaning, while weak regulators and osmoconformers did not perform this behaviour. Although *L. wurdemanni* did not increase grooming of the antennules, there was also no reduction in the performance of this behaviour. This shrimp species may be able to tolerate reductions in salinity for periods of time. Future studies are needed to ascertain the osmoregulatory physiology of *L. wurdemanni.*

CHAPTER VII

SUMMARY

Although most species of crustaceans are gonochoristic, there exist several representatives that are hermaphroditic. Hermaphroditism has been reported in several species of decapod crustaceans, especially within the Infraorder Caridea. These shrimp typically display forms of protandric hermaphroditism, with the majority of individuals initially maturing as males, but later developing into females. Variations on this type of hermaphroditism have been observed in several species, with individuals retaining male characteristics throughout their lifetime, or maturing directly as females.

Until recently, shrimp belonging to the genus *Lysmata* were considered to be protandric hermaphrodites. Several recent studies, however, have indicated that these shrimp instead possess a unique sexual system. Bauer (1998) found that in *Lysmata wurdemanni*, individuals initially mature as males, but become functional simultaneous hermaphrodites as they age and grow. In this system of protandric simultaneous hermaphroditism, smaller individuals reproduce as males, and possess a testis in addition to external characteristics typical of caridean males. With time, however, the ovarian portion of the ovotestis develops, a reduction occurs in the size of the external male characteristics, and the individual becomes capable of reproduction through female function as well as male. As in most caridean shrimp, mating is associated with moulting. Although *L. wurdemanni* can mate as a male during the intermoult period, breeding as a female only occurs for a short time immediately after moulting.

The operational sex ratio of *L. wurdemanni* is very male biased. While every small *L. wurdemanni* and every intermoult hermaphrodite can potentially mate as a male partner, the number of 'females' is determined by the number of recently moulted individuals. Since *L. wurdemanni* do not appear to moult *en masse*, the number of 'females' encountered in a population at any point in time is very low. In addition, moulting can occur without vitellogenic oocytes being present within the ovaries, and thus not all recently moulted individuals will be able to produce viable embyros. Under these conditions, it is expected that there will be much intra-sexual competition for access to potential mates. Selection should favour mechanisms and behaviours which permit individual *L. wurdemanni* to quickly identify and locate potential mates.

Crustaceans are known to use chemical cues in the initiation and maintenance of feeding behaviours, habitat selection, aggression and mating behaviour. The reproductive behaviours of several decapod crustacean species have been found to be influenced by sex pheromones, which can provide individuals with information about gender, moult stage, and reproductive receptivity.

In an experiment to examine the use of chemical cues in *L. wurdemanni*, conditioned stimulus water was collected from individuals of three distinct reproductive conditions. These three solutions and a control were pumped into the four corners of a testing tank containing a single *L. wurdemanni*. The responses of test animals indicate that chemical cues can be used by members of this species to distinguish among the different reproductive conditions. These shrimp spent a greater proportion of time near the corner to which the solution collected from a recently moulted and reproductively

ready *L. wurdemanni* (H4) was pumped. In addition, test animals physically manipulated almost exclusively the plastic nozzle used to pump this H4 solution.

The increased response directed towards the H4 solutions suggested that any sex pheromone found in *L. wurdemanni* was produced by the maturing ovary. Previous studies have implicated the hormone methyl farnesoate (MF) in the stimulation of testicular growth, as well as in the maturation of the ovaries of several crayfish, crab and shrimp species. To determine if MF might be a key component of *L. wurdemanni* sex pheromone, increasing concentrations of MF were pumped into a small testing tank and the behavioural responses of a test shrimp recorded. *L. wurdemanni* did not perform any reproductive actions towards any of the MF solutions, or to any control solution. This result indicated that MF alone was not used as an indicator of reproductive condition. It was hypothesized that a combination of MF and crustecdysone (a hormone associated with moulting in crustaceans) may provide individuals with information about both moulting stage and reproductive receptivity.

Inter-sexual competition is expected to increase in biased operational sex ratios. When this ratio is male-biased, selection is expected to favour monogamy or mate guarding. These activities occur in a wide range of taxa, including several decapod species. Although *L. wurdemanni* does not display the sexual dimorphism typically associated with mate guarding, the extreme male-biased operational sex ratio suggests that this behaviour should occur.

The occurrence of both pre-and post-copulatory mate guarding activities was examined in *L. wurdemanni* at two competition densities. Copulation duration was

found to be influenced by the number of intermoult competitors present, as durations recorded in the low density condition (only 1 competitor present) was significantly higher than in the high density situation (5 competitors present). When competition was high, intermoult individuals approached the recently moulted H4 shrimp more often than after mating. Similarly, intermoult shrimp followed, physically contacted and remained within 1 body length of the H4 individual more often in the pre-copulatory period than in the post-copulatory session. These differences were not observed in the low density condition. There was almost no aggression observed between intermoult individuals. H4 shrimp were observed to perform greater numbers of escape behaviours after copulation had occurred in both density conditions, but approached intermoult conspecifics only in the low density condition.

Density was also observed to affect a number of reproductive behaviours performed before and after mating. When competition density was high, intermoult individuals may have reduced the number of pre-copulatory approaches made towards the receptive individual. Similarly, there was no difference observed in the total duration of pre-copulatory following behaviour.

The observations that intermoult shrimp spent relatively little time within 1 body length of the H4 individual, and that almost no aggression occurred in any replicate of this experiment indicate that mate guarding does not occur in *L. wurdemanni*. It was hypothesized that reproductive success might be greatest if individuals performed a tactic of pure searching during the intermoult phase, and performed multiple escape responses immediately after mating through female function.

L. wurdemanni with considerable epibiota attached were discovered while collecting shrimp for use in the above studies. Although epibiota is often encountered upon the surfaces of some crustaceans, it is rare to observe the fouling of quick-moving and frequently grooming shrimp. Epibiota found on *L. wurdemanni* were identified as epistylid ciliates, bryozoans (*Membranipora* sp.), and the estuarine barnacle *Balanus improvisus*. Barnacles were predominantly attached to the dorsolateral surfaces of shrimp, and to the carapace and abdomen in particular. An analysis of barnacle size indicated that the largest barnacles were between 20 and 26 days old. Precipitation data was obtained for the 90 day period prior to barnacle settlement, as well as for the 26 day period between settlement and collection. During both periods, the collection site received 121-135% more precipitation than average, a increase in freshwater that would have certainly reduced the salinity of the coastal region. Data obtained for three historical reports of shrimp fouling also indicated that fouling may occur after decreases in salinity.

The effect of salinity change on grooming behaviour was examined by exposing *L. wurdemanni* to a rapid decrease in salinity, followed by small increases. It was expected that *L. wurdemanni* would either decrease grooming behaviours entirely, or that the pattern of grooming would be altered. An analysis of specific grooming activities found that a general decrease in grooming behaviour did not occur, but that specific body areas were instead cleaned less often. Individual *L. wurdemanni* were observed to preferentially groom body regions that possessed chemoreceptors (e.g. pereiopods, maxillipeds, antennules), but reduced the grooming of the carapace and

abdomen. The fouling of body surfaces and chemosensory body regions may affect the ability of individual intermoult *L. wurdemanni* to successfully perform a pure searching mating tactic.

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APPENDIX

Several videoclips are mentioned within the text of this dissertation, and are used to illustrate testing conditions or behaviours observed. All clips were digitally captured from video feeds and then processed using Adobe Premiere. Descriptions of each clip are as follows:

Cleaning.avi—numerous *L. wurdemanni* swarm and subsequently groom human fingers.

Reflection.avi—illustrates how the use of reflective tape glued to the carapace of H4 individuals permit their identification under infrared illumination. The position of an H4 individual within the tank can be identified by the occasional but regular reflection from the attached tape. The H4 observed on the clip is followed by an intermoult H1.

Follow.avi—an H1 intermoult shrimp can be viewed following directly behind a near-moult H4 conspecific.

Vicinity.avi—an H1 intermoult shrimp remains within one body length of a near-moult H4 conspecific. Individuals are parallel to each other, and can be observed moving in tandem.

MateHigh.avi—moulting and mating under high density conditions. The H4 individual can be observed moulting, with mating occurring shortly after ecdysis occurs. Copulation is brief, and interference occurs when two H1 shrimp attempt to mate simultaneously with the recently moulted H4.

MateLow.avi—moulting and mating under low density conditions. The H4 individual can be observed moulting (ending with a tailflip out of the old exoskeleton). An intermoult H1 approaches this recently moulted shrimp, and mating occurs. Copulation is extensive in duration (compare with MateHigh.avi).

H4Escape.avi—Escape responses performed by an H4 after mating. The H4 individual can be observed "tailflipping" when approached or touched by H1 conspecifics. Activity in this clip is fairly slow; H4 escape responses are typically much more rapid.

Death.avi—this videoclip illustrates how freshly moulted H4 individuals may be killed if multiple matings occur. The clip begins shortly after the H4 shrimp has moulted and mated. This shrimp can be observed "spinning", and is quickly swarmed by several H1 conspecifics. Several copulations are attempted, and it is believed that the physical manipulations performed

by H1 competitors during this interaction severely damaged the H4 individual, resulting in this shrimp's death.

Grooming.avi—grooming behaviours were filmed under infrared illumination. This videoclip demonstrates what was visible during filming, and also illustrates the performance of antennular and maxilliped grooming.

VITA

Tuhin Giri
993 Kipling Avenue
Etobicoke, Ontario
M9B 3L3 CANADA

Educational Background

B.Sc. (Zoology), University of Toronto (Trinity College)	1990-1995
M.Sc. (Zoology), University of Toronto	1995-1997
Ph.D. (Zoology), Texas A&M University	1997-2003

Publications

Giri, T. & Wicksten, M. K. 2001. Fouling of the caridean shrimp *Lysmata wurdemanni* (Gibbes, 1850) by the barnacle *Balanus improvisus* Darwin, 1854 and other epibionts. *Crustaceana,* **74**, 1305-1314.

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Teaching Experience

Animal Behaviour (Toronto)	Animal Communication (Toronto)
Evolution & Population Genetics (Toronto)	Biological Imaging (TAMU)
Electron Microscopy (TAMU)	Embryology (TAMU)
Introductory Biology (TAMU)	Marine Biology (TAMU)