LARVAL TYPES, COURTSHIP AND MATING BEHAVIORS, AND THE COSTS

ASSOCIATED WITH EXCLUSIVE MALE PARENTAL CARE IN THE SEA SPIDER

ACHELIA SIMPLISSIMA (PYCNOGONIDA)

by

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A THESIS

Presented to the Department of Biology
and the Graduate School of the University of Oregon
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"Larval Types, Courtship and Mating Behaviors, and the Costs Associated with Exclusive Male Parental Care in the Sea Spider *Achelia simplissima* (Pycnogonida)," a thesis prepared by Zair P. Burris in partial fulfillment of the requirements for the Master of Science degree in the Department of Biology. This thesis has been approved and accepted by:

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Title: LARVAL TYPES, COURTSHIP AND MATING BEHAVIORS, AND THE

COSTS ASSOCIATED WITH EXCLUSIVE MALE PARENTAL CARE IN THE

SEA SPIDER *ACHELIA SIMPLISSIMA* (PYCNOGONIDA)

Approved: _____
                            Alan Shanks

In all species of pycnogonids (sea spiders) males care exclusively for the

offspring, making this group essential for studies on sex roles, sexual selection, and the

evolution of parental investment. Unfortunately, little is known about pycnogonid

mating patterns, larval development, or the costs associated with parental care. The

mating habits of both male and female *Achelia simplissima* were studied experimentally

and reveal that both sexes routinely mate multiple times and have multiple mates.

Parental males experience higher frequencies of predator attacks and epibionts and a

lower rate of movement as compared with nonparental males. However, parental males

are harder to dislodge than nonparental males and suffer no change in feeding frequency

as a result of parental care. The external morphology of the first larval stage of *Achelia*

simplissima was described using SEM photos and compared with other larval

pycnogonids. Morphological characteristics suggest a "parasitic" mode of postembryonic development.

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CHAPTER I

INTRODUCTION

Pycnogonids are slow moving marine arthropods that can be found crawling over the muddy bottom of the deep sea, clinging tightly to intertidal cnidarians, or actively swimming through the water column (Morgan *et al.,* 1964; King, 1973; Arnaud & Bamber, 1988). Sea spiders exhibit a number of interesting characteristics: most are parasites during part of their lifecycle, females are often larger than males, the males brood the embryos using a pair of specialized legs, and in both sexes guts and gonads extend into each leg (Cole, 1904; King, 1973). In spite of these unusual and extraordinary features, little is known about sea spiders in general and very few studies have dealt with American species (Child, 1992; Bain & Govedich, 2004).

Pycnogonids are among the few marine invertebrates exhibiting exclusive male-care (Child, 1992). Because of this uniparental care and that females are often larger than males, sea spiders are hypothesized to be sex-role reversed, that is, to have intense female-female competition for mates (Shuster & Wade, 2003; Bain & Govedich, 2004). This idea has never been tested directly; however, a single study of the genetic mating system of *Pycnogonum stearnsi* has provided evidence that at least one species of sea spiders may have conventional sex-roles (Barreto & Avise, 2010). Yet, the little information available on sea spider mating behaviors, such as which sex courts, suggests a variety of possible mating strategies. For instance, in two closely related species,

Propallene saengeri and *Propallene longiceps*, the former has intense female competition during courtship that leads to 'physical combat' (Bain & Govedich, 2004), while the latter has male courtship involving stroking of the female with his ovigerous legs (Nakamura & Sekiguchi, 1980).

Although sea spiders could be very useful for testing parental investment hypotheses and sexual selection theories (Shuster & Wade, 2003), courtship and mating behaviors have been witnessed for only a handful of species (Arnaud & Bamber, 1988; Bain & Govedich, 2004). Current knowledge is based mainly on laboratory observations that have become generalizations for the group as a whole (Bain & Govedich, 2004). During mating, the female transfers her eggs to the male, who fertilizes them externally and forms them into balls that are then attached to his specialized ovigers (Nakamura & Sekiguchi, 1980). Although males have been recorded carrying up to 14 egg masses simultaneously, it is not known if they have mated once or multiple times, and with a single female or multiple females (King, 1973). Even less is known about female mating behaviors, since most mating studies have focused almost entirely on the male (Reviewed in Bain & Govedich, 2004).

Mating patterns are likely very diverse in the pycnogonids, species differ in the number of egg masses they carry, in the number of eggs in each egg mass, and in the size of their eggs. For instance, males of *Pycnogonum rickettsi* have been found carrying only a single large egg mass full of thousands of small eggs (pers obs)(Figure 1A). Males of *Achelia gracilipes* carry at least 16 egg masses, but only 6-8 large eggs in each (pers obs)(Figure 1B). *Achelia chelata* has been found carrying over 30 egg masses, with 10-

20 small eggs in each (pers obs)(Figure 1C). Depending upon how these species partition

their eggs into egg masses, *P. rickettsi* may be shown to have a monogamous mating

pattern, while *A. chelata* may mate 15 or even 30 times during a breeding period.
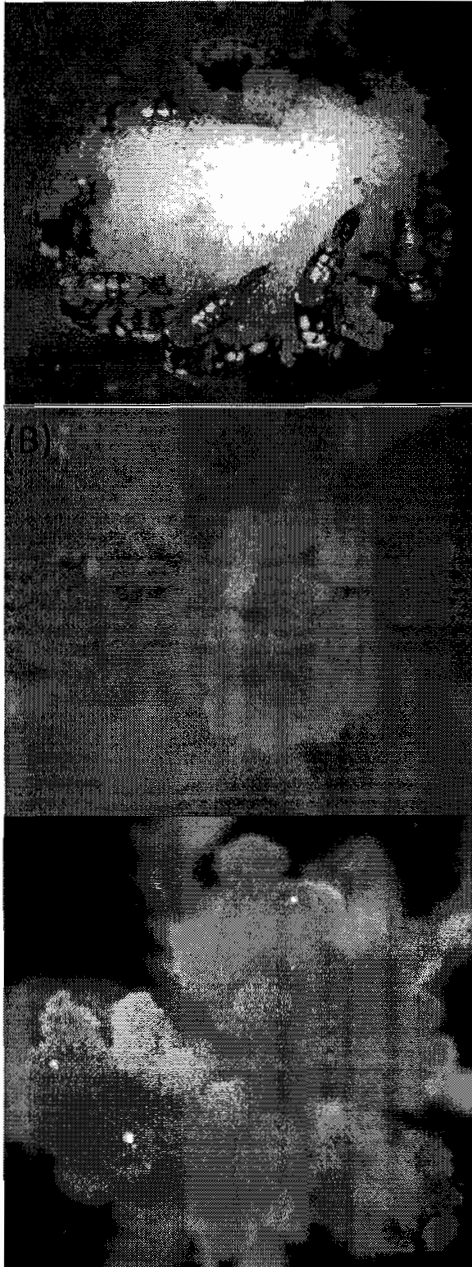
Figure 1: Ventral view of male sea spiders with egg masses. **(A)** *Pycnogonum rickettsi* carrying one large egg mass. **(B)** *Achelia gracilipes* carrying at least 16 egg masses with 6-8 large eggs in each. **(C)** *Achelia chelata* with over 30 egg masses, with 20-30 small eggs in each.

The personal cost to males of providing prolonged care to young has never been quantified (Bain & Govedich, 2004; Barreto & Avise, 2008). Reports of males carrying so many egg masses that they are "barely visible" suggest that parental males may have reduced foraging, lower mobility, increased predation, and a higher susceptibility to dislodgment than nonparental males (Cole, 1904; Stock, 1954; Arnaud & Bamber, 1988; Bain & Govedich, 2004). The length of time a male spends brooding, and hence the magnitude of the costs imposed on him, is dependent upon the species' mode of postembryonic development. For instance, a larva may leave the male immediately after hatching and become encysted in a hydroid ('encysted' mode of development), or a larva may stay attached to the male after hatching ('attaching' mode), until it becomes a juvenile half the size of the adult (Tomaschko *et al.*, 1997; Bain, 2003). A species with an 'attaching' mode of development is likely to have more significant parental care costs than a species with an 'encysted' development. Although postembryonic developmental mode is not known for most species of pycnogonids (Bain, 2003), it can often be inferred from the larval morphology (Bogomolova & Malakhov, 2003, 2004; Bogomolova, 2007; Cano and Lopez-Gonzalez, 2009).

The small intertidal pycnogonid *Achelia simplissima* Hilton 1939 is a convenient organism for studies on sea spiders (Figure 2A). This species is sexually dimorphic and animals can be easily sexed by the size and shape of their ovigers. Similarly, the transparent cuticle of this species allows the white eggs of reproductive females to be visible in their femurs (Figure 2B). *Achelia simplissima* occurs in relatively high densities at a ±1m intertidal level underneath rocks where it feeds on the spirorbid worm

(Spirorbis bifurcates). This thesis provides the first experimental study of the mating

system of both male and female sea spiders (Chapter 2), as well as evidence of significant

costs to brooding males as a result of parental care (Chapter 3). Larval morphology is

examined in detail using SEM and is compared with that of other larval pycnogonids in

order to infer the mode of postembryonic development (Chapter 4).
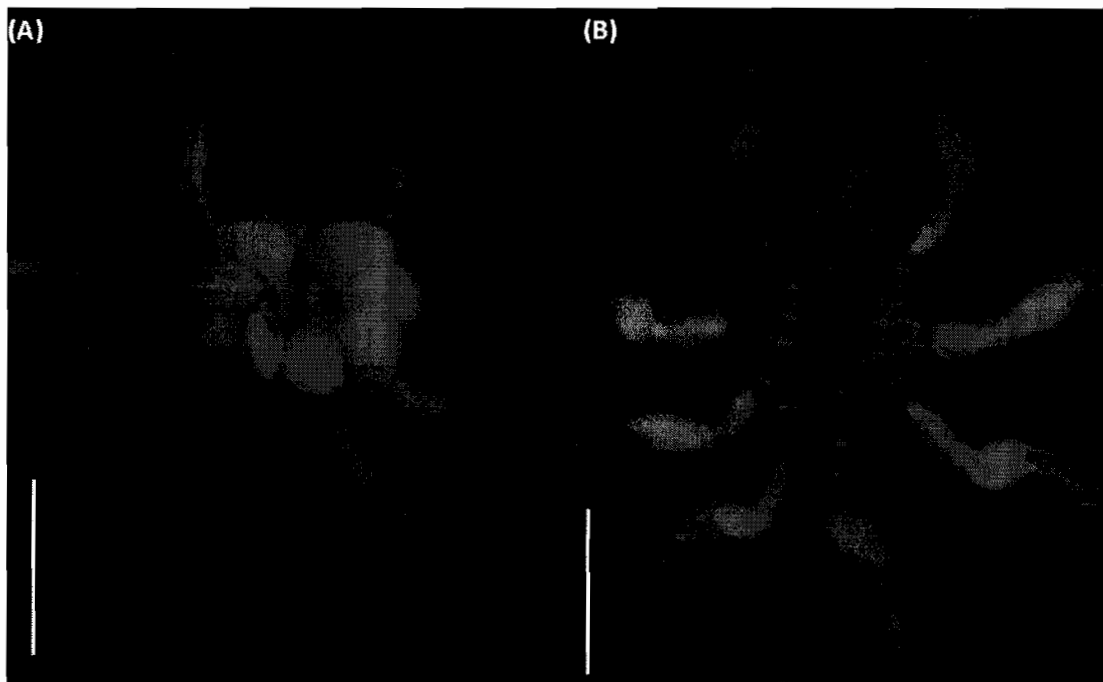
Figure 2. Adult *Achelia simplissima.* **(A)** Ventral view of male carrying eight egg masses, four on each oviger (scale bar = 1mm). **(B)** Dorsal view of a reproductive female with white eggs in each leg.

CHAPTER II

THE MATING SYSTEM OF THE SEA SPIDER *ACHELIA SIMPLISSIMA*

Introduction

Pycnogonids, the sea spiders, are a small group (1300 species) of marine invertebrates that exhibit a number of unique characteristics, among them a pair of specialized legs for carrying their embryos (King, 1973; Child, 1979; Arnaud & Bamber, 1988). In all species of pycnogonids, the males care for the offspring by carrying and actively aerating the egg masses (King, 1973; Arnaud & Bamber, 1988; Bain & Govedich, 2004a). In many species of pycnogonids, females are larger than males, which, coupled with their uniparental care, make them an interesting group for studies on sexual selection, sex-roles, and parental investment (King, 1973; Ridley, 1978; Shuster and Wade, 2003; Bain & Govedich, 2004a). Unfortunately, sea spiders have been overlooked and understudied because of their small size, cryptic coloration, and often patchy distribution. Few studies have been done on sea spider courtship and mating behaviors. The little that is known is often generalized to the group as a whole (Arnaud & Bamber, 1988; Bain & Govedich, 2004a). For instance, it is assumed that pycnogonids breed during the spring and summer, although only a handful of studies have focused on sea spider reproductive periodicity (Jarvis & King, 1975, 1978; Arnaud & Bamber, 1988;

Bain & Govedich, 2004a). Additionally, although information on competition for mates is nonexistent for most species, it is generally believed that males initiate courtship (King, 1973; Arnaud & Bamber, 1988; Bain & Govedich, 2004a). Most studies of pycnogonid mating have focused entirely on the male, providing little information on female mating behavior. Still, observations of eggs remaining in females even after mating events have led to the assumption that females mate multiple times and have multiple mates (Sanchez, 1959; King & Jarvis, 1970; King, 1973; Bain & Govedich, 2004a). To date, a single genetic study of *Pycnogonum stearnsi* has demonstrated routine multiple female matings in a sea spider (Barreto & Avise, 2010), but this has never been investigated directly.

Observations of males carrying many egg masses at once have also led to the belief that males often mate multiple times and with multiple females (King, 1973; Arnaud & Bamber, 1988; Bain & Govedich, 2004a). Unfortunately, few studies have actually carried out mating experiments to test these assumptions (Nakamura & Sekiguchi, 1980; Barreto & Avise, 2008, 2010). Instead, workers have inferred multiple matings from the different developmental stages of the egg masses carried by a male (King & Jarvis, 1970; Barreto & Avise, 2009). Yet the length of embryonic development may be highly variable and is only suggestive of multiple mating events, not multiple mates. There may be other reliable predictors of mating events and mate number, such as the number of egg masses or the positions of the egg masses on the ovigers. However, mating observations demonstrate considerable differences among species in the number of egg masses laid during each mating event (King, 1973; Bain &

Govedich, 2004a). For example, the females of *Propallene longiceps* lay two egg masses per mating, while *Pycnogonum litorale* routinely lay one large egg mass (Jarvis & King, 1972; Nakamura & Sekiguchi, 1980). It is unknown whether males continue adding eggs to already existing egg masses, or whether eggs are partitioned into individual masses after each mating event. Determining whether a male carrying fourteen egg masses has mated fourteen, seven, or two times would be valuable for future research on pycnogonids.

The number of matings and mates has only been determined for male pycnogonids, and in only two species (Barreto & Avise, 2008, 2010). Physical traits such as trunk size and oviger length did not explain male mating success, making behavioral traits during courtship potentially very important in determining reproductive success (Barreto & Avise, 2008, 2010). In many fish species with paternal care, females often prefer to mate with parental males over nonparental males (Ridley & Rechten, 1981). Similarly, brood size in pycnogonids could be influential during courtship and mating events. Determining the reproductive success of male pycnogonids, as well as the physical or behavioral traits associated with success, could provide insight into the direction of sex-roles in this group.

There is likely a variety of mating strategies in the pycnogonids, with differences in reproductive periods, in courtship behaviors, in the number of egg masses laid per mating event, and in the number of mates a female or male has during a mating period (King, 1973; Arnaud & Bamber, 1988; Bain & Govedich, 2004a). By documenting the fundamental mating behaviors and strategies of the intertidal sea spider *Achelia*

simplissima Hilton 1939, this study provides the groundwork for future studies on pycnogonid sex roles, parental investment, and sexual selection. These are the first observations of pycnogonid courtship and mating for any species in the family Ammotheidae and the first study to experimentally examine both male and female mating behaviors in any pycnogonid.

Materials and Methods

Animals were collected between January 2009 and March 2010 from Middle Cove and North Cove, Cape Arago, Oregon (43° 18'N latitude, 125° 25'W longitude). Animals were sexed based on femur size and oviger shape. Males and females were separated and kept on rocks with their food source, the spirorbid worm *Spirorbis bifurcates,* and used within two weeks of collection. Females were deemed gravid if white eggs were present in at least one femur. Males were considered reproductive if they were carrying eggs or if their ovigers were completely developed.

Description of Mating

Time-lapse photography was used to determine mating behavior and the number of egg masses laid during each mating event. Fifteen pairs of reproductive male and female pycnogonids were placed in culture dishes in a seawater table on small (4cm^2) rocks inhabited with spirorbid worms until mating occurred. Rocks were changed every week and water was changed every three days. Four males were allowed to mate with multiple females in order to determine if they added newly laid eggs to already existing

egg masses. Animals were photographed every minute until mating finished. Images were compiled in ImageJ. The time of day when mating occurred, the sex of the pycnogonid that initiated mating, the length of the mating event, and the number of new egg masses were recorded. Two pairs of animals were video-taped in real time during mating events to describe egg transfer and to determine behaviors indicative of a readiness to mate. One pair was interrupted during egg transfer to determine if fertilization occurs while the eggs are still on the female or after they were transferred.

Reproductive Period

Male and female sea spiders were collected during February, May, June, July and November 2009, and January and March 2010 to determine the reproductive period of *Achelia simplissima*. The percentages of gravid females and egg-carrying males in the population were recorded, as well as the number of egg masses each male carried. Pictures of the ovigers and the dorsal side of the trunk were taken of fifteen brooding and fifteen nonbrooding males to determine if physical characteristics (trunk area and oviger length) are indicative of male mating success. Images were analyzed in ImageJ and measured to the nearest 0.01 mm. Single-factor ANOVAs were run to test for differences in brooding and nonbrooding male characteristics.

Eggs were dissected out of the femurs of ten females, examined unstained, classed by size and color (Schmidt, 1971) as previtellogenic (lightly opaque and less than 0.125 mm in diameter) or vitellogenic (white and greater than 0.125 mm in diameter), and counted to determine if eggs mature all at once. Twenty-one males carrying between 1

and 7 egg masses (72 total egg masses) were chosen and the number of eggs in each egg mass was determined. A linear regression was performed and an equation for predicting the number of eggs carried by a male based on egg mass number was calculated.

Male and Female Mating Behavior

To determine whether both males and females mate multiple times and have multiple mates, ten males were each paired with two gravid females and allowed to mate for two weeks. Similarly, ten females were each paired with two reproductive males to determine female mating patterns. Animals were kept in culture dishes as above and checked twice daily for mating events. The number of mating events, the number of mates, and the time between mating events were recorded. Individual female femurs were photographed before and after mating to determine which female had mated and from which femur(s) the eggs were released. An additional study was added after females mated with only one male, to determine if this was due to mate fidelity or differences in male quality. Four females were each placed with four males and allowed to mate for 2 weeks. Matings, mates, and time between matings were also recorded.

To determine whether females prefer to mate with parental males over nonparental males, two choice experiments were run. First, both a nonparental male and a parental male (carrying between 2-9 egg masses) were placed with a gravid female as above. Second, two parental males, one carrying few (1-2) and the other carrying many (4-9) egg masses, were paired with a gravid female as above. Fifteen replicates were done for each experiment and animals were left until a mating event occurred or two

weeks had passed. The amount of time to mate and the male that had mated were recorded. An analysis of variance (ANOVA) was run to test for differences in times between matings for both experiments.

A total of thirty-two males were allowed to mate multiple times (a total of 53 times) with females and the placement of egg masses on the ovigers was recorded. To test whether egg mass development accurately reflects the order in which egg masses were laid, all 53 egg masses were checked daily for hatching. Egg masses were considered as hatched when they were no longer carried by the male and at least half of the mass had hatched. The time between when egg masses were laid and when they hatched (with regard to the previous egg mass) were recorded to determine if egg mass hatching times are good predictors of when egg masses were laid.

Results

Description of Mating

All matings occurred between evening and early morning (6pm to 8am). After every mating event, males were found with a single new egg mass. Of the males that mated multiple times, a new egg mass was found after each mating event. Eggs from a new mating event were not partitioned into multiple egg masses or added to already existing egg masses.

Males showed no apparent behavioral signs indicating their readiness to mate. However, all females that mated exhibited a 'pumping' motion during courtship. While 'pumping,' a female pushed her body away from the substrate and then towards the

substrate every 10-20 seconds (see supplemental "pumping" video). In the majority of mating events (12/19), the female initiated courtship by approaching the male, climbing on his dorsal side, pumping, and then leaving. The female approached and left the male multiple times before the male responded by pulling her underneath him. After orienting himself so that they both faced the same direction, the male then looped his ovigers underneath the female's ovigers. When pairs took this position, mating always occurred. On average, animals remained in this position for 202 minutes ($\pm$34 minutes SE, range 113 to 541 minutes), and the female continued 'pumping' throughout. On several occasions a second female was observed 'pumping' and often climbed underneath the mating pair and remained there for many minutes (up to 22) before leaving. This female never released eggs.

The mating female released eggs on her ventral side, where she held them with her ovigers in a loose ball. All eggs were released within 15 minutes of each other. The male then crawled over the front of the female, so that his proboscis was near the substrate and his back legs were holding on to the female's trunk. Using her ovigers, the female passed the eggs to the male, who took them with his ovigers. Three to six minutes later, the female would leave. The male then takes up to 10 minutes to pack the eggs into a tight ball. When mating was interrupted during egg transfer, the female was left with the eggs, which she eventually dropped. These eggs failed to develop, suggesting that fertilization does not occur until the eggs are passed to the male.

Reproductive Period

A total of 295 adult males and 216 adult females were collected. Both sexes were reproductive during all months studied (Figure 1A). On average, 93.4% (±3.5 SE) of females sampled were gravid, while most males (75.8%, ±7.4 SE) were carrying between one and twelve egg masses (Figure 1B). The majority (74%, ±4.3 SE) of males with egg masses carried fewer than 6 egg masses; less than 1% (±0.5 SE) carried 12 egg masses (Figure 1B). The frequency distribution of egg masses carried by males (ave.= 2.74 egg masses per male, variance: 2.72) did not fit a Poisson distribution (chi-sq=177.3, d.f=6, p<.001) (Figure 1B). There was no statistical difference in the oviger lengths of brooding (1.81 mm, ±0.02 SE) and nonbrooding males (1.78 mm, ±0.03 SE)(F=1.18, p=0.28), or in the size of the trunk (brooding: 0.95 mm², ±0.03 SE; nonbrooding: 0.93mm², ±0.04 SE)(F=0.15, p=0.69).
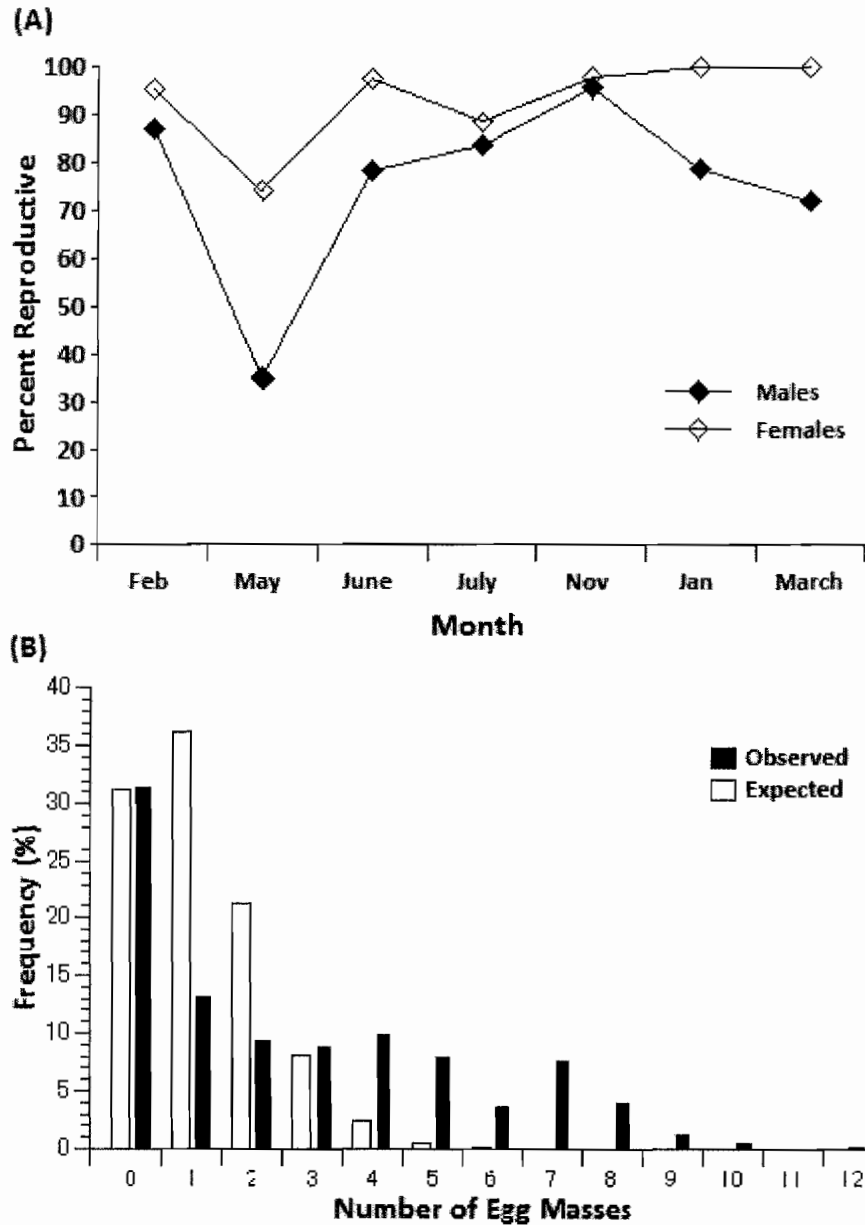
Figure 1: Breeding period of female and male sea spiders. **(A)** Percentage of gravid females (open diamonds) and egg carrying males (closed diamonds) in the population. The majority of males and females were reproductive during all months sampled. **(B)** Observed (black bars) versus expected (open bars) average frequency distribution of the number of egg masses carried by males. The majority of brooding males (74%) carried fewer than 6 egg masses simultaneously, while less than 1% of males carried 12 egg masses. The observed distribution did not fit the expected Poisson distribution (chi-square= 177, p<0.001).

The number of eggs in an egg mass differed considerably within and between individuals (range 89 to over 1250), but on average egg masses held 693 eggs (±32.8 SE)(Figure 2). The regression equation "Number of eggs = 18.22 ± 687.895(number of egg masses)" fit the data well (R^2= 0.84).
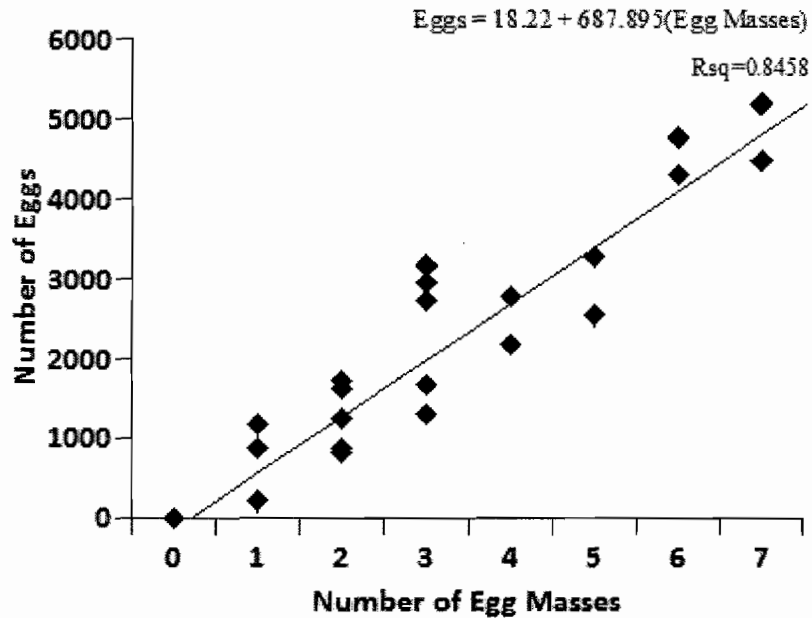
Figure 2: The number of eggs carried by a male predicted by the number of egg masses carried. Males carried as few as 211 eggs with a single egg mass to over 5000 eggs with 7 egg masses. Egg masses held, on average, 693 eggs.

On average, females had 949 (±122.4 SE; n=10) eggs present with a range of 376-1573 eggs. Females held between 15 and 255 eggs in each femur (average 127, ±17.1 SE; n=10). Both previtellogenic and vitellogenic eggs were present in each femur (Figure 3A), but vitellogenic eggs were slightly more abundant (50.4%, ±6.3 SE) than previtellogenic eggs (49.6%, ±6.3 SE). Almost one third of a female's vitellogenic eggs (30.3%, ±5.78 SE) and almost one quarter of all previtellogenic eggs (24%, ±1.34 SE) were present in a single femur (Figure 3B).
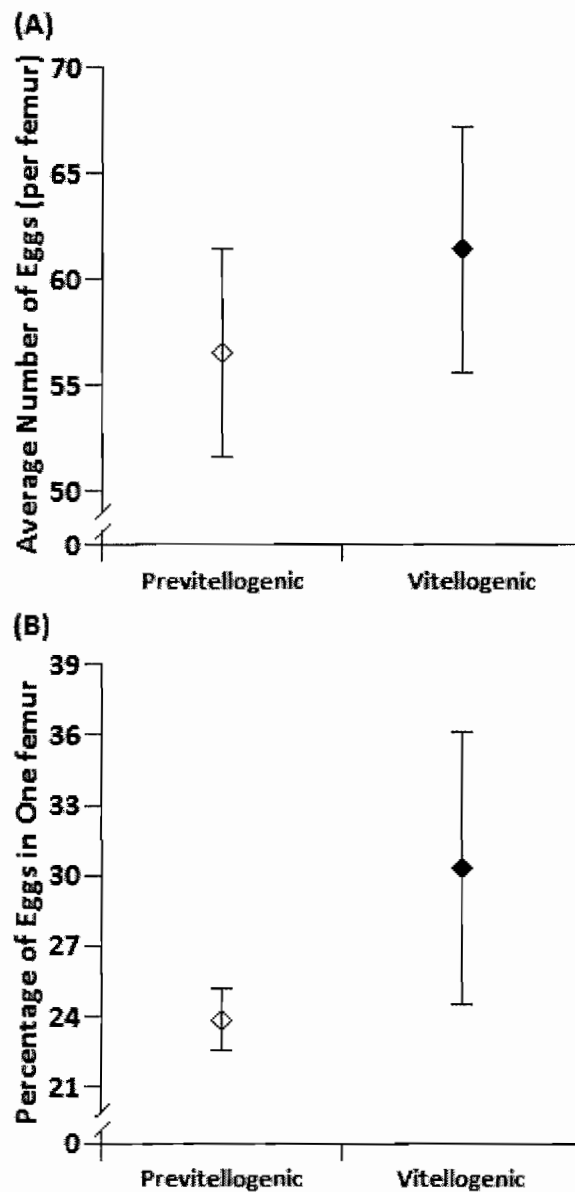
Figure 3: Previtellogenic and vitellogenic eggs present in female femurs. (**A**) Average number of previtellogenic and vitellogenic eggs present in each femur. Both previtellogenic and vitellogenic eggs were present in each femur, with slightly more vitellogenic eggs (61.4 eggs, ±5.8 SE) present than previtellogenic (56.5, ±4.9 SE). (**B**) Percentage of the total previtellogenic and vitellogenic eggs present in a single femur. Almost one third of a female's vitellogenic eggs (30.3%, ±5.78 SE) and almost one quarter of all previtellogenic eggs (24%, ±1.34 SE) were present in a single femur.

Male and Female Mating Behavior

Both males and females mated multiple times and had multiple mates. In the male mating study, all ten males mated after 2 weeks; 70% of males mated multiple times and with both females (Figure 4A). Two males mated three times and two males mated four times. On average, males took less time to mate a second time (32.6 hours, ±6.7 SE) than their first (51 hours, ±9.96 SE). Individual males re-mated in as little as 12 hours. The majority (75.9%, ±12.8 SE) of the females released many eggs from many femurs during a mating event. Females rarely released eggs from only a single femur (3.7%, ±3.7 SE) or from all femurs (20.3%, ±11.7 SE), instead releasing the majority of eggs from 3 to 5 femurs.

When ten females were each paired with two males, only 60% of females mated. However, 5/6 of those females mated twice and with the same male that they had mated with previously (Figure 4B). Females never mated with both males. On average, females took significantly longer to start mating (116 hours, ±27.3 SE) and to re-mate (86.4 hours, ±19.5 SE) than males in the previous study (single factor ANOVAs: F=7.1, p=0.019; F=8.8, p=0.014) . When there were four males present, every female mated and 3/4 of those females mated twice and with different males.
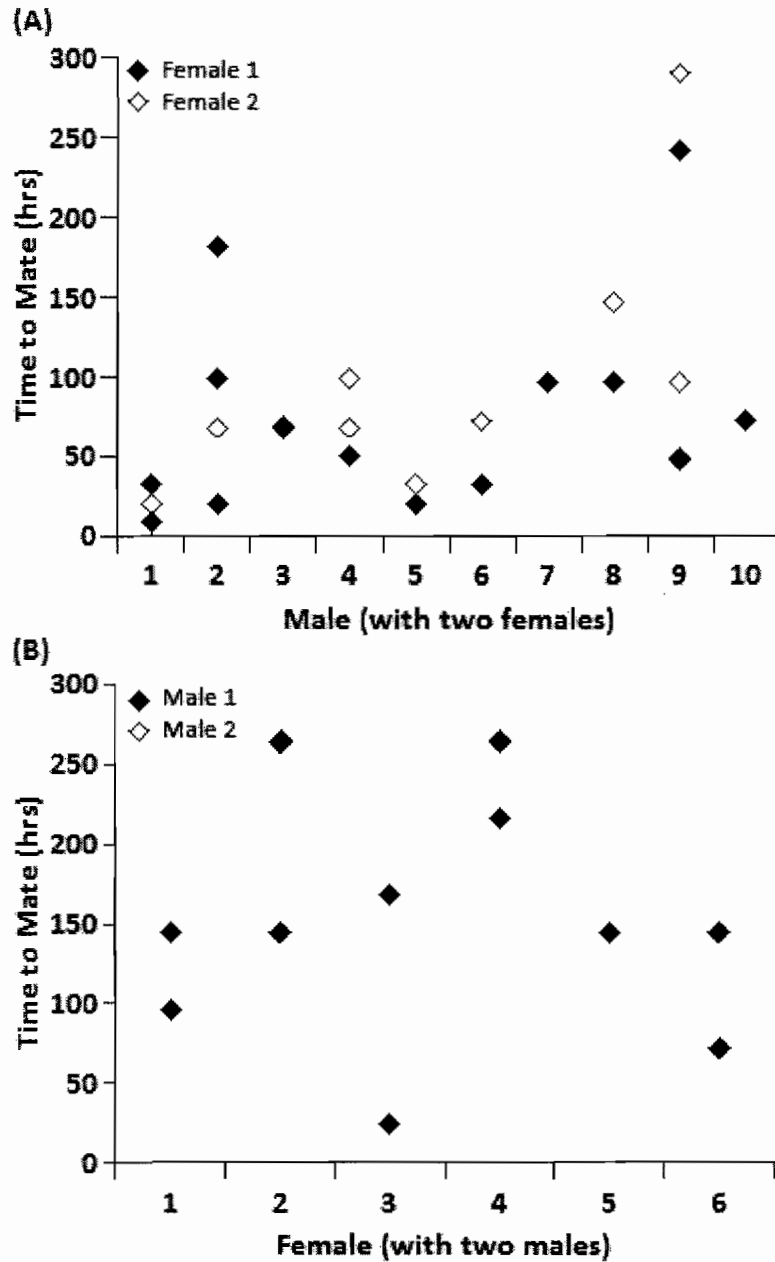
Figure 4: Number of mates and time (hours) between matings for male and female sea spider mating experiments. **(A)** Single males paired with two gravid females. 70% of males mated multiple times (up to 4) and with multiple females (up to 3 times with the same female). Males re-mated in as little as 12 hours (ave. 32.6 hours, ±6.7 SE). **(B)** Single females paired with two males. 50% of females mated multiple times with the same male. Females never mated with the second male. Females averaged 86 hours between re-matings (±19.5 SE).

When females were offered a choice to mate with parental and nonparental males, eleven of the fifteen females mated and, of those that mated, 91% mated with nonparental males (Figure 5A). When females were given the choice between parental males with few egg masses (1-2) and parental males with many egg masses (4-9), the majority of females (82% of the 11 that mated) mated with those carrying few egg masses (Figure 5A). On average, mating occurred significantly faster when nonparental males were present (152.7 hours, ±34.29 SE) than when two parental males were present (283.6, ±29.98 SE)(p=0.009, F=8.26)(Figure 5B).
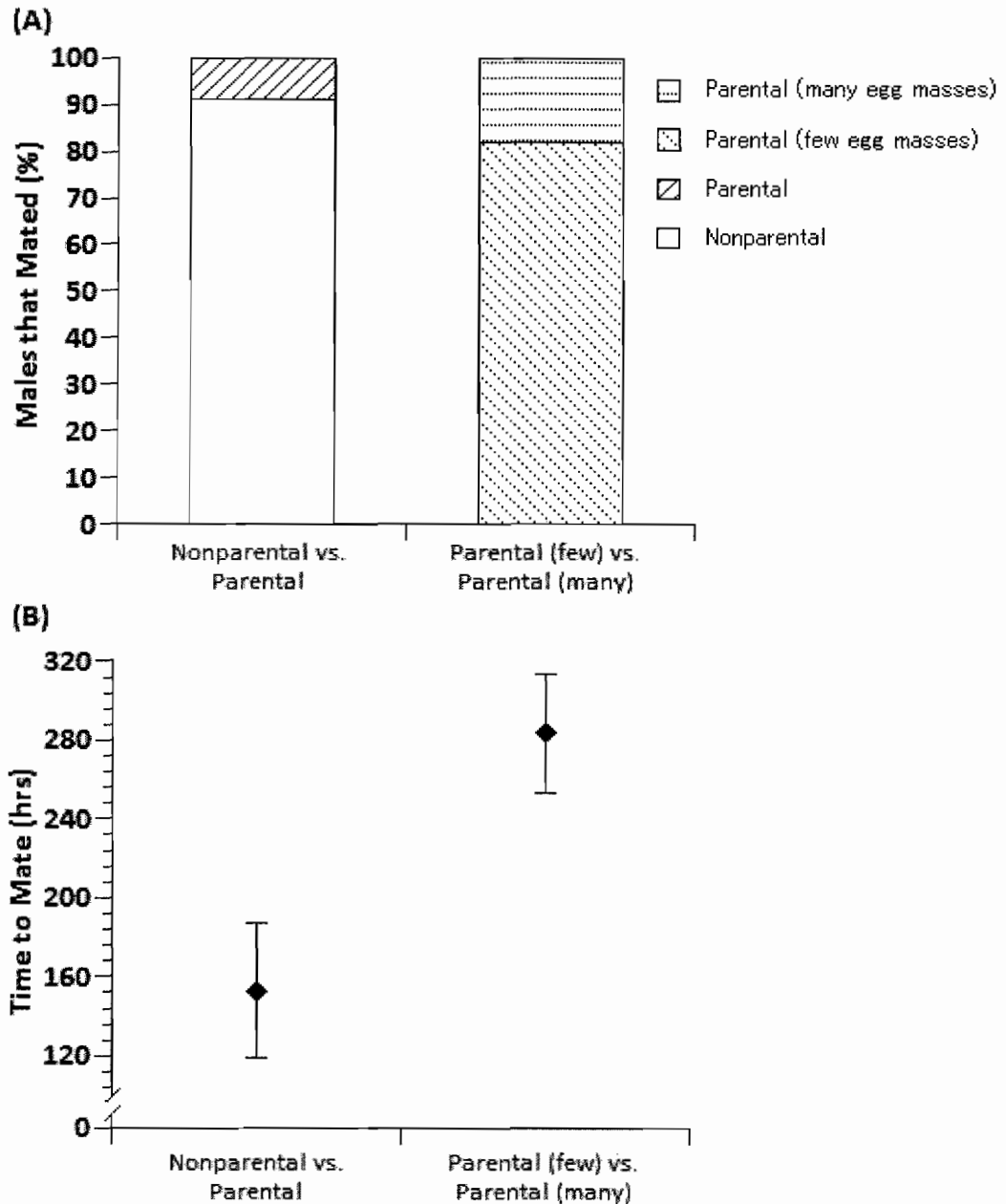
Figure 5: Female mate choice between nonparental and parental males and parental males carrying many or few egg masses. **(A)** Percentage of nonparental (open bar) and parental (hashed bars) males that females mated with. 91% of females mated with nonparental males. When given the choice between parental males carrying few (hashed right bar) or many (horizontal bar) egg masses, 82% of females mated with males carrying few egg masses. **(B)** Average female mating times when nonparental and parental males were present or when only parental males were present. Females mated in significantly less time when nonparental males were present (152.7 hours, ±34.29 SE) than when only parental males were present (283.6, ±29.98 SE)(ANOVA F=8.26, p=0.009).

Head

oviger
base

tip

1   5   2

3   4

Abdomen
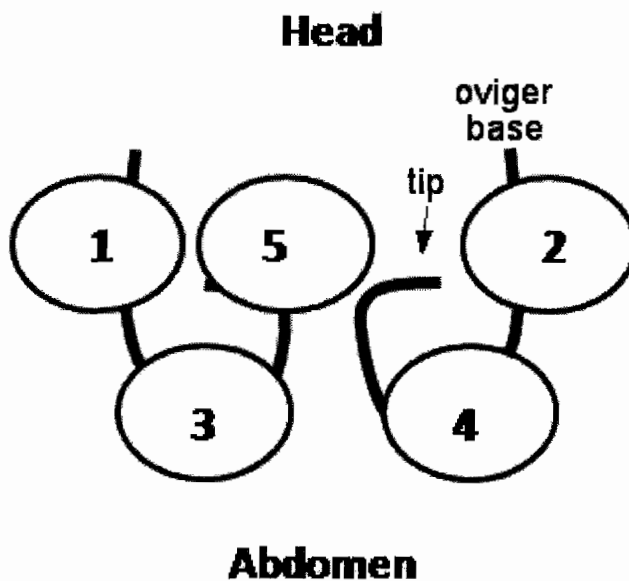
Figure 6: Placement (left or right oviger) and positioning (distal or basal) of egg masses by males. Most males (69%) placed the first egg mass on the right oviger, all males placed the second egg mass on the opposite oviger, and the majority (82%) placed the third egg mass back on the original oviger. Egg masses were added distally to the ovigers so that the most fully developed (first laid) egg masses were at the base of the ovigers and the least developed (youngest) at the tips of the ovigers.

Males exhibited a strong behavioral pattern when placing newly laid egg masses on their ovigers (Figure 6). Males alternated between placing new egg masses on the left and right ovigers, with the majority (69%) placing the first egg mass on the right oviger. Every male placed the second egg mass on the opposite oviger, and 82% of males placed the third egg mass on the original oviger. Newly laid egg masses were placed distally, so that the most developed (oldest) egg masses were found at the base of the ovigers and the least developed (youngest) at the tips. All egg masses hatched in the order in which they were laid; however, there were significant differences in egg mass hatching times within

and between males. Egg masses hatched in as little as 7 days, while others took as long as 36 days, for an average of 18.9 days (±1.14 SE). Males carrying 1 to 4 egg masses did not differ significantly in the amount of time they spent parenting (ANOVA F=1.28, p=0.3). On average, males carried one egg mass for 22.3 days (±2.2 SE), two egg masses for 24 days (±2.3 SE), three egg masses for 23 days, and 4 egg masses for 32.7 days (±2.9 SE). Relative hatching times were not good predictors of when egg masses had been laid (R^2= 0.06)(Figure 7). For instance, egg masses laid 2 days apart hatched an average of 7.4 days apart (±1.43 SE), instead of the predicted 2 days. Similarly, egg masses laid 13 days apart hatched an average of only 3 days apart (±2 SE), instead of the predicted 13 days.
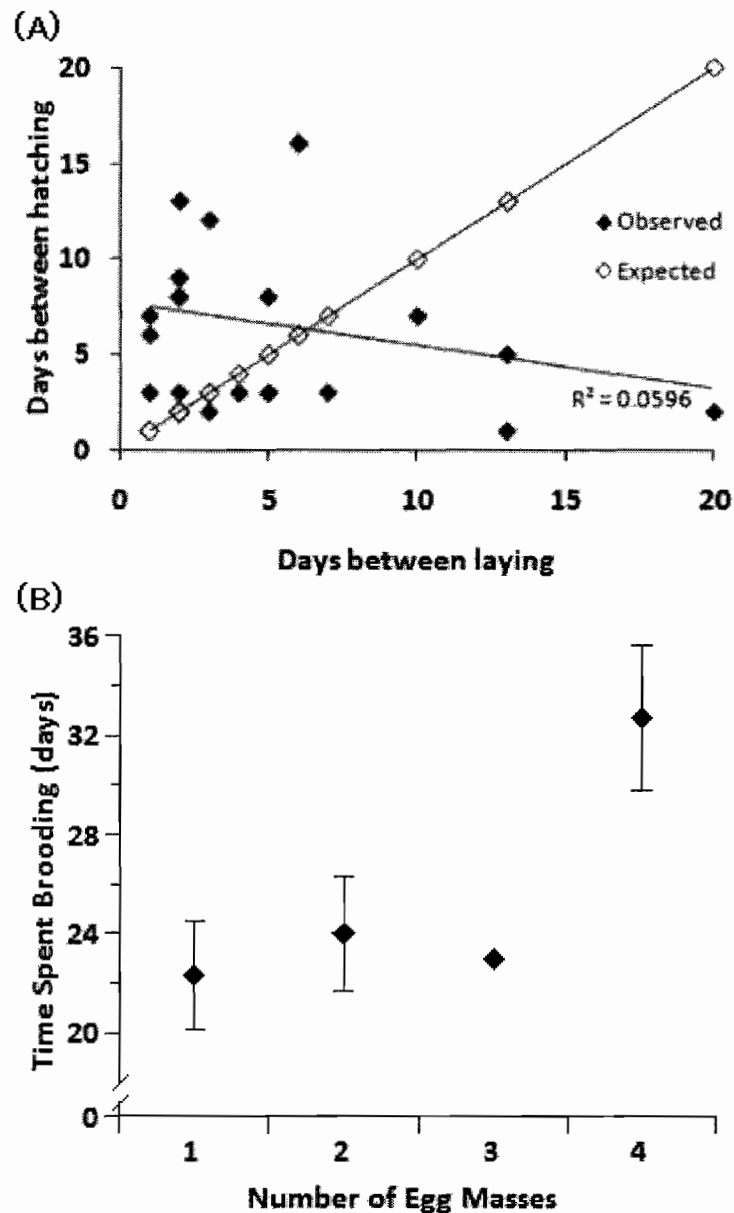
Figure 7: Egg mass hatching times (days). (A) The predicted (open diamonds) and observed (closed diamonds) relationship between when egg masses were laid and when they hatched. Hatching times were highly variable and the observed values did not fit the predicted line. (B) Average (±SE) amount of time (days) males spent brooding by number of egg masses carried. Brooding times did not differ significantly for males carrying 1, 2, 3, or 4 egg masses. On average, males carried one egg mass for 22.3 days (±2.2 SE), two egg masses for 24 days (±2.3 SE), three egg masses for 23 days, and 4 egg masses for 32.7 days (±2.9 SE).

Discussion

This is the first experimental study on the mating system of pycnogonids to address female as well as male mating patterns. *Achelia simplissima* mates year round, generally during the night, and both males and females mate multiple times and have multiple mates. Often, females of *Achelia simplissima* actively initiate courtship by 'pumping' while approaching a male. This is the first record of behavior indicating a readiness to mate for any species of sea spiders. While *A. simplissima* females are not the exclusive initiators of courtship, female courtship has been witnessed for only one other pycnogonid, *Propallene saengeri,* in which female-female aggression was also detected (Bain & Govedich, 2004b). However, no competition between females was observed in *A. simplissima.*

Although this is the first observation of mating for a pycnogonid in the family Ammotheidae, the mating process is similar to that described for other species in terms of mating position, mating duraation, and egg mass placement. Several species of pycnogonids, *Parapallene avida* and species of *Pycnogonum*, mate in the same position as *A. simplissima*, with the male on the back of the female and both sexes facing the same direction (Hooper, 1980; Nakamura & Sekiguchi, 1980). Mating in several other species, *Anoplodactylus lentus, Phoxichilidium tubulariae,* and *Endeis laevis,* differs only in that the two animals face opposite directions (Jarvis & King, 1972; Nakamura & Sekiguchi, 1980; Bain & Govedich, 2004a). Unlike species of the genus *Pycnogonum,* which take up to 5 weeks to mate, most pycnogonids require between 30 minutes (*Phoxichilis laevis*) and 4.5 hours (*Parapallene avida*) to mate (Jarvis & King, 1972; Hooper, 1980;

Nakamura & Sekiguchi, 1980; Bain & Govedich, 2004a). The duration of mating in *A. simplissima* is also fairly short, averaging only 3.5 hours.

Every mating event in *A. simplissima* resulted in a single egg mass; males did not partition eggs into multiple egg masses or add new eggs to already existing egg masses. Therefore, each egg mass is indicative of a mating event, meaning that males carrying 12 egg masses have mated twelve times. Apart from *Pycnogonum litorale* and *P. stearnsi*, *A. simplissima* is the only sea spider studied that does not partition its eggs into multiple egg masses (Jarvis & King, 1972; Bain & Govedich, 2004a; Barreto & Avise, 2010). For example, *Endeis laevis, P. longiceps, P. avida, Nymphon gracile* partition eggs into two egg masses after mating (Jarvis & King, 1970, 1972; Hooper, 1980; Nakamura & Sekiguchi, 1980; Arnaud & Bamber, 1988; Bain & Govedich, 2004a). Females often mated multiple times (up to 3) with the same male during experiments, demonstrating that, while indicative of the number of mating events, the number of egg masses a male carries is not a reliable predictor of mate number.

The placement of egg masses on the male is similar to what was hypothesized for the closely related *Ammothella hilgendorfi* (Barreto & Avise, 2008) and *Tanystylum brevipes* (Cole, 1901b), but opposite to what has been observed for other pycnogonid species. Males of *A. simplissima* placed egg masses on alternating ovigers, most often starting with the right oviger, and always added new egg masses distally. In all other species where placement is known, new egg masses are placed at the base of the ovigers so that the oldest (most developed) egg masses were found on the tips of the ovigers (Cole, 1901a; Nakamura & Sekiguchi, 1980; Bain & Govedich, 2004a; Barreto & Avise,

2008). The behavioral patterns of egg mass placement coupled with developmental stage is an accurate and much faster method than using hatching times for predicting the order that egg masses were laid. Although egg masses generally hatched in the order in which they were laid, hatching times were not predictive of when an egg mass was laid relative to other egg masses. Differences in hatching times are probably most affected by the number of eggs in the egg mass, since the amount of time males spent brooding 1, 2, 3, and 4 egg masses did not differ significantly. It is possible that with a large egg mass, the eggs in the center are receiving less oxygen than those eggs towards the outside (Strathmann & Strathmann, 1995), resulting in slower developmental rates for those inner eggs.

The high variance in the number of eggs each egg mass holds appears to be due to the number of ripe eggs in a female's femurs, and not an early termination of mating events or limit to the amount a male can hold. The number of mature eggs in each female femur varied considerably, with a high proportion of the vitellogenic eggs found in a single femur. Females did not release all vitellogenic eggs during each mating event, instead releasing some eggs from only 3 to 5 femurs. However, females of many other species of sea spiders release a set number of eggs during a mating event and from only certain femurs. For instance, *P. longiceps* releases two eggs from each femur during mating, *P. avida* releases 5-8 eggs from each femur, *E. spinosa* releases all eggs from only a single femur, and *P. litorale* releases all mature eggs from all femurs (Sanchez, 1959; Jarvis & King, 1972; Hooper, 1980; Nakamura & Sekiguchi, 1980). Unlike *E. spinosa*, whose eggs mature all at the same time (Nakamura & Sekiguchi, 1980), the eggs

of *A. simplissima* mature at different times within and between females, allowing females to mate many times over a long period of time.

Achelia simplissima is one of only two sea spiders that are known to reproduce year round (the other is *Ammothella longipes,* Munilla, 1980). For instance, *Endeis laevis* has two reproductive periods (from May to October and again from February to March)(Jarvis & King, 1975), *Parapallene avida* mates during the summer and autumn months (Hooper, 1980), and *Anoplodactylus angulatus* and *Phoxichilidium virescens* have been found carrying eggs only during autumn and winter (Jarvis & King, 1978). Since the main food sources for many pycnogonids are hydroids, which are often seasonal in abundance, the continuous breeding of *Achelia simplissima* could be explained by a year round availability of food. This species feeds on the spirorbid worm *Spirorbis bifurcates* which is abundant year round where *A. simplissima* is found. Similarly, the larval food source of *A. simplissima*, which is believed to be the same as the adult (pers. obs), may also play a role in supporting a year round reproductive cycle. For instance, in *P. litorale*, reproduction is synchronized with the abundance of the hydroid upon which the larva feeds (Wilhelm *et al*, 1997).

While the majority of male *Achelia simplissima* were reproductive year round, there was an unusually high frequency of males carrying either no egg masses or many egg masses (up to 12) as compared to random expectations. This suggests that some males may have a behavioral or physical advantage over others in acquiring mates. Similarly, when given the choice between two nonparental males, females only mated with one male, sometimes many times. Unfortunately, as in *A. hilgendorfi* (Barreto &

Avise, 2008), neither trunk size nor oviger size were significantly different in parental and nonparental males. What is more, no male-male competition was observed during courtship. It is also unlikely that differences in success are due to female encounter rates since most females (93.5%) in the population were gravid at any one time and this species occurs in high densities (ave. 50.1/m², ±8.3 SE, pers. obs). Rather, other physical or behavioral traits are likely to explain differences in male reproductive success. For instance, the number of egg masses a male is carrying appears to affect his mating success. When both a nonparental and parental male were present, the females mated with the nonparental males the majority of the time. Additionally, when both were parental males, the females mated with the males carrying the fewer egg masses. It appears that the act of parenting (carrying egg masses) either reduces a male's willingness or ability to mate or decreases his attractiveness to females. Since most males (99%) in the population were carrying many fewer than the maximum number of 12 egg masses, it is probably not likely that males were unwilling to mate. While carrying egg masses, parental males move much less frequently than nonbrooding males during the hours that mating occurs (see Chapter 3) and, hence, may encounter females less often. However, during these experiments, both parental and nonparental males were given the same access to females, so reduced encounter rates does not appear to be the cause of the difference in mating observed in the experiment. It is possible that parenting reduces a male's attractiveness to females; female mate choice may play a large role in a parental male's future mating success.

Even though *Achelia simplissima* has exclusive paternal care, female-biased sexual dimorphism, and females often initiate courtship, this species does not appear to be sex-role reversed. No female-female competition was observed during mating experiments and females appear to be the limiting sex in terms of reproduction. Females took much longer to mate and to re-mate than males, suggesting that it takes females longer to ripen eggs than it takes males to make more sperm. The operational sex ratio (the number of available reproductive males and females in the population), which is used to indicate the direction of sex-roles, is likely never female biased in this species (Clutton-Brock & Vincent, 1991; Andersson, 1994). Even though a male has mated, he is still considered reproductive in the population because he can carry multiple broods simultaneously. In addition, males are likely to never accumulate eggs fast enough to fill up the space on their ovigers since 99% of males in the population were carrying fewer than the maximum number of egg masses observed. According to the number of eggs a female has available in her femurs, she would never be able to produce more eggs than a male could hold, allowing more males than females to be available to mate at any one time. Similarly, the length of both mating and brood-carrying is not lengthy in this species, making it unlikely that *A. simplissima* is sex-role reversed.

CHAPTER III

THE COSTS OF EXCLUSIVE MALE PARENTAL CARE IN A PYCNOGONID

Introduction

Parental investments, from basic gamete production to complex care of young after hatching, result in cost to the parents (Clutton-Brock, 1991). One of the most costly investments is parental care (Keenleyside, 1979; Clutton-Brock, 1991), behavior by the parent that increases the number or quality of young (Trivers, 1972; Wittenberger, 1981). Often, the degree of parental care provided by the two sexes is uneven, with many species exhibiting uniparental care (Trivers, 1972; Ridley, 1978). In species where only one parent provides care, the job is typically filled by the female; males are rarely the sole care-givers (Trivers, 1972; Ridley, 1978). One of the few groups of marine invertebrates to exhibit exclusive male parental care is the pycnogonids (Child, 1992). Male sea spiders carry embryos ventrally, out of reach of predators, and actively ventilate them by swinging their specialized ovigerous legs back and forth (Bain, 2003). Reports of males carrying so many egg masses that they are "barely visible" suggest that parenting costs do exist (Cole, 1904; King, 1973). However, parenting costs have never been examined in any species of pycnogonid (Barreto & Avise, 2008).

This study explores four potential parenting costs in the small (1mm long) intertidal sea spider *Achelia simplissima* Hilton, 1939. First, parental males may be more visible to predators since the bright white embryos carried by this species disrupts its cryptic coloration. Moreover, predators that would not normally prey on a sea spider may be more inclined to if yolky eggs are present. Avoiding detection by predators is likely very important for pycnogonid survival, since they move slowly and have only an exoskeleton for protection (King, 1973). Second, parental pycnogonids may be more susceptible to epibionts than nonparental sea spiders. Since pycnogonids use their unique pair of ovigerous legs for grooming in addition to carrying embryos (Bain, 2003), males may not be able to use these ovigers to keep themselves free of epibionts when covered with egg masses. Sea spiders stop molting once they become adults (King, 1973) making the presence of an epibiont potentially very costly for future survival. Third, parental males may experience reduced mobility or a change in movement patterns making them more apt to come in contact with predators, less likely to encounter gravid females, or reduce their feeding frequency. Sea spiders of the species *Achelia simplissima* feed on sessile spirorbid worms (*Spirorbis bifurcatus* Knight-Jones, 1978), requiring movement on the part of the pycnogonid to obtain the next meal. Finally, brooding males may be more susceptible to dislodgment than males not carrying eggs. While aerating embryos, brooding males are generally farther from the substrate than nonbrooding males (pers obs), and may experience increased drag due to the attached egg masses. Aerating egg masses could deplete energy reserves of parental males, making them prone to dislodgment.

Parental care has been shown to be considerably costly for many animals (Lack, 1968; Verner & Willson, 1969; Thornhill, 1976; Kleiman, 1977; Shine, 1980; Thomas & Zeh, 1984; Zeh & Smith, 1985; Clutton-Brock, 1991; Schwarzkopf & Shine, 1992; Balshine-Earn, 1995; Smith & Wootton, 1995). The parental costs to male sea spiders have never been quantified but are likely to be significant (Barreto & Avise, 2008). Examining these costs would be valuable for studies of sexual selection, sex-role hypotheses, and the evolution of paternal care. This study explores the costs associated with parental care in the pycnogonid *Achelia simplissima.*

Materials and Methods

All animals were collected from Middle Cove or North Cove, Cape Arago, Oregon (43° 18'N latitude, 125° 25'W longitude), between January 2009 and March 2010. Animals were sexed based on femur size and oviger shape. Nonbrooding males and brooding males were separated and kept on rocks with their food source. Brooding males used in experiments were carrying between 3 and 9 egg masses. Nonbrooding males were deemed mature based on trunk size and degree of oviger development. Since it is also possible that these nonbrooding males could have recently cared for embryos, all males were held in the lab for two weeks before use to minimize any residual effects that parental care might have on subsequent experiments. In all experiments, brooding and nonbrooding males of similar trunk lengths were used. All averages are given ± SE.

Predation

There are few records of sea spiders as prey for other animals (King, 1973).

However, the intertidal distribution of *Achelia simplissima*, although limited to high on

the shore (±1 m), overlaps with that of many potential predators. Therefore, a variety of

potential predators were used in order to determine those most important, if any. Three

species of shore crabs (*Hemigrapsus oregonensis, H. nudus,* and *Pachygrapsus*

crassipes), the isopod *Gnorimosphareoma oregonensis*, tidepool fishes (*Xiphister sp,*

Apodichthys flavidus, Oligocottus maculosus), the tidepool shrimp *Heptacarpus*

sitchensis, and the starfish *Leptasterias aequalis* were tested as potential predators.

These animals are generalist predators and are of small enough size to potentially prey

upon *A. simplissima*. Potential predators were collected from South Cove, Oregon, and

held for three days without food before use.

Live male pycnogonids, two with and two without egg masses, were allowed to

attach to small rocks (1 cm²) held in place with clay in culture dishes and offered to

individual predators. Pycnogonids and predators were observed interacting and

behaviors were documented. After six hours the number of uneaten sea spiders was

recorded and predators were determined. Seven individuals of each predator type were

used.

To test whether brooding males are preyed upon more frequently than

nonbrooding males, subsequent feeding trials were conducted with the predators

determined from the previous experiment (the tidepool fish and shrimp). Both a

brooding and nonbrooding male were allowed to settle on a rock for 10 minutes as

described above. One predator was added and the time and order in which sea spiders were eaten were recorded. The number of attacks on each sea spider was recorded, as well as the amount of time the predator spent feeding. Animals were watched continuously until both males were eaten or until one hour (for shrimp predation) or three hours (for fish predation) had passed. Ten shrimp and 35 fish were used.

Dislodgment

To determine if brooding males of *Achelia simplissima* are more easily dislodged from rocks than nonbrooding males, a re-circulating flow tank was used (110 X 16 X 18cm; designed according to Vogel & LaBarbera, 1978) to accurately control water speeds. A small rock (1cm²) was attached with clay to the top of a rod extending 10cm into the water column. Flow rates in front of the rod were determined by timing particles traversing a known distance.

Achelia simplissima is very thigmotropic, attaching to anything it encounters (pers. obs). Males were allowed one minute to attach to the rock with no water current. Water speeds were slowly augmented over 5.5 minutes to a maximum speed of 86cm/s. The experiment started with a water speed of 10cm/s, which was held constant for 30 seconds, to ensure that the water reached the correct speed. The water speed was then increased over the next 30 seconds to 29.4cm/s and held constant for another 30 seconds. Water speeds were then increased over 30 seconds to 38.5cm/s, 50cm/s, 68cm/s, and 86cm/s, with 30 second pauses at each speed. Trials continued until the male was completely dislodged from the rock or until the maximum speed was reached and held for 30 seconds. The speed and time at which an animal was dislodged were recorded.

Dislodgment trials were repeated four times using the same 15 brooding and 14 nonbrooding males, with 24 hours between experiments. One brooding male was not included after the first trial because an egg mass hatched, leaving him carrying only two egg masses. Therefore, only 14 brooding males were used in the later three trials. The percentage of animals dislodged, the average water speed at dislodgement, and the average time to dislodgment were calculated for each trial. Paired t-tests were used to test for differences in average dislodgment frequencies, time, and water speeds between brooding and nonbrooding males.

Movement and Feeding

Time-lapse photography was used to document sea spider movement and feeding frequency. A small rock (12cm^2) was divided into two parts using clay and a brooding and nonbrooding male were placed on opposite sides of the clay wall. Animals were allowed 45 minutes to explore their surroundings. Sea spiders were kept on a 12 hour dark/12 hour dim light period and photographed every minute for 48 hours. Image sequences were analyzed using ImageJ and distances were measured to the nearest hundredth of a millimeter. For each male, distance traveled was measured and average movement per hour was calculated. The number of frames a male moved was recorded as a proxy for the percentage of time a male spent moving. The frequency of feeding and the percent of time (number of frames) spent feeding were also recorded for each male. A male was considered feeding if his proboscis was inserted into the tube of the spirorbid worm. Twelve brooding and twelve nonbrooding males were used. Separate paired t-tests assuming equal means were run for brooding and nonbrooding males to determine

day and night movement patterns. To test for differences in movement and feeding, Wilcoxon Signed-Ranks Tests were performed.

Epibionts

Brooding and nonbrooding males collected during the months of February, June, and November 2009 and January and March 2010 were checked immediately for the presence of epibionts. The type, number, and placement of epibionts on each male were recorded and frequencies by month were determined for brooding and nonbrooding males. Frequencies of sessile and mobile epibionts were also calculated. Differences in the overall frequency of epibionts for brooding and nonbrooding males were tested with a Wilcoxon Signed-Ranks Test.

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

Predation

Sea spiders were only missing or damaged in containers with the tidepool fish *Oligocottus maculosus* and the tidepool shrimp *Heptacarpus sitchensis*. Therefore, only these two predators were used in the choice experiments.

Only 8.6 percent (3/35) of fish *Oligocottus maculosus* preyed upon sea spiders, but in all cases both the brooding and nonbrooding males were consumed within the first 35 minutes of the experiment (Figure 1). Brooding males were always consumed before nonbrooding males, within the first 12 minutes (Figure 1). Nonbrooding males were consumed between 10 seconds and 23.5 minutes later (Figure 1). The fish did not handle the males before consuming them; pycnogonids were usually consumed during the first attack made by a fish.
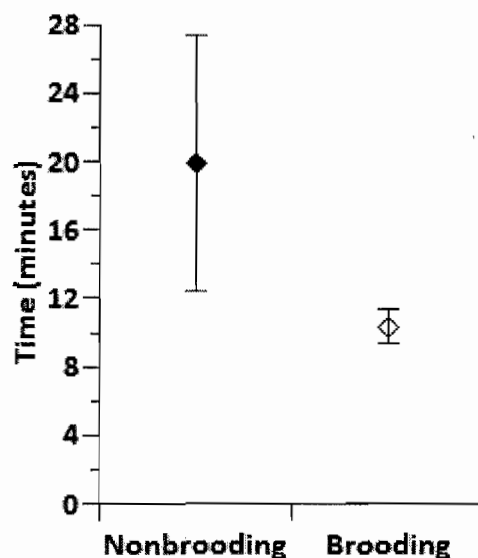
Figure 1: Fish *(Oligocottus maculosus)* predation on nonbrooding and brooding sea spiders. Both types of males were consumed by fish, but brooding males were always consumed before nonbrooding males (Average brooding: 10.33 minutes, ±1.01 SE; Average nonbrooding: 19.89 minutes, ±7.45 SE).

Only egg masses were consumed by the tidepool shrimp. Shrimp attacked brooding males first and much more frequently than nonbrooding males (Figure 2). Shrimp attacked 90% of brooding males at least once, while only 10% of nonbrooding males were attacked (Figure 2A). Brooding males were attacked 3.2 times on average (range 1 to 7 times) and nonbrooding males were attacked on average 0.2 times (Figure 2B). Shrimp attacked brooding males an average of 2.7 times (±0.36 SE) before consuming egg masses. Brooding sea spiders were seen actively moving away from shrimp before and after an attack. All sea spiders that were attacked were removed from the rock and then dropped by the shrimp after the attack. Shrimp attacks lasted between 1 and 5 seconds.

The majority of shrimp (80%) consumed sea spider egg masses (Figure 3). Shrimp fed for as few as 41 seconds to more than 8 minutes, but averaged 3 minutes and

46 seconds (Figure 3A). In the majority of cases, the shrimp succeeded in consuming the entire brood during one feeding event. However, 3/8 shrimp (37.5%) re-attacked brooding males multiple times (3.6 attacks on average), consuming eggs missed during the earlier feeding attacks (Figure 3B). By the end of the experiment, only one brooding male that had been attacked had eggs (approx. 20) left on his ovigers. No sea spider had any obvious damage as a result of shrimp attacks; all pycnogonids had two complete ovigers and all eight legs at the end of the experiment.
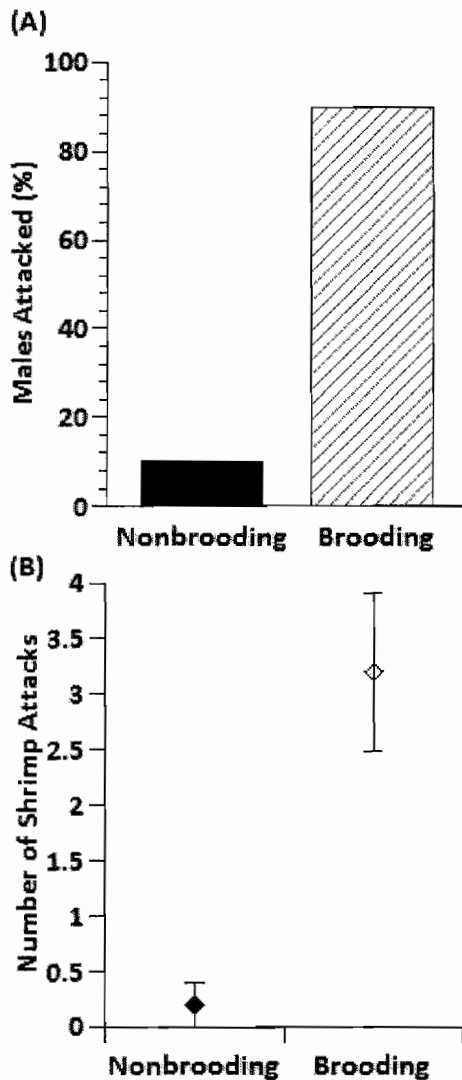
Figure 2: Shrimp (*Heptacarpus sitchensis*) attacks on nonbrooding and brooding sea spiders. (**A**) Percentage of brooding (hashed bars) and nonbrooding (open bars) sea spiders attacked by shrimp. Almost all (90%) brooding males were attacked, while only 10% of nonbrooding males were attacked. (**B**) Average number of shrimp attacks on brooding and nonbrooding males. Shrimp attacked brooding males more often than nonbrooding males (Average brooding: 3.2 attacks, ±0.7 SE; Average nonbrooding: 0.2 attacks, ±0.2 SE).
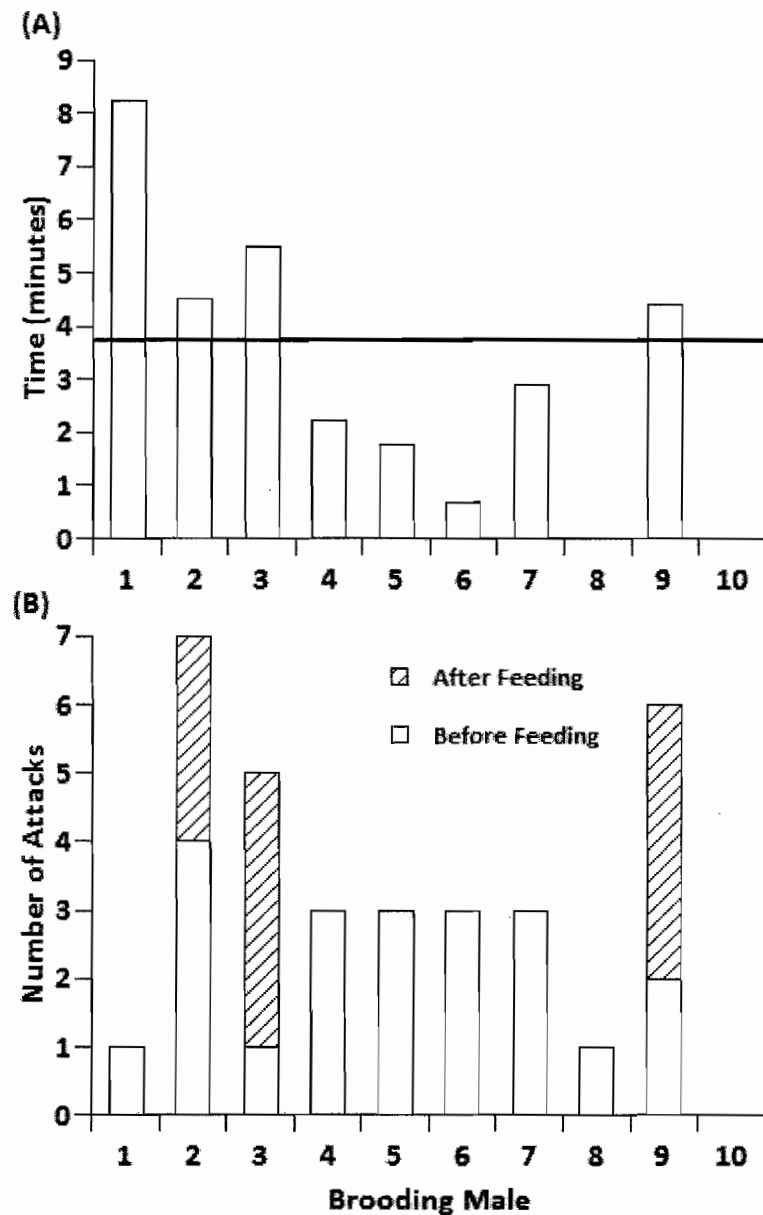
Figure 3: Shrimp (*Heptacarpus sitchensis*) consumption of sea spider egg masses. (**A**) Average (black line) time shrimp spent feeding on egg masses. The majority (80%) of shrimp consumed egg masses and averaged 3.77 minutes (±0.85 SE) to feed. (**B**) Number of shrimp attacks before and after egg masses were consumed. On average, shrimp attacked 2.7 times (±0.36 SE) before consuming eggs, 37.5% re-attacked brooding males after consuming eggs.

Dislodgment

Contrary to the expectation, nonbrooding males were dislodged from rocks more easily than brooding males. On average, half (52%, ±3.4 SE) of nonbrooding males were dislodged during the experiment, while less than one third (30%, ±2 SE) of brooding males were dislodged (Figure 4A). About 85% of all dislodged males were dislodged at speeds below 40 cm/s and within the first 2.5 minutes of the experiment. Nonbrooding males were dislodged at lower average water speeds (35.5cm/s, ±1.09 SE) than brooding males (39.7cm/s, ±2.27 SE) during all four trials (Figure 4B). In all trials, nonbrooding males were dislodged in less time on average (113 seconds, ±4.52 SE) than brooding males (130 seconds, ±9.24 SE) (Figure 4C). Differences, though, in water speed and time at dislodgment between males are only weakly significant (Paired t-tests: t=-2.15, p=0.06 and t=-2.3, p=0.052). However, differences in the percentage of brooding and nonbrooding individuals dislodged are statistically significant (Paired t-test: t= 4.96, p=.007).
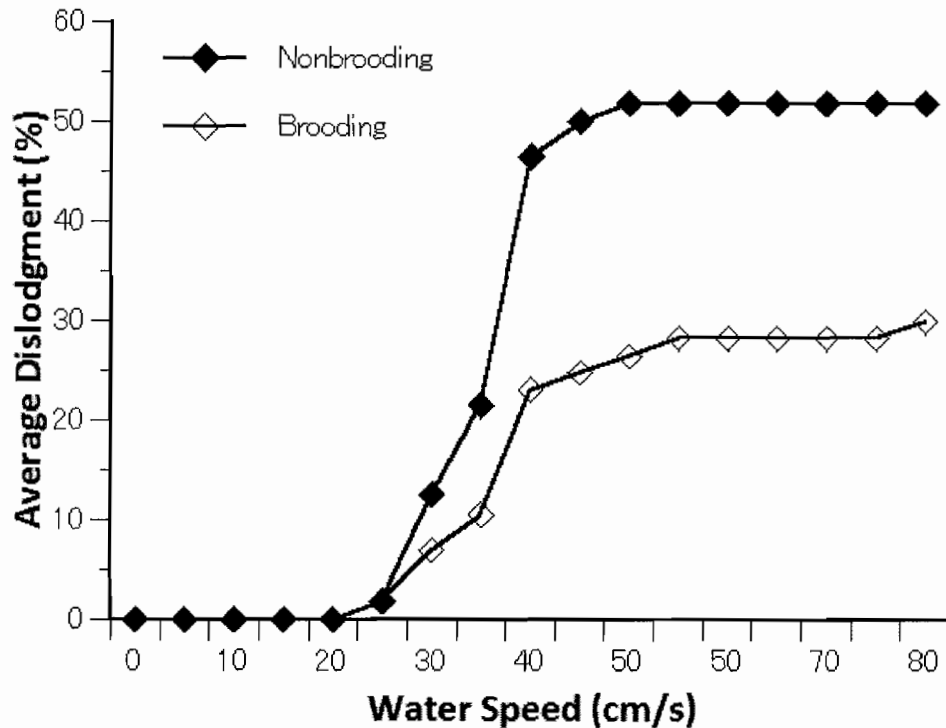
Figure 4: Average percent dislodgment of nonbrooding and brooding sea spiders by water speed (cm/s). Nonbrooding males were dislodged significantly more often than brooding males (nonbrooding: 52%, ±3.4 SE; brooding: 30%, ±2.0 SE). Nonbrooding males were also dislodged at lower water speeds (35.5cm/s, ±1.09 SE) compared to brooding males (39.7cm/s, ±2.27 SE).

Movement and Feeding

Nonbrooding males covered more distance and moved more frequently than brooding males. On average, nonbrooding males moved 4.14 mm/hr compared to 1.17 mm/hr for brooding males (Figure 5A). Nonbrooding males spent 5.5% of the time moving (or 2 hours and 38 minutes) while brooding males spent 1.8% of the time moving (or 54 minutes) (Figure 5B). Differences in both the distance traveled and frequency of movement were statistically significant (Wilcoxon tests: z=2.41, p=0.008; z=2.18, p=0.015).

Night and day movement patterns were detected, but only for nonbrooding males (Figure 6). Nonbrooding males moved significantly more during the day (from 6am until 6pm), increasing movement from 1.8mm/hr to 6 mm/hr at night (Figure 6A). The peak in movement occurred between midnight and 1 am (Figure 6B). Brooding males, however, did not show this pattern, traveling about 1 mm/hr both day and night (Figure 6). Differences in day and night movement were statistically significant for nonbrooding males but not brooding males (Paired t-tests: t-stat=-3.56, p=.002 and t-stat=-1.27, p=0.12). Similarly, nonbrooding males moved significantly farther than brooding males during both day and night periods (Paired t-tests: t-stat= -1.82, p=.048 and t-stat= -4.35, p<.001).
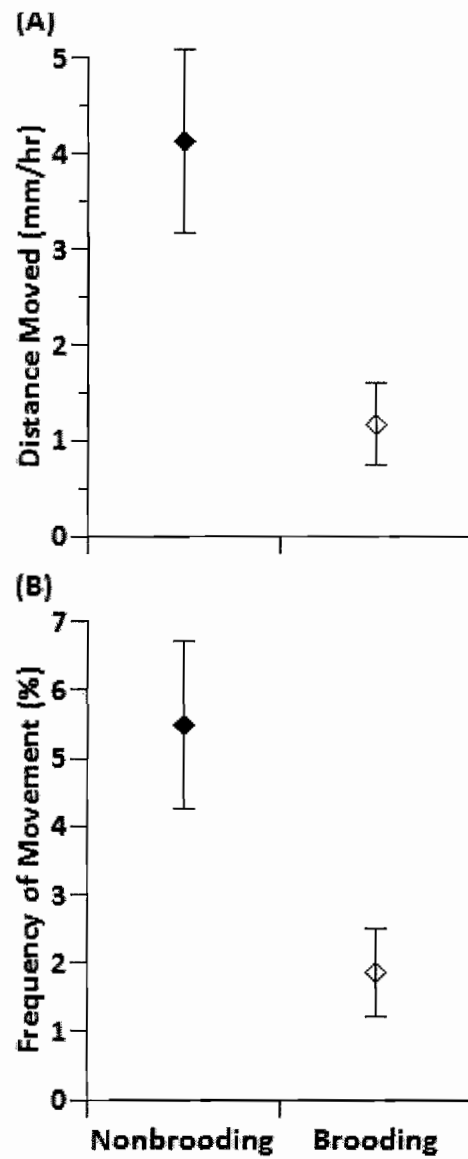
Figure 5: Movement patterns of nonbrooding and brooding sea spiders. (**A**) Average distance moved. Nonbrooding males moved farther (4.14 mm/hr, ±0.97 SE) than brooding males (1.17 mm/hr, ±0.43 SE). (**B**) Frequency of male movement. Nonbrooding males moved more frequently (5.5% of the time, ±1.22 SE) than brooding males (1.8%, ±0.64 SE).
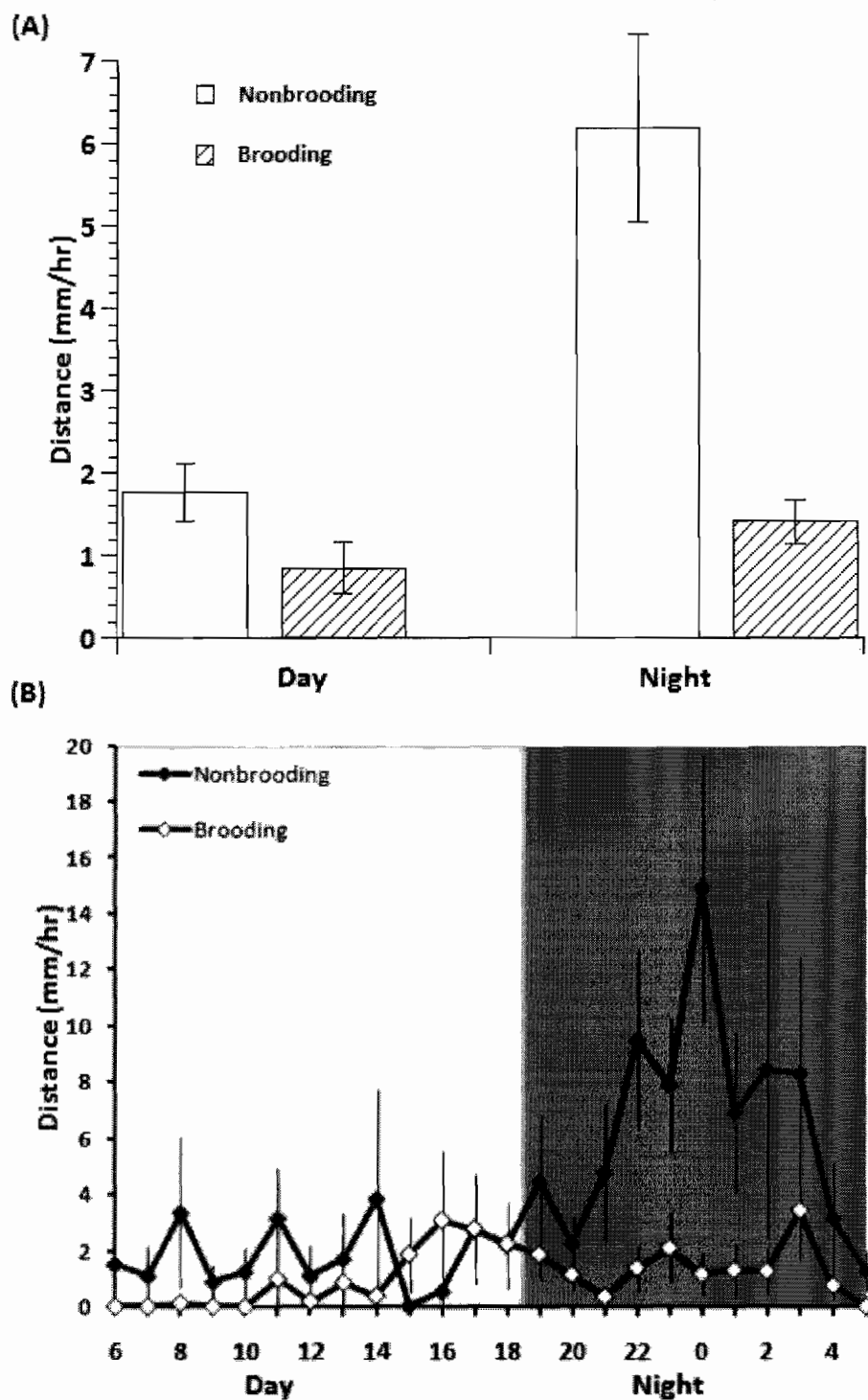
Figure 6: Day and night movement patterns of nonbrooding and brooding sea spiders. (**A**) Average distance travelled during day and night for nonbrooding and brooding males. Nonbrooding males moved significantly more during the night (6mm/hr, ±1.13 SE) than the day (1.8mm/hr, ±0.35 SE). Brooding males moved the same distance both day and night (about 1mm/hr, ±0.3 SE). During both day and night, nonbrooding males moved significantly more than brooding males. (**B**) Average distance travelled by hour for nonbrooding and brooding males. Nonbrooding males had a peak movement of 16mm/hr between midnight and 1 am.

Brooding and nonbrooding males did not differ significantly in the amount of time spent feeding (Wilcoxon test: z=0.14, p=0.44). On average, nonbrooding males spent 4% of the time feeding (almost 2 hours during the 48 hour trial), while brooding males fed for 3.7% of the time (1 hour and 46 minutes) (Figure 7A). Brooding males fed an average of 1.44 times per day for an average of 35.6 minutes per feeding event, while nonbrooding males fed 1.19 times per day for an average of 54.6 minutes per feeding event (Figure 7B,C). However, these differences were not significantly different (Wilcoxon tests: z=-.06, p=.47; z=-.45, p=.33).
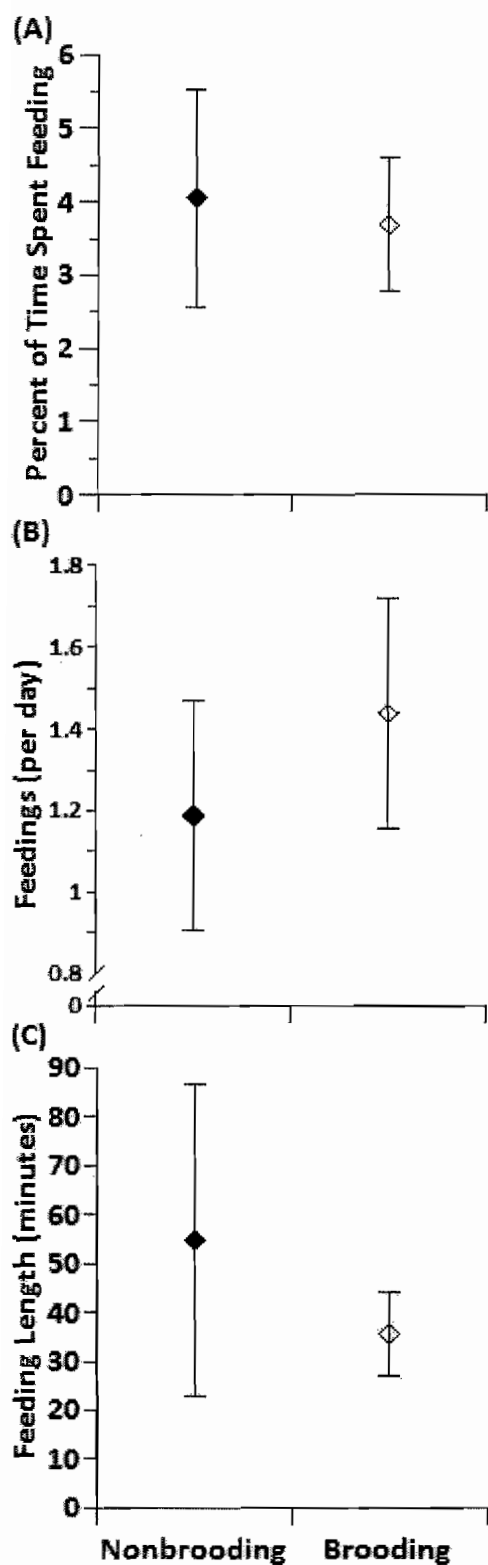
Figure 7: Feeding patterns of nonbrooding and brooding sea spiders. (**A**) Average percentage of time spent feeding by nonbrooding (open bars) and brooding males (hashed bars). There was no difference in the percentage of time spent feeding by nonbrooding (4%, ±1.5 SE) and brooding males (3.7%, ±0.9 SE). (**B**) Average number of feeding events by brooding and nonbrooding males per day. There was no difference in the number of feeding events for nonbrooding (1.19 feedings, ±0.28 SE) and brooding males (1.44, ±0.28 SE). (**C**) Average time spent per feeding event for males. Nonbrooding males fed for slightly longer periods (54.6 minutes, ±31.7 SE) than brooding males (35.6, ±8.5 SE).

Epibionts

Four types of epibionts (a branching bryozoan *Crisia sp.*, the foraminiferan *Cibicides lobatulus*, a nematode, and a Halacarid mite) were found on males of *Achelia simplissima*. A total of 281 brooding males and 83 nonbrooding males were examined, with a total of 34 brooding males and only 3 nonbrooding males found carrying epibionts. On average, epibionts were present on 15% of the brooding male population while only 3.3% of nonbrooding males had an epibiont during any month (Figure 8A). Brooding males had a statistically higher frequency of epibionts than nonbrooding males (Wilcoxon test: W=15, p=.05).

All four types of epibionts were found on brooding males, while the mobile epibionts (mites and nematodes) were never present on nonbrooding males (Figure 8B). The majority (66.7%) of epibionts found on nonbrooding males were foraminiferans, while 70% of epibionts on brooding males were foraminiferans or mites (Figure 8B). The highest rates of epibionts occurred during January 2010 for nonbrooding males, and in March 2010 for brooding males. Most males had only a single type of epibiont present. Mites (0.8mm long) and nematodes were found on either the ovigers or the egg masses themselves. Branching bryozoans were mainly found growing around the legs of the animal, while foraminiferans occurred primarily on the dorsal side of the trunk.
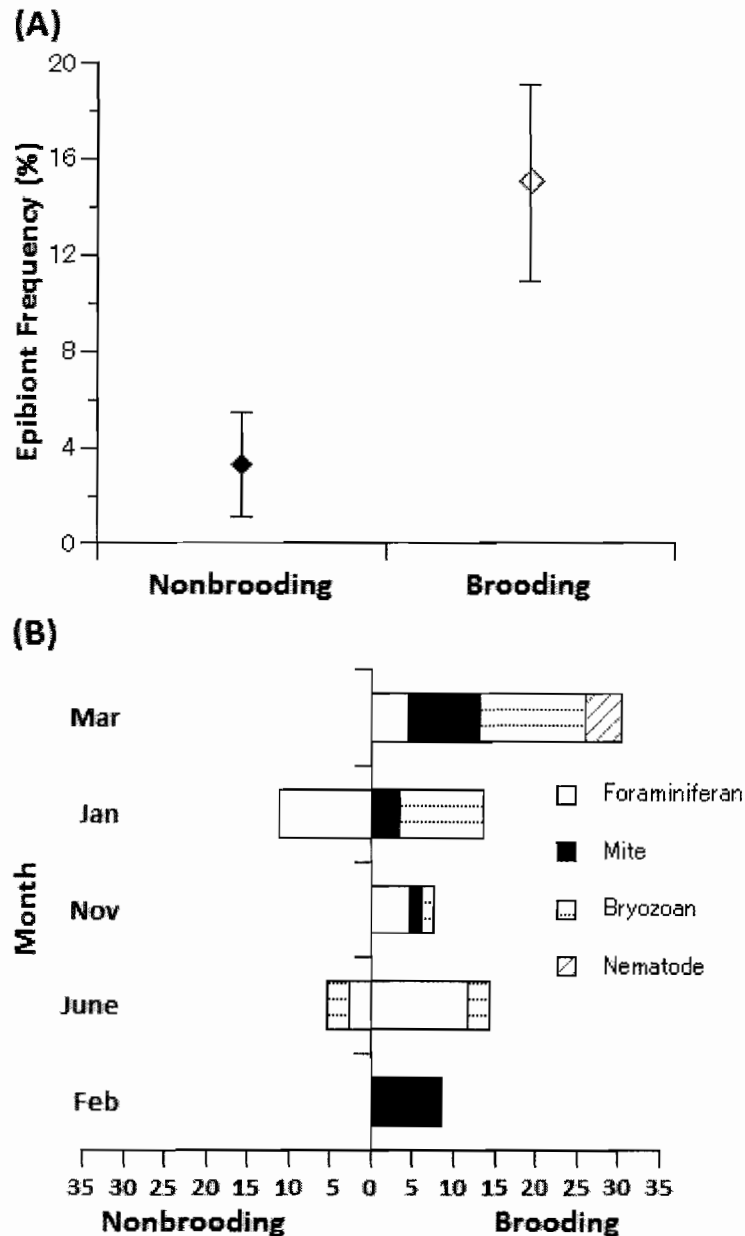
Figure 8: Epibiont presence on nonbrooding and brooding sea spiders. (**A**) Average frequency (%) of epibionts on nonbrooding and brooding males. Significantly more brooding males (14.96%, ±4.1 SE) had epibionts present than nonbrooding males (3.3%, ±2.2 SE) (Wilcoxon W=15, p=0.05). (**B**) Relative abundance of epibionts on nonbrooding (left of axis) and brooding (right) males by month. Brooding males had a higher frequency of epibionts than nonbrooding males in all five months. Brooding males were found with all four types of epibionts, while nonbrooding males never had nematodes or mites present. Foraminiferans were the main (66.7%) epibionts on nonbrooding males and both foraminiferans and mites were equally common (35%) on brooding males.

Discussion

This is the first study to address the costs associated with male parental care in pycnogonids and the first observation of predation on sea spider eggs. Of the seven potential predators assayed, only the tidepool fish *Oligocottus maculosus* consumed adult pycnogonids, but did so infrequently (only 3 of 35 fish). However, the common tidepool shrimp *Heptacarpus sitchensis* routinely preyed on *Achelia simplissima* egg masses. The egg masses are held underneath the male pycnogonid, in a position difficult for predators to reach. As such, the shrimp has to remove the pycnogonid from the substrate and physically hold it in order to feed on its egg masses. Interestingly, the shrimp predator catches the pycnogonid and consumes only its egg masses, leaving the adult sea spider unharmed. However, it is not known if the pycnogonid, after becoming dislodged by the shrimp, is able to reattach to the substrate before being removed from his habitat by water currents. Therefore, further studies are required to determine if shrimp egg predation actually lowers the brooding male's chance of survival through dislodgment. However, shrimp egg predation is likely to instead impose a reproductive cost for brooding males and their female partners. It is not uncommon to find multiple brooding males of *A. simplissima* living under the same rock (pers. obs.), allowing a shrimp to potentially consume large amounts of eggs from many different males. Since shrimp consumed the majority, if not all, of the pycnogonid eggs, then predation could have a substantial impact on a male's individual reproductive success and on the success of the population as a whole.

Nonbrooding males were more prone to dislodgment than brooding males, possibly due to a behavioral difference between nonbrooding and brooding males. Brooding males may attach themselves better or hold on tighter to the substrate. *Achelia simplissima* are commonly found on the underside of rocks where they would be exposed to much slower currents than those used in this experiment. However, rocks are commonly flipped over in the intertidal, making it possible that males may experience these current speeds during some point in their life. Brooding males carrying between 3 and 9 egg masses were used in this study, so it is unknown whether males carrying fewer egg masses would give the same results. It is possible that males with 1-2 egg masses are more easily dislodged because of unevenly distributed masses. Still, it appears that caring for young may have a positive effect on individual survival by increasing the force needed for dislodgment.

Brooding sea spiders covered less distance and moved less frequently than nonbrooding males, especially at night. Since mating and courtship take place only during the night and early morning in this species (pers. obs.), nonbrooding males are likely to encounter gravid females that are ready to mate more often than brooding males. Parental care often reduces access to mates, resulting in less frequent matings while caring for offspring (Bateman, 1948; Manica & Johnstone, 2004). Decreased movement had no impact on the feeding frequency of brooding males, suggesting that nonbrooding males are mainly moving, not to forage, but to find mates. Many species exhibit male mate-seeking movement patterns during the reproductive season (Howard, 1980; McRae *et al*, 1981; Gibbons, 1986; Hunt & Nault, 1991). In spiny lobsters, for instance, males

increase movement during both the day and night while searching for mates

(MacDiarmid *et al*, 1991). By moving significantly less during the night, brooding males

may be missing out on new mating opportunities. Additionally, unlike many fish species

whose brooding males are more attractive to females than nonbrooding males (Ridley &

Rechten, 1981; Unger & Sargent, 1988; Knapp & Sargent, 1989; Forsgren *et al.,* 1996;

Forsgren, 1997), females of *Achelia simplissima* mate more often with nonbrooding

males when given the choice (pers. obs). By decreasing their movement and

attractiveness to females, brooding males, although already reproductively successful,

may experience *future* reproductive costs as a result of parental care.

There was a disproportionately higher presence of epibionts on brooding than on

nonbrooding males and mobile epibionts (nematodes and mites) were only ever found on

brooding males. Epibionts occurred individually on males, were relatively small, and

were rarely located on the dorsal trunk of the male where they would be in a position to

be beneficial to the pycnogonid by camouflaging or covering him. Rather than

providing benefits, epibionts are more often detrimental to their host by increasing

dislodgment frequency or decreasing mobility (Dayton, 1973; Paine, 1979; Buschbaum &

Reise, 1999). Nonbrooding *Achelia simplissima* may have fewer epibionts than brooding

males because they are able to clean themselves more effectively with grooming ovigers

that are not covered with egg masses. Mobile epibionts, present exclusively on brooding

males, were always found on the ovigers or directly on the egg masses. This indicates

that these epibionts may be attracted to the egg masses and arrive only after the male

begins parenting in order, perhaps, to feed on the eggs or larvae. Egg predators are not

uncommon in marine habitats and many have been shown to cause high egg mortality in their arthropod hosts (Kuris & Wickham, 1987; Torchin *et al*, 1996; Williams, 2000). If these mobile epibionts are indeed consuming eggs, then their presence may be reproductively costly to brooding males. Since epibionts were found year round, and, since sea spiders stop molting as adults, the presence of epibionts may have long-term consequences for brooding males even after the eggs hatch. For instance, the sessile branching bryozoan was often found growing around the brooding male's legs, presumably restricting or even inhibiting movement of the appendages. The presence of this epibiont could potentially lower a male's mobility, reduce his foraging, or increase his susceptibility to dislodgment.

This is the first study to demonstrate that costs do exist for sea spiders as a result of parental care. Brooding males of *Achelia simplissima* experienced higher frequencies of epibionts and attacks by egg predators, both of which are likely to affect his reproductive success. Compared to nonbrooding males, brooding males also had decreased movement rates which, although not affecting feeding frequency, may decrease their chances of subsequent matings. However, brooding males are not consumed by predators more frequently than nonbrooding males, and may benefit from a decreased chance of dislodgment. Although parental care costs do exist for *A. simplissima*, they are minimal, possibly due to the mating strategies of this species. Fertilization occurs only after the eggs are transferred to the male, allowing him to be certain that he is putting energy into caring only for his offspring. Because of their unique ovigerous legs, male pycnogonids are able to carry multiple broods simultaneously, allowing them to mate

multiple times with multiple females. This species also reproduces year round, unlike

most species of sea spiders (King, 1973; Bain & Govedich, 2004), and so a missed

mating opportunity may not be as costly to a male's reproductive success as it otherwise

could be. What is more, parental care is not extensive, since embryonic development is

relatively short and the larvae do not remain attached to the male as in other pycnogonids

(Bain & Govedich, 2004, pers. obs.). Determining the magnitude of costs imposed by

paternal care in other sea spiders could provide valuable information for studies on sexual

selection, sex roles, and the evolution of paternal care.

CHAPTER IV

LARVAL MORPHOLOGIES AND POTENTIAL DEVELOPMENTAL MODES OF EIGHT SEA SPIDER SPECIES (ARTHROPODA: PYCNOGONIDA) FROM THE SOUTHERN OREGON COAST

Introduction

Pycnogonids (the sea spiders) are a small group of marine invertebrates that display significant diversity in larval development (Bain, 2003; Bogomolova, 2007). However, complete larval development (from hatching through formation of adult appendages) has not been described for the vast majority (>98%) of pycnogonids, making their developmental *modes* unknown as well (Bain, 2003).

Bain (2003) proposed four modes of development. The most common form, the 'typical protonymphon' development, involves a free-moving six-legged larva that acquires its adult limbs sequentially (King, 1973; Behrens, 1984; Bain, 1991; Okuda, 1940; Vilpoux & Waloszek, 2003; Bain, 2003). Larvae following the 'atypical protonymphon' pathway develop all eight adult legs simultaneously while inside the mantle cavities of molluscs or on sedentary polychaetes (Ohshima, 1933; Arnaud, 1978; Ogawa & Matsuzaki, 1985; Salazar-Vallejo & Stock, 1987; Bain, 2003). The 'encysted' postembryonic mode of development is characterized by a larva that develops in the

gastrocoel of cnidarians and becomes free-moving with the first juvenile stage (Hilton, 1916; Lebour, 1945; Russel, 1990; Bain, 2003; Lovely, 2005). The 'attaching' developmental mode involves a larva that has only one pair of appendages (as opposed to three) and remains attached to the male throughout most of its development (Meinert, 1899; Hooper, 1981; Nakamura, 1981; Bain, 2003). Recently, an additional developmental mode was described, the 'lecithotrophic protonymphon,' in which the larva remains on the male for a longer period than the 'attaching' larva and has two pairs of reduced larval appendages (Bogomolova & Malakhov, 2006; Bogomolova, 2007; Cano & Lopez-Gonzalez, 2009).

Although Bain's terminology for pycnogonid postembryonic development is commonly used in the literature, it confuses larval type with mode of development and should be modified (Cano & Lopez-Gonzalez, 2009). The terms 'typical protonymphon,' 'atypical protonymphon,' and 'encysted larva' suggest that morphological differences exist between the larvae following these developmental patterns. However, Bain insists that there are only two types of larvae (the attaching larva and the protonymphon), and that these three development modes all share the same protonymphon. This implies that a sea spider's developmental mode cannot be determined based on characteristics of the first larval stage. However, several studies have shown that a species' developmental mode can often be inferred from the larva's morphology (Bogomolova & Malakhov, 2003, 2004; Bogomolova, 2007; Cano & Lopez-Gonzalez, 2009). Unfortunately, larval morphology has not been described for most species of sea spiders (Bain, 2003).

This paper illustrates the variety of larval morphologies within and between three Families (Phoxichilidiidae, Ammotheidae, and Pycnogonidae) and five genera of intertidal sea spiders. An alternative terminology is proposed for the developmental modes of pycnogonids based on morphological characteristics of larvae with known developmental patterns. The larval morphologies of eight pycnogonid species are described for the first time and used to infer postembryonic development mode. Insight into pycnogonid postembryonic developmental modes can lead to a better understanding of larval dispersal and host preferences, adult biology and distribution, and the phylogenic position of the sea spiders.

Materials and Methods

Males with egg masses of the species *Pycnogonum rickettsi* Schmitt, 1934 were collected during January 2009 from North Cove, Cape Arago, Oregon. Specimens of *Pycnogonum stearnsi* Ives, 1892 were collected in March 2009 from Asilomar, Monterey, California. Egg-carrying males of both species were found on the columns of the sea anemone *Anthopleura xanthogrammica* Brandt, 1835. Specimens of *Achelia gracilipes* Cole, 1904 were collected from bryozoans (*Crisia sp.*) at Sunset Beach, Cape Arago, Oregon during April 2009. Males of *Achelia simplissima* Hilton, 1939 and *Eurycyde spinosa* Hilton, 1916 were found on rocks with large aggregations of the spirorbid worm *Spirorbis bifurcates* Knight-Jones, 1978 at North Cove, Cape Arago, Oregon during January 2010. All other species were collected at Lighthouse Beach, Cape Arago, Oregon in June 2009. Specimens of *Achelia chelata* Hilton, 1939,

Nymphopsis spinosissima Hall, 1911, and *Anoplodactylus viridintestinalis* Cole, 1904 were found on the sandy tops of large boulders. Males carrying egg masses were kept in Petri dishes and checked daily for hatched larvae.

Scanning electron microscopy (SEM) was used to examine the first postembryonic stages of all species. Upon hatching, larvae were relaxed in 7.5% MgCl for twenty minutes and fixed in osmium tetroxide and sea water according to protocol. Larvae were then dehydrated in an ascending elthyl alcohol series (10, 30, 50, 70, 85, 95, 100%), immediately critical point dried, and coated with gold. Larvae were examined with a Tescan Vega SBU scanning electron microscope. Larval characteristics, including the shape and size of the body, proboscis, mouth, spines, appendages, and cheliphores, as well as the presence of pores and sensilla, are described and measured from SEM images. For each species, body size was measured in ten larvae prior to dehydration. There was no difference in average measurements of body size before and after dehydration; therefore, measurements for all other characteristics were made from SEM images. For *Anoplodactylus viridintestinalis*, the quality of the preps did not allow for descriptions of all characteristics. However, this species is included because it exhibits a distinctive larval morphology.

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

The first larval stage in all studied species is characterized by having three pairs of appendages, the cheliphores and II and III appendages (Figure 1A). The appendages are typically tripartite. The most anterior and largest pair of larval appendages are the cheliphores. They point forward over the proboscis and terminate with a set of chelae, or

claws, which are directed downwards. Unless specified, the proboscis is held horizontal to the body, directed forward, and cannot be seen from the dorsal side as it is hidden by the cheliphores. The edges of the larval body hang over the bases of the II and III appendages. These two appendages are always similar in length and ornamentation; therefore, characteristics of the II and III appendages will only be described once but will refer to both appendages.

Figure 1. Larva of *Achelia gracilipes.* **(A)** Side view of larva (scale bar: 50 μm) and quadfurcate sensillum (arrow), **(B)** tripartite mouth with raised lip (arrow) ending in a single denticle (scale bar: 5 μm), **(C)** Cheliphores and spinning spines (scale bar: 50 μm), **(D)** tip and tooth of terminal article of larval appendages (scale bar: 20 μm); **(I)** cheliphore, **(II)** second larval appendage, **(III)** third larval appendage, **(1)** spinning spine, **(2)** proboscis, **(3)** fixed chela finger, **(4)** moveable chela finger, **(5)** trifurcate smooth sensillum.

Family Ammotheidae

GENUS *ACHELIA*

Larval morphology of Achelia gracilipes (n=15)

The body is rounded and 110-130 µm in length (measured as the distance between the base of the proboscis and the abdomen) (Figure 1A). The cuticle of the larval body is wrinkled (a possible artifact of the dehydration process). The proboscis is conical in shape, 65 µm long, and 60 µm wide at the base. The mouth is unopened, tripartite, and 13 µm in diameter (Figure 1B). There is a thin, smooth, raised lip around the mouth that ends in a single pointed denticle (Figure 1B).

The basal article of the cheliphores is 60 µm in length and is slightly wider than long (Figure 1A, C). There is a spinning spine located on the distal external edge of the article. It is the same length (85 µm) as the chelae (Figure 1A, C). The hooked fingers of the chelae overlap when closed. The inner edges of the moveable fingers have a number of small sharp denticles (Figure 1C), while the outside edges bear a single small denticle. The fixed finger bears a single large denticle located on the inner edge. On the dorsal and ventral sides, there are multiple slit-like pores located at the base of the cheliphores, as well as at the base of the chelae. The pores are about 1.5-2 µm long located in a circular 3 µm diameter depression of the cuticle.

The basal articles of the II and III appendages are much smaller (20 µm in length) than the second articles (50 µm) (Figure 1A). The terminal claw is 80 µm long with one or two large teeth located on the inside edge halfway up the article (Figure 1D). There is

a short (20 µm) spine located at the base of the appendages. There are pores located on the ventral side of the articles.

Nine sensilla were found in the larva, between 25-30 µm long each (Figure 2). On the anterior dorsal side of the body there is an unpaired median sensillum (Figure 2A). At the ventral posterior end of the body there is a pair of sensilla. These three sensilla are quadfurcate. Two pairs of trifurcate posterior dorsolateral sensilla and slit-like pores are located behind the second and the third pair of appendages on the dorsal side of the body (Figure 2A, B). In between this last pair of sensilla, is a pair of simple sensilla (Figure 2A). Located above each of these simple sensilla is a slit-like pore (Figure 2B). The pores and sensilla are distributed bilaterally symmetrical. In the center of the dorsal side of the body is a pair of 6 diamond-shaped pores. These pores are not slit-like, but are depressions that are 3-4 µm long (Figure 2A).
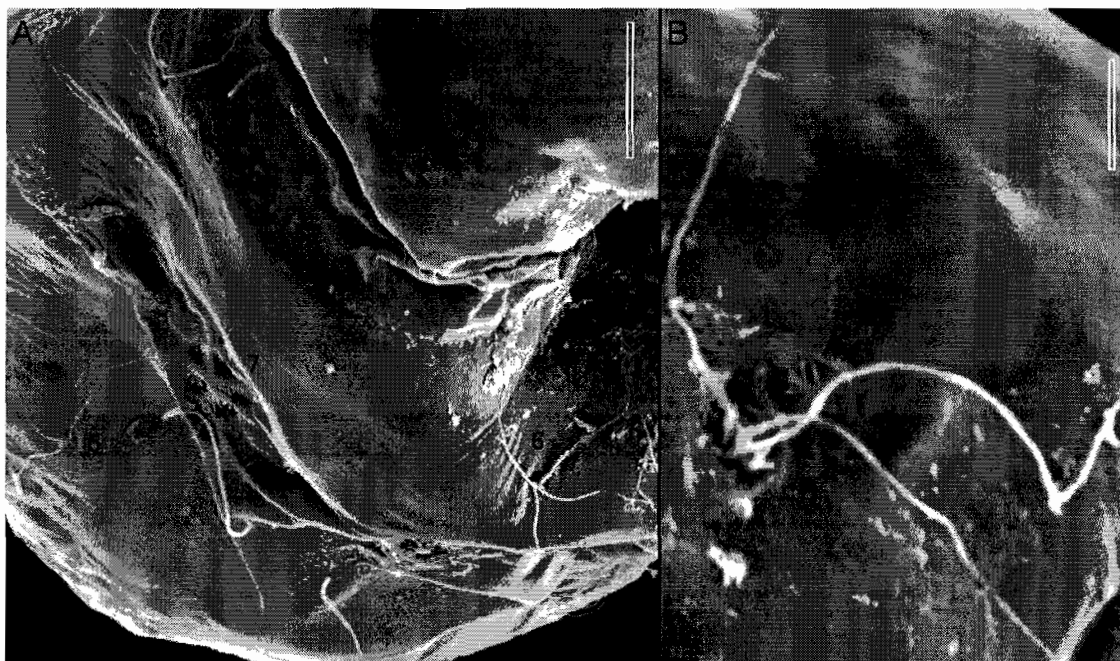
Figure 2. Dorsal sensilla and pores of *Achelia gracilipes*. **(A)** Trifurcate sensillum **(5)**, median dorsolateral quadfurcate sensillum **(6)**, cluster of 6 dorsal pores **(7)** (scale bar: 20 μm) **(B)** Trifurcate sensillum and slit-like pore (arrow) (scale: 5 μm).

Larval morphology of Achelia chelata (n=15)

The body is 105-115 μm in length and is more circular than ovoid (Figure 3A). The conical proboscis is 45 μm in length and 30 μm at the widest part. The mouth is circular and on average 14 μm in diameter (Figure 3C). It is closed in a distinct Y-shape. The surrounding raised lip bears up to ten denticles.

The cheliphores are smooth with the moveable finger rounded, and the fixed finger ending in a sharp hook (Figure 3B). The basal article is 60μm in length and the chelae are 100 μm long. There is no spinning spine present.

There are short (50-60 μm) spines at the base of the II and III appendages. The basal article of the appendages is very short (16 μm) and has one slit-like pore (2 μm) located distally on the ventral side. The second article, which has two slit-like pores on its ventral side, is 50 μm long. The terminal claw is almost twice as long as the second article (90-100 μm). The surface of the claw is sparsely decorated with thin denticles. At about 1/3 of the length from the tip there is one large tooth on the ventral side of the claw. The tip of the claw is bifurcate.

Five sensilla were found in the larva, between 10-15 μm long each. On the anterior dorsal side of the body there is an unpaired median sensillum. There is a pair of posterior dorsolateral sensilla located behind the third pair of appendages on the dorsal side of the body. At the ventral posterior end of the body there are paired sensilla. The dorsally located sensilla are smooth and bifurcate, while the ventrally located sensilla are trifurcate. Located above each of these sensilla is a slit-like pore.
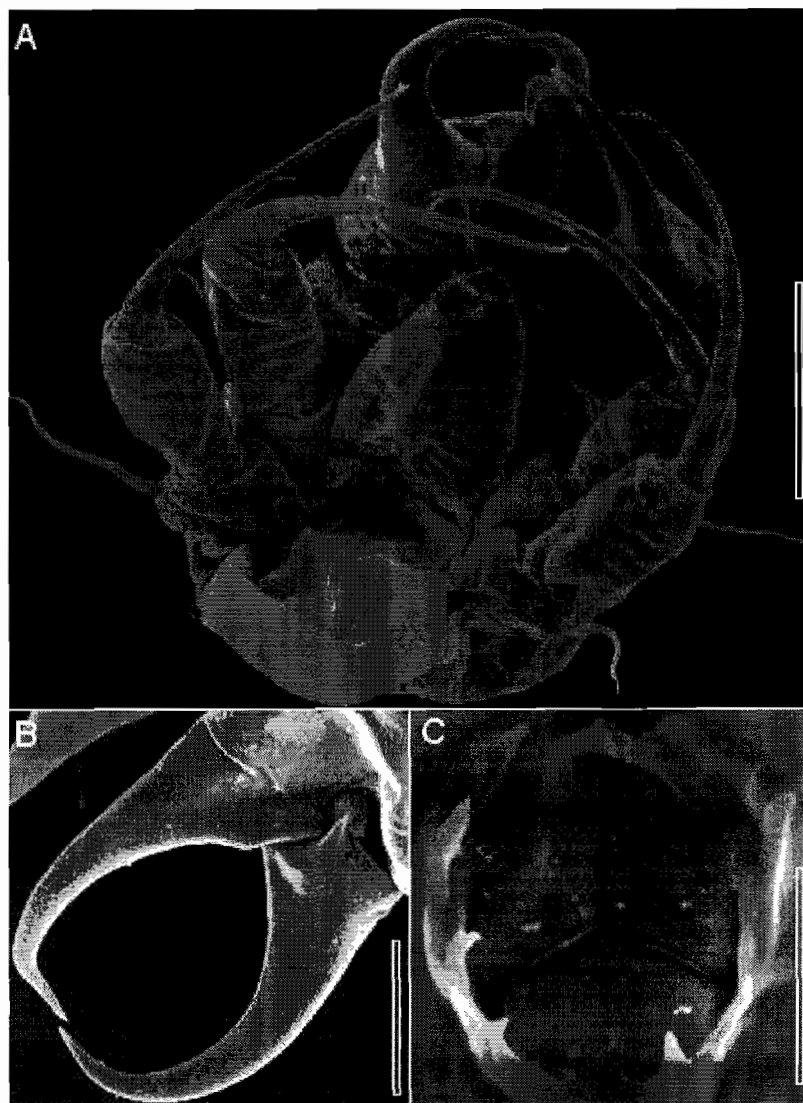
Figure 3. *Achelia chelata* larva. **(A)** Ventral view (scale bar: 50 μm), **(B)** fixed and moveable finger of chela (scale bar: 30 μm), **(C)** tripartite closed mouth and denticles on lip (scale bar: 10 μm)

Morphology of Achelia simplissima (n=10)

The body is much smaller than the previous two species, only about 33 μm long (Figure 4A). The proboscis is about 17 μm long, and 15μm wide at its base (Figure 4A). It is cylindrical in shape, tapering slightly to the end with a diameter of 11 μm. The mouth (5 μm in diameter) is open and tripartite. The three surrounding lips are smooth, without denticles (Figure 4D).

The basal article of the cheliphore is wider than it is long (15μm long and 21μm wide) (Figure 4B). The chelae are almost twice the length of the basal article (26 μm in length). The chelae are rounded, with overlapping fingers when closed. The fingers are smooth, except for one small tooth on the inner edge of the fixed finger. There are no spinning spines present.

The II and III appendages have a basal article 6 μm long, and a second article and terminal claw 16 μm long each (Figure 4A). The appendages are without denticles. About one quarter of the way from the tip of the claw is a small denticle (Figure 4C). The tip of each claw is bifurcate. There is a pore on the inside of the distal edge of the second article of the II and II appendages. There is a short (6-10 μm) spine at the base of each leg (Figure 4A).

The larva has one large median dorsolateral pore that is not slit-like. There was no evidence of sensilla anywhere on the larva.
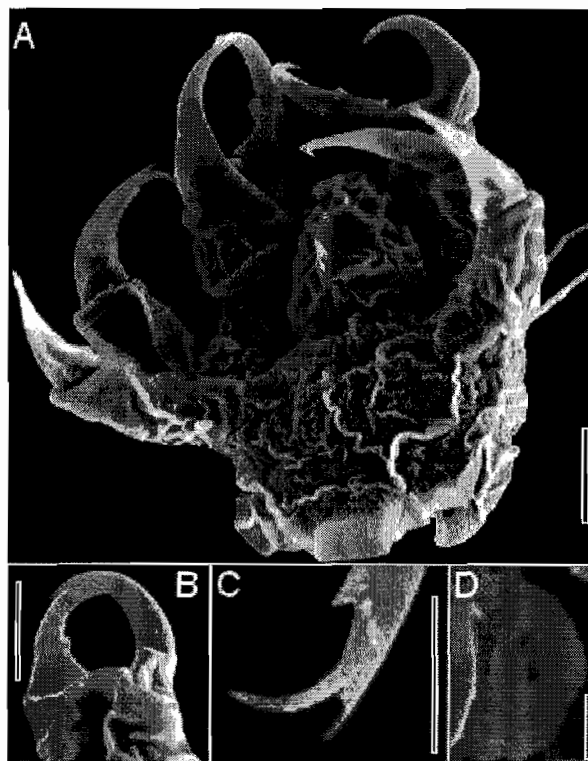
Figure 4. *Achelia simplissima* larva. **(A)** Ventral view (scale bar: 10 μm), **(B)** chela (10 μm), **(C)** tip and teeth on terminal article of II and III appendages (5 μm), **(D)** tripartite mouth (5 μm)

GENUS *NYMPHOPSIS*

***Larval morphology of* Nymphopsis spinosissima (n=15)**

The larval body is rectangular; 140-150 μm long (Figure 5A). The proboscis is very short (55-65 μm) and rounded; it does not even reach the chelae (Figure 5A, D-E). The tripartite mouth is 15 μm in diameter and is open (Figure 5D). The surfaces of the lips are smooth and at their crevices there are three pores of proboscis glands.

The cheliphores are almost twice the length of the body, 290-300 μm long (Figure 5A). Cheliphores are set slightly apart so that the proboscis is visible from the dorsal side (Figure 5E). The basal article is 85-90 μm long, while the chelae are just over 200 μm long (Figure 5C). At the base of the first article is a very thin reduced spinning spine, 22-26 μm long (Figure 5A). The shape of the chelae is ovoid and the fingers do not overlap when closed. The inside blade of each finger bears 11-13 teeth.

The appendages II and III are not tripartite; instead they are quadripartite (Figure 5A). The basal article of the II and II appendages is 22 μm long, and bears a short spine at the base (65-80 μm). The second article is between 40-45 μm long. The third and extra article is very long (100 μm). It is covered by a different sort of cuticle compared to the rest of the body. The surface of the cuticle has many small entwined threads (Figure 5A). The fourth article, the claw, is curved inwards and is 95 μm long. The claw has two rows of pointy denticles, one on either side of the blade (Figure 5B). They are between 7-10 μm long and there are seven in each row. At the base of the inner edge of the claw is a small (35 μm) spine.

There are seven sensilla; each is bifurcate (Figure 5E). The sensilla are 15-25 μm long. There is an unpaired sensillum located just behind the proboscis on the dorsal side of the larva (Figure 5E). There is a pair of dorsolateral sensilla and slit-like pores located just behind the II appendages. Located 1/3 of the way from the posterior end of the body and in line with the middle of the cheliphores is another pair of sensilla and slit-like pores on the dorsal side of the larva. The final pair of sensilla is located ventrally at the posterior end of the larva.
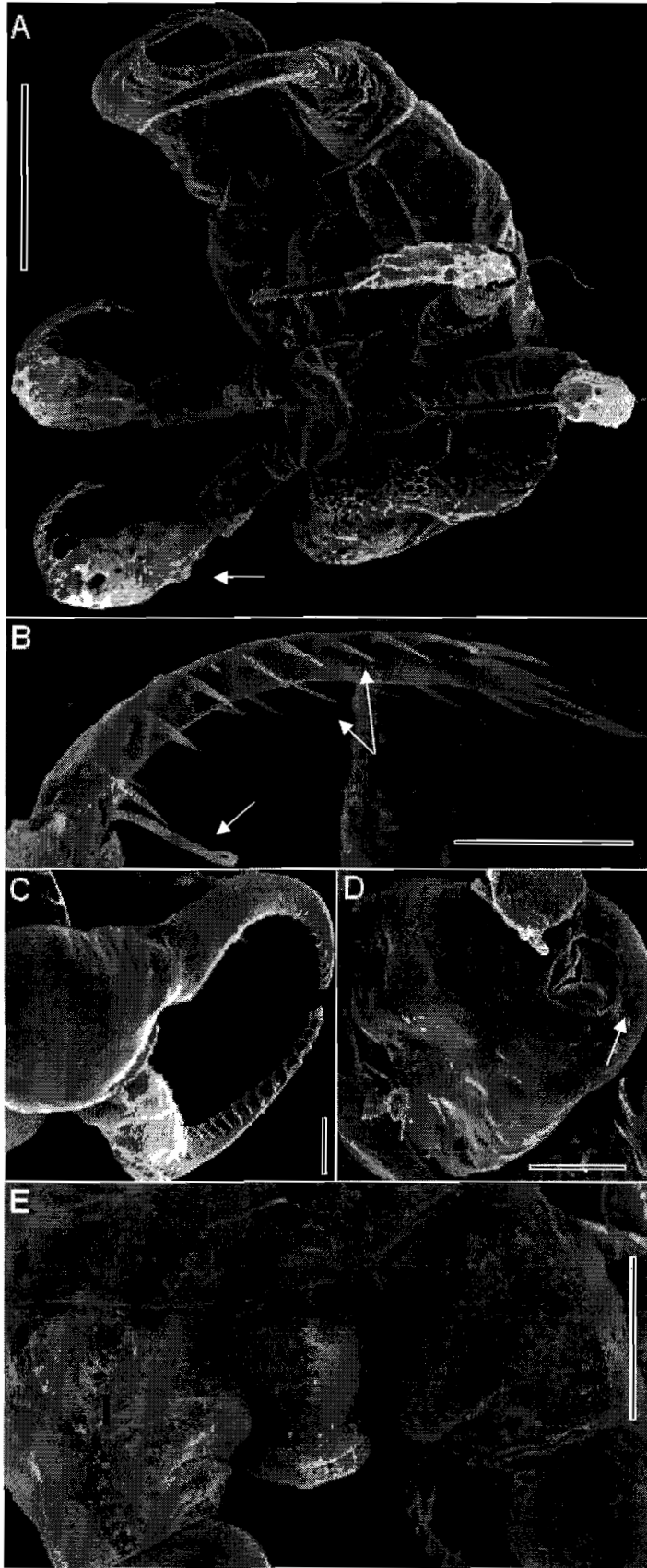
Figure 5. *Nymphopsis spinossisima* larva. **(A)**Ventral view showing II and III appendages with four articles (scale bar: 100 μm), **(B)** terminal article of the II and III appendages showing basal spine and two rows of denticles (20 μm), **(C)** chela showing ornamentation (20 μm), **(D)** proboscis and tripartite mouth with glands at each corner (arrow) (20 μm) **(E)** view of proboscis from dorsal side with cheliphores offset and a median dorsolateral unpaired bifurcate sensillum (8) (50 μm).

GENUS *EURYCYDE*

***Larval morphology of* Eurycyde spinosa (n=10)**

The larva is slightly wider than long (115 μm, 110 μm), and rectangular in shape (Figure 6A). The proboscis is conical, 45 μm long and 50 μm wide at its base. The mouth is 13 μm in diameter. There are at least four indents just below the rim of the mouth that are 3-4 μm across. The mouth is tripartite, with pores at the corners of the three lips (Figure 6D). The lips lack denticles.

The cheliphores are 85-95 μm long. The chelae are half of their size and have a large (65-70 μm) spinning spine (Figure 6B). The inner side of the fixed finger has a tooth 1/3 of the way from the tip. The moveable finger has many small denticles on the bottom half of the inner edge (Figure 6B).

The II and III appendages are 150-180 μm in length. The first article is half the length of the second article (45 μm long). At the base of this article is a thin but long spine (30-50 μm). The terminal article is twice the length of the second article (90-115 μm)(Figure 6C). The inner blade has many short denticles, and a long (10-13 μm) tooth halfway from the base.

There were seven bifurcate sensilla (19-24 μm long) found on the dorsal side of the larva (Figure 6E). They are in the same position as in the larvae of *Nymphopsis spinosissima*. There were no pores found.

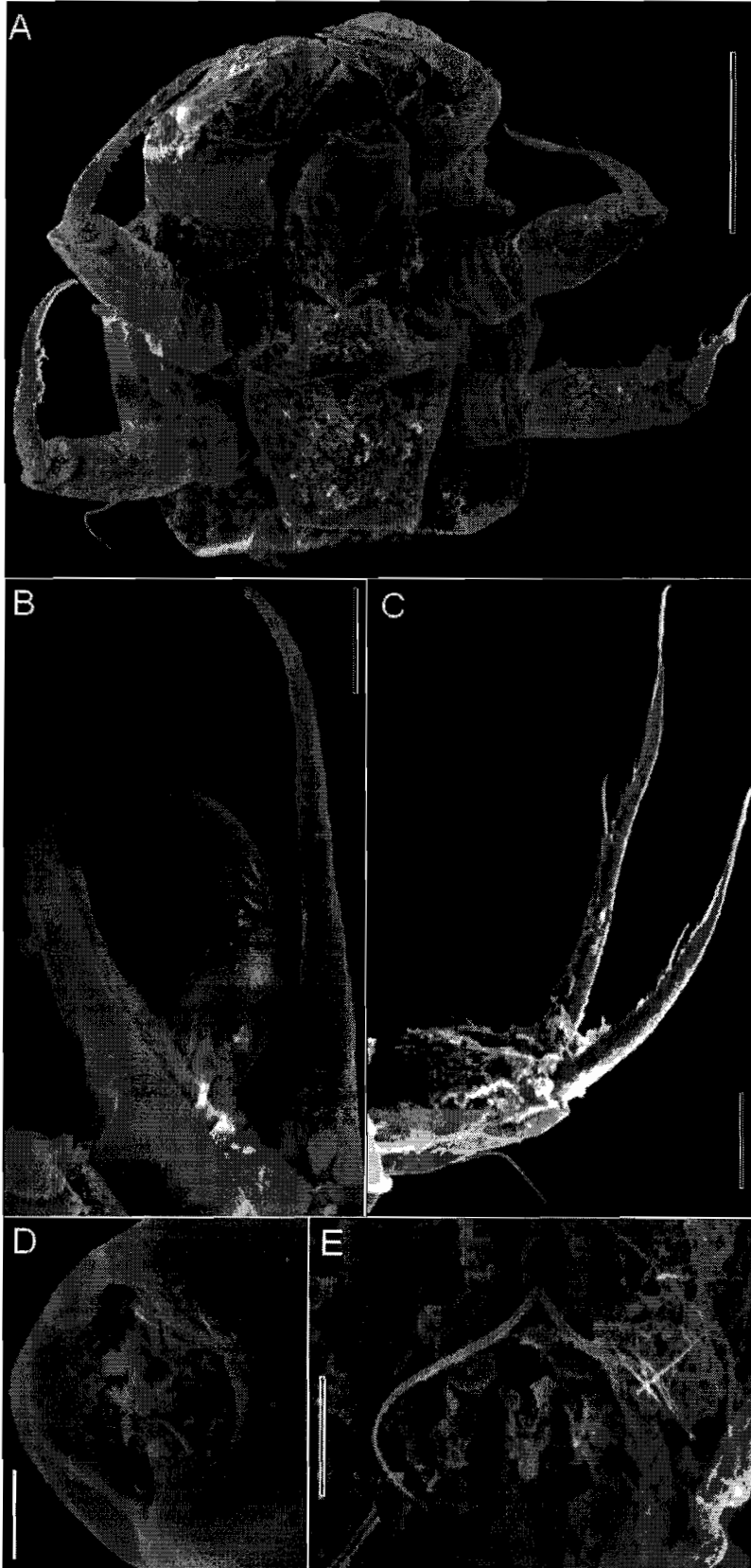**Figure 6.** *Eurycyde spinosa* larva. **(A)** Ventral view (scale bar: 50 µm), **(B)** chela with tooth and spinning spine (10 µm), **(C)** II and III appendages (20 µm), **(D)** mouth (5 µm), **(E)** bifurcate sensilla on dorsal posterior (5 µm).

Family Pycnogonidae

GENUS *PYCNOGONUM*

Larval morphology of Pycnogonum stearnsi (n=20)

The larval body is dorsally rounded, ventrally flat, and slightly wider than long. The length of the body is 70-80 μm and the width is 70-100 μm (Figure 7A). The cuticle is wrinkled dorsally but smooth ventrally. There is a centrally located pore on the ventral side of the body and also one on the dorsal side. The proboscis is cone shaped and ends in a sharp point (Figure 7A). It is 33-40 μm long and 23 μm in diameter at the widest point. Lips are not visible.

The cheliphores are almost as long as the body (60-65 μm). The basal article is cylindrical and wider than long. At the distal edge of each basal article is a spinning spine (70 μm long) that extends out past the chelae (Figure 7A). On the ventral and dorsal sides of the basal article is a large pore (5 μm long). The chelae are almost half the width of the basal article. The movable finger is hook-shaped, tapering to a point, and almost twice as long as the fixed finger (Figure 7B). It has a series of thin spines along the inner and outer edges (Figure 7B). The distal inside edge of the fixed finger has one blunt tooth (Figure 7B).

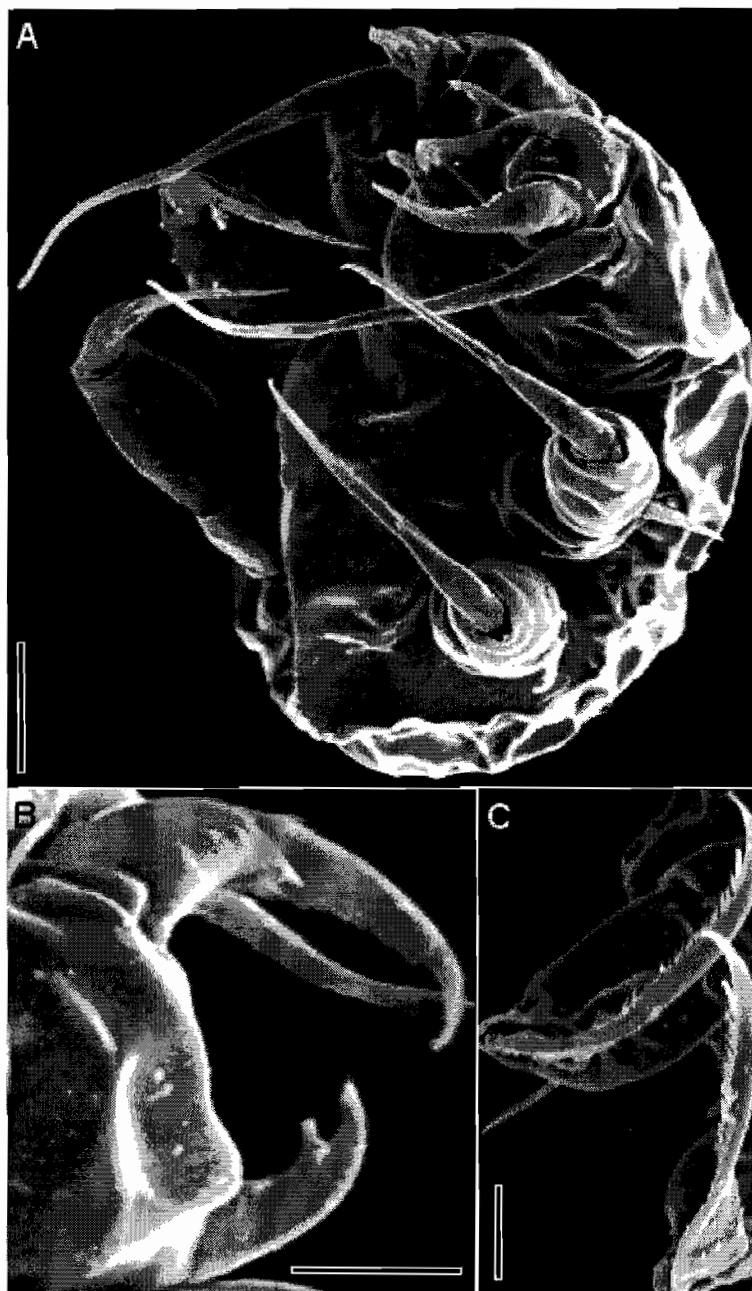**Figure 7.** *Pycnogonum stearnsi* larva. **(A)** Ventral view of larva and proboscis (scale bar: 20 μm), **(B)** chela with spinning spine and short fixed finger (10 μm), **(C)** terminal articles of II and III appendages showing fine denticles (10 μm)

The II and III appendages are 90-100 μm in length. The basal article is the shortest (15 μm long) but widest (22 μm) article of each appendage. At the distal end of this article is a smooth spine (30 μm in length). On the ventral side of each basal article is a large pore. The second article is narrower than the first but about 30 μm long. The terminal article is 50-55 μm long. It tapers to a point and has a slight curve towards the ventral side of the larva. The inside edge of the claw is ornamented with a dense row of thin denticles (Figure 7C). The outside edge is smooth.

There were five bifurcate sensilla on the dorsal side of the larva. At the anterior end of the body is an unpaired median sensillum. There is a pair of smooth sensilla located behind the cheliphores. At the posterior end of the body behind the III appendages is another pair of smooth sensilla, with a large pore located between them. There are two large pores, not slit-like, located at the posterior of the larva, below the final pair of sensilla.

Larval morphology of Pycnogonum rickettsi (n=20)

The larval morphology of *Pycnogonum rickettsi* is very similar to that of *Pycnogonum stearnsi*. The larval body is rectangular in shape and slightly larger (90-105 μm long and 100 μm wide) than that of *P. stearnsi* (Figure 8A). There is no ventrally located pore present. However, the cuticle is also wrinkled on the ventral side. The proboscis is cylindrical in shape and almost half the length of the body (50 μm long and 32 μm wide) (Figure 8A, D). The tip of the proboscis tapers slightly to a blunt point 20

μm in diameter. The mouth (4 μm in diameter) is open and surrounded by a single raised lip (Figure 8E).

The cheliphores are also larger (80-90 μm long) than those of *P. stearnsi* (Figure 8B). The basal articles have two pores, one ventral and the other dorsal. The spinning spine on the distal end of the basal article is the longest of any of the species described. It is more than two times the length of the body (200-250 μm long). The chelae are large, and both fingers have thin spines on the inner blades (Figure 8B). The fixed finger has three teeth, two on the inner edge and one near the tip of the outer edge. The fixed finger ends in a strong hook (Figure 8B).

The II and III appendages are 120-140 μm in length (Figure 7C). The basal article is 15-20 μm long, the second article is 30-40 μm long, and the terminal article is 80-95 μm long. There is a long coiled spine (150-280 μm long) at the base of each basal article. There is a single denticle on the inner edge of the terminal article about 1/3 from the tip (Figure 8C).

There were three sensilla on the dorsal side of the larval body. There is one trifurcate sensillum located at the anterior end of the larva. There are paired bifurcate sensilla located behind the III appendages. There is one large pore between this pair of sensilla, and two more large pores just posterior to them. There are two large pores located behind each of the II appendages.
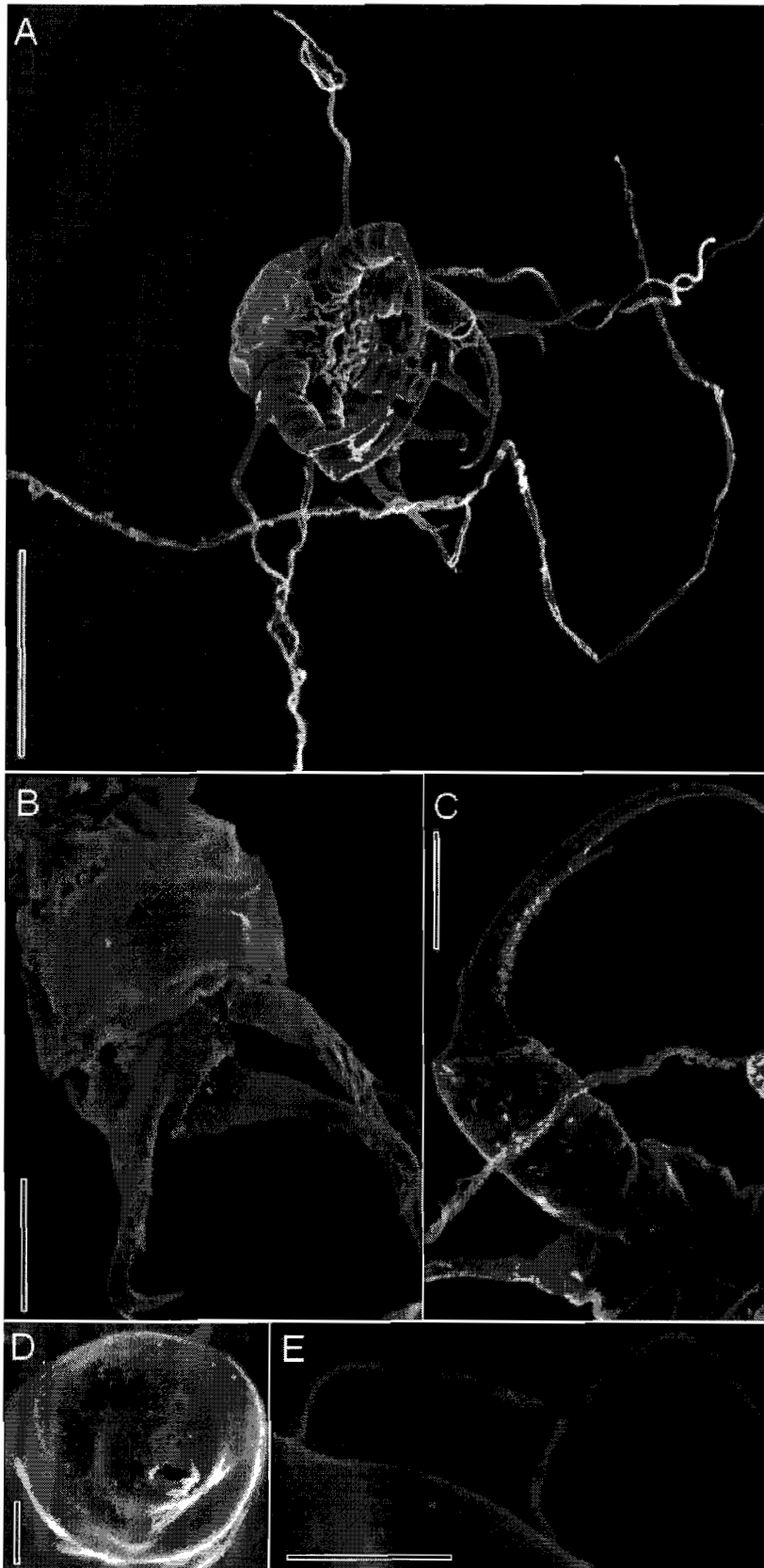
Figure 8.
Pycnogonum rickettsi larva. **(A)** Ventral view showing long cheliphore spinning spines and II and III appendage spines (scale bar: 100 μm), **(B)** chela with sharply hooked fixed finger and large pore on the basal article of the cheliphore (arrow) (20 μm), **(C)** terminal article of II and III appendages showing denticle (20 μm), **(D)** open mouth showing single raised lip (10 μm), **(E)** trifurcate sensilla (5 μm).

Family Phoxichilidiidae

GENUS *ANOPLODACTYLUS*

***Larval morphology of* Anoplodactylus viridintestinalis (n=10)**

This larva is very small, with a body length of 26-36 μm (Figure 9A). The mouth is circular with no hint of a tripartite form. The mouth appears to be open and is 1.5 μm in diameter (Figure 9C). The external surface of the mouth was smooth with straight edges without any denticles.

The cheliphores are rounded, and the chelae (20 μm) are smooth and without ornamentation (Figure 9B). The movable finger has a slight hook at the end. There are no spines at the base of any of the appendages. Instead, the terminal articles of the appendages are long (100μm), smooth threads instead of claws (Figure 9A). With these threads, the legs can be four times the length of the larval body.

There was no indication of pores or sensilla on the larval body.

Figure 9. *Anoplodactylus viridintestinalis* larva. **(A)** Side view showing filamentous strands as the terminal articles of the II and III appendages (scale bar: 20 μm), **(B)** chela (5 μm), **(C)** open mouth (5 μm).

Discussion

Dogel (1913) suggested three forms of pycnogonid postembryonic development based on the 'way of life of the larva' (free-moving ectoparasites, endoparasites, and feeding lecithotrophically while on the adult male). Bain (2003) proposed four modes of development, three of which ('typical protonymphon,' 'encysted larva' and 'attaching') correspond to those suggested by Dogel. Bain defined the fourth mode, the 'atypical protonymphon,' based on three criteria: the number of larval appendages (three pairs or one pair), the larva's way of life (parasitizing, encysting, or attaching) and the formation of the adult walking legs (sequential, simultaneous or partially simultaneous). While Bain's criteria are more inclusive than Dogel's, the terminology confuses larval morphology with developmental mode. Bain's terms imply that the larval mode of development cannot be determined from protonymphon morphology. However, it has been shown that pycnogonid species having the same mode of development also have larvae with similar morphologies (Bogomolova & Malakhov, 2003, 2004, 2006; Bogomolova, 2007; Cano & López-González, 2009). Therefore, I propose new terms for the larval types and postembryonic developmental modes of pycnogonids. The term 'ectoparasitic' will replace 'typical protonymphon' since larvae undergo development as ectoparasites on cnidarians (King, 1973; Behrens, 1984; Bain, 1991; Okuda, 1940; Vilpoux & Waloszek, 2003; Bain, 2003). 'Endoparasitic' will be used in place of 'atypical protonymphon' because larvae develop while inside bivalves and sedentary polychaetes (Ohshima, 1933; Arnaud, 1978; Ogawa & Matsuzaki, 1985; Salazar-Vallejo & Stock, 1987; Bain, 2003). The term 'encysting' replaces 'encysted larva' and

'attaching' will be used instead of 'attaching larva.' Finally, 'prolonged attaching' will be used in place of 'lecithotrophic protonymphon' because these larvae remain on the male for much longer than those following the 'attaching' mode of development (Bogomolova & Malakhov, 2006; Bogomolova, 2007; Cano & Lopez-Gonzalez, 2009).

Drawings and descriptions of larvae for which developmental modes are known reveal four morphological characteristics indicative of a species' pattern of development. The first and most obvious characteristic is larval body size. Large larvae (>300 μm) are typical of the 'attaching' and 'prolonged attaching' developmental modes. Because these larvae are non-feeding while they develop, their large sizes may be due to their substantial yolk reserves (Nakamura, 1981; Bain, 2003; Bogomolova & Malakhov, 2006; Bogomolova, 2007; Cano & López-González, 2009). Similarly, very small feeding larvae (<80 μm) are typical of the 'encysting' mode of development (Meinert, 1899; Bain, 2003; Lovely, 2005). Second, the number and development of larval appendages may also suggest the mode of development. For example, all species shown to have the 'attaching' mode of development have a larva with the II and III appendages absent (Bain, 2003). Although Hooper (1980) described an attaching larva with all appendages present, it is evident that this 'larva' is actually at a very advanced stage of development. Similarly, all larvae with reduced II and III appendages, as in the species *Ammothea glacialis* Hodgson, 1907 and *Nymphon grossipes* Fabricius, 1794, have been shown to follow the 'prolonged attaching' mode of development (Bogomolova & Malakhov 2003; Bogomolova, 2007; Cano & López-González, 2009). In larvae following these developmental modes, the function of attachment to the male is by the cheliphores or

spinning spines and larvae are not free-moving until the end of development. Therefore, fully developed II and III larval appendages do not appear to be functionally necessary in these larvae (Cano & López-González, 2009). Third, the presence or absence of a spinning spine is also indicative of a species' postembryonic mode of development. Species shown to have the 'ectoparasitic' mode of development always have larvae with spinning spines that may be used in attachment (Okuda, 1940; Behrens, 1984; Vilpoux & Waloszek, 2003; Bogomolova & Malakhov, 2006; Gillespie & Bain, 2006). For example, the species *Pycnogonum litorale* Ström, 1762 (family Pycnogonidae) and *Tanystylum orbiculare* Wilson, 1880 (family Ammotheidae) both follow the 'ectoparasitic' development and have long spinning spines that they use as 'safety lines' to return to their host if they become dislodged (Russel, 1990; Bain, 2003; Vilpoux & Waloszek, 2003). Both the larvae of *Nymphonella tapetis* Ohshima, 1927 and *Ammothella spinifera* Cole, 1904 lack spinning spines, presumably because attachment organs are not needed for an endoparasitic lifestyle (Ohshima, 1933; King, 1973; Salazar-Vallejo & Stock, 1987). Finally, the morphology of the terminal articles on the II and III appendages is suggestive of a species' postembryonic mode of development. For instance, only species shown to have an 'encysting' development have larvae with modified terminal articles: in the place of claws they have long filamentous strands up to five times the length of their bodies (Hilton, 1916; Lebour, 1945; Bain, 2003; Lovely 2005). A larva with characteristics of the encysting mode of development has been found in the plankton, making it possible that these long strands are an adaptation to the pelagic environment by decreasing the rate of larval sinking (Malakhov & Bogomolova, 2001).

They may also be provisional structures for movement to the larva's hydroid host

(Russel, 1990). When considered together, these four morphological characteristics (size,

number and development of appendages, presence/absence of spinning spines,

modifications of the terminal appendage articles) can be used to indicate a sea spider's
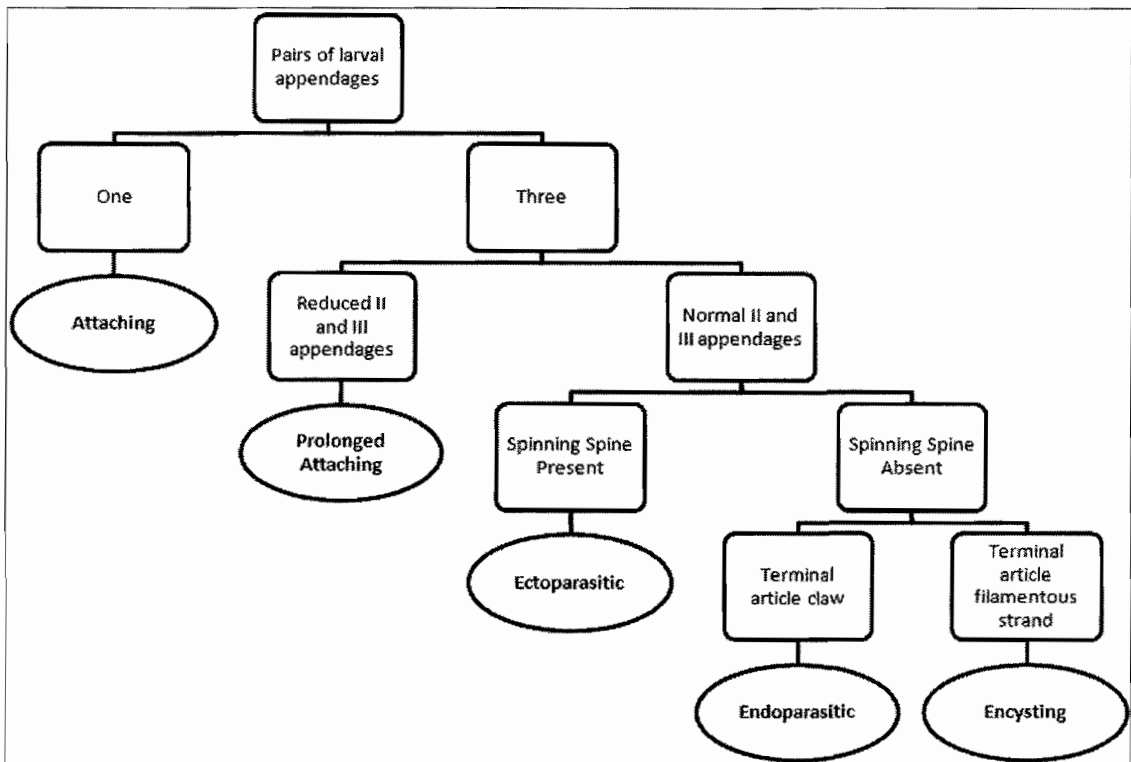
mode of postembryonic development (Figure 10).

Figure 10. Predicted postembryonic development mode based on larval characteristics
(number of larval appendages, degree of development of larval appendages,
presence/absence of spinning spine, and morphology of the terminal article of the II and
III appendages). Larval characteristics are in boxes and developmental modes are in
ovals.

The larvae of *Achelia gracilipes, Eurycyde spinosa, Pycnogonum rickettsi* and *Pycnogonum stearnsi* have morphologies characteristic of the most common developmental pathway, the 'ectoparasitic' mode (Figures 10 & 11). These larvae are of a medium size (70-130 μm long), the three pairs of larval appendages are not reduced, the II and III appendages end in claws (not filamentous strands), and spinning spines are present. Morphologically, these larvae are very similar to the larvae of *Nymphon brevirostre* Hodge, 1863, *Nymphon micronyx* Sars, 1888, *Nymphon longitarse* Krøyer, 1844, *Tanystylum orbiculare, Tanystylum duospinum* Hilton, 1939, *Ammothea alaskensis* Cole, 1904 and *Pycnogonum litorale,* which have been shown to have the 'ectoparasitic' mode of development (Morgan, 1891; Okuda, 1940; Russel, 1990; Tomaschko *et al.,* 1997; Wilhelm *et al.,* 1997; Bain, 2003; Vilpoux & Waloszek, 2003; Gillespie & Bain, 2006; Bogomolova, 2007). Therefore, based on body size, number of larval appendages, presence of a terminal claw and of spinning spines, it is likely that the larvae of *P. rickettsi, P. stearnsi, A. gracilipes* and *E. spinosa* undergo an 'ectoparasitic' developmental mode (Figures 10 & 11).

The family Ammotheidae exhibits the most diversity in postembryonic development modes of all eight pycnogonid families; both the 'ectoparasitic' and 'endoparasitic' modes of development are typical of species in this family (Bain, 2003). It is unsurprising that larvae of *Achelia chelata* and *Achelia simplissima* display significant differences in morphology from the larvae of *Achelia gracilipes.* The generally small body sizes (less than 100 μm long) and absence of spinning spines suggest that these larvae follow an 'endoparasitic' mode of development instead of an

'ectoparasitic' mode (Figures 10 & 11). The life history of the juveniles and adults of *A. chelata* supports an 'endoparasitic' mode of development. Juveniles and adults of *A. chelata* have been found parasitizing the mantle cavities of molluscs. (Benson & Chivers, 1960; personal observation of the author). Although larvae have not been found inside the hosts, reproductive adults were present. Larvae could have been overlooked because of their size. Small size coupled with a lack of spinning spines suggests that *A. simplissima* and *A. chelata* undergo an 'endoparasitic' postembryonic mode of development (Figures 10 & 11).
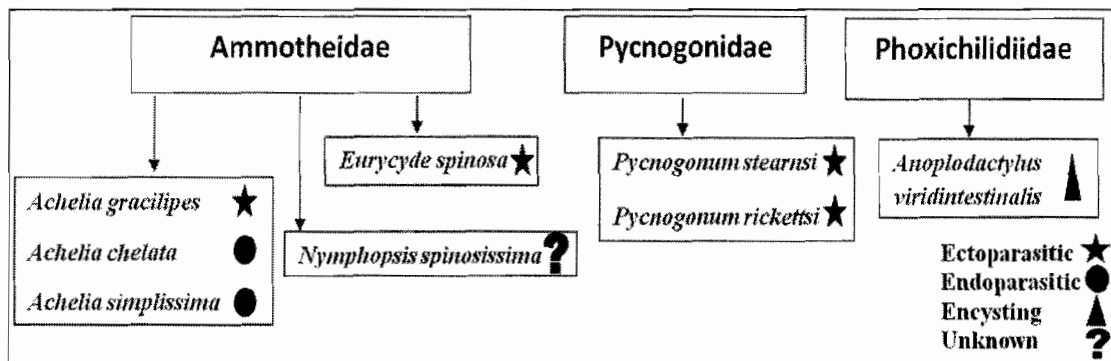
Figure 11. Predicted mode of development for eight species of pycnogonids based on larval morphology, showing within and between family and genera differences. The five species examined in the family Ammotheidae exhibit larval morphologies indicative of two types of postembryonic development ('ectoparasitic' and 'endoparasitic'). The two species of larvae from the family Pycnogonidae both display characteristics suggestive of an 'ectoparasitic' postembryonic development. *Anoplodactylus viridintestinalis* from the family Phoxichilidiidae has a larva indicative of the 'encysting' developmental mode.

Larvae of the species *Anoplodactylus viridintestinalis* from the family

Phoxichilidiidae show characteristics suggestive of an 'encysting' mode of development

(Figures 10 & 11). The size of the larval body is extremely small (30 μm) compared to

most other pycnogonid larvae (King, 1973). In addition, larvae of *A. viridintestinalis*

have terminal articles modified into strands that are up to four times the length of the

body. All other species of the genus *Anoplodactylus* that have been described follow the

'encysting' developmental pathway and have a small larva with modified terminal

articles and no appendage spines (Hilton, 1916; Lebour, 1945; Bain, 2003; Lovely,

2005).

Larvae of the species *Nymphopsis spinosissima* have some unusual morphological

characteristics, but most notably, an extra article on both the II and III larval appendages.

There are no descriptions in the literature of a larva with quadriarticulated appendages.

The number and development of larval appendages seems to be correlated with

developmental mode (Bogomolova & Malakhov, 2006; Bogomolova, 2007; Cano &

López-González, 2009). Therefore, the modified larval appendages of *N. spinosissima*

may be an important characteristic indicative of a new form of larval development.

Additionally, the larval cheliphores of *N. spinosissima* are unusually positioned: offset on

either side of the body, they allow the short proboscis to be seen from the larva's dorsal

side. Although present, the spinning spine is much reduced. All terminal articles show an

unusual amount of ornamentation. There are no larval descriptions for any species of the

genus *Nymphopsis*; however, no other larvae in the literature have offset cheliphores and

quadriarticulated, heavily ornamented appendages (Bain, 2003) (Figure 11).

Unfortunately, the larvae of *N. spinosissima* did not develop past the first larval stage, and so further research will need to be conducted to determine if this species does indeed follow a new mode of development.

Larvae of most species of sea spiders have not yet been described (Bain, 2003). Future research describing larval morphologies and developmental modes will provide a better understanding of which larval characteristics are most helpful in determining postembryonic developmental modes. Larval morphology suggests that two types of postembryonic development (ectoparasitic and endoparasitic) are evident in members of the genus *Achelia*. Results suggest that the 'ectoparasitic' mode of development is present in both the families Ammotheidae and Pycnogonidae. The 'encysting' development typical of the family Phoxichilidiidae is suggested for the species *Anoplodactylus viridintestinalis*. This paper is the first description of larvae from these eight species of Oregon pycnogonids.

CHAPTER V

CONCLUSIONS

The results of this study suggest that the intertidal pycnogonid *Achelia simplissima* reproduces year round and mainly at night. In addition, both males and females mate multiple times and have multiple mates. Males of this species do not partition eggs from a single mating event into multiple egg masses, and they do not add new eggs to already existing egg masses. As a result, the number of egg masses a male is carrying corresponds to the number of times that he has mated, but not necessarily to the number of mates. There was no female-female competition for mates observed, but females did exhibit specific 'pumping' behavior to initiate mating. Egg masses varied considerably in the number of eggs they held and in their length of embryonic development. Egg masses hatched in order of when they were laid on the male, however, these relative hatching times are not good indicators of when the egg masses were laid.

Parental care costs to male pycnogonids are not extreme in this species, but may include an increased susceptibility to fish and shrimp egg mass predation. In addition, parental males also had an increased frequency of epibionts and decreased movement patterns which may reduce their encounter rate with gravid females. However, feeding frequency was not affected by parental care and parental males are harder to dislodge than nonparental males.

A review of the literature suggests a correlation between larval morphology and developmental modes. I propose new terminology for the postembryonic development modes of sea spiders ('ectoparasitic,' 'endoparasitic,' 'encysting,' 'attaching,' and 'prolonged attaching' modes). This study suggests that based on the presence of filamentous strands in the first larval stage of *Anoplodactylus viridintestinalis*, this species follows an 'encysting' mode of development. The presence of spinning spines in the larvae of *Pycnogonum stearnsi, P. rickettsi, Achelia gracilipes,* and *Eurycyde spinosa*, suggests that these species follow an 'ectoparasitic' mode of development. The absence of spinning spines in the first larval stages of *A. simplissima* and *A. chelata* suggests that these species have an 'endoparasitic' development. The unusual larval morphology of *Nymphopsis spinosissima* may suggest a new mode of postembryonic development.

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