Life history patterns, social group structure, and mating system of Pederson cleaner shrimps *Ancylomenes pedersoni*

by

Jessica Anne Gilpin

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Approved by

Nanette E. Chadwick, Chair, Associate Professor of Biological Sciences
Raymond T. Bauer, Professor Emeritus of Biological Sciences,
University of Louisiana at Lafayette
Dennis DeVries, Professor of Fisheries, Aquaculture and Aquatic Sciences
Sharon Hermann, Assistant Professor of Biological Sciences

Abstract

Cleaner organisms perform key functional roles in reducing rates of parasitism in marine communities. Pederson cleaner shrimps *Ancylomenes pedersoni* are major cleaners of reef fishes in the tropical western Atlantic, and form obligate symbioses with host sea anemones. They usually occur in social groups of several different-sized individuals per host; a size-dependent social hierarchy has been proposed to structure these groups, but no quantitative data exist to support this idea. Information about their life history traits and mating system also would contribute to understanding how symbiosis impacts life history evolution in crustaceans, but little is known about patterns of growth and reproduction in this anemoneshrimp.

I quantified growth, sexual reproduction, senescence, mortality and social interactions in individuals of *A. pedersoni* under laboratory conditions, and their abundance and population size structure, including group dynamics, on coral reefs at St. Thomas, U.S. Virgin Islands. Individuals grew rapidly when young, then slowed their growth after reaching sexual maturity at ~ 6 months. Individuals were gonochoric with sexual dimorphism, as shown through long-term growth measurements and experimentally-manipulated groups of shrimps observed for extended periods to document sexual habits. Females were larger than males, and exhibited continuous reproductive cycles in the laboratory. Prior to death at < 2 years, members of both sexes exhibited senescence during which they ceased reproducing, shrank (females only), changed body coloration, and decreased their activity levels over ~ 1-4 weeks.

Field populations were abundant and composed mostly of juveniles during both years

examined. Populations appeared to be stable, with individuals reaching maximum yield at ~ 4 months of age. On some host sea anemones in the field, shrimp social groups contained individuals that were similar in body size. As such, Pederson shrimps formed loosely-structured social groups, which did not exhibit rigid structure in terms of their relative body sizes on each host sea anemone. However, these shrimp social groups were spatially structured; the distances of individuals from the host anemone tentacles decreased significantly with shrimp body size. Large individuals (usually gravid females) occupied habitat on the anemone tentacle crown, while smaller group members perched on the surrounding substrate, with the smallest individuals located the farthest distance from the host. Under laboratory conditions, these shrimps engaged in size-structured behavioral dominance hierarchies, in which large individuals excluded smaller ones from access to resources (food and habitat). Large females both approached and cleaned client fish models (potential sources of food in the form of ectoparasites), and also attacked and chased smaller shrimps (thus excluding them from prime positions in the culture tank), significantly more frequently than did smaller females, males, and juveniles. Behavioral interactions explained observed microhabitat use patterns of shrimps on hosts in the field.

I conclude that a size-based behavioral dominance hierarchy in social groups of Pederson shrimps allows large individuals to monopolize food resources in the form of client fish ectoparasites, and also to occupy habitat space near the centers of host anemones which provides more shelter than do peripheral microhabitats. I conclude that obligate symbiosis with large sea anemones, and cleaner mutualism with reef fishes, both contribute to explaining aspects of the life history of Pederson shrimps, especially their apparent mating system of pure-search polygynandry. This life history information also provides a scientific basis for sustainable fishery management and aquaculture of this key coral reef organism.

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List of Abbreviations

CL	Carapace Length
WM	Wet Mass
PMTL	Precisely Measured Total Length
VETL	Visually Estimated Total Length

Chapter 1

Cleaner shrimps: ecology, biology, and fisheries management

Ecological roles of cleaner shrimps on coral reefs

Cleaners and their clients form intimate relationships that are mutually beneficial to both parties, and thus are a form of symbiosis or facultative interaction (reviewed in Boucher et al., 1982). Cleaners prey upon parasites that graze on the tissues of hosts but do not kill the hosts (Kuris and Lafferty, 2000; Lafferty and Kuris, 2002). Many types of invertebrates function as cleaners, but shrimps are the most diverse. Of 130 known species of marine cleaners, shrimps contribute over 40 species and are the main invertebrate cleaners on coral reefs (Côté, 2000). Studies on cleaning symbiosis have focused mainly on the cleaner fishes (Bshary, 2003), but research on cleaner shrimps is growing (Mahnken, 1972; Chockley and Mary, 2003; Chockley *et al.*, 2008; Silbiger and Childress, 2008). Cleaner shrimps belong to 20 genera in five families (Gnathophyllidae, Hippolytidae, Palaemonidae, Penaeidae, Stenopodidae) within the order Decapoda (Becker and Grutter, 2004). There are seven infraorders within Decapoda, and some of the major cleaner shrimps, such as banded coral shrimps *Stenopus hispidus* and Pederson shrimps *Ancylomenes pedersoni* belong to the infraorders Stenopodidea and Caridea (> 3,400 species; De Grave and Fransen, 2011) respectively (Bauer, 2004). Cleaner shrimps are most diverse in the tropical Indo-Pacific region, where eight species, including four endemics are found (Côté, 2000). Many of the other cleaner shrimp species occur in the Gulf of California,

tropical east Atlantic, tropical west Pacific, and Australia, while the Caribbean region contains six species of cleaner shrimps, including three endemics (Côté, 2000). Pederson cleaner shrimps and banded coral shrimps are two major shrimp cleaners in the Caribbean Sea (Bauer, 2004). These two shrimps are major removers of fish parasites as demonstrated in both mesocosms and field behavioral studies, in contrast to spotted cleaner shrimps *Periclimenes yucatanicus* (Bunkley-Williams and Williams, 1998; McCammon *et al.*, 2010), which more rarely clean fishes (Titus *et al.*, 2017).

In laboratory experiments, the Caribbean Pederson cleaner shrimps removed ectoparasitic juvenile cymothoid isopods *Anilocra haemuli* from French grunts before they had negative impacts on fish health in the form of damaged tissues, bacterial infection, reduced hematocrit, and death (Bunkley-Williams and Williams, 1998; Sikkel *et al.*, 2005; Östlund-Nilsson *et al.*, 2005; Sikkel *et al.*, 2011). This shrimp species cleans the members of at least 16 different families of fishes (Wicksten, 1998; Huebner and Chadwick, 2012a), which indicates potentially broad impacts on fish diversity by members of this species since fish rely on this service.

Pederson cleaner shrimps, banded coral shrimps, and spotted cleaner shrimps all associate with large Caribbean coral reef sea anemones: corkscrew anemones *Bartholomea annulata*, giant or rosetip anemones *Condylactis gigantea*, and sun anemones *Stichodactyla helianthus* (Bauer, 2004). The combination of a cleaner shrimp and associated anemone forms a cleaning station, where the shrimp may perch on the tentacles or oral disc of the anemone, and are immune to the stinging nematocysts of the host (Mihalik and Brooks 1997). Cleaner shrimps that associate with anemones use them as the center of their cleaning stations, where client fishes approach to have their ecotoparasites removed (McCammon *et al.*, 2008). Reef fishes use sea anemones as visual cues to locate cleaning stations and to engage in cleaning interactions with

shrimps (Silbiger and Childress, 2008; Huebner and Chadwick, 2012b). Some commercially important fishes such as groupers even center their territories around anemones with cleaner shrimps (Sluka *et al.*, 1997). As a result of their ability to host cleaners and act as visual signals that draw in client fishes, the anemones also receive nutrients for their symbiotic algae in the form of waste products (excreted ammonia) from their crustacean symbionts and visiting clients (Cantrell *et al.*, 2015).

In addition to the visual cues provided by host anemones, some cleaner shrimps also exhibit behaviors such as rocking dances and antenna vibrations that advertise their cleaning services to potential client fishes (Becker *et al.*, 2005; Chadwick *et al.*, 2008). These visual cues help client fishes find cleaning stations and receive the benefits of parasite removal.

Ornamental fisheries for cleaner shrimps

Cleaner shrimps are in popular demand for the ornamental aquarium trade (Hardin and LeGore, 2005) and their abundance on reefs is in decline, as evidenced by declining catch numbers despite intense efforts by collectors in the Caribbean (Hardin and LeGore, 2005; LeGore *et al.*, 2005; Nelsen, 2008; Rhyne *et al.*, 2009). The striking coloration patterns, cleaning behavior, and symbiotic nature of caridean shrimps, as well as their "reef safe" characteristics, including not harming fish or other invertebrates in aquaria, cause shrimps from the genus *Periclimenes* to be very popular ornamental organisms (Calado, 2009). Information on the life histories of cleaner shrimps and effects of their removal are needed to provide quantitative scientific evidence to support sustainable fisheries, especially caridean shrimps such as Pederson and spotted cleaner shrimps (Calado, 2009). Pederson cleaner shrimps are the most well know

cleaner species in the Caribbean (Calado, 2009) and their collection is not monitored closely. Based on this information, revised permit and catch limits could allow populations of these key organisms to remain stable on reefs.

Fish abundance and diversity on some coral reefs have both declined significantly over the past few decades, with a dramatic increase in the number of grazers and a decrease in other types of fishes that are heavily collected for the aquarium trade (Paddack *et al.*, 2009; Rhyne *et al.*, 2009). Fishing for sport, food, and ornamental aquaria all have negatively impacted reef organisms (Andrews, 1990; Wabnitz, 2003; Rhyne *et al.*, 2009). Landings of ornamental marine invertebrates as well as vertebrates are increasing, but regulation of these fisheries is static (Rhyne *et al*., 2009). Only a limited amount of data is available on the import and export rates of marine ornamental shrimps, and current rates of trade for these organisms appear to be underestimated (Calado, 2009).

The natural abundances and dynamics of key organisms like cleaners need to be determined, so that human impacts on reef ecosystems, such as through the aquarium trade, can be understood, and management policies developed that place limits on collecting (Hardin and LeGore, 2005).

Life history, social structure, and population dynamics of cleaner shrimps

An understanding life history patterns and population dynamics is important for the management of species; these processes are known for some cleaner shrimps such as banded coral shrimps in the Caribbean (Chockley and St. Mary, 2003), but have not been quantified for the major Caribbean cleaner, Pederson cleaner shrimps. Life history traits that remain unknown

for this species include details on growth, size or age at maturity, lifespan, the duration of the reproductive cycle (e.g., length of embryo incubation), details of the mating system, and aspects of their population structure (e.g., whether females or males are dominant in social groups). Information about population size structure also is needed to assess rates of recent recruitment, and the age structure and stability of field populations. Knowledge of these traits is important for fisheries managers, so they can determine how many and which sexes/sizes of individuals should be collected while still maintaining sexually viable populations.

Finally, the dominance structure of social groups of these shrimps on each sea anemone host remains unknown, and is important for understanding their rates of feeding and migration among hosts. Pederson shrimps form aggregations of up to 12 individuals per host anemone, in which individuals appear to be of different body sizes and to consist of both genders (Mahnken, 1972). However, the structure of these groups in terms of dominance interactions, habitat segregation on the host, and impacts of dominance hierarchies on shrimp access to fish clients has not been quantified.

Overview of dissertation chapters

This dissertation presents new information concerning several aspects of the biology of Pederson shrimps: life history characteristics including growth, sexual reproduction, senescence and mortality (Chapter 2), the dominance structure of social groups on sea anemones (Chapter 3), and analysis of the sexual mating system including rates of embryo production (Chapter 4). A major application of these results is presented in the form of recommendations for sustainable

fishery management, based on the examined aspects of the biology of this important coral reef organism.

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Chapter 2

Life history traits and population structure of Pederson cleaner shrimps *Ancylomenes pedersoni*

Biological Bulletin **(in press)**

Abstract

Cleaner organisms perform key functional roles in reducing rates of parasitism in marine communities. Pederson cleaner shrimps *Ancylomenes pedersoni* are major cleaners of reef fishes in the tropical western Atlantic, and form obligate symbioses with host sea anemones. Information about their life history traits would contribute to understanding how symbiosis impacts life history evolution in crustaceans, but little is known about patterns of growth and reproduction in this anemoneshrimp. We quantified growth, sexual reproduction, senescence and mortality in individuals of *A. pedersoni* under laboratory conditions, and their abundance and population size structure on coral reefs at St. Thomas, U.S. Virgin Islands. Von Bertalanffy growth curves were fit to the data to determine age-size relationships, and the Beverton-Holt model was used to estimate mortality rates and size at maximum yield. Individuals grew rapidly when young, then slowed their growth after reaching sexual maturity at ~ 6 months. Individuals were gonochoric, with males attaining significantly smaller body sizes and shorter lifespans than did females. Prior to death at < 2 years, members of both genders exhibited senescence during which they ceased reproducing, shrank (females only), and decreased their activity levels over ~

1-4 weeks. Field populations were abundant and composed mostly of juveniles during both years examined. Populations appeared to be stable but highly dynamic in terms of individuals, reaching maximum yield at 4 months of age. We conclude that obligate symbiosis with large sea anemones and cleaner mutualism with reef fishes both contribute to explaining aspects of the life history of Pederson shrimps, especially their apparent mating system of pure-search polygynandry. This life history information also provides a scientific basis for sustainable fishery management and aquaculture of this key coral reef organism.

Introduction

Life history patterns illustrate biological tradeoffs that contribute to understanding organismal evolution. Beneficial changes in one type of trait may lead to detrimental changes in another (Stearns, 1989), such as the tradeoff between growth rate and organismal age and size at reproduction. Knowledge of life history patterns also provides insight into factors that influence population dynamics, such as lifespan and maximal rates of body growth. This type of information enhances understanding about how ecological interactions including obligate symbioses shape the evolution of life history traits including mating systems within a group of related organisms (reviewed in Baeza and Thiel, 2007).

Despite the importance of knowledge about life history traits, such information is lacking for most shrimps on coral reefs. Characteristics such as investment into growth versus reproduction, and patterns of recruitment and mortality, vary widely among shrimp species (Bauer, 2004; Baeza and Piantoni, 2010). In the Caribbean Sea, these processes have been examined for ecologically-important banded coral shrimps *Stenopus hispidus* which clean

ectoparasites from reef fishes (Chockley and Mary, 2003), and are in demand for the ornamental aquarium trade (Lin, 2005). However, most aspects of life history and reproductive biology have not been quantified for Pederson cleaner shrimps *Ancylomenes pedersoni* (Chace, 1958), which serve as the main cleaners of reef fishes in some parts of the Caribbean Sea and Florida Keys, more so even than do cleaner fishes (Mahnken, 1972; Wicksten, 1995; Bunkley-Williams and Williams, 1998; Titus *et al.*, 2017). Individuals of *A. pedersoni* associate in obligate symbiosis with large sea anemones, including corkscrew anemones *Bartholomea annulata*, rosetip anemones *Condylactis gigantea*, and sun anemones *Stichodactyla helianthus* (Bauer, 2004), which they use as the centers of cleaning stations that attract client fishes for ectoparasite removal (McCammon *et al.*, 2010). Cleaning stations are spatially and temporally dynamic on coral reefs, because cleaner shrimps may migrate nocturnally among host anemones (Chadwick *et al.*, 2008), and some host anemones have short lifespans (~1 yr; O'Reilly and Chadwick, 2017). Information on anemoneshrimp life history patterns is important for gaining insight about both ecological variation, such as the spatial and temporal stability of cleaning stations, and about evolutionary implications in terms of how obligate symbioses shape species traits.

Anemone-associated cleaner shrimps are key players in a complex mutualistic network that influences the ecology and evolution of several types of reef organisms (Cantrell *et al.*, 2015). Reef fishes use sea anemones as visual cues to locate cleaning stations and then engage in cleaning interactions with resident shrimps (Silbiger and Childress, 2008; Huebner and Chadwick, 2012a); as such, some commercially-important groupers center their territories around anemones with Pederson cleaner shrimps (Sluka *et al.*, 1997). Due to their ability to host cleaners and act as visual signals that draw in client fishes (Huebner and Chadwick, 2012a), the anemones also may receive nutrients in the form of waste products (excreted ammonia and

eliminated feces) from their crustacean symbionts and visiting clients, thus contributing to efficient nutrient cycling on coral reefs (Cantrell *et al.*, 2015; also known for goby-shrimp symbiosis, Kohda *et al.*, 2017). Pederson cleaner shrimps consequently provide several types of ecological services in reef ecosystems, and knowledge of their life history patterns is needed for understanding the dynamics of these mutualistic networks. Large sea anemones host a wide diversity of obligate crustaceans, some of which are cleaners, on both Caribbean and Indo-Pacific coral reefs (Nizinski, 1989; Chadwick *et al.*, 2008; Colombara *et al.*, 2017). Comparison of life history traits among these symbiotic crustaceans would enhance our understanding of life history evolution in symbioses (Baeza and Thiel, 2007).

Data on life history characteristics also form a fundamental scientific basis for the development of conservation management strategies to apply to species at risk (Calado, 2009; Fenner, 2012; Dee *et al.*, 2014). The striking coloration patterns, cleaning behavior, symbiotic habit, and "reef safe" characteristics of Pederson cleaner shrimps cause them to be popular in the ornamental trade (Calado, 2009), putting them at widespread risk of over-collection. Populations of rapidly-expanding invasive Pacific red lionfish *Pterois volitans* in the Caribbean Sea appear to target shrimps including cleaner shrimps for predation, putting these species at risk of complete elimination from some reef systems (Faletti and Ellis, 2014; Tuttle, 2017). Life history information about this species therefore is needed also to guide their conservation management (Hardin and LeGore, 2005).

Major life history traits that remain unknown for Pederson cleaner shrimps include growth rate, size and age at sexual maturity, mortality rate (longevity) and various aspects of population structure (i.e., proportion and role of females versus males in social groups). Within the diverse shrimp Infraorder Caridea (> 3,400 species; De Grave and Fransen, 2011), some

aspects of these traits are known for other species (reviewed in Bauer, 2004; Baeza and Thiel, 2007; Prakash *et al.,* 2017). Other tropical carideans have short life spans of ~ 4-5 months to < 1 year due to their ability to mature quickly and reproduce intensively, and they attain small adult body sizes of only 1-8 mm carapace length (CL, length from posterior edge of the eye orbit [eyestalk] to the middorsal posterior edge of the carapace or cephalothorax; the most commonly-used measure of caridean body size; 9 species examined; Bauer, 2004). Breeding in tropical carideans may occur continuously throughout the year, with females producing a brood after each molt phase, which appears to occur approximately weekly for *A. pedersoni* (Mahnken, 1972).

Here we report rates of growth, molt interval, sexual reproduction, senescence and mortality of *A. pedersoni* under laboratory conditions. We also quantify the size and age structure of populations on coral reefs at St. Thomas, US Virgin Islands, then construct mortality curves and life tables based on the combined laboratory and field data. We compare these life history patterns with those of other symbiotic crustaceans, and examine how they fit the predictions of an evolutionary model (Baeza and Thiel, 2007) to draw conclusions about how symbiosis shapes their life history. We also provide recommendations for ornamental fisheries management and aquaculture of this species, to support sustainable aquarium trade practices for this key coral reef organism.

Methods

Organismal collection and laboratory culture

In the laboratory we examined 92 Pederson cleaner shrimps *Ancylomenes pedersoni* cultured in closed system aquaria at Auburn University for 4 years (January 2013 - December 2016), with some individuals surviving for up to 21 months during that period. Individuals were collected by hand from shallow nearshore marine habitats at Marathon Key, Florida Keys, USA, or purchased from commercial collectors in Florida (Dynasty Marine and Sea Life Inc., Marathon, Florida, USA). All shrimps were transported to Auburn University within a few days of collection, in aerated buckets by car (if collected by the authors), or by overnight shipping (if ordered from commercial collectors). Shrimps arrived to the laboratory 3-4 times per year to replace individuals lost due to mortality (see below), resulting in 8-25 individuals cultured during any given period. The 10 arrival periods were: June 2012 (prior to start of the study, N = 3 individuals), January 2013 (10), February 2014 (12), May 2014 (15), September 2014 (12), February 2015 (4), October 2015 (6), March 2016 (9), June 2016 (11), and September 2016 (10).

Upon arrival to the laboratory, each shrimp was assigned an individual identification code, its body size measured (see below), and its reproductive condition determined as: (1) Female (N = 56, CL = 3.4-6.1 mm), based on the presence of incubating embryos, mature ovaries, or expanded abdominal pleura if there were no incubating embryos or mature ovaries (defined as female breeding dress: large size of the region below the abdomen due to enlarged ventral surface of skeletal plates on the first three abdominal segments; Bauer, 2004). Changes in the first four pairs of pleopods (increase in length and development of setae for attaching

embryos to the abdomen; Chace, 1958) were not used as characters because they were difficult to visualize without damaging the live shrimps; (2) Male (N = 19, CL = 3.4-4.1 mm): based on medium-sized narrow body, lack of oocytes or female breeding dress, and fertilization of co-housed females after they arrived to the laboratory. Presence of an appendix masculina (Prakash *et al.,* 2017) was not used to confirm male identity, because it was difficult to observe without killing and preserving the shrimps; or (3) Juvenile (N = 17, CL < 3.3 mm): based on small body size, absence of oocytes or breeding dress, and lack of subsequent fertilization of co-housed females (after Bauer, 2004).

Initially (January 2013 – May 2014), shrimps were cultured in social groups of 1-5 individuals per tank (similar to social group sizes per host sea anemone in the field; Chace, 1958; Mahnken, 1972). They were identified individually based on their body size and reproductive status relative to the other shrimps in each small social group. Each tank was 75 L with a 75 L sump (150 L per tank system) maintained with an aquarium light, protein skimmer filter, and heater, that had been developed to mimic coral reef conditions for reef fish culture (details in Roopin and Chadwick, 2009; Huebner *et al.*, 2012; Cantrell *et al.*, 2015). Later (June 2014 – December 2016) shrimps were cultured in the same types of social groups in smaller tanks that did not contain sumps or overhead aquarium lights (40 L, with external hanging filter driven by a small pump, Aqua-Tech Power Filter 5-15, Spectrum Brands), because initial observations indicated that (1) shrimps did not require large water volume and high flow, and (2) they survived better in smaller tanks with less flow because they were less likely to become sucked into sumps. In both types of tanks, all shrimps grew continuously and females regularly produced egg clutches. Throughout the 4-yr study, water temperature was 23.5-26.5°C (slight seasonal change with ambient air temperature) and salinity 33-37 ppt, both within the range of conditions

on coral reefs. About 40% of the seawater in each tank was changed monthly using artificial seawater mixed from DI water and Instant Ocean brand Sea Salt Mix (Spectrum Brands). In the small tanks, lighting was provided by overhead ceiling lights in the laboratory room. Each tank was covered with rigid plastic grating (15 mm grid size, economy grade polystyrene; Louvered Ceiling Light Panel, PLASKOLITE) that was trimmed to fit the tank top, to reduce shrimp mortality due to their accidentally jumping out of the tank. All shrimps were fed every other day to saturation with fish food pellets (Formula One and Formula Two Pellets, Ocean Nutrition, Newark, CA), which caused them to grow rapidly, up to 2 mm CL per month in small individuals. Each tank contained a small unglazed ceramic pot for shelter. The shrimps performed normal behaviors such as cleaning signals (Becker *et al.*, 2005), molted frequently (every 8-16 days, see below), and grew throughout the study, indicating healthy condition.

Body size parameters

Carapace Length (CL; see above) was measured to the nearest 0.1 mm each month using vernier calipers, by carefully removing each shrimp from its culture tank with a hand net and transferring it to a plastic petri dish (88 x 13 mm) containing ~ 8 mm depth seawater (to keep the shrimp submerged but unable to locomote), then viewing it under a dissecting microscope at 10x power. The presence of oocytes in the ovaries or of incubated oocytes or embryos on the abdomen was noted (after Allen, 1966; Bauer, 1986, 1991; Prakash *et al*., 2017). Water in the petri dish remained at ambient room temperature. Each shrimp then was removed from the petri dish using a thin spatula, blotted with Kimwipes to remove excess water, placed on a tarred electronic balance (Fisher Scientific A-160), wet mass (WM) recorded to the nearest 0.0001g,

and returned to its culture tank. Each shrimp was out of the culture tank for < 3 min each month for these measurements, and exhibited normal behavior almost immediately (within 1 min) upon return to the tank, so did not appear to experience long-term stress from these procedures.

Near the beginning of the study, two additional measures of body size were determined: precisely-measured total length (PMTL, anterior tip of rostrum to posterior tip of telson, measured in petri dishes as described above, N = 35 individuals), and visually-estimated total length (VETL, a less stressful form of total length measurement from rostrum to telson estimated while shrimps remained inside culture tanks, using vernier calipers placed ~ 1 cm distant from each shrimp, N = 26 individuals). Relationships were determined among all four measures of body size (CL, WM, PMTL, VETL), to allow alternate measures to be used during field studies where CL may be difficult to quantify. Computer image analysis from photographs was not used to size the shrimps, because preliminary observations indicated that the dimensions of their small translucent bodies were not clearly visible in photographs, under either field or laboratory conditions.

Sexual maturity

Juveniles were observed daily for signs of sexual maturity without removing them from culture tanks. Gender in sexually-maturing juveniles was determined to be female as soon as individuals developed paired ovaries with pink/orange/beige oocytes visible in the cephalothorax, posterior to the eyestalks and dorsal to the cardiac and pyloric stomach (after Bauer, 1986), or began to brood oocytes or embryos under the abdomen. Juveniles were recognized as males as soon as they appeared to fertilize co-housed females, evidenced by the

females commencing to brood embryos instead of unfertilized oocytes (females co-housed only with other females or with juveniles never brooded embryos). Body size at maturity was assessed for each maturing shrimp.

Molt interval

To determine the molt interval (period between each shedding of the exoskeleton) of adult shrimps, 4 individuals [2 female, 2 male] were selected randomly in culture tanks on August 30, 2016, and individually tagged on the carapace (modified after Nizinski, 1989). Twelve more individuals [6 female, 6 male] were selected randomly and tagged on September 15, after the initial tagging method proved to be effective, plus 2 more females on October 12 (N = 18 total; 10 female, 8 male). To tag shrimps, small circles (1.6 mm diameter) of colored plastic sheeting (Avery, CCL Industries) were cut using a standard small hole punch and quartered to make them smaller (~ 0.8 mm diameter each). Then each selected shrimp was removed from its culture tank, placed in a petri dish, and blotted with a Kimwipe to remove excess water. A drop of super glue (Loctite Super Glue brand, Loctite) was applied to the plastic sheeting half-circle, and the sheeting was placed carefully onto the dorsal anterior section of the carapace posterior to the eyestalks, using fine tweezers and taking care to not place on the white and violet color patches or on areas of segmentation. The glue was allowed to dry for 20 seconds, then the shrimp was returned to its culture tank. Each shrimp was out of its tank for 3 min and did not appear to experience excessive stress from tagging (except for 3 individuals damaged by excess glue application; see Results). The remaining individuals behaved normally (slow beating of

pleopods, locomotion around the tank, loss of stress coloration) within 3 min after return to the tank, similar to their responses after monthly body size measurements (see above).

Tagged shrimps continued to produce oocytes (if female) and to fertilize females (if male) throughout the molt observation period of 3 months (August 30 – November 30, 2016). The 18 tagged shrimps examined for intermolt duration also continued to be observed for rates of growth, reproduction, senescence and mortality. A different tag color was applied to each shrimp that was cohoused with other tagged shrimps, for a total of 4 tag colors employed on 18 shrimps in 6 tanks. Tagged shrimps were observed daily until each molted and shed its tag along with the molted exoskeleton. Promptly after each molt, a new tag was reapplied so that the next full molt interval could be tracked. This procedure was repeated for ~ 30 days on each tagged shrimp, or until the durations of ~ 3 molt cycles were determined. Tagging was used only for these 18 shrimps examined for molt interval, and not for all 92 laboratory-cultured individuals, because it was needed only to detect when each exoskeleton had been molted; all other shrimps were identified clearly by their relative body size and sexual condition, within small social groups of 1-5 shrimps cultured per tank.

Senescence and mortality

When shrimps died in the laboratory, the cause of mortality was determined as: (1) accidental (ie: sucked into tank filter, jumped out of tank), (2) natural (due to senescence, see below), or (3) unknown (shrimps disappeared or carcass found with no evidence of accident or prior signs of senescence). Senescence was characterized by up to 5 processes observed during 1-4 weeks prior to natural death: (1) body shrinkage: decrease in CL and/or wet mass; (2) change

in body color from mostly transparent to opaque (also occurred 1-2 days prior to each molt, but lasted up to 1-2 weeks during senescence); (3) reduced activity level: slower or less frequent locomotion, cleaner signaling behaviors, and/or interaction with other shrimps, general listlessness; (4) reduced feeding: lower food consumption, fewer foraging attempts to locate food in the tank; and (5) reduced or ceased sexual reproduction: reduction in the volume of oocyte or embryo masses, or complete cessation of oocyte production (in females) or of fertilization of the oocyte masses carried by co-habiting females (in males; senescence was not observed in any juveniles).

After a shrimp died, its body was removed from the culture tank and size at death was determined (both CL and WM), unless the carcass had become disfigured or dismembered (from being sucked into the tank filter, partially consumed by tank mates, etc.).

Population size and age structure

The field component of the study examined shrimps during two summers (July 2015 and August 2016) on coral reefs at ~ 6 m depth in Brewers Bay, St. Thomas, U.S. Virgin Islands (18°19' N, 64°59' W). This study site was selected because it contained abundant corkscrew sea anemones *B. annulata* with associated Pederson cleaner shrimps *A. pedersoni,* and was located adjacent to the University of the Virgin Islands MacLean Marine Science Center for logistical support of scuba diving operations (site description in Huebner and Chadwick, 2012a, b). We deployed six 50-m transect lines (three each year), with transects placed haphazardly in reef areas that appeared to contain high abundances of *A. pedersoni*. As such, the field data reported here reflect population size structure in areas of potentially maximal shrimp abundance. An area

extending 1 m to the right and left of each transect line was examined, resulting in 100 m^2 area examined per 50-m belt transect (three transects per year x 100 m^2 per transect = 300 m^2 total examined per year). For each individual of *A. pedersoni* observed inside the transects (N = 135 in 2015; N = 176 in 2016), precisely-measured total length (PMTL) was measured, and female status was recorded based on the presence of oocytes or embryos (as described above; males and juveniles could not be separated in the field). A different reef area was examined each year, and most individuals of *A. pedersoni* live only ~1 year (see Results), so the data from 2015 and 2016 were considered to be independent samples, resulting in a total sample size of 311 shrimps examined in the field.

To assess PMTL, each shrimp was collected from its host sea anemone using a small hand net and carefully transferred underwater to a ziplock plastic bag. Water was squeezed out of the bag to decrease volume and immobilize the shrimp without harming it, and a vernier caliper was used to measure PMTL through the bag. This provided greater accuracy than estimation of shrimps sizes while on their host anemones (i.e.: VETL), because their profiles were clearly visible through the clear plastic, and they were unable to swim around. Carapace length (CL) was not measured in the field, because it was too difficult to view the posterior edge of each shrimp carapace underwater. Each shrimp was separated from its anemone for < 5 min; after return to the host, all remained on their host anemones for at least the duration of the dive, as known for these shrimps following similar manipulations during previous field studies (Huebner and Chadwick, 2012a). They exhibited low stress due to this procedure as evidenced by normal swimming and almost immediate return to slow ventilation rates upon return to the host (as described above for laboratory shrimps). This procedure appeared to cause less stress to shrimps

than did the laboratory size measurements, possibly because the shrimps remained fully submerged.

To compare with the population size structure of shrimps in the field, we also assessed the population size structure of shrimps in the laboratory, from the body sizes of individuals upon their arrival to the laboratory from field collection (N = 92, see above). Thirteen of the newly-arrived individuals were excluded from this analysis because their reproductive status was not recorded upon arrival to the laboratory (N = 79 for laboratory population size structure).

Statistical analyses

Relationships among body size parameters were assessed using correlation analyses. Correlation equations were not anchored at y-intercept = 0, because they reflected body size relationships during the benthic life stage, which in crustaceans differ from those during earlier larval phases (after Gregati and Negreiros-Fransozo, 2007; Hayd and Anger, 2013; Paschoal *et al.*, 2013; Silva *et al.*, 2014).

Shrimp growth rates were determined by selecting a 1-month growth period for each laboratory-cultured individual to which we could assign gender (male or female; no juveniles), because preliminary analyses indicated that growth rate varied with gender. Some individuals thus were excluded from growth analysis because they remained juveniles (N = 6), died < 1 month after arrival to the laboratory (N = 6), had not been in the laboratory for a full month by the end of the study (N = 6), or represented apparent measurement errors (N = 3; total number removed from analysis = 21; 23.7 % of all shrimps), resulting in 71 individuals analyzed for growth rates (58 females and 13 males). Growth periods were selected to include initial sizes

which spanned the entire observed range of laboratory body sizes (2.0 – 6.7 mm CL). Shrimps were grouped into 4 size classes based on their body size at the start of the selected growth period: small (2.0 – 2.9 mm CL, N = 13 females and 4 males), medium (3.0 – 3.9 mm CL, N = 14 females and 5 males), large (4.0-4.9 mm CL, N = 15 females and 4 males), and very large (> 5 mm CL, N = 16 females; no males reached this body size). A fifth size class was added for comparative analyses of population size structure, because field populations also contained some very small individuals (< 2.0 mm CL). The smallest shrimps that arrived to the laboratory were > 2.0 mm CL, so we assumed that most individuals < 2 mm CL comprised either very small juveniles or planktonic larvae that had not yet recruited to the benthos (Strathmann, 1977).

Variation in growth rate with body size and gender was analyzed with 2-way ANOVA. Linear regressions assessed variation in growth rate with body size separately for each gender, for application to the von Bertalanffy growth function and Beverton Holt population dynamics model (after Guest, 1979; Chadwick-Furman *et al.*, 2000; see Results for details of model applications). Molt intervals were determined for 5 females and 8 males (N = 13; 4 females and 1 male died during the molt study so did not provide complete data sets), with the mean molt interval per individual calculated from N = 3 - 8 molts examined for each individual. A t-test was applied to analyze variation in molt interval with gender. Results are presented as means ± one standard error unless indicated otherwise.

Results

Body size parameters

All 4 measures of body size were highly correlated (Fig. 2.1). Visually-estimated total length (VETL) correlated with precisely measured total length (PMTL), but was somewhat smaller than VETL (correlation significantly different from $y = x$, $p < 0.001$; Fig. 2.1A). Both measures of total length increased linearly with carapace length (CL, Fig. 2.1B,C), while wet mass increased exponentially with CL (Fig. 2.1D).

Growth, sexual maturity, and molt interval

Sexually-mature individuals remained either male or female for the duration of their lifespans, with no transitional individuals (exhibiting both male and female traits) observed. Growth rate varied significantly with body size but not with gender, and there was no interaction effect (Table 2.1). Small females grew the most rapidly, medium-sized and large ones more slowly, and most of the very large females shrank slightly (Fig. 2.2). Most females attained a maximum body size of ~ 5.8 mm CL, but a few outliers (4 individuals) reached up to ~ 6.7 mm CL.

Males also grew most rapidly when small, and decreased their growth rate when they became medium-sized (Fig. 2.2). Their mean growth rates were more rapid than those of females in each of these two size classes, but the difference was not significant (t-test, $p = 0.48$ and 0.16 respectively). Upon reaching large body size, males almost completely ceased growing, and

grew significantly more slowly than did large females (t-test, $p < 0.001$). In contrast to females, males never attained the very-large size class, reaching maximum body sizes of only ~ 4.0 mm CL.

The rate of reduction in growth rate with body size did not vary significantly with gender (test of difference between 2 regression slopes, $p = 0.11$). However, males appeared to exhibit more rapid initial mean growth and a steeper decline in rate with size than did females (Fig. 2.3), and males reached significantly smaller adult body sizes than did females (t-test, $p < 0.001$). Males did not shrink near the end of their lifespans, in contrast to females (Fig. 2.3). Variation with gender in size at sexual maturity could not be assessed statistically due to small sample sizes (3 females and 1 male); most individuals arrived to the laboratory when already sexually mature, or matured during periods when they were not being observed daily, so their body sizes at maturity could not be determined precisely. However, size at maturity varied little among the examined individuals (3.3, 3.3, 3.5, and 3.4 mm CL, respectively), indicating that they reached sexual maturity at ~ 6 months of age according to the growth model (see below).

The growth constant K in the von Bertalanffy growth function was 0.16 for females and 0.32 for males (Fig. 2.3). The maximum expected CL for females ($L_\infty = 5.52$ mm; Fig. 2.4A, grey line) was similar to the maximum body size observed under laboratory conditions (5.7 mm CL, Fig. 2.4B), but was substantially larger than that observed under field conditions (only 4.4 mm CL, Fig. 2.5). The maximum expected CL for males ($L_\infty = 4.13$ mm; Fig. 2.4A, black line) also was similar to the maximal body size observed under laboratory conditions (4.1 mm CL, Fig. 2.4B), and only slightly larger than that observed in field populations (3.9 mm CL, Fig. 2.5).

Lifetime growth curves according to the von Bertalanffy growth model:

$$L_t = L_\infty (1 - e^{-Kt})$$

in which L_t = shrimp length at age t, L_∞ = maximum expected carapace length, K = growth constant, and t = shrimp age, were calculated as L_t (mm, female) = 5.52 $(1-e^{-0.16t(\text{months})})$ and L_t (mm, male) = 4.13 $(1-e^{-0.32t(\text{months})})$. Females thus reached maximal body size at ~24 months and males at ~10 months of age (Fig. 2.4A). Juveniles grew rapidly, then growth rate decreased at sexual maturity when individuals were ~ 3 mm CL and 6 months of age in both sexes (see below for details). Observed age-specific growth curves were similar to the modeled growth curves, however under laboratory conditions some individuals appeared to live longer than expected (Fig. 2.4B).

Thirteen of the tagged shrimps (8 male, 5 female) completed at least three complete molt cycles, while five shrimps died before three rounds of their molt cycles were observed (three died accidentally, apparently due to excess glue applied during the tagging process; two died naturally following a period of senescence). The molt interval was significantly longer in females than in males (t-test, t = 6.34, p < 0.001; females: 13.8 ± 0.6 days, range = 9 – 16 days, N = 5; males: 10.1 ± 0.3 days, range = 8 – 15 days, N = 8). Females molted asynchronously, in that the 5 females observed shed their exoskeletons on different dates than did other cohoused females.

Senescence and mortality

Of the 92 shrimps cultured in the laboratory, 21.7% died prematurely due to accidents, 17.4% disappeared, and 18.5% remained alive at the end of the study. The rest (42.4%) died of natural causes at the end of their apparently maximal lifespans. All of the juveniles that died, disappeared with no prior evidence of senescence (died of unknown causes).

All cases of natural death were preceded by 1-4 weeks of senescence, in which one or more senescence processes were observed, usually at least three (see Methods). The most commonly observed senescence processes involved changes in body color from mostly transparent to opaque, and changes in behavior including reduction of food consumption. Other processes were observed only occasionally because they occurred mostly in females and required frequent monitoring to detect: body shrinkage in very large females (detectable only through monthly size measurements), cessation of sexual reproduction (detectable only via daily recording of changes in brood mass size for senescing females, or continued lack of incubated embryos in females co-housed with senescing males).

Of the 75 individuals which died under laboratory conditions from all causes, lifespan varied significantly with gender (juvenile, male, female; ANOVA, $F = 7.85$, $p < 0.001$). Lifespan was significantly longer for both females and males compared to juveniles ($p < 0.01$ for both), but not for females compared to males ($p = 0.60$; Tukey multiple comparisons tests). Laboratory lifespan was ~ 1.5 years for females (19.2 ± 1.6 months, range: 5.2 - 40 months, N = 59; including 7 remarkable females who lived > 3 years), slightly < 1 year for males (11.6 ± 1.8 months, range 5.4 – 15.4 months, N = 7), and only a few months for the juveniles who died in the laboratory (4.5 ± 0.4 months, range 3.0 – 5.9, N = 9).

Population structure and mortality curves

The population size structure on coral reefs at St. Thomas was dominated by small individuals during both years examined, with the number of individuals in each size class decreasing exponentially with both body size and age (Fig. 2.5&6). The smallest individuals

detected on host sea anemones were ~ 0.96 mm CL (converted from field-measured PMTL using the equation in Fig. 2.1), and the largest were ~ 4.4 mm CL for females (carrying oocytes or embryos) and ~ 4.0 mm CL for males (large male status inferred from estimated size at maturity, see above, and the absence of oocytes or embryos, Fig. 2.6). In contrast, the population size structure of shrimps newly arrived to the laboratory (i.e., collected from reefs in Florida) was dominated by medium to large individuals (3.0 – 4.9 mm CL), and contained some very large females (5.0+ mm CL, Fig. 2.5). Most female shrimps in the field population commenced brood production at > 3 mm CL or 6 months of age, similar to those cultured in the laboratory.

During 2015, fewer small individuals were observed in the field than in 2016, but population size structure did not differ significantly between years (t-test, t = 0.71, p = 0.48). Age-frequency distributions indicated that the population was dominated by young individuals during both years (Fig. 2.6). Almost the entire population (97.0 - 98.7 %, depending on the year) was estimated to be < 10 months old, with the oldest females ~ 12 months and males ~ 8 months of age. Combined data for both years yielded a similar age frequency distribution to the data from each year separately (Fig. 2.6).

Instantaneous rates of mortality according to the Beverton Holt model:

$$N_t = N_0 \, e^{-Zt}$$

where N_t = # individuals at age zero, N_0 = # individuals remaining at age t, Z = instantaneous rate of annual mortality, t = shrimp age, and age classes below 2 months excluded as underrepresented in field samples (Chadwick-Furman *et al.*, 2000), were calculated as:

2015: $N_{t(\text{months})} = 143 \, e^{-0.355t}$

2016: $N_{t(\text{months})} = 129 \, e^{-0.252t}$

2015 and 2016 : $N_{t(\text{months})} = 150 \, e^{-0.4105t}$

The mortality slopes indicated that 50% of individuals died by ~2 months of age during both years, and 100% mortality occurred by ~12 and ~15 months of age during each year respectively (Fig. 2.7), somewhat shorter than the shrimp lifespans observed under laboratory conditions (see above). Maximum population yield in terms of mass per recruit was achieved by 4 months of age, which was near the age at sexual maturity (Table 2.2).

Discussion

General comments

Life history patterns of symbiotic crustaceans, including their reproduction and mating systems, may be predicted in part by the level of predation pressure they experience, and traits of their symbiotic hosts (Baeza and Thiel, 2007). This modelling approach predicts that crustaceans which experience low predation and associate with hosts that are abundant, relatively large, and structurally complex, will develop a mating system of pure-search polygynandry, in which they form aggregations comprised of small males and large females on each host. The biology of Pederson shrimps *Ancylomenes pedersoni* supports these predictions, in that members of this species occupy sea anemone hosts that may be abundant, relatively large (~10-14 cm maximum diameter, O'Reilly and Chadwick, 2017; vs. *A. pedersoni* ~ 4.4 mm CL maximum, present study; size ratio at least 20:1), and structurally complex (containing at least 3 types of crustacean microhabitats, similar to other large reef anemones; Khan *et al.,* 2004; Colombara *et al*., 2017). In addition, Pederson shrimps are cleaning organisms, and thus are largely immune to predation by reef fishes who visually recognize the status of cleaners and avoid consuming them (Côté,

2000). We show here that female *A. pedersoni* become significantly larger than males in both laboratory and field populations. Previous studies have demonstrated that they form mixed-size aggregations on each anemone host (Mahnken, 1972), and that individuals may participate in host-switching (Mascaró *et al.*, 2012), possibly as a form of mate searching.

We further predict that other cleaner shrimp species which occupy large symbiotic hosts will exhibit a similar life history pattern of small body size relative to the host, social aggregations comprised of small males and larger females on each host, and high levels of mobility with host switching. These predictions are supported by the aspects of life history known thus far for other cleaner shrimps symbiotic with sea anemones (Nizinski, 1989; Chadwick *et. al*, 2008) and scyphozoan medusas (Filho *et. al.,* 2008).

We also demonstrate here that Pederson shrimps have lifespans similar in duration to those of their host sea anemones (~1-2 yr, O'Reilly and Chadwick, 2017). According to our age-size model, laboratory-cultured individuals appear to live longer than those in the field, possibly due to enhanced resource availability (food, space) and reduced risks (predation, storms, etc.) in the laboratory relative to field conditions. Comparison of population size structure between the field and laboratory indicates that large brooding females may be disproportionately collected from the field, highlighting the risk of overfishing the largest reproductively-active individuals from coral reefs. The field population in Florida from which our laboratory individuals were collected also may naturally contain larger shrimps and/or more females than the field populations we observed in the Virgin Islands.

Differences in characteristics between the laboratory and field individuals examined here could be caused by other factors including genetic divergence between the source populations. However, recent genetic studies have determined that *A. pedersoni* from the Virgin Islands are

more genetically similar to those from Florida, than are some populations within Florida to each other (Titus and Daly, 2015). Laboratory shrimps also were maintained without sea anemones and without access to fish (and fish parasites for food), and they were maintained in an artificial environment with artificial food. Field studies examining growth and reproductive rates would provide interesting data for comparison with the laboratory data presented here. However, these types of field studies will be challenging to conduct because of the technical difficulties in maintaining identification tags on these small, frequently-molting shrimps, combined with their potentially high mobility and their location on coral reefs accessible only via scuba diving.

Individuals of *A. pedersoni* seem to thrive on the Caribbean reef examined here, as evidenced by the high proportion of small individuals observed at St. Thomas during both years, indicating potentially high recruitment. This apparently stable population structure is similar to patterns observed previously for anemoneshrimps in Bermuda (Nizinsky, 1989), Mexico (Colombara *et al.*, 2017), and elsewhere in the U.S. Virgin Islands (Mahnken, 1972).

All of the examined body size parameters were highly correlated, indicating that size can be assessed fairly accurately from visual estimates using calipers or rulers held near but not touching the shrimps, without removing them from their tanks (under laboratory conditions) or host sea anemones (under field conditions). Wet mass increased as a squared rather than cubed function relative to the 1-dimensional measure of body length, contrary to expectation as mass is a 3-dimensional measure of body size. This pattern may have occurred because these shrimps possess an elongated, laterally-compressed body shape (~ 2-dimensional) rather than a more spherical body shape (3-dimensional). Our results validated carapace length (CL) as a standard measure to describe body size in *A. pedersoni*, similar to the use of this measure for other shrimp species (Bauer, 1986), and showed that CL can be inferred from other measures of body size.

Photography followed by computer image analysis also has been used successfully to quantify the body size parameters of some shrimps (Thiel *et al.* 2010; Ory *et al.* 2012; Rojas *et al.* 2012). However, this method may have limited application for some species, especially those with very small translucent bodies that are difficult to visualize in photographs against laboratory and field backgrounds, such as in the present study.

Growth and reproduction

The prominent sexual dimorphism of small males and large females observed here in *A. pedersoni* is similar not only to that of other cleaner shrimps symbiotic with cnidarians (Nizinski, 1989; Chadwick *et al.,* 2008; Filho *et al*., 2008), but also other types of aggregation-forming anemoneshrimps (Baeza and Piantoni, 2010), and free-living species with pure-search mating systems, such as Sitka shrimps *Heptacarpus sitchensis* and daggerblade grass shrimps *Palaemonetes pugio* (Bauer, 2004). Female *A. pedersoni* may become larger than males due to fitness benefits associated with large female body size, given that the number of incubated embryos increases with shrimp body size (Goy, 1990; Bauer, 1991; Anger and Moreira, 1998). The largest individuals in field populations, which were sexually-active females during both years examined, are likely to contribute substantially to larval production, and may function as the most important individuals for larval recruitment and maintenance of local populations, similar to the pattern known for other reef organisms (Black *et al.*, 1991; Swearer *et al.*, 2002; Dixon *et al.*, 2017). Both the larger body size and brighter body coloration of females relative to males may cause them to attract more client fishes (or to monopolize the clients that arrive, due to the behavioral dominance of large females over smaller shrimps; J Gilpin, unpubl data), and

consume more fish parasites, allowing them to obtain more energy stores for reproduction than do smaller shrimps within each social group.

Some individuals of *A. pedersoni* appear to be highly mobile and to migrate frequently among host anemones (N Chadwick, unpubl data; also observed for *P. longicarpus* on Red Sea reefs, Chadwick *et al.*, 2008). Their high mobility may occur in part because fish clients potentially could transport them among hosts, and the hosts themselves may exhibit high turnover in the form of frequent recruitment and mortality (O'Reilly and Chadwick, 2017). As such, males may not be able to guard stable harems of females, and thus not benefit from relatively large body size. Instead, males appear to adopt a strategy of small size and high mobility, in which they move among social groups of females and fertilize them, as occurs in some other crustaceans that migrate among symbiotic hosts to locate mates (Patton *et al.*, 1985; Correa and Thiel, 2003; Thiel *et al.*, 2003; van der Meij, 2014; Baeza *et al.*, 2015). Pederson shrimps also do not exhibit fidelity to a single species of host sea anemone, and have the ability to migrate and acclimate to several types of reef anemones (Mascaró *et al.*, 2012). Pure searching is a common mating system in some other gonochoric caridean shrimps (Bauer, 2004; Baeza and Thiel, 2007). Additionally, male *A. pedersoni* do not exhibit sexually-selected traits including exaggerated weaponry or robust body shapes that would aid in competition with other males. They reach sexual maturity quickly, which allows them potentially to spend most of their lifespans fertilizing many females. As they remain small, males are able to divert energy that would otherwise be spent on growth, instead into frequent locomotion to locate new females. Finally, because females molt asynchronously every ~ 14 days, males potentially can fertilize batches of oocytes frequently, especially if they switch mates. A mating system of small mobile males and large, possibly less mobile females also appears to occur in some crabs that form

obligate symbioses with large cnidarians. Female coral pit crabs (Cryptochiridae) on massive stony corals are large and remain immobile in excavated pits, while males are small and move among the coral pits fertilizing females (Simon-Blecher and Achituv, 1997; Simon-Blecher *et al.*, 1999; van der Meij, 2014). Likewise, female coral gall crabs (also Cryptochiridae) are large and become imprisoned in galls that branching stony corals form around them, while males are highly mobile and visit numerous corals to fertilize the resident females (Kotb and Hartnoll, 2002; Johnsson *et al.*, 2006; Vehof *et al.*, 2016). Quantification of the rates of gender-specific Pederson shrimp mobility among sea anemones in the field would add valuable information about their mating system.

Maximal body size for *A. pedersoni* is similar to that of the closely-related species *Periclimenes anthophilus* (CL = 1.4 – 4.3 mm; Nizinski, 1989) which also lives on sea anemones and is endemic to Bermuda (Titus *et al.*, 2017). In contrast, *A. pedersoni* individuals become only half as large as those of free-living Caribbean shrimps *Lysmata wurdemanni* which also form aggregations and may clean reef fishes (CL = 3.5 - 9.8 mm; Nizinski, 1989; Bauer and Holt, 1998). The relatively small body size of *A. pedersoni* and other shrimps which form obligate associations with sea anemones may relate in part to microhabitat space constraints on their hosts (Colombara *et al.*, 2017). We were not able to quantify sex ratio in the field because we did not collect and microscopically examine individuals. However, because individuals of this species reach sexual maturity at ~3.5 mm CL, and the majority of individuals above this size were females in the field population, the sex ratio appears to be biased toward females, as known for other shrimps that form aggregations on sea anemones (Nizinski, 1989; Baeza and Piantoni, 2010).

Lifespan and senescence

The life span of *A. pedersoni* (< 2 years) and attainment of sexual maturity within ~ 6 months after fertilization are similar to patterns in other small carideans, such as the subtropical and temperate freshwater shrimp *Palaemonetes paludosus* which reaches sexual maturity at 2-3 months and lives for only 1 year (Beck and Cowell, 1976). The protandrous hermaphroditic marine shrimp *Rhynchocinetes uritai* also occurs on tropical coral reefs and is similar to these gonochorisitic species, in that small males transform into larger females before death at ~ 3 years of age (Bauer and Thiel, 2011). The lifespans of shrimps in the tropics are much shorter than at higher latitudes, where shrimps can live well over 2 years and have strong seasonal constraints on reproduction (Bauer, 1992; Costa-Souza *et al.* 2017).

Our observations of senescence in relatively large, old individuals, including body shrinkage, behavioral changes, and reproductive cessation, constitute the first description of these processes in caridean shrimps. Limnadiidid clam shrimps *Eulimnadia texana* also senesce (Weeks *et al.*, 1997), but all other carideans examined thus far, such as sea grass shrimps *Latreutes pymoeus* and Monaco shrimps *Lysmata seticaudata*, appear to die without exhibiting senescence beforehand (Calado and Narciso, 2003; Penha-Lopes *et al.*, 2007). Senescence in *A. pedersoni* is similar to that in *E. texana*, in that sexual reproduction slows or ceases during the final weeks of life. However, individuals of *E. texana* are even shorter-lived, reaching sexual maturity in only 4-7 days, then commencing senescence after 2 weeks of age, and dying at only < 4 weeks old (Weeks *et al.*, 1997). In contrast, senescence extends much longer in *A. pedersoni* in that it extends ~ 2-4 weeks near the end of a 1.5 - 2 year lifespan.

We did not determine whether body shrinkage during senescence always corresponded with a final molt, because we did not tag a large number of individuals for molt cycle analysis due to the technical difficulty of the tagging process. However, the 2 tagged individuals that exhibited senescence (see Results) both shrank slightly (~ 0.1 mm CL) after the final molt, and died 1 day later. Body shrinkage likewise did not universally follow the production of a final brood, as some females continued to reproduce during body shrinkage. Shrinkage would make the most adaptive sense if females mobilized their final reserves (resulting in body shrinkage) for a terminal reproductive event. More extensive studies on patterns of reproduction during senescence and body shrinkage are needed to clarify relationships among these processes.

The occurrence of senescence allows prediction of the imminent death of individuals, which could be a useful characteristic for aquaculture. The removal of clearly senescing individuals from culture tanks would avoid tank fouling due to subsequent corpse decomposition, especially for large crustaceans, and allow autopsy information to be obtained immediately following death.

Molt interval

Molt intervals in *A. pedersoni* are similar to those of other tropical carideans such as green snapping shrimps *Alpheus normanni* (molt interval range 9 -12 days; mean 10 days) and Manning grass shrimps *Thor manningi* (range 6 -10 days; mean 8 days; Bauer, 2004), both of which are similar in adult body size to *A. pedersoni* (4.4 – 7.8 mm and 0.8 – 2.1 mm CL, respectively; Bauer, 1986; Kim and Abele, 1988). Molt interval increases with age and body size in crustaceans, and can be much more lengthy for species with large body size or those occurring

in cold water habitats where growth rate is retarded (Bauer, 2004). All female carideans brood embryos (Burkenroad, 1947) and do not commence each molt cycle until the embryos hatch, so their molt interval is constrained by the duration of their embryo maturation. Similar to body growth rates, the maturation rates of brooded embryos range widely among carideans, from one to a few weeks in tropical hippolytid shrimps *Hippolyte curacaoensis* (Hart, 1980; Bauer, 1989, 1991, 1992) to several months in high-latitude shrimps such as northern prawns *Pandalus borealis* (Bergström, 2000).

The tagging process used here to follow molt cycles appeared to damage some shrimps, as 16.7% of tagged individuals (N = 18; see Results) died following the accidental application of excess glue to the very small carapace area. This mortality reflects the technical challenge of tagging these delicate organisms. However, this type of tag application remains a viable albeit time-consuming method to identify individuals and track their molt cycles, at least under laboratory conditions, as most individuals survived multiple rounds of tagging.

Coral reef management applications and conclusions

The life history and population patterns presented here for cleaner shrimps provide a foundation for scientifically-based recommendations to support their conservation management. This application of life history information is especially important because increasing demand for marine ornamental organisms threatens many coral reef species with overfishing and local extinction (LeGore *et al.*, 2005; Rhyne *et al.*, 2009, 2014). Collection restrictions based on shrimp body size can be effective in maintaining sustainable fisheries, because shrimp in certain size classes are reproductively active and important for stable populations. Based on our results,

we recommend that individuals of *A. pedersoni* which are < 3.5 mm CL (= 18.3 mm TL; not reproductively mature) be protected from collection so that they can grow to adult size and eventually produce larvae to contribute to population replenishment (Wood, 2001; Calado, 2006). We also recommend that very large individuals (> 5.0 mm CL or 24.1 mm TL) not be collected because they likely are important suppliers of larvae for benthic recruitment. This recommended ban on the collection of the largest individuals is similar to that applied to many other reef organisms including major fishes, in which the largest individuals produce the majority of larval supply (reviewed in Birkeland, 2015). This collection restriction would end the destructive practice of apparently collecting mainly large females, as evidenced by the population structure of newly-collected individuals reported here. We accordingly suggest a slot limit to allow the collection of limited numbers of medium-sized individuals of *A. pedersoni* (after Birkeland, 2015). Based on our laboratory methods, and the rapid growth and frequent reproduction of the individuals cultured here, we suggest that the juvenile to adult stages may be cultured easily with minimal infrastructure. In contrast, culturing of the larval stages is likely to be the greatest challenge to commercial production of cultured Pederson shrimps to supply the ornamental trade.

We conclude that Pederson cleaner shrimps exhibit life history characteristics similar to those of other tropical caridean shrimps, in that they grow rapidly and mature quickly, reach small adult body size, and have short lifespan. They form large stable populations at some reef sites, and exhibit the first-known senescence for caridean shrimps. Their reproductive patterns support the predictions of conceptual modeling based on the effects of ecological factors such as predation pressure and symbiotic host traits. They occupy abundant, relatively large, and structurally-complex hosts and experience low predation pressure due to their cleaner status; this

ecological framework may cause them to form mixed-gender aggregations of small males and larger females on each host and engage in host-switching, as part of a mating system of pure-search polygynandry. This life history pattern appears similar to that of other cleaner shrimps symbiotic with large hosts. However, further research is needed to elucidate the life history of symbiotic cleaner shrimps to allow more robust comparisons. The information presented here adds to the growing body of literature concerning the life history of caridean shrimps, as a basis for understanding the ecology and evolution of these key marine invertebrates.

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Table 2.1. Two-way ANOVA results for variation in growth rate with body size and gender in Pederson cleaner shrimps *Ancylomenes pedersoni* under laboratory conditions.

	DF	SS	MS	F	p
Body size	3	2.125	0.708	25.201	< 0.001
Gender	1	0.034	0.034	1.216	0.274
Body size * Gender	2	0.084	0.084	2.974	0.058

Table 2.2. Life table for Pederson cleaner shrimps *Ancylomenes pedersoni*. Bolded month indicates the age at maximum population yield in terms of mass per recruit, based on field population data from 2015 and 2016. Age at sexual maturity was estimated for both males and females at ~ 6 months. See text for details.

Age (months)	Carapace Length (mm)	Wet Mass (g)	Survival	Yield (g/rec)
0	0	0	1.00	0
1	0.79	0.002	0.66	0.0016
2	1.46	0.009	0.44	0.0039
3	2.04	0.018	0.29	0.0051
4	**2.54**	**0.028**	**0.19**	**0.0053**
5	2.96	0.039	0.13	0.0050
6	3.32	0.049	0.085	0.0042
7	3.64	0.059	0.057	0.0034
8	3.91	0.069	0.037	0.0025
9	4.14	0.077	0.025	0.0019
10	4.33	0.085	0.016	0.0014
11	4.50	0.092	0.011	0.0010
12	4.65	0.098	0.0073	0.00072
13	4.77	0.104	0.0048	0.00050
14	4.88	0.109	0.0032	0.00035
15	4.97	0.113	0.0021	0.00024
16	5.04	0.117	0.0014	0.00016
17	5.11	0.120	0.0009	0.00011
18	5.17	0.123	0.0006	0.00008
19	5.22	0.125	0.0004	0.00005
20	5.26	0.127	0.0003	0.00003

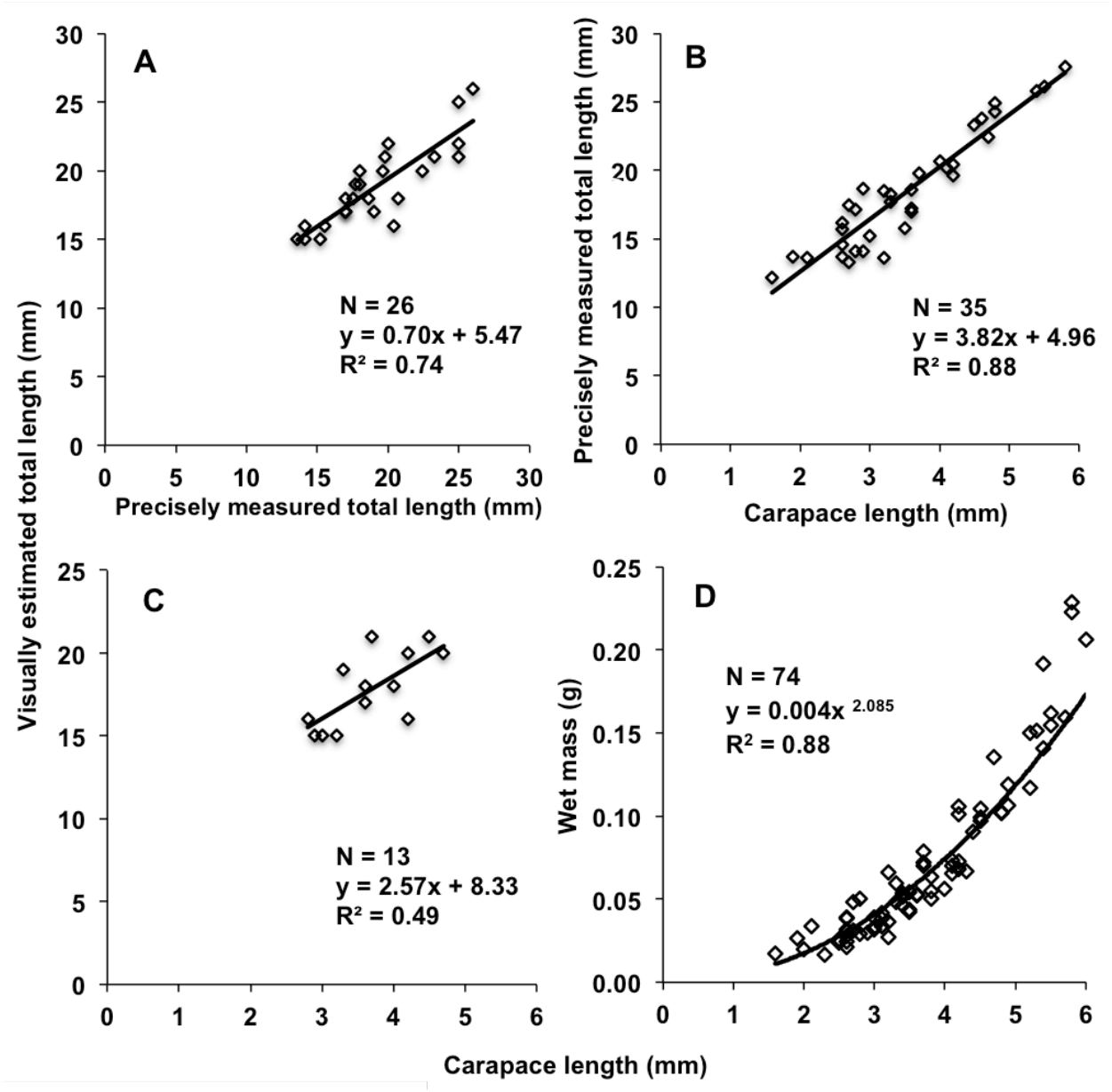

Figure 2.1. Relationships between body size parameters of Pederson shrimps *Ancylomenes pedersoni*: **(A)** Precisely measured total length (PMTL) vs. visually estimated total length (VETL). Note that VETL somewhat under-estimated precise body length, especially for large individuals; **(B)** PMTL vs. carapace length (CL); **(C)** VETL vs. CL; **(D)** Wet mass (WM) vs. CL. Note the tight correlations among all body size measures examined. See text for details.

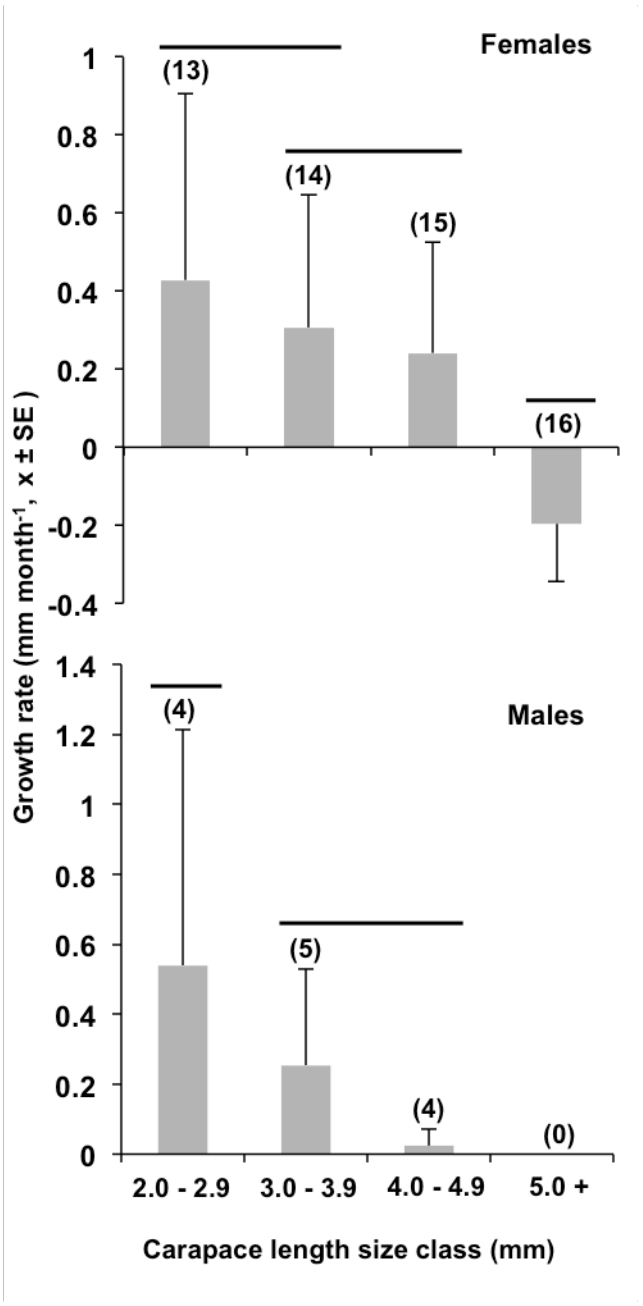

Figure 2.2. Variation in growth rate among size classes of Pederson shrimps *Ancylomenes pedersoni* for **(A)** females and **(B)** males. Size classes within each gender that did not differ significantly in growth rate are joined by a bar. Sample sizes are shown in parentheses.

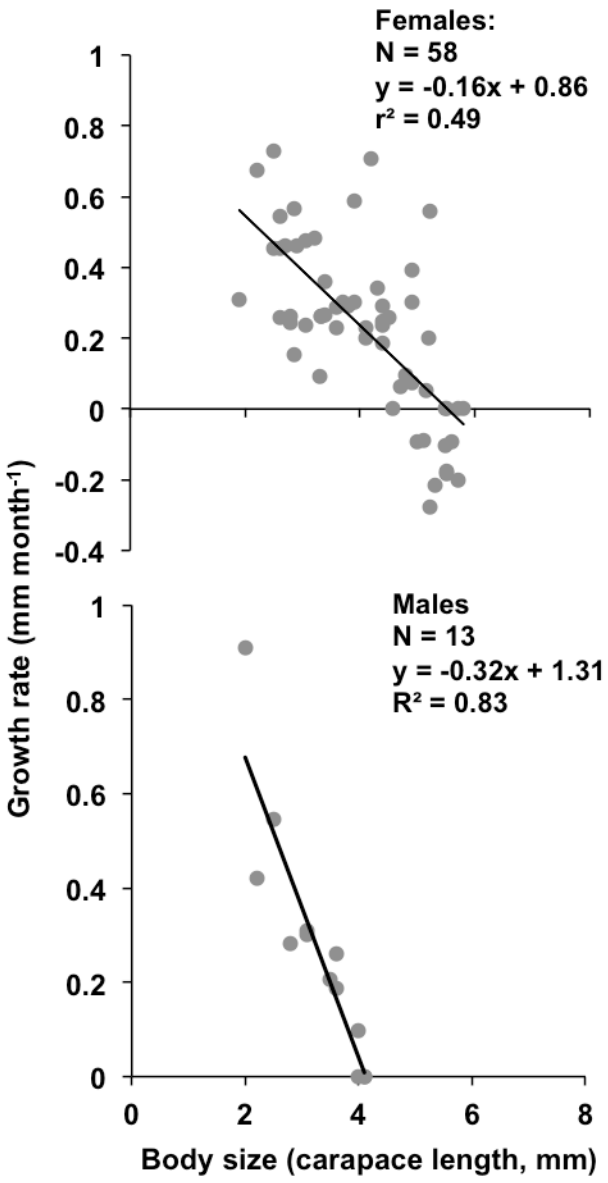

Figure 2.3. Variation in growth rate with body size for Pederson shrimps *Ancylomenes pedersoni*, **(A)** females and **(B)** males. Note that males appeared to decrease their growth rate more rapidly with body size than did females, but that after reaching large size (> 5.3 mm carapace length), only females shrank prior to death.

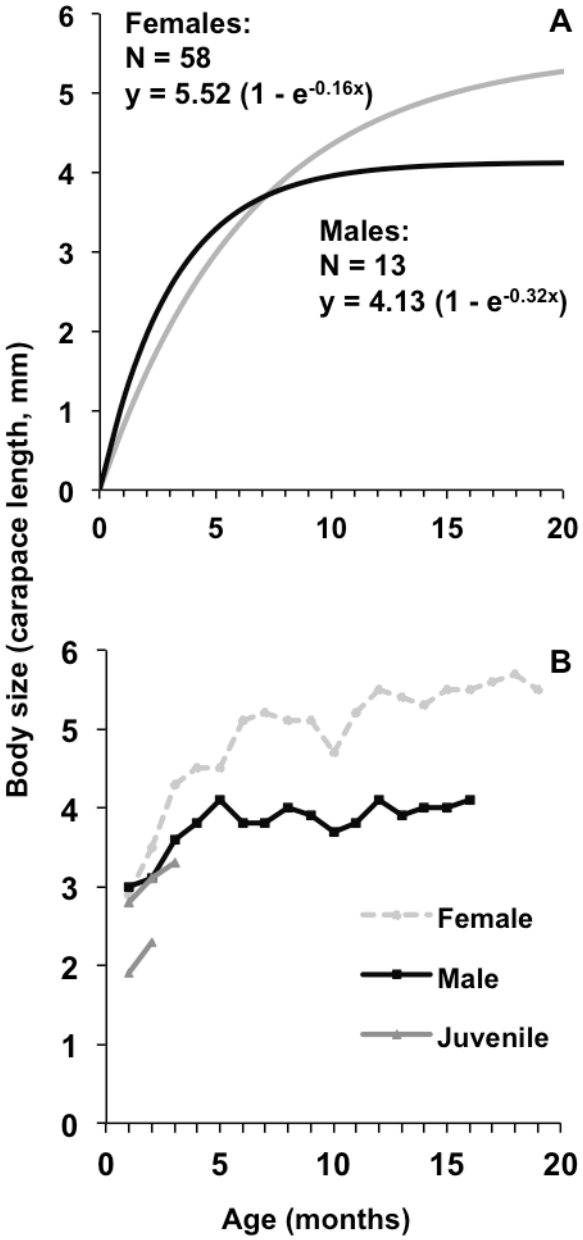

Figure 2.4. Growth patterns of Pederson shrimps *Ancylomenes pedersoni* under laboratory conditions: **(A)** Age-specific growth curves for females and males. **(B)** Growth trajectories and laboratory lifespans of representative individuals: two juveniles (dark grey lines) who increased rapidly in body size and then died of accidental causes before reaching maturity; one male (black line); one female (light gray line). Both adults died of natural causes following senescence; note the female shrinkage prior to death. Note that both were already ~ 5 months old (3 mm carapace length) upon arrival to the laboratory based on Fig. 3, and that their growth patterns followed a similar trajectory to the growth curves shown in A.

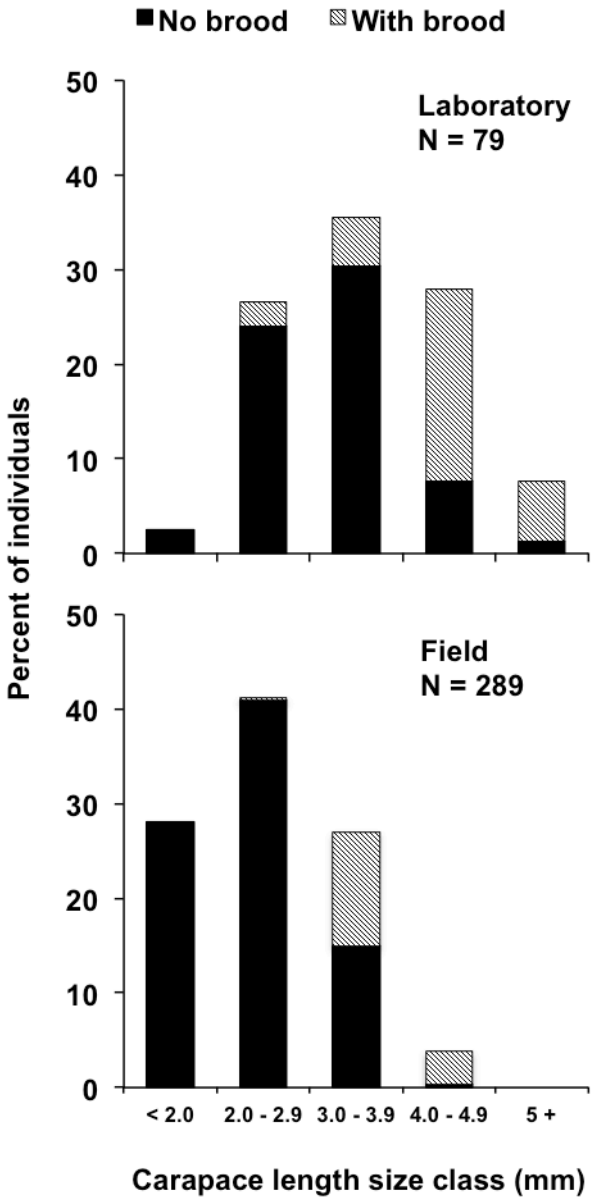

Figure 2.5. Variation in the population size structure of Pederson shrimps *Ancylomenes pedersoni* under laboratory versus field conditions. Laboratory data represent the body sizes of individuals upon arrival to Auburn University after field collection in the Florida Keys (N = 79; 13 laboratory individuals excluded from analysis because their reproductive activity was not recorded upon arrival). Field data represent the body sizes of individuals in populations on coral reefs at St. Thomas, U.S. Virgin Islands, from surveys conducted during 2015 and 2016 (both years combined). Individuals are grouped into four size classes based on carapace length (CL), similar to Fig. 3. The percent of individuals in each size class and the percent carrying brood masses (either oocytes or embryos) are shown. Note that few individuals < 3 mm CL carried broods. See text for details.

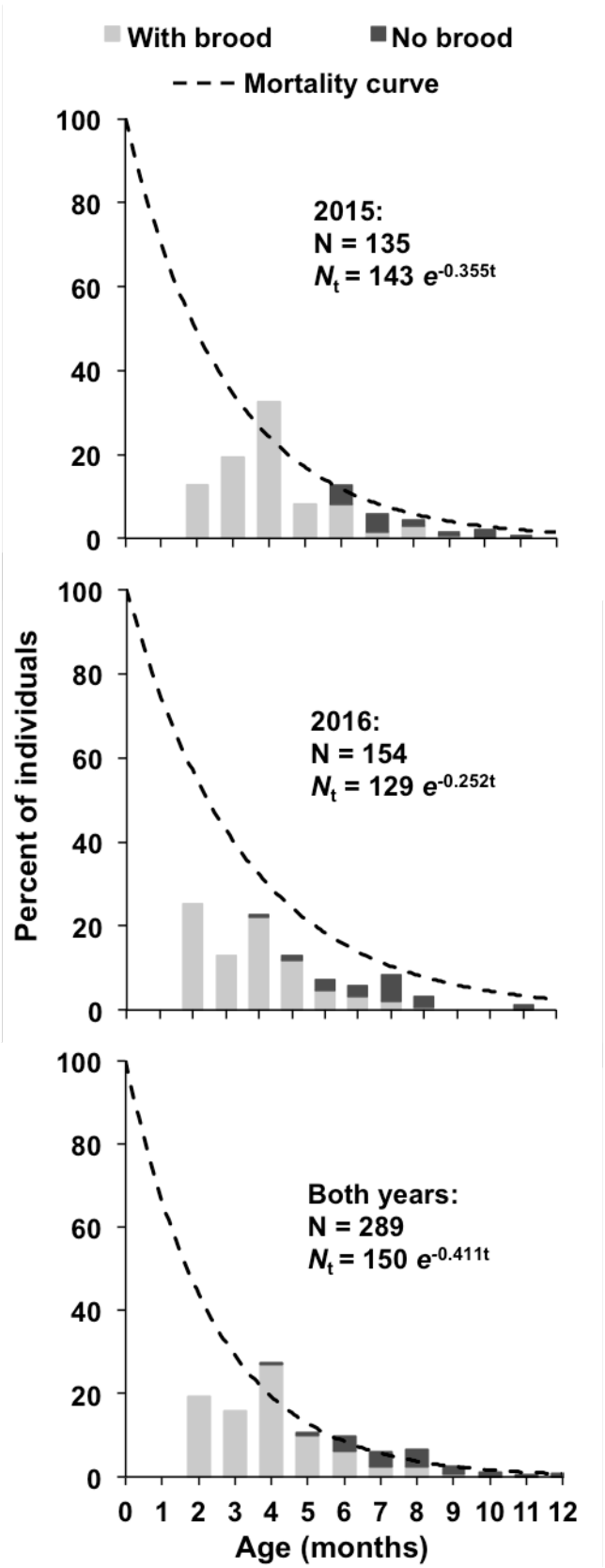
With brood
No brood
Mortality curve
2015:
N = 135
$N_t = 143\ e^{-0.355t}$
2016:
N = 154
$N_t = 129\ e^{-0.252t}$
Both years:
N = 289
$N_t = 150\ e^{-0.411t}$
Percent of individuals
Age (months)
0 1 2 3 4 5 6 7 8 9 10 11 12
0 20 40 60 80 100

Figure 2.6. Population age structure and mortality curves of Pederson shrimps *Ancylomenes pedersoni* on coral reefs at St. Thomas, U.S. Virgin Islands. A. 2015. B. 2016. C. Both years combined. Note the dearth of individuals < 2 months old, probably because they were still in the planktonic larval phase (< 1 mm carapace length, Figs. 3 and 5). The age at female sexual maturity (brood mass production) corresponded to ~ 6 months (3.3 mm body size), similar to the age and size at maturity in the laboratory population, when growth also began to level off at the beginning of the adult phase (Fig. 4A).

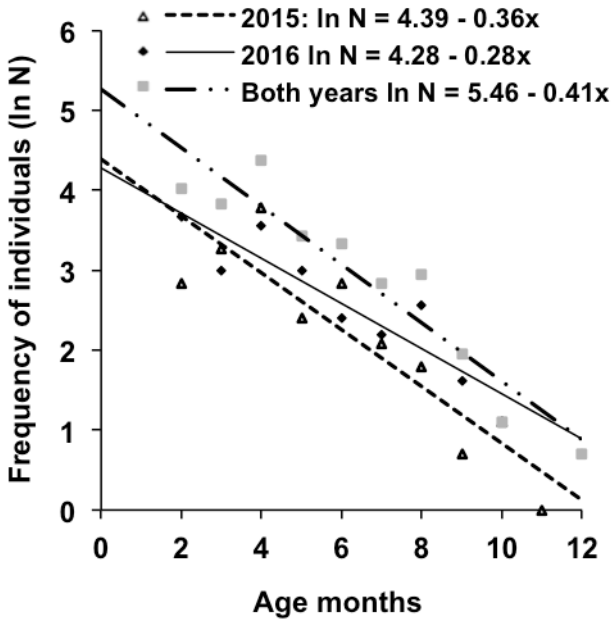

Figure 2.7. Mortality rates of Pederson shrimps *Ancylomenes pedersoni* on coral reefs at St. Thomas, U.S. Virgin Islands, estimated from mortality curves in 2015 (dashed line; white triangles), 2016 (solid line; black diamonds), and both years combined (dot-dash line; grey squares). Note that few individuals survived > 1 year under field conditions (Fig. 6), in contrast to lifespans potentially almost twice as long under laboratory conditions (Figs. 4 and 5).

Chapter 3

Social structure of Pederson cleaner shrimps *Ancylomenes pedersoni*

Abstract

Pederson cleaner shrimps *Ancylomenes pedersoni* form obligate associations with large sea anemones on Caribbean coral reefs and provide important cleaning services to reef fishes, but major aspects of their biology remain unknown. They usually occur in social groups of several different-sized individuals per host anemone; a size-dependent social hierarchy has been proposed to structure these groups, but no quantitative data exist to support this idea. I examined characteristics of Pederson shrimp social groups under both field and laboratory conditions, and determined how rates and types of social dominance behaviors varied among group members, in terms of: (1) exploitation competition (signaling toward and approaching food sources in the form of fish clients), and (2) interference competition (direct aggression in the form chases and attacks toward conspecifics). Based on these data, I constructed dominance hierarchies for this species, and modeled the types of environmental factors that impact social group dynamics. On coral reefs at St. Thomas, USVI, shrimps occurred in social groups of 1 to 9 individuals (3.5 + 0.20 individuals per group, x+SE). The body size ratio between adjacent individuals ranged 0.02 to 2.87 and did not differ significantly from a random distribution, indicating that some social groups contained individuals that were very similar in body size. Shrimp distance from host

anemone tentacles decreased significantly with body size; large individuals occupied habitat on the tentacle crown, while smaller group members perched on surrounding substrate up to 8.5 cm from the host. Large individuals > 4.0 mm CL usually (90% of individuals) were gravid females, with smaller individuals (3.0 to 4.0 mm CL) being males, and the smallest individuals (0.77 to 3.0 mm CL) juveniles. Many social groups (48%, N = 68) consisted of at least 1 gravid female and some mix of males and juveniles, while the remainder contained only females or only non-egg-bearing shrimps. In experimental social groups of 5 shrimps per laboratory tank, individuals exhibited a size-based hierarchy in terms of most behavioral interactions. The largest (alpha) female in each group was dominant behaviorally over the next-largest (beta) female, and they in turn dominated smaller females (gamma, delta, etc.), males and juveniles. Large shrimps also tended to interact most frequently with client fish models, however some medium and small individuals also signaled toward and attempted to clean the models. When individuals were moved among groups, their dominance positions changed according to their relative body sizes in the newly-formed groups. I conclude that Pederson shrimps form structured social groups that do not exhibit strict size structure on each host sea anemone. However, under laboratory conditions they display size-structured behavioral dominance hierarchies in which large individuals exclude smaller ones from access to resources (food and habitat). These behavioral interactions explain their observed microhabitat use patterns on the symbiotic host, and may allow large individuals to monopolize food resources in the form of client fish ectoparasites.

Introduction

Corkscrew sea anemones *Bartholomea annulata* are common, large-bodied Caribbean

cnidarians that host a variety of associated organisms on, within, and beneath their tentacles (Mahnken, 1972). One species of ectosymbionts found on and within the tentacles of corkscrew anemones is the Pederson cleaner shrimp *Ancylomenes* (*Periclimenes*) *pedersoni.* Pederson shrimps occupy a variety of Caribbean sea anemone host species, including rosetip sea anemones *Condylactis gigantia*, branching sea anemones *Lebrunia danae*, and sun sea anemones *Stichodactla helianthus*. Pederson shrimps are reported to perch on the host oral discs and tentacles of corkscrew anemones (Mahnken, 1972). They are also known to occupy the reef crevices where these anemones attach their pedal disks (Limbaugh *et al.*, 1961). These large cnidarian hosts serve as visual cues that attract reef fishes which then engage in cleaning interactions with the Pederson shrimps (Huebner and Chadwick, 2012a). On branching anemones *L. danae*, the Pederson shrimps typically perch on reef substrate within a few cm of the diurnally-expanded fronds of these anemones, or rest atop the fronds (Herrnkind *et al.*, 1976). The shrimp-anemone complex forms a cleaning station, where shrimps consume parasites and decayed tissue from the body surfaces of fishes that visit the anemone (Bunkley-Williams and Williams, 1998; Côté, 2000). Pederson shrimps tend to aggregate in social groups ranging from 1-10 shrimps per host anemone (Mahnken, 1972), with individuals in each social group varying in body size (Mahnken, 1972; Mascaró *et al.*, 2012). Some researchers have speculated that the size of Pederson shrimps social groups depend on habitat space availability on the anemone hosts, as well as on the abundance of the local shrimp population; space limitation (host anemone abundance and body size) is thus thought to be more important in this species than in other non-group-forming shrimps associated with Caribbean anemones (Mahnken, 1972; Nizinski, 1989). A size-dependent social hierarchy has been proposed to structure the Pederson shrimps social group that occupies each anemone (Mahnken, 1972), but no quantitative data exist to support this

idea.

Many studies have focused on the functions of cleaning stations in coral reef ecosystems, including the role of cleaner organisms in enhancing reef fish health (Bunkley-Williams and Williams, 1998; Bshary, 2003; Becker and Grutter, 2004, 2005; Bshary *et al.*, 2007) and several experimental demonstrations have explored how fishes use cleaning stations (Chapuis and Bshary, 2009; Huebner, 2010; Huebner and Chadwick, 2012b). However, analyses of behavioral interactions with cnidarian hosts, and of the life histories of cleaning organisms are lacking, outside of host preference studies (Guo *et al.*, 1996; Silbiger and Childress, 2008; Mascaró *et al.*, 2012), acclimation studies (Levine and Blanchard Jr, 1980) and one population biology study of non-cleaner shrimps *Pereclimenes anthophilus* which are closely related to Pederson shrimps (Nizinski, 1989). The only life history study on Pederson shrimps indicates that this species has separate sexes in which females are larger than males, and that individuals live for ~1 year under laboratory conditions (Gilpin and Chadwick, in press). The structure of population size-classes of this species on coral reefs is dominated by small individuals, with the number of individuals in each size class decreasing exponentially with both body size and age (Gilpin and Chadwick, in press). No quantitative studies have examined the social group structure of Pederson cleaner shrimps, and the possibility of dominance hierarchies within these groups. The available literature on these shrimps mentions general group sizes (Mahnken, 1972), but no study has measured shrimp sizes and their precise locations of microhabitat use on or near host anemones.

Interference competition may occur among members of social groups of this species (Mascaró *et al.*, 2012), however studies examining aggressive interactions among Pederson shrimps are lacking. Winston and Jacobson (1978) hypothesized that responses to the "aggressive state" of other group members may be more important than individual recognition in

invertebrate social hierarchies. Among arthropods, various studies have quantified aggressive behaviors and how they structure social dominance hierarchies, for example in crickets (Alexander, 1961), hermit crabs (Courchesne and Barlow, 1971) and crayfish (Bovbjerg, 1953; Rubenstein and Hazlett, 1974; Copp, 1986; Issa *et al.*, 1999). These studies have shown clear formation of dominance hierarchies within arthropod social groups, based on results of encounters between individuals, with large males usually taking the dominant role.

Body size is a major determining factor in the outcome of conflicts among conspecific organisms (Bovbjerg, 1953; Ranta and Lindström, 1992; Pavey and Fielder, 1996). Relatively large individuals of Pederson shrimps in Florida, USA are collected for the aquarium trade (Gilpin and Chadwick, in press), probably because they both occupy more prominent positions on tips of anemone tentacles and closer to anemones compared to smaller shrimps within each social group (J. Gilpin, per obs). In addition, large body sizes makes detection easier compared to small individuals. The prominent habitats occupied by large individuals could result from their successful competition for space in the form of better positions on the host, to access client fishes and thus obtain food in the form of parasites and decayed tissue (Mascaró *et al.*, 2012). Thus, both the body size and microhabitat use (prominent position on the host) of large versus small Pederson shrimps could indicate a structured social hierarchy within the social group that occupies each anemone host. Additional information, including the level of behavioral aggressiveness of each individual (willingness to fight with other shrimps, a form of interference competition), also would contribute to understanding whether dominance hierarchies exist in this species. It is possible also that the large shrimps in each social group exhibit exploitation competition in the form of signaling to and approaching fish clients more frequently than do small shrimps, which would allow them to monopolize food resources (in the form of fish

ectoparasites) before small individuals can access them.

We tested the hypothesis that *A. pedersoni* form dominance hierarchies based on size, sex and/or behavioral interactions. We tested this hypothesis by examining characteristics of social groups under both field and laboratory conditions, and determined relative rates and types of both cleaning-related behaviors (signals and approaches toward fish clients) and aggressive behaviors (approaches and initiating contact toward conspecifics) within Pederson shrimps social groups. The results of this study allowed us to construct dominance hierarchies, and to model the environmental factors that may influence these hierarchies.

Methods

Field observations

Field observations were conducted during July 2015 and August 2016, on coral reefs in Brewers Bay, St. Thomas, U.S. Virgin Islands, at ~ 6 m depth (18°19' N, 64°59' W; detailed site descriptions in (Gilpin and Chadwick, in press; Huebner and Chadwick, 2012a; b). I deployed six 50-m transect lines (three each year) haphazardly in reef areas that contained high abundances of corkscrew sea anemones and associated individuals of Pederson shrimps. The data reported here represent patterns in areas of potentially maximal abundance for this shrimp species in this bay. An area extending 1 m to the right and left of each transect line was examined, resulting in 100 m^2 area examined per 50-m belt transect.

For each corkscrew anemone observed inside the transects (2015: N = 109; 40 with Pederson shrimps; 2016: N = 140; 76 with Pederson shrimps), I approached close enough to

detect associated Pederson shrimps (2015: N = 135 shrimps; 2016: N = 154), but took care to not touch or otherwise disturb the shrimps or anemones. I recorded the number of shrimps on each anemone host, and type of microhabitat that each occupied on the host body. Host bodies were classified into 5 zones (modified after Khan *et al.*, 2004): 1) anemone column, 2) inner tentacle crown, 3) outer tentacle crown, 4) hard substrate adjacent to anemone, 5) soft substrate adjacent to anemone (Fig. 3.1). For shrimps that occurred on substrate adjacent to the anemone, I also recorded distance to the nearest anemone tentacle tip, using a Vernier caliper measured to the nearest millimeter. The body size of the anemone also was determined using a Vernier caliper, taking care to not touch the anemone or to cause tentacle contraction. Tentacle crown length and width were measured in mm for calculation of tentacle crown surface area (TCSA, after (Hirose, 1985; Hattori, 2002; Huebner *et al.*, 2012; O'Reilly and Chadwick, 2017). I then carefully collected each of the shrimps from the host anemone using a small hand net, and transferred them underwater to a ziplock plastic bag, which allowed for close examination of the shrimps through the clear plastic. I used a caliper to measure shrimp body size as precisely measured total length (PMTL; Gilpin and Chadwick, in press), and recorded the presence of any oocytes or developing embryos to indicate female status. Photographic analysis of body size was not used, because these shrimps are too small and translucent to easily visualize them in photographs taken in the field against reef and sand backgrounds.

A different area was examined on reefs in the bay during each year. Most corkscrew anemones (O'Reilly and Chadwick, 2017) and Pederson shrimps live only ~1 year (Gilpin and Chadwick, in press), so the data from 2015 and 2016 were considered to be independent samples, resulting in total sample sizes of 116 anemones and 289 shrimps examined (see Gilpin and Chadwick, in press for details of field sampling methods).

Laboratory experiments

Laboratory experiments were conducted at Auburn University during August 2016 – May 2017. The shrimps observed had been cultured as part of a previous laboratory study on life history patterns (details of animal collection and culture in Gilpin and Chadwick, in press). For the present study, shrimps were cultured in small social groups of 4-5 individuals in per tank, in 6 tanks (40 L each; 27 shrimps total). Laboratory social groups were set up to mimic typical social group structure in the field; the social group size (4-5 shrimps per group) was within the range of natural social group sizes observed at the field site (range = 1 to 9 shrimps), but slightly larger on average (mean group size in the field = 3.5 individuals; median = 3.0; not including solitary individuals). This relatively large group size was used because preliminary observations indicated that social groups of < 4 individuals per tank did not evoke many aggressive interactions (J. Gilpin pers. obs.). This social group size also included enough individuals to allow the formation of clear dominance hierarchies, but few enough so individuals could be identified in tanks based on their relative body sizes. Each group included 1-4 females, 1 male, and 0-2 juveniles, all at least 0.2 mm different in carapace length between adjacent group members (CL; similar to minimum difference in body size between adjacent group members in the field: mean = 0.58 mm CL, + 0.53 mm CL standard deviation, see below for details). I chose a difference of 0.2 mm to maximize closeness in size between individuals in order to increase the likelihood of dominance interactions. The gender compositions chosen were based on personal observations that social groups including males led to more interactions between non-male individuals and thus increasing the likelihood of behavioral interactions.

Each culture tank was lit by standard ceiling lights in the culture room and contained an

external hanging filter driven by a small pump (Aqua-Tech Power Filter 5-15, Spectrum Brands). High-output lights hung over each tank were not used here, in contrast to previous culture methods for anemones and shrimps in the laboratory, because photosynthetically active radiation (PAR) did not have to be high to support sea anemone photosynthesis (reviewed in Cantrell et al. 2015). In each tank, a red clay pot was placed in the middle to simulate three-dimensional reef structure and to provide an anemone-sized shelter space. Individuals were fed every 2 days with fish food pellets (Formula One and Formula Two Pellets, Ocean Nutrition, Newark, CA); they grew steadily and sexually reproduced regularly under these culture conditions, as reported previously (Gilpin and Chadwick, in press), indicating healthy condition.

Social groups were established in August 2016 and allowed to acclimate for two weeks, then initial behavioral observations were conducted during August to November 2016 (N = 6 tanks of 1 social group per tank, each consisting of 4-5 individuals per group = 27 shrimps total). The tanks contained social groups structured as either: (1) 5 individuals each (N = 3 tanks of 15 shrimps total); 4 females [alpha, beta, gamma, and delta, ranging from large to small in body size] and 1 male; or (2) 4 individuals each (N = 3 tanks of 12 shrimps total); either 3 females [alpha, beta, and gamma, ranging from large to small in body size] and 1 male (1 tank), 2 females [alpha and beta ranging from large to small in body size], 1 male and 1 juvenile (1 tank); or 1 female, 1 male and 2 juveniles (1 tank).

Shrimp mortality during August – December 2016, due mostly to natural causes (senescence followed by end of natural lifespan, see Gilpin and Chadwick, in press) caused the number of individuals to decrease to only 14 by December. Then in January 2017, 10 individuals were moved among tanks to create 2 new social groups (N = 2 tanks of 1 social group per tank, each consisting of 5 individuals per group = 10 shrimps total). Tanks observed during the second

study thus contained 2 social groups of 5 individuals each: 4 females (alpha, beta, gamma, and delta ranging from large to small in body size) and 1 male. The remaining 4 individuals were placed in a third tank and not included in the experiment.

All shrimps observed during the second 5-month study (January – April 2017) comprised a subset of those observed during the initial 5-month study (August – December 2016). Individuals were moved among the tanks in January to create new social groups each comprising of 5 shrimps that differed in adjacent body sizes, as well as similar in social group size, gender composition, and relative body sizes within each group, as in the initial study.

Three shrimps in the second study remained in the same type of social group and the same relative size position within that group, as they had during the initial study. The remaining 7 shrimps were placed into different relative body size positions in the newly-created groups for the second study.

In order to create groups of varying sized individuals, all shrimps were measured for carapace length using a vernier caliper, assigned to a body size class [Large: > 5.0 mm, Medium: 4.2 – 4.9 mm; Small: < 4.2 mm], and sexed. The groups were then formed by randomly selecting Large, Medium, and Small females from the pool of individuals, until three groups each with a clear size hierarchy of Alpha, Beta, Gamma, and Delta females and one male (3.3 to 4.0 mm CL) were formed in August 2016. Individuals not used in these three groups then were placed in the three remaining tanks in August 2016. In January 2017, the two groups were created using the random choice method described above, with the remaining shrimps cultured in a third tank not observed for social behaviors.

After the creation of social groups, I made behavioral observations on each group to assess social structure. Nine types of behaviors were characterized for shrimps interactions with

each other and with client fish models, ordered from the most subordinate to most dominant behaviors, in terms of conspecific interactions: (1) retreat from conspecific (locomote away), (2) no response to conspecific (no change in position), (3) approach conspecific (chase; locomote toward), and (4) initiate contact with conspecific (usually with chelae; attempt to pinch); and client fish interactions: (1) retreat from client, (2) no response to client, (3) signal to client (body rocking and/or antenna vibration, after (Becker *et al.*, 2005; Chapuis and Bshary, 2010), (4) approach client (locomote toward), (5) clean client (jump onto fish model and climb over model surface). These dominance ratings of behaviors were selected because previous studies categorized increasing levels of approach toward, raising appendages toward, and using appendages to pinch conspecifics, as expressions of relative social dominance among crustaceans (Bovbjerg, 1956; Issa *et al.*, 1999). Additionally, increasing levels of engagement with client fishes (approach, signal, clean) have been described as expressions of relative dominance in social groups of other cleaner organisms (Mascaró *et al.*, 2012).

To examine shrimp responses to client fish as a component of their dominance in social groups, I created a visual model of a fish client indicative of a small adult (13.5 cm). I obtained from the internet a high resolution photograph of a common Caribbean fish client (Blue Tang *Acanthurus coeruleus*, Huebner and Chadwick 2012a), and printed and laminated it so that it was water resistant. A thin wire was attached to the top of the model so that I could lower it into the shrimp tanks during behavioral trials. I used a fish model, because this type of model is likely to evoke natural responses from shrimps, in that they are color blind and have coarse spatial resolution (Caves *et al.*, 2016), which may cause them to recognize 2-dimensional photographic models as fish clients.

Prior to all behavioral trials, I starved the shrimps in each focal tank for 2 days, to ensure

that they were hungry enough to potentially engage with the client fish model, because cleaner shrimp motivation to signal and clean increases with hunger level (Chapuis and Bshary, 2010). Starting in August 2016, six tanks were randomly chosen each day for behavioral trials to observe cleaning behaviors in the presence the fish model, and one tank was chosen to quantify shrimp social interactions and signaling behaviors in the absence of the fish model. From January - April 2017, both tanks were tested each trial day. Before observing each tank, a cardboard blind with a viewing hole was set up for five minutes of acclimation time to restrict environmental stimuli. For all the trials, the model then was submerged into the selected tank for five minutes.

During each fish trial, the model was placed inside the tank near the shrimps without blocking the view of the shrimps to the observer. The fish model was placed initially on the tank bottom, resting on the sand ~10 cm away from a focal shrimp, without any movement to observe initial shrimp reaction to a motionless model. If there was no observed reaction to the model, or if other shrimps in the group were not near the model, the fish model was moved closer, but no closer than 2-3 cm from any shrimp, so as not to "chase" the shrimps with the model. As such, the distance that the fish model was presented to shrimps was ~ 2-10 cm; shrimps appeared to perceive and react to the fish models when they were up to 10 cm away.

From August – December 2016, 15 behavioral trials with the fish model were conducted per tank. This method collected a total of 90 samples (15 trials per tank x 6 tanks), not including the control trials, which were N = 12 trials total (2 trials per tank x 6 tanks; total of 102 observational periods recorded on a total of 28 shrimps in 6 tanks). From January - April 2017, each tank was exposed to the fish model 15 times, ie: 15 trials were conducted on each tank. I collected a total of 30 samples (15 trials per tank x 2 tanks). For observations with no model

present, both tanks were observed 10 times (N = 20 total; 10 trials per tank x 2 tanks). As such, a total of 50 observations were recorded on a total of 10 shrimps in 2 tanks.

Data analysis

Field population analysis

The body sizes of sea anemones occupied vs. those unoccupied were compared between the 2 sample years using 2-way ANOVA, to assess size differences between anemones with and without Pederson shrimps, as well as anemone size differences between the data collection years. Linear regression analyses were used to describe covariation in shrimp and anemone characteristics (variation in shrimp social group size with anemone body size, variation in shrimp body size with distance from anemone, and variation in microhabitat use among shrimp size classes).

Field social hierarchy analysis

For all shrimps observed in the field, I ranked the individuals (ranks 1-9) within each social group (i.e., all shrimps that co-occurred on each host sea anemone), based on their relative body sizes (PMTL), with the largest shrimp in each group ranked #1. I then determined the ratio of PMTL of individuals adjacent in rank within each group (PMTL rank N/PMTL rank $N + 1$, after (Buston and Cant, 2006). Individuals with rank N were considered dominant to all individuals with ranks greater than N, based on preliminary observations. I also analyzed

whether the body size ratios of individuals adjacent in rank were non-randomly distributed. This allowed us to determine whether individuals were evenly-spaced in terms of their body size ratios, and whether there was a minimum size ratio between adjacent individuals, in which very similarly-sized individuals did not co-occur within the same social group. I compared the observed distribution of size ratios with a random distribution of size ratios expected under a null model. I calculated 92 body size ratios of individuals that were adjacent in rank within each observed social group of shrimps during 2015, and 81 body size ratios in 2016, to obtain an observed distribution of ratios each year. I created null distributions of 9200 (2015) and 8100 (2016) ratios using a Monte Carlo procedure in R. For this procedure, I randomly selected individuals from the pool of 119 available individuals (in the same 27 groups) in 2015 and 123 available individuals (in the same 42 groups) in 2016 (not including shrimps that were alone on anemones), and combined them into social groups according to the observed distribution of social group sizes. I then calculated the body size ratios of individuals adjacent in rank from this null population. The procedure was iterated 100 times, generating an expecting random distribution of ratios to which the observed ratios were compared.

Laboratory experiment analysis

To compare the frequencies of social behaviors exhibited by shrimps in different social hierarchy positions, ANOVA analyses with Tukey multiple comparisons of means were used. These analyses described variation among shrimps in different social hierarchy positions, in their frequencies of behavioral interactions both with fish client models (signals, approaches, cleans,

and retreats), and their social interactions with other shrimps (approaches, initiated contacts, and retreats).

Results

Field observations

During both 2015 and 2016, corkscrew sea anemones exhibited stable population size structures, in which the population consisted of many small individuals and few large ones (Fig. 3.2). Less than half of the very small sea anemone hosts contained shrimps during both years, while almost all of the largest ones did during one of the years examined (2016). The body size of host sea anemones did not vary significantly between the 2 years examined (2-way ANOVA, $F = 0.982$, $p = 0.323$), but did with the presence of Pederson shrimps (2-way ANOVA, $F = 24.641$, $p = <0.001$).

The number of Pederson shrimps that occupied each anemone (i.e., shrimp social group size) varied widely among anemones, but did exhibit some trends. Some of the occupied anemones of all body sizes contained only 1 – 4 shrimps, even among the largest anemones. However, the smallest occupied anemones (up to 25 cm^2 TCSA) each contained a maximum of only 4 shrimps during both years, while larger anemones each hosted up to 6-9 shrimps, leading to a significant increase in shrimp social group size with host anemone body size during 2016 ($F = 35.89$, $P < 0.0001$), but not during 2015 ($F = 0.6307$, $P = 0.432$; Fig. 3). There appeared to be a maximum group size for shrimps (~ 6-9 individuals maximum per anemone) even in very large

anemones; as anemone body size reached ~ 100 cm^2 TCSA, the shrimp group sizes reached an asymptote.

Shrimp body size within each social group decreased significantly with distance from the host anemone, during both years (2015: $F = 5.56$, $P < 0.05$; 2016: $F = 9.33$, $P < 0.01$, Fig. 3.4). The largest shrimps in each social group resided either among the anemone tentacles (zero distance from the host, microhabitat zone 3, Fig. 3.1), or on adjacent substrate up to only 2 cm distant from the tentacle crown. In a few cases, large shrimps occurred up to 4 cm distant from the anemone tentacle crown, in microhabitat zones 4 and 5 (Fig. 3.1). In contrast, the smallest shrimps (<10 mm PMTL) in each group rarely contacted the host tentacles, and perched on adjacent substrate up to 8.5 cm distant from the host. The types of microhabitats occupied by shrimps accordingly depended on shrimp body size. During 2016, large shrimps (> 21 mm PMTL) resided significantly more frequently in contact with the host, where they perched on tentacles of the inner and outer tentacle crown (occupied by 20.9 and 45% of large individuals in 2015 and 2016 respectively, N = 18 and 43 respectively, Fig. 3.5) than did small shrimps (< 11 mm PMTL), which more frequently occurred on soft or hard substrate near the host (0.0 – 8.5 cm distant from the host tentacle tips; 2016: $F = 20.9$, $p < 0.0001$; not significant in 2015: $F = 3.69$, $p = 0.0568$). During both years, most shrimps occupied soft or hard substrate near the host (zone 4 and 5; 86.7 % in 2015 and 72.1 % in 2016), with the rest perching in contact with the anemone tentacle crown (zones 2 and 3); none occurred along the host column (zone 1).

The observed distribution of body sizes between adjacent individuals within each social group did not differ significantly from random during both years examined (Fig. 3.6; Kolmogorov-Smirnov test: (2015) $D(2) = 0.0671$, $p = 0.807$; (2016) $D(2) = 0.0936$, $p = 0.484$). Similar to the randomly-generated ratios, which ranged 1 to 4 during both years, the observed

body size ratios did the same, indicating that individuals in each social group exhibited a wide range of relative sizes of adjacent members during both years with many adjacent ranks being close to 1:1. These patterns show that Pederson shrimps co-occur in distinctly random groupings of individuals in terms of their relative body sizes, and that individuals very close in body size can occur together within social groups.

Laboratory experiments

Exploitation competition behaviors toward the client fish model

Alpha females exhibited the highest frequencies of positive interactions with client fish models; 67.8% of all alpha female interactions with client fish models were positive (Fig. 3.7). These large females signaled toward, approached, and attempted to clean the fish model during most of their interactions with the model. In contrast, shrimps in each of the 5 other types of social positions (beta, gamma, delta females, males and juveniles) often ignored the model, or retreated from them; members of each of these 5 groups exhibited < 60% positive interactions with the fish model. Shrimp body size did not correlate significantly with position in the social hierarchy, in terms of exploitation competition behavior toward the fish model ($F = 3.67$; $p = 0.376$). However, the percent of positive interactions with the fish model varied significantly with position in the social hierarchy (ANOVA: $F = 2.86$; $p < 0.05$). Pairwise comparisons revealed that alpha females interacted with the fish model significantly more frequently than did male shrimps ($p < 0.05$), with no significant difference among all other social positions (Figs. 3.7 and 3.8).

Interference competition behaviors toward conspecifics

Pederson shrimps interacted frequently with each other in the laboratory-created social groups (Table 1 and Fig. 3.9). Similar to their high frequencies of approach to the client fish model (a form of exploitation competition behavior toward their food source), the alpha female shrimps in each social group also frequently exhibited interference competition behaviors toward conspecifics, in the form of social dominance behaviors. The most frequent type of interaction toward other social group members was to approach toward them (= 64.6% of all alpha female interactions with conspecifics). They approached conspecifics significantly more frequently than did all other types of shrimps (ANOVA: $F = 9.38$; $p < 0.0001$; pairwise comparisons revealed significant differences in the percent of approaches by alpha females compared to the approach frequencies of shrimps in all other positions: beta, gamma, delta, male 1, male 2, and juveniles; $p < 0.05$). In contrast, the shrimps in all other social positions (smaller females, males, and juveniles) exhibited approach behavior in less than half of their social interactions (< 50% of conspecific interactions by each type of shrimp), and their frequencies of approach did not differ significantly from each other (pairwise comparisons; $p = 0.42$ to 1.00; Fig. 3.9).

In terms of initiating physical contact with conspecifics, all types of females exhibited fairly high frequencies of contact with other shrimps in their social groups (31.2% of all interactions by alpha females, 34.2% of interactions by beta females, 26.0% of those by gamma females, and 31.25% of interactions by delta females). Contacts consisted mostly of individuals approaching and contacting conspecifics with their chelae; pinching the antennae and forelimbs of the receiving shrimp. In some cases, the receiver reciprocated and contacted the initiator in the form of pinching antennae and forelimbs of the initiator. In contrast to females, the males and

juvenile shrimps rarely initiated contact with conspecifics (Fig. 3.9). However, these patterns were not statistically significant; the percent of conspecific interactions involving physical contact did not vary significantly with social position (ANOVA: $F = 1.24$; $p < 0.32$).

Both male and juvenile shrimps exhibited high frequencies of socially subordinate behaviors. Juvenile shrimps retreated from conspecifics in all of their observed cases of social interaction (100% of juvenile conspecific interactions). Both the larger (male 1) and smaller male shrimps (male 2) in each group also frequently retreated during interactions with conspecifics (77.8% and 73.7% of interactions with other group members, respectively). Conversely, all types of females only rarely retreated from conspecifics (range = 4.1 to 44.7% of the social interactions exhibited by each type of female). As such, the frequency of interactions resulting in retreat varied significantly with social position (ANOVA: $F = 2.82$; $p < 0.05$). Pairwise comparisons revealed that larger males (male 1) retreated during a significantly higher percentage of their social interactions than did alpha females. Even though juveniles exhibited higher frequencies of retreat (in 100% of cases) than did larger males (male 1; 77.8% of cases), their retreat frequencies did not differ statistically from those of alpha females, likely due to very small sample size of juvenile interactions.

Discussion

General comments

I demonstrate here that on Caribbean coral reefs, Pederson shrimps form social groups that do not appear to have a rigid size ratio structure on host sea anemones on Caribbean coral

reefs. However, social groups are significantly spatially structured, in that large individuals occupy more central habitat on host anemones than do smaller ones, which are relegated to peripheral habitat on substrate around the anemone. Under laboratory conditions shrimps exhibit strongly size- and gender-based behavioral dominance hierarchies, in which large females display both exploitation and interference competition behaviors more frequently than do smaller subordinate group members. Large females appear to frequently signal towards, approach, and attempt to clean model client fishes (exploiting their potential food source of fish ectoparasites), and also approach and contact smaller group members (an expression of interference competition for habitat space). Conversely, smaller females, males, and juveniles all exhibit fewer positive interactions with fish models, and more subordinate social behaviors in the form of retreats from conspecifics, than do large alpha females. These encounters allow larger shrimps to occupy more dominant social positions which may result in their securing prime microhabitat locations on the tips of host sea anemone tentacles. This spatial resource monopolization in turn likely grants them prime access to client fishes that visit the shrimp-anemone complex, also known as a cleaning station. However, obtaining larger sample sizes in both field and laboratory settings for these types of interaction data may reveal somewhat different patterns in this system, and should be explored in future studies.

Field observations

The field observations of corkscrew sea anemones in Brewer's Bay, St. Thomas USVI revealed a population dominated by small individuals indicative of a stable population with high recruitment. Many corkscrew sea anemones hosted Pederson shrimps, but there were unoccupied

anemones so these shrimps are not space limited by the number of anemones in the population. This pattern is similar to that observed for closely-related obligate anemoneshrimps, including the symbiotic cleaner shrimps *Periclimenes anthophilus* which do not reside on every anemone on a reef (Nizinski, 1989).

Pederson shrimp groups are hypothesized to congregate in regions with prime access to reef fishes for cleaning interactions (Mahnken, 1972). In addition, large anemones, which are known to visually attract client fishes (Huebner and Chadwick 2012a) hosted larger shrimp social groups than did small anemones. The large social groups observed here, of up to 9 individuals per anemone, are unusual for crustaceans that associate with sea anemones; most crustacean species on cnidarian hosts occur as a pair of mated individuals (Duffy, 1996; Baeza *et al.*, 2002; Hirose, 2012). Their small body size relative to the host, their cleaning habit, and other ecological factors may cause Pederson shrimps to occur in mixed-gender social groups (reviewed in Gilpin and Chadwick, in press). Likewise, squat anemone shrimps *Thor amboinensis* have small body sizes and are sequential hermaphrodites that can live in groups of up to 11 individuals per host (Baeza and Piantoni, 2010). Other anemoneshrimps on *B. annulata* hosts, such as snapping shrimps *Alpheus armatus*, live in male-female breeding pairs and reside below the host anemone column (Knowlton, 1980).

Large anemones serve as conspicuous visual signals which attract client fishes (Huebner and Chadwick, 2012a), possibly driving in part the observed increase cleaner shrimp abundance with host anemone size that was documented in the current work. Although the size of shrimp social groups was not found to significantly correlate with host anemone body size in 2015, it did in 2016; the small number of shrimps on small host anemones also may indicate microhabitat space limitation due to small host size, with a maximum number of shrimps that can associate

with a given size of anemone. In addition, the strong relationship between shrimp size and distance from the host anemone suggests behavioral habitat partitioning according to body size in this species. Inhabiting the tentacle tips of the host anemone may allow the larger, usually breeding female shrimps to gain enhanced protection from predators. Their positioning also may be key to drawing in reef fish clients. If a reef fish client comes to a cleaning station, the most dominant, central individual will probably be the first to access food in the form of fish ectoparasites. Studies on the nutritional status of cleaner shrimps of varying sizes on host anemones have not been completed, but would enhance understanding of how relative position in the social groups of these shrimps ultimately impacts their fitness. Juveniles and other small individuals (such as males and small females) may be tolerated in social groups because they use fewer resources (habitat space, food items) than do large individuals, as described for spider crabs *Inachus phalangium* on corkscrew sea anemones (Wirtz and Diesel, 1983).

The broad range of shrimp sizes found within groups, with the size ratios between adjacent ranking individuals not differing significantly from that of a randomly-mixed population, indicates that social groups in this species are only loosely organized by body size. I conclude that Pederson shrimps may randomly colonize into and emigrate from individual anemones, but that their behavioral interactions within any single group may lead to dominance hierarchies based on their relative sizes and genders. These shrimps have the ability to migrate frequently between anemones, as do some other cleaner shrimps (Chadwick et al. 2008; reviewed in Gilpin and Chadwick, in press), which may in part explain why they do not exhibit highly size-structured groups.

Laboratory experiments

The behavioral patterns of Pederson shrimps in dominant positions (Alpha females) within the laboratory-formed social groups showed the highest occurrence of positively interacting with model reef fish, indicating they are likely to signal to and jump on client fishes to participate in cleaning behaviors. This behavior allows them to monopolize food resources before other shrimps in the social group are able to consume them.

The largest females also most frequently approached subordinate individuals in the social group, indicating that they may earn their dominant positions by forcing the retreat of subordinates. The high rates of beta females initiating contact with subordinate individuals also shows that they also conflict with others, which allows them to maintain their mid-level position in the hierarchy. Conversely, the high rates of retreat by males and juveniles reveal their clearly subordinate status in relation to the more dominant individuals. These behavioral patterns provide a mechanism whereby these shrimps maintain their spatial partitioning of microhabitat areas relative to the anemone body, which were observed in the field. Work of this nature has not been completed previously on symbiotic cleaner shrimps, and indicates a highly structured group pattern with likely major consequences to the dynamics of fish-cleaner interactions. Future studies should focus on dominance behaviors by individuals within the social groups of other types of cleaner shrimps, for comparison.

General conclusions

Overall, the host use patterns and social group structure of Pederson's shrimps indicate a social hierarchy among individuals based on behavioral interactions, sexual status, and body size. The recognition of aggressive states that lead to linear dominance hierarchies has been hypothesized for invertebrates (Winston and Jacobson, 1978), and is thought to structure crayfish hierarchies (Copp, 1986). In Pederson shrimps, reproductively active females are the most dominant individuals on each host anemone, with males and juveniles acting as subordinates. This social hierarchy supports the idea of Pederson shrimps being highly mobile as well, because the subordinate individuals may migrate frequently among anemones to search for better access to resources (mates, food, habitat space). Future work should focus on the mobility of these shrimps in the field, and how migration rates vary among individuals that differ in social status.

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Table 3.1. Number of instances in which individuals of each social type (ordered by relative body size and gender, from most dominant [alpha female] to most subordinate [smallest juvenile = juv 2]) initiated each type of behavioral interaction with other individuals in the social group.

	Receiving Position								
Initiating Rank	**Behavior type**	**Alpha**	**Beta**	**Gamma**	**Delta**	**Male 1**	**Male 2**	**Juv 1**	**Juv 2**
Alpha	**Approach**		10	9	2	7	2	1	
	Initiate contact		6	5	1	1	1	1	
	Retreat			1	1				
Beta	**Approach**			1	0	5	2		
	Initiate contact	2		1	1	6	3		
	Retreat	9		7	1				
Gamma	**Approach**	1	5		4	10	1		
	Initiate contact	3	2		1	6	1		
	Retreat	9	1		3	2	1		
Delta	**Approach**	1	1	1		1			
	Initiate contact	1	1	1		2			
	Retreat	3	1	3					
Male 1	**Approach**			3			1		
	Initiate contact	2	1	1			1		
	Retreat	6	6	11	3		2		
Male 2	**Approach**					1			
	Initiate contact	1	1			2			
	Retreat	7	4	1		2			
Juv 1	**Approach**								
	Initiate contact								
	Retreat	1							
Juv 2	**Approach**								
	Initiate contact								
	Retreat								

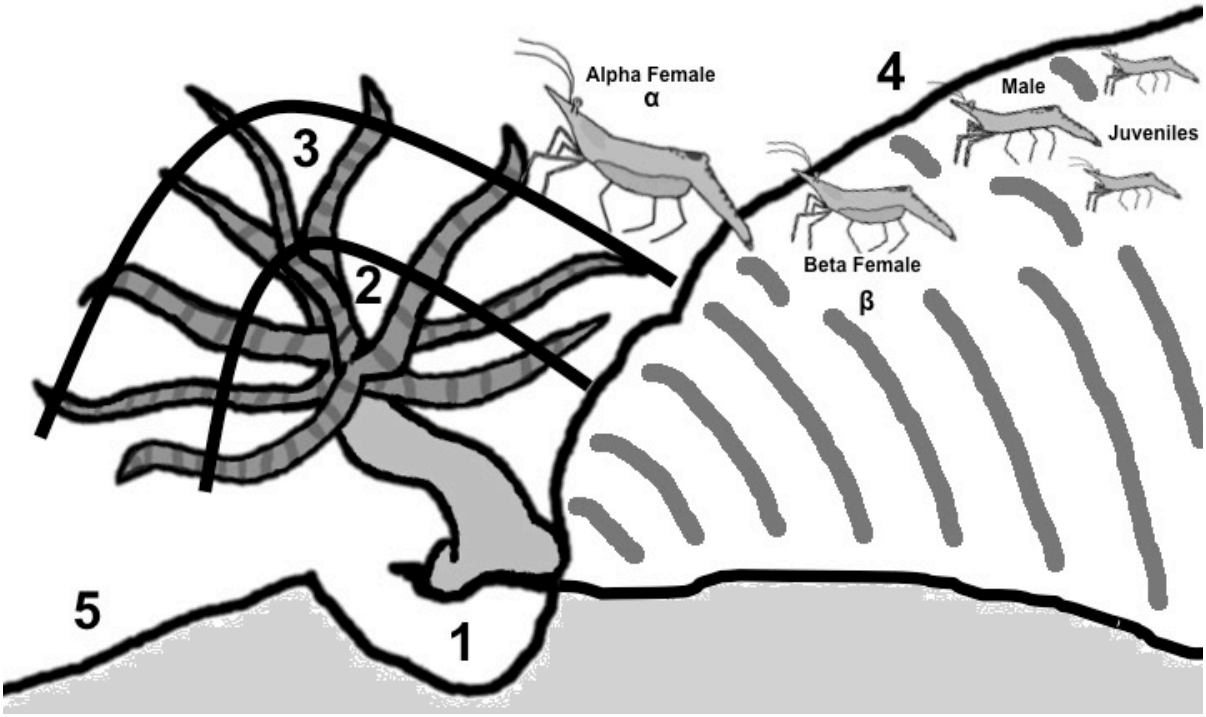

Figure 3.1. Microhabitats on host sea anemones *Bartholomea annulata* that were surveyed for the presence of Pederson cleaner shrimps *Ancylomenes pedersoni* on coral reefs at St. Thomas, U.S. Virgin Islands. Microhabitats were classified into 5 zones on or near the host anemone body; 1: anemone column, 2: inner tentacle crown, 3: outer tentacle crown, 4: hard substrate adjacent to anemone, 5: soft substrate adjacent to anemone. Shown is a typical social group of Pederson shrimps, with individuals utilizing microhabitats according to their relative body sizes and genders within the group. The largest female (alpha female) tends to occur in zone 3, perched on the anemone tentacle tips. All other group members occupy zones 4 and 5, with the second largest female (beta female) residing close to but not touching the anemone, and smaller individuals (gamma and delta females if present, males, and/or juveniles) occurring at significantly greater distances from the host (see text for details).

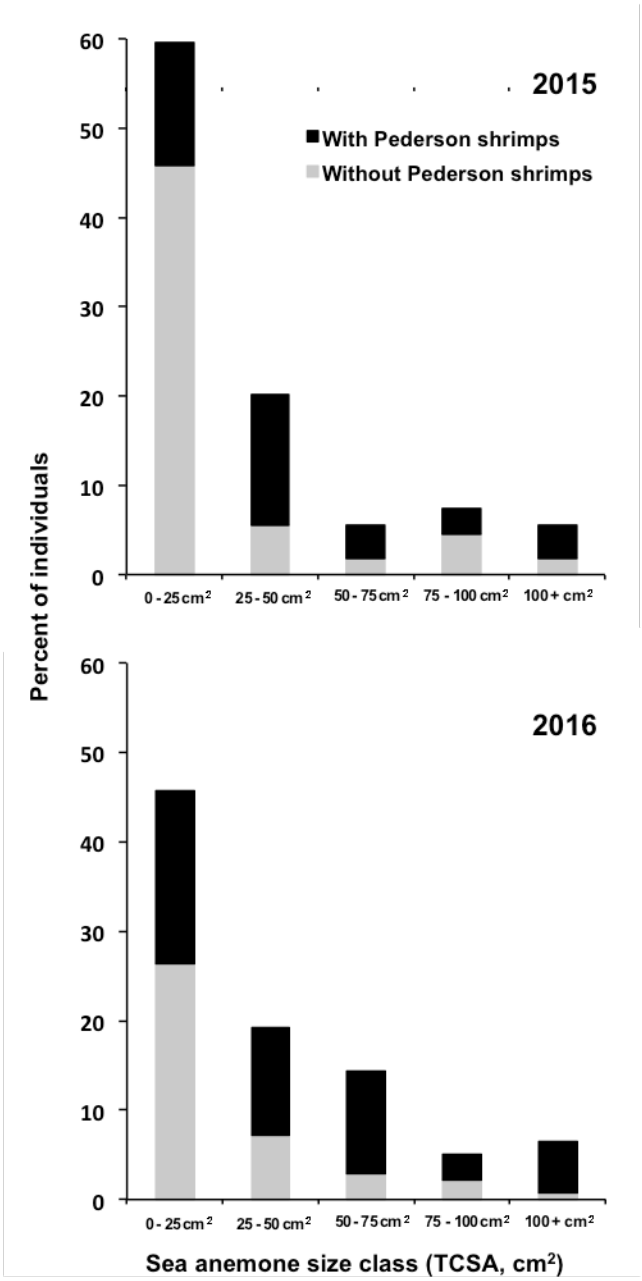

Figure 3.2. Population size structure of corkscrew sea anemones *Bartholomea annulata* on coral reefs in Brewer's Bay, St. Thomas, U.S. Virgin Islands, during July 2015 (N = 109) and August 2016 (N = 140), and proportion of anemones in each size class that were occupied by Pederson cleaner shrimps *Ancylomenes pedersoni*. TCSA = Tentacle crown square area. Individuals of Spotted cleaner shrimps *Pereclimenes yucatanicus* also occupied the anemones but were rare (2015: 0.9%; 2016: 9.3% of anemones); other crustacean associates were not censused here.

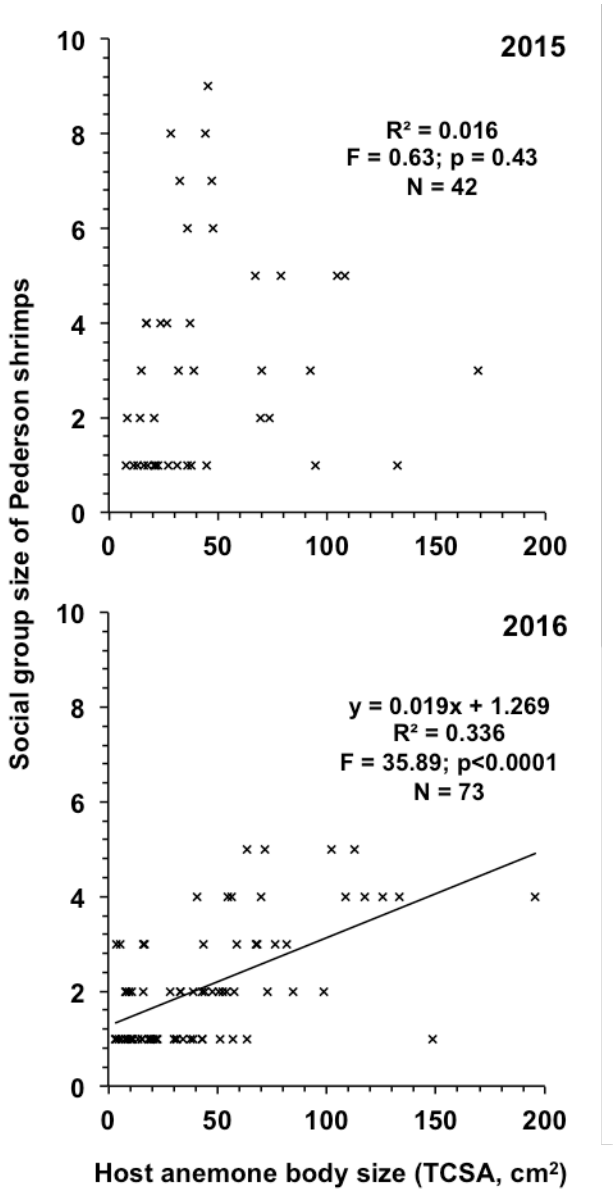

Figure 3.3. Variation in the social group size of Pederson shrimps *Ancylomenes pedersoni* with the body size of host sea anemones *Bartholomea annulata*, during July 2015 and August 2016 on coral reefs in Brewers Bay, St. Thomas, US Virgin Islands. TCSA = Tentacle crown square area. Note that the relationship exhibited a significant trend only during 2016.

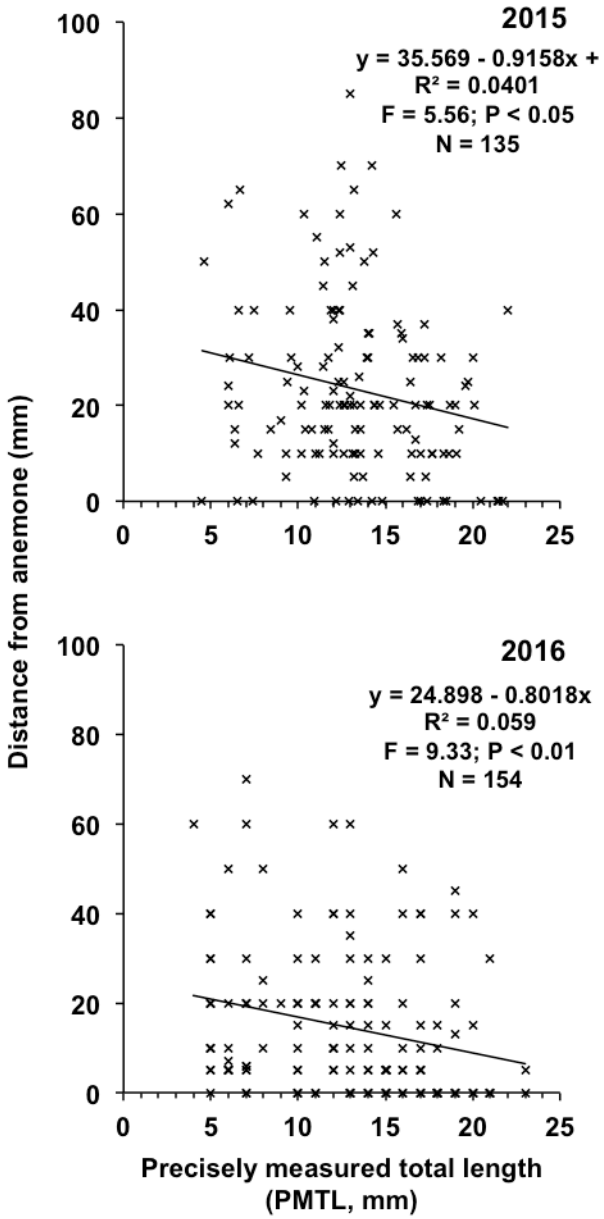

Figure 3.4. Variation in the distance of Pederson shrimp *Ancylomenes pedersoni* from host sea anemones *Bartholomea annulata* with shrimp body size (PTML), during July 2015 and August 2016 on coral reefs at Brewers Bay, St. Thomas, US Virgin Islands. PMTL = Precisely measured total length.

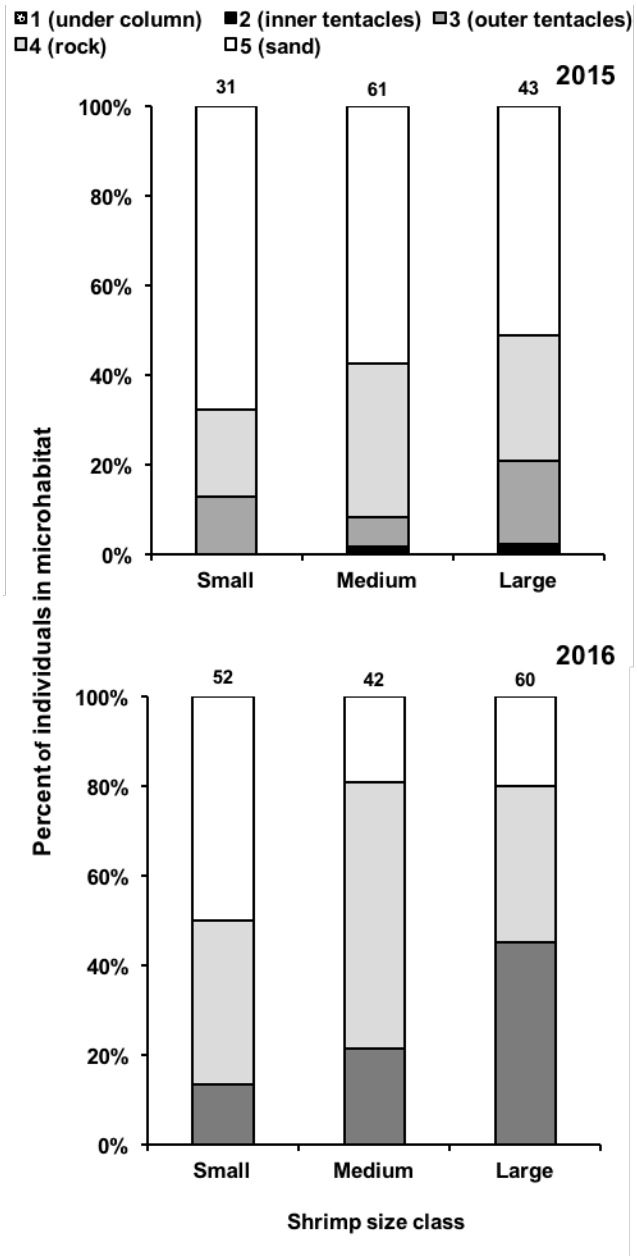

Figure 3.5. Microhabitat zones occupied by Pederson shrimps *Ancylomenes pedersoni* associated with corkscrew sea anemones *Bartholomea annulata* at Brewers Bay on St. Thomas, U.S. Virgin Islands during July 2015 and August 2016. Legend numbers indicate microhabitat zones on host sea anemones, as described in detail in Figure 1; bars indicate the percent of individuals in each size class within each zone (Small: <11mm carapace length; Medium: 11-15 mm; Large: >15 mm. The numbers at the top of each bar are the sample sizes of shrimps in each size class.

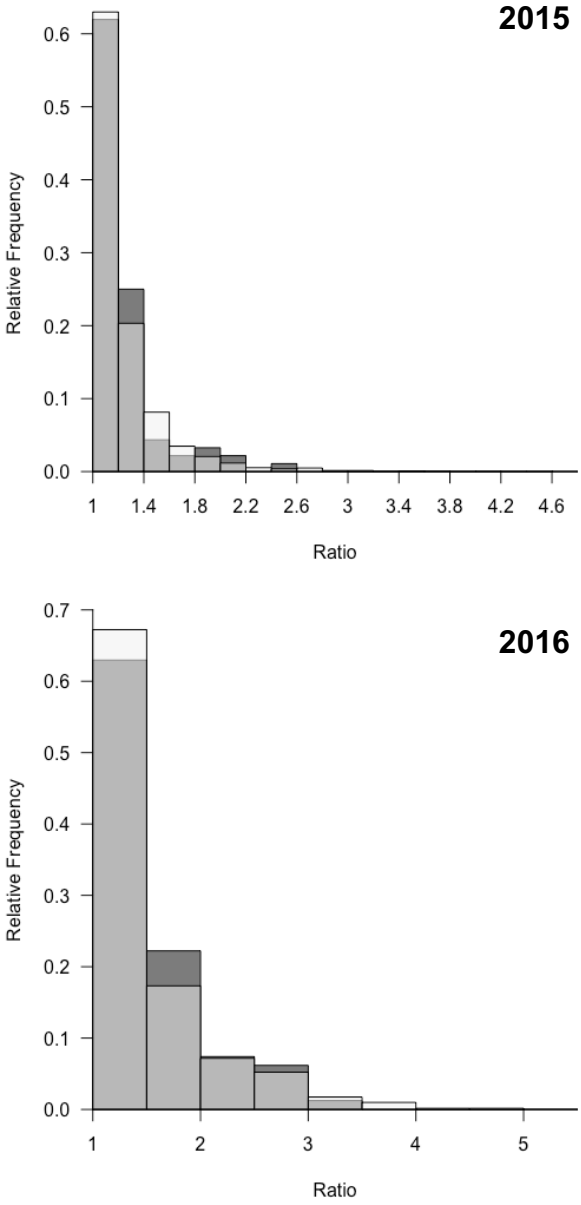

Figure 3.6. Observed distribution of body size ratios of total lengths of individuals adjacent in rank (dark grey and light gray), within social groups of Pederson shrimp *Ancylomenes pedersoni* on host sea anemones *Bartholomea annulata*, during July 2015 and August 2016, on coral reefs at Brewers Bay, St. Thomas, US Virgin Islands and the distribution of ratios expected under a null model generated using Monte Carlo procedure (white and light gray). Light gray represents area where two distribution overlap; they were not significantly different during both years from expected ratios based on randomly-generated distributions (see text for details).

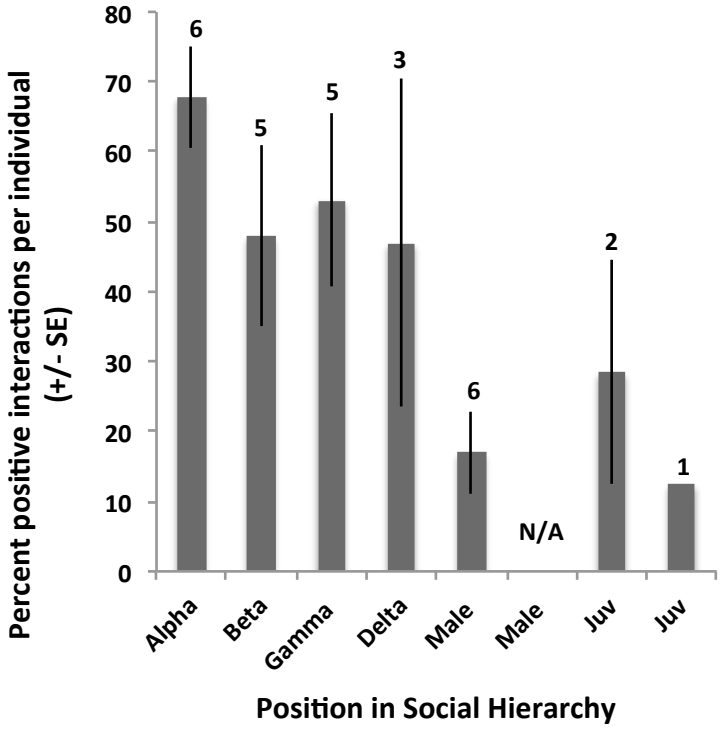

Figure 3.7. Percent of positive interactions with fish models per individual under laboratory conditions. Positive interactions include instances where a shrimp signaled, approached, and/or cleaned the fish model.

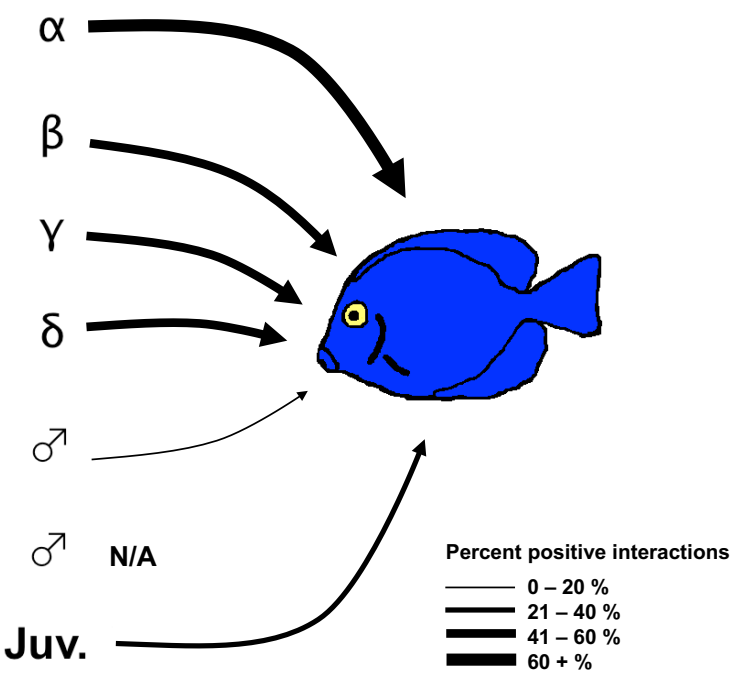

Figure 3.8. Percent positive interactions with fish model per individual under laboratory conditions. Positive interactions include instances where a shrimp signaled, approached, and/or cleaned the fish model. Line width indicates percentage of positive interactions between social hierarchy positions and client fish model.

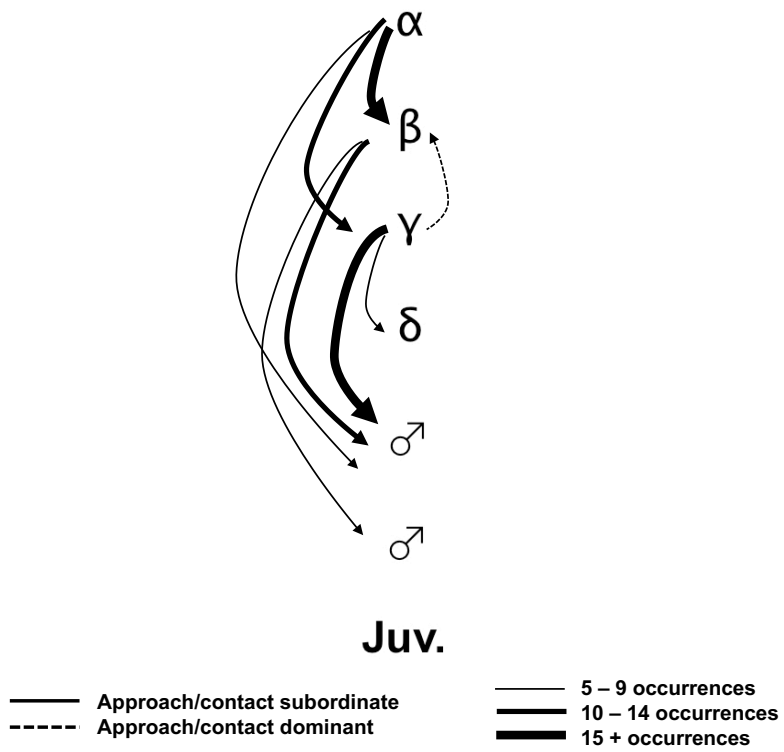

Figure 3.9. Number of occurrences of individuals in each social hierarchy position interacting dominantly with each other (i.e., approaching or initiating contact with other shrimps) during laboratory trials. Only interactions that occurred more than 5 times are shown. Note that shrimps in the top hierarchy positions tended to interact dominantly with shrimps in the lower hierarchy positions, with one exception of a reversal interaction between the gamma and beta females. The alpha, beta, gamma, and delta individuals are breeding females ordered by decreasing body size. Males tend to be smaller than the smallest breeding females, and also are ordered by decreasing body size, while juveniles (Juv) are the smallest individuals in the social group.

Chapter 4

Mating, sexual system and reproductive rates of Pederson cleaner shrimps *Ancylomenes pedersoni* under laboratory conditions

Abstract

Caridean shrimps, which include Pederson cleaner shrimps *Ancylomenes pedersoni*, are a diverse group of organisms with varied sexual systems. Sex change and/or simultaneous hermaphroditism occur in some carideans, and some of these shrimps also are proven cleaners, including scarlet striped cleaner shrimps *Lysmata amboinensis* and *L. grabhami*, and fire shrimps *L. debelius*. Thus, sex change and hermaphroditism are fairly common among caridean shrimps, but have not been observed in Pederson cleaner shrimps. As an organism with commercial and ecological importance, it is surprising that there are few life history studies on Pederson shrimps. The phases of their reproductive cycle (e.g., length of embryo incubation) and their mating system are not characterized. The present study documented continuous reproductive cycles for female shrimps and added evidence to support the pure-search polygynandry mating system hypothesized for these shrimps. Experimentally manipulated groups of shrimps (female-female pairings, female-male pairings, all female groups, all male groups, mixed sex groups) were observed for extended periods to document the sexual habits of this species. Individuals appear to be gonochoric (separate sexes) with no evidence of sex change or hermaphroditism. Detailed observations of senescence and cessation of reproduction near the end of the lifespan added

further evidence concerning this caridean shrimp characteristic, which has not been studied in other caridean species. These data will be used to inform fisheries managers and aid in setting restrictions on collecting of this organism, which provides an important cleaning service to coral reef fishes.

Introduction

Pederson cleaner shrimps *Ancylomenes pedersoni* are common ectosymbionts of giant sea anemones such as corkscrew sea anemones *Bartholomea annulata* and rosetip or giant sea anemones *Condylactis gigantea* in the Caribbean. These shrimps and their associated anemones act as cleaning stations, which reef fish recognize visually and approach to have their ectoparasites removed (Huebner and Chadwick, 2012). In addition to the reef services provided by *A. pedersoni*, they are also "reef safe" ornamental organisms that do not harm fish or other invertebrates in aquaria, and thus are popular in the aquarium trade (Calado, 2009). As an organism with commercial and ecological importance, it is surprising that there are few life history studies on Pederson cleaner shrimps. Recent work (Gilpin and Chadwick, in press) has described growth and mortality rates, population structure, and some aspects of reproduction in this species. However, the phases of their reproductive cycle (e.g., length of embryo incubation) and their mating system have not been characterized.

The caridean shrimps (infraorder Caridea), which include *A. pedersoni* are a diverse group of organisms with varied sexual systems. The ability to change sex from male to female (protandry) has been reported for > 30 species of carideans (an infraorder of Decapoda) including the seaweed shrimp species *Hippolyte inermis* (Chiba, 2007). The Manning grass

shrimp *Thor manningi*, another caridean, is classified as partially protandric, as half of the population consists of males that never change sex, and half consists of males that change into females (Bauer, 2004). Simultaneous hermaphroditism occurs in some carideans of the family Hippolytidae, including members of the genera *Exhippolysmata* and *Lysmata* (Bauer, 2000; Bauer, 2006). Some of these shrimps also are proven cleaners, including the scarlet striped cleaner shrimps *Lysmata amboinensis* and *L. grabhami*, and fire shrimps *L. debelius*. Thus, sex change and hermaphroditism are fairly common among caridean shrimps, but have not been observed in Pederson cleaner shrimps. Knowledge of sexual traits is important for fisheries managers, so they can determine how many and which sexes/sizes of individuals should be collected to maintain sexually viable populations.

Typically, caridean shrimps (including the major Caribbean cleaner shrimp species *A. pedersoni*) mate immediately after the female molts, with external fertilization of a spawned egg mass, which then is incubated under the mother's abdomen for a several days (Bauer, 2004). Before the egg mass is spawned by the female, ova develop internally posterior to the eyestalks and dorsally from the cardiac and pyloric stomach. In general, larger females produced larger broods (more embryos) than do smaller females, as seen in manning grass shrimps (Bauer, 1986). Shrimp brood sizes may vary widely, from < 10 embryos per brood in small (1.1 mm CL) pontoniine shrimps *Fennera chacei* (Bruce, 1976), to 35,000 embryos per brood in large (48 mm CL) pandalid shrimps *Heterocarpus laevigatus* (King and Butler, 1985). Breeding seasons are continuous all year for most species (Mahnken, 1972).

I conducted laboratory observations and completed experiments to test hypotheses about the sexual system of Pederson cleaner shrimps, including the possibility of sperm storage, multiple spawning events, continuous reproduction, and sex change in this species. Experimental

manipulations of groups of shrimps (female-female pairings, female-male pairings, all female groups, all male groups, and mixed sex groups) were observed for extended periods to document their sexual habits. My assumption was that if egg clutches were produced and the development of embryos occurred with no males present, then this would show that the females do not require recent mating with males. This could suggest that females are capable of storing sperm, or that they are simultaneous or sequential hermaphrodites, or that they could possibly reproduce by parthenogenesis or cloning. Other groupings, such as all male groups, also were observed for sex change. The data collected here may be used to inform fisheries managers and aid in setting restrictions on collecting of this organism, which provides an important cleaning service to coral reef fishes.

Methods

The present study was conducted at Auburn University from June 1, 2014 to December 2016. Individual Pederson cleaner shrimps (N = 29) were purchased from commercial collectors, and cultured as described in Gilpin and Chadwick (in press). Gender also was assessed using the same criteria as described in Gilpin and Chadwick (in press).

Determination of sexual system

To determine the mating system in this species, individual females (n = 21) were used sequentially to up to 6 treatments, with treatment order based on which other types of shrimps were available for treatments: each was cultured either alone (N = 13), with one juvenile (N = 7),

one male (N = 10), another female (i.e., two females together, N = 15), a small group of females (up to 6 individuals total; N = 8), or a small group of females with one male (up to 6 individuals total; N = 8). Each treatment lasted for at least 3 egg production cycles (~ 20 days per egg cycle x 3 cycles = at least 60 days per treatment). This allowed enough time for clear establishment of any patterns of fertilization, egg production, sex change, etc. in response to each treatment, before treatments were changed. Males also were cultured alone (N = 2), with one female (N = 10 as above), or with a group of females (N = 8 as above). Only a few shrimps were subjected to all-male treatments (N = 2) to determine the possibility of hermaphroditism in this species, including sequential (protandry; protogyny) or simultaneous (individuals functioning as both sexes during the same time period), as males are smaller than females (Gilpin and Chadwick, in press).

All females usually were observed every 1-2 days, however occasionally there were 2-3 days between observations. In addition, no observations were made during December 15, 2014 - January 10, 2015. During each observation, the presence/absence of oocyte and embryo masses was recorded (to quantify the duration of reproductive cycles), as well as any changes in body coloration or unusual behaviors (to track senescence as described in Gilpin and Chadwick, in press). After each female had incubated an egg mass under her abdomen for at least 3 days, she was placed in a petri dish with tank water and visually examined under a dissecting microscope, to confirm if eggs had been fertilized and begun to develop into embryos. Egg/embryo stages were characterized as: no development (only yolk seen), eye development (eye spots visible on embryos), and full development (eyespots as well as body systems developing) (Wehrtmann, 1990). Each examination took approximately 3 minutes and water temperature rose by $<1^{o}$ C. Once shrimps were returned to their respective tanks, they exhibited normal behavior almost

immediately (< 1 minute), indicating that the procedure did not generate long-term stress (after Gilpin and Chadwick, in press).

In addition, I used photographic analysis to quantify the developmental rate (in days) of fertilized, incubating embryos on the abdominal region on a subset of females (N = 2 females) and the rate of removing unfertilized incubated oocytes (N = 2 females, reared without a male). Photographs of developing embryos and oocytes were taken daily throughout the reproductive cycle using a DSLR camera (Cannon T3i) and high power compound microscope (Nikon Alphaphot YS). This process took ~ 5 minutes but, as described above, did not put shrimps under long-term stress.

Observations from the manipulative treatments revealed the frequency of egg production in an ideal environment. This included the average time for development of oocytes before ovulation in the region posterior to the eyestalks and dorsal to the stomach, before the oocytes dropped down to the abdominal region for incubation. The average time the female incubated either fertilized embryos or unfertilized oocytes in the abdominal region was also quantified.

To determine the size of brooded embryo masses, I measured the length, width, and height of embryo masses on preserved specimens (measured in millimeters using ImageJ; N = 6 shrimps). Individual *A. pedersoni* that had died during transport to the laboratory from the field were preserved in 95 % ethanol. I multiplied the linear dimensions of the brood mass in each preserved specimen to obtain an approximate brood mass volume. In addition to measuring the volume of the embryo masses, I made direct counts of embryos on preserved specimens (N=6) to determine the total number of embryos in the embryo masses of each dead *A. pedersoni*. Because the dead shrimps were suspended in ethanol, they decolorized and their tissues became cloudy

and opaque. Thus, for embryo counting, it was necessary to remove the embryos from the shrimp abdomen using tweezers, and then count the excised embryos.

Data analysis

Duration of reproductive cycles

To compare the durations of ovarian maturation as well as incubation of fertilized embryos and unfertilized oocytes, averages of at least 3 production cycles were calculated for each individual female in each treatment. Using these averages, ANOVA analyses with Tukey multiple comparisons of means were used to compare the average duration of ovulation and incubation among treatments. These analyses described variation in reproductive habits among shrimps in different social situations.

Senescence analysis

To analyze variation in senescence traits of females and the cause of death (natural: lived out entire lifespan; unnatural: deceased due to accidents before their lifespan was complete; see Gilpin and Chadwick, in press for details), I applied t-tests. The end of female reproductive activity was characterized by the last day each female was observed with mature ovaries (last ovarian maturation) and with incubating embryos or oocytes beneath her abdomen (last incubation) before death. The number of days before death that these characteristics were observed was then compared among females that experienced natural vs. unnatural deaths. Results are presented as means ± one standard error unless indicated otherwise.

Results

Determination of sexual system

In the manipulative experiments, females were observed to incubate developing embryos under their abdomens only in the treatments that included male shrimps. In treatments that lacked a male (i.e., in which females were: (1) alone (n = 13), or (2) with a juvenile (n = 5), (3) with another female (n = 15), or (4) part of a small group of females (n = 8); up to 6 individuals total), females were never observed to brood developing embryos. All females within each treatment that included a male (n = 18) were successful in brooding fertilized embryos. Treatments with only males (either alone (n = 2), or with another male; n = 2) did not lead to sex change (protandry), even after extended periods of observation (up to 6 weeks). Females that did not occur with males (i.e., alone (n = 13) or with other females (n = 23) or juveniles; n = 5) continued to produce oocytes regularly, but in all cases the oocytes did not develop into embryos.

Duration of reproductive cycles

The duration of each female's ovulation cycle did not vary significantly among treatments (ANOVA, $F = 1.16$, $p = 0.34$; Fig. 4.1A). The average duration of maturation of ovaries per individual ranged from 7.5 – 15.5 days with an average of 10.97 ± 0.20 days. The duration that females incubated oocytes or embryos below their abdomens however varied significantly among treatments (ANOVA, $F = 65.27$, $p < 0.0001$; Fig. 4.1B). Incubation time was

significantly longer in treatments with a male present, in which the embryos became fertilized (Table 4.1). The treatments of male/female pairing and social groups of females with one male did not differ significantly in embryo incubation period ($p = 0.42$, Tukey multiple comparisons tests). The average duration of incubation for fertilized embryos was 12.32 ± 0.69 days, while the duration for incubation of unfertilized oocytes was ~ 4-fold shorter, only 3.12 ± 0.52 days. Information from daily tracking of embryonic development matched these estimated durations of incubation. Fertilized embryos developed for 12 days on the two females observed for details on stages of embryo development, before the embryos hatched to become free-swimming larvae (Fig. 4.2). In contrast, most of the unfertilized oocytes disappeared within 5 days from the abdomens of the two females observed which brooded unfertilized oocytes (Fig. 4.3).

Pederson shrimp brood volumes

Analysis of the preserved individuals of *A. pedersoni* revealed that the shrimps were moderate in their body sizes (CL 3.1 – 4.5 mm) compared to other caridean shrimps, and that they brooded 24 – 117 embryos per shrimp (Table 4.2). The embryo brood volumes ranged 4.50 – 6.20 mm^3. A weak relationship between female size and clutch size was found ($r^2 = 0.22$, $p = 0.29$, $N = 7$ shrimps, $y = 33.54x - 55.6$).

Senescence

Of the 21 females cultured in manipulative experiments, 16 died of natural causes during daily observations. The remaining 5 individuals died from unnatural causes (see Gilpin and

Chadwick, in press for details). Senescence of female reproductive cycles was observed for all (100%) of the 16 individuals that died naturally. Females, on average ceased ovulation 30.93 ± 5.47 days before their natural deaths, which was significantly longer than the number of days that ovarian maturation ceased before unnatural deaths (average: 2.40 ± 1.17 days; t-test, $t = 5.10$, $p < 0.0001$; Fig. 4.4). As such, females that died unnaturally (e.g., due to tank accidents, etc.) continued to reproduce until shortly before death). In contrast, females that died naturally at the end of their natural lifespans appeared to senesce in terms of reproductive activity, in that they ceased reproduction up to a full month before death. The final incubations of these females concluded on average 25.44 ± 5.18 days before natural death, which was also significantly longer that the pattern for females that died from unnatural deaths (average: 3.2 ± 1.98 days; t-test, $t = 4.01$, $p < 0.0001$; Fig. 4.4).

Males also showed signs of reproductive senescence, in that they ceased to fertilize the oocytes of cohoused females (as evidenced by cessation of fertilized embryo production by the females) on average 37.17 ± 7.10 days before their natural death ($N = 6$ individuals). In contrast, the one male that was observed to die unnaturally remained sexually active almost until it died (last fertilization only 5 days before unnatural death, Fig. 4.4). These differences were not compared statistically because only one male died unnaturally during the manipulative treatments that were observed daily. In addition to cessation of reproductive activity, shrimps also changed their body coloration from transparent to translucent before natural deaths. On average, females became opaque 20.17 ± 8.15 days before natural death, while males averaged 14.33 ± 6.98 days (Fig. 4.5).

Discussion

General comments

Evidence presented here from manipulative laboratory treatments of varied social groups confirm that cleaner shrimps *Ancylomenes pedersoni* are gonochoric (separate sexes) and appear to engage in pure-search polygynandry (as hypothesized in Gilpin and Chadwick, in press). Females were unable to produce fertilized embryos without males present for copulation. Sex change either from female to male (protogyny) or male to female (protandry) was not observed at any point during this 2.5 year study. Hermaphroditism was also ruled out as a component of the Pederson cleaner shrimp mating systems, because ample time was allowed in treatments for these changes to occur for both male and female shrimps, and they were not observed. Within group treatments where a male was present, all females incubated fertilized embryos throughout the treatment period. This type of mating system is not unusual for caridean shrimps (reviewed in Gilpin and Chadwick, in press).

Duration of reproductive cycles

Detailed studies of reproductive patterns on caridean shrimps, especially ecologically and commercially important species such as Pederson cleaner shrimps, are vital for understanding their life history. Pederson shrimps are continuous brooders in tropical environments, which allows individuals to produce large numbers of offspring (Bauer, 2004). The current study

generated estimates of clutch sizes for female shrimps and for the duration of reproductive cycles, so that estimates of total female reproductive ability (lifetime fitness) can be obtained.

Patterns of senescence

Included here are observations on senescence in relatively large, old individuals and details on the timing of reproductive cessation in both females and males. As described in Gilpin and Chadwick (in press), patterns of reproductive senescence in Pederson cleaner shrimp are similar to that in *Eulimnadia texana*, in that sexual reproduction ceases during the final weeks of life. However, individuals of *E. texana* are more short-lived, reaching sexual maturity in only 4-7 days, then commencing senescence after 2 weeks of age, and dying at only < 4 weeks old (Weeks *et al.*, 1997). In contrast, senescence extends much longer in *A. pedersoni*, in that it begins ~ 2-4 weeks near the end of a 1.5 - 2 year lifespan. No other caridean shrimps have been shown to senescence, but the literature on the subject is limited, as only sea grass shrimps *Latreutes pymoeus* and Monaco shrimps *Lysmata seticaudata*, have been observed to die without exhibiting senescence beforehand (Calado and Narciso, 2003; Penha-Lopes *et al.*, 2007). Future studies on caridean shrimp senescence are needed to expand the knowledge base concerning senescence processes in these shrimps. Senescence is an interesting life history trait, because although I observed reproductive cessation patterns under ideal laboratory conditions, older shrimps may play a key role in nutrient cycling within the cleaning mutualism these shrimps take part in. As shrimps age, they become easy targets for predators, but also for host anemones and cohabitating shrimps. Cannibalism of cohabitating shrimps has been observed in peppermint

shrimps *Lysmata wurdemanni* at high densities (Baldwin and Bauer, 2003), but no quantitative study has been completed on cannibalism rates on senescing shrimps.

General conclusions

Overall, the reproductive patterns of Pederson cleaner shrimps indicate a gonochoric species with a pure-search polygynandrous mating system, due to their ecological and life history patterns. Senescence as described in Pederson cleaner shrimps is unknown for other caridean shrimps, and could play a key role in the life history of this organism. Future studies on life history of Pederson cleaner shrimps in the field are necessary to compare to results of laboratory studies, in order to confirm the extent to which the patterns observed in a controlled laboratory setting also occur in nature.

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Table 4.1. ANOVA results comparison of the duration of embryo/oocyte incubation rate with social group treatment type in Pederson cleaner shrimps *Ancylomenes pedersoni* under laboratory conditions. * indicates significance at $\alpha = 0.001$.

Treatments	p
♀ group – alone	0.94
♀ pair - alone	1.00
♀/♂ pair - alone	< 0.001*
group w/ ♂ - alone	< 0.001*
♀ pair - ♀ group	0.97
♀/♂ pair -♀ group	< 0.001*
group w/ ♂ - ♀ group	< 0.001*
♀/♂ pair - ♀ pair	< 0.001*
group w/ ♂ - ♀ pair	< 0.001*
group w/ ♂ - ♀/♂ pair	0.42

Table 4.2. Means, standard errors and ranges of carapace lengths, embryo numbers, and embryo mass volumes for 6 preserved individuals of female Pederson cleaner shrimps *Ancylomenes pedersoni.*

Carapace length (mm)		**Embryo number**		**Embryo mass volume (mm^3)**	
mean ± SE	range	mean ± SE	range	mean ± SE	range
3.85 ± 0.19	3.10-4.50	73.57 ± 13.57	24-117	5.36 ± 0.23	4.50-6.20

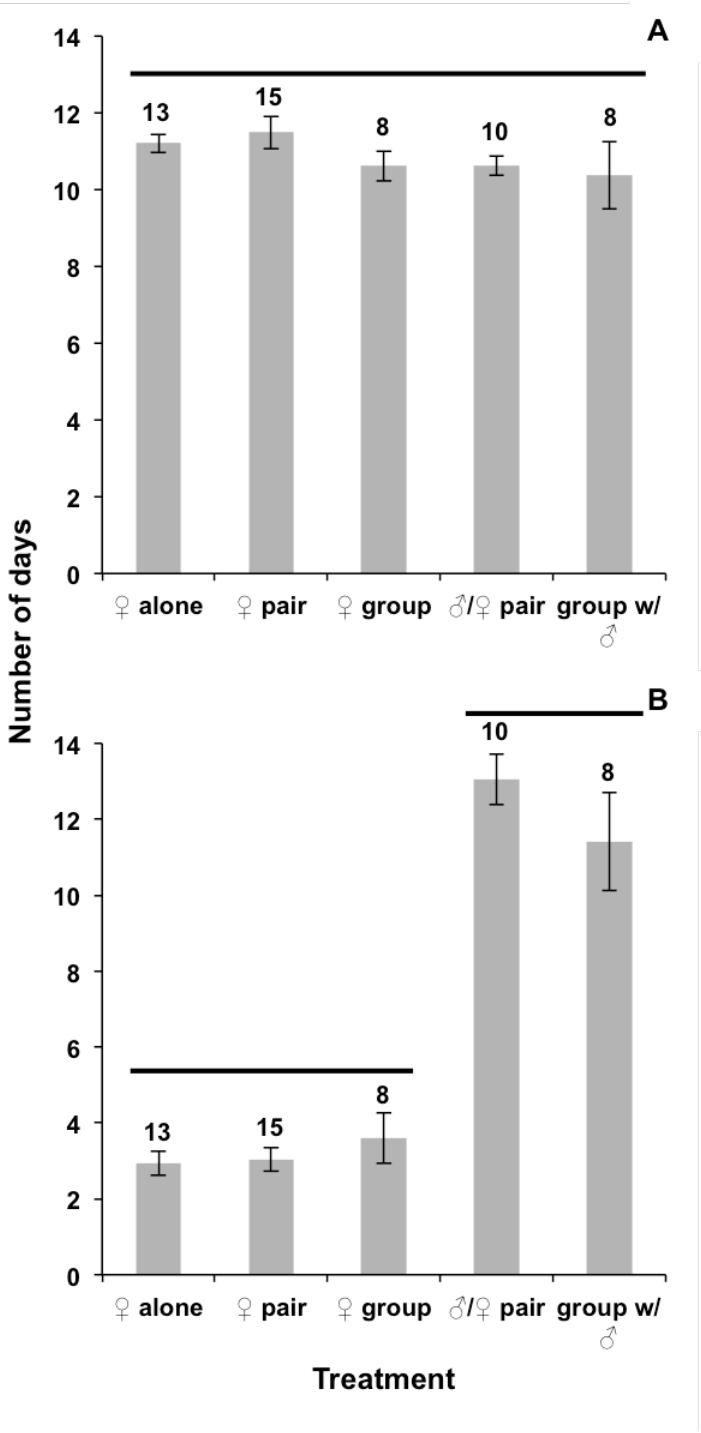

Figure 4.1. Variation in the duration of the reproductive cycle with social group treatment type in Pederson cleaner shrimps *Ancylomenes pedersoni* under laboratory conditions. A. Duration of oocyte maturation in ovaries (prior to ovulation and movement of oocytes to beneath the abdomen). B. Duration of incubation of oocytes or embryos below abdomen. Sample sizes are indicated above the bars and significantly different treatments are shown by varying lines above the bars. Ovulation time during all treatments was not significantly different, while incubation below the abdomen was significantly different for treatments that included a male.

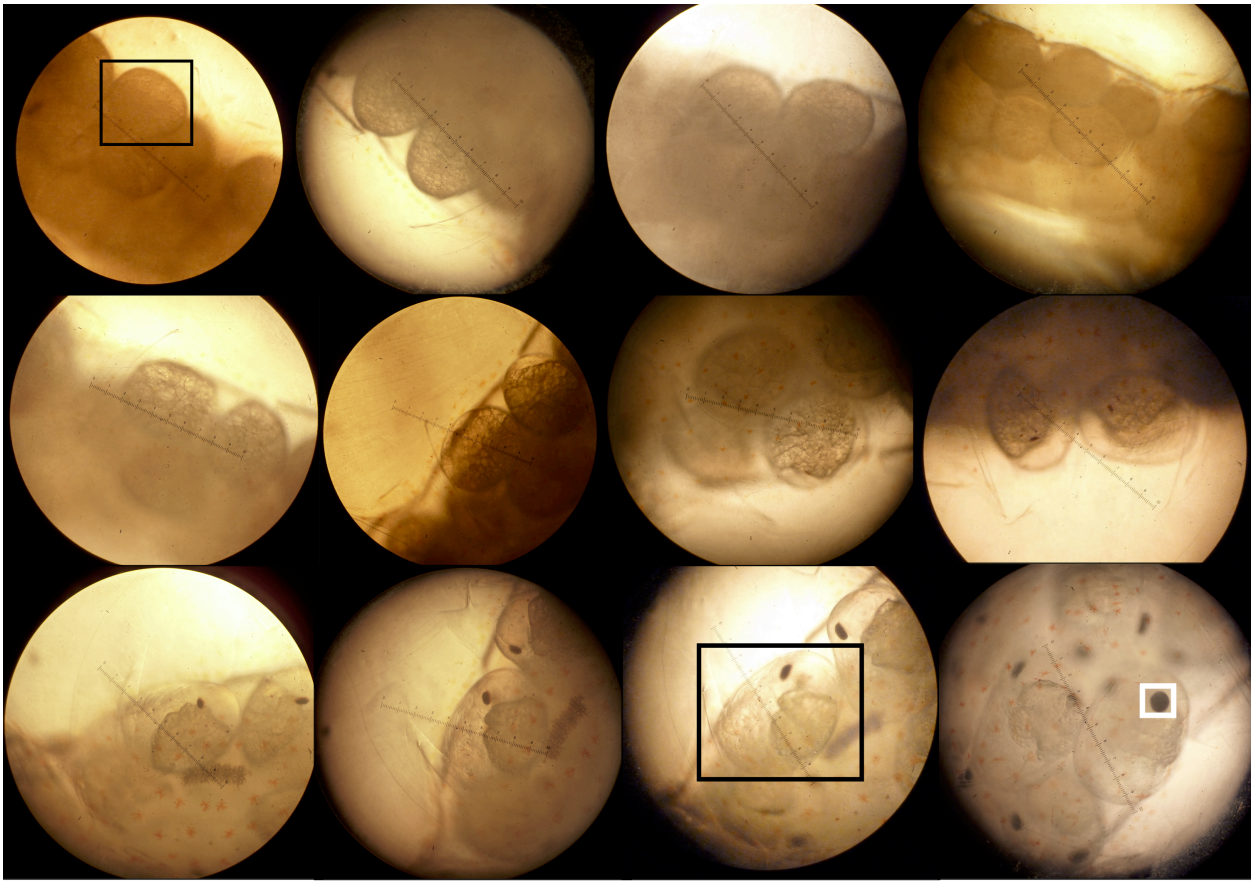

Figure 4.2. Daily photographic analysis of embryonic development of Pederson cleaner shrimps *Ancylomenes pedersoni* under laboratory conditions. Fertilized embryos required 12 days to develop during incubation below the female's abdomen, before they hatched as free-swimming larvae. Black boxes indicate entire fertilized embryos, and the white box indicates a developing eyespot of an embryo.

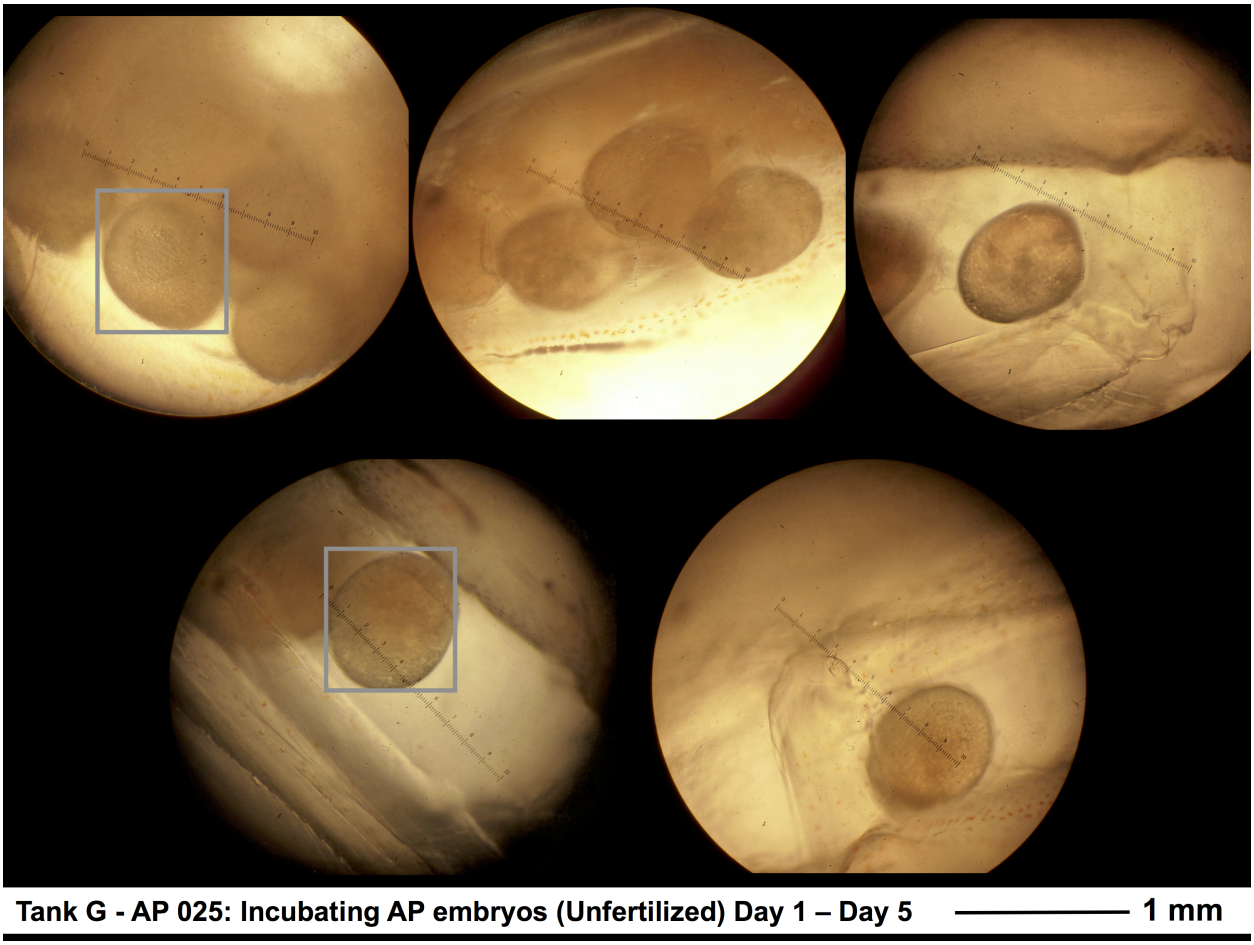

Figure 4.3. Daily photographic analysis of unfertilized oocytes incubated under the abdomen of female Pederson cleaner shrimps *Ancylomenes pedersoni* under laboratory conditions. Most unfertilized embryos were removed from the female's abdomen by day 5. No signs of embryonic development were visible during this period. Grey boxes indicate entire unfertilized oocytes.

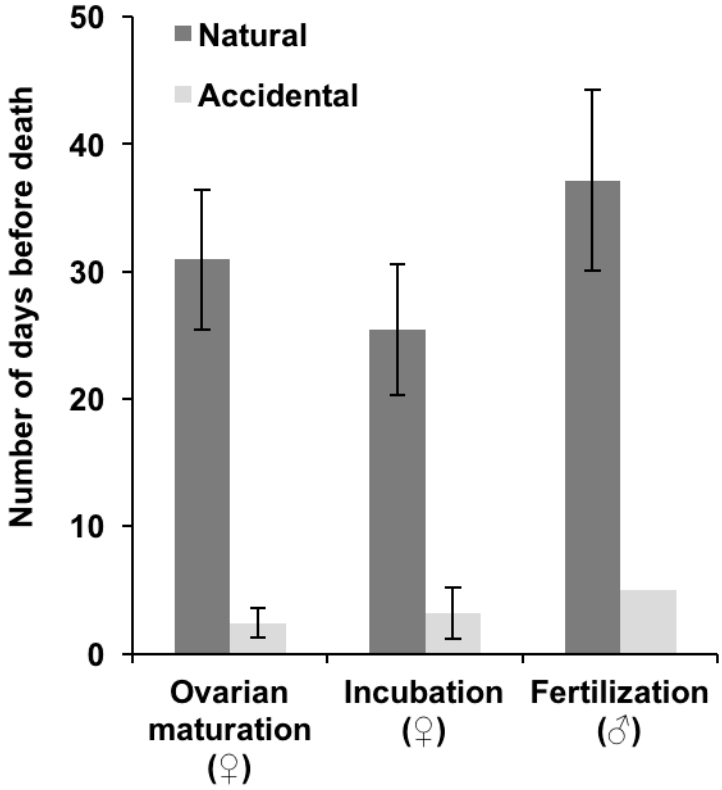

Figure 4.4. Reproductive cessation of female and male Pederson cleaner shrimps *Ancylomenes pedersoni,* for individuals that died naturally versus unnaturally under laboratory conditions. The duration of cessation of female ovarian maturation and oocyte incubation before natural deaths was significantly longer than before unnatural deaths. Males also suppressed fertilization of females before natural deaths. Sample sizes are indicated above the bars.

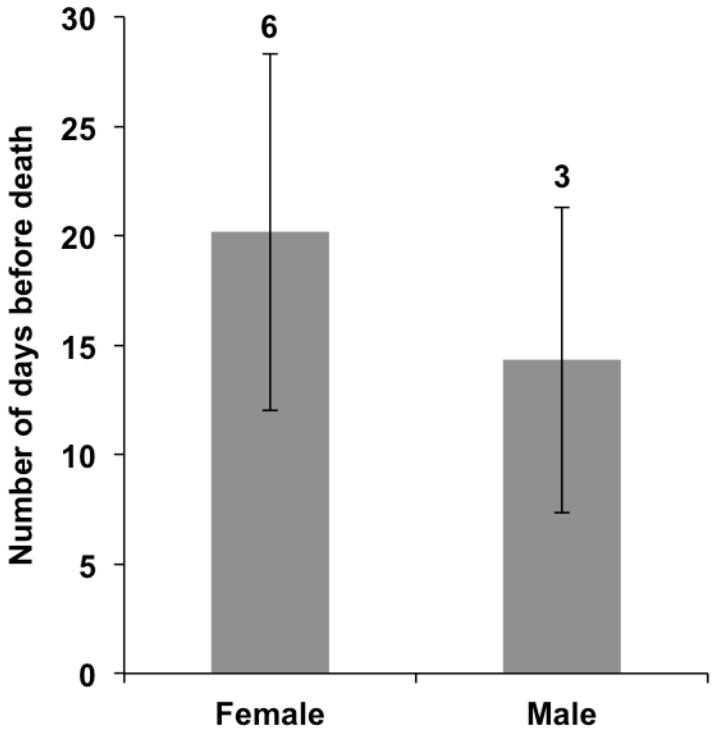

Figure 4.5. Number of days before natural death during which body coloration began to change from transparent to translucent for Pederson cleaner shrimps *Ancylomenes pedersoni* under laboratory conditions. Males and females both participate in body coloration change before natural deaths. Sample sizes are indicated above the bars.