Dispersal of Pederson cleaner shrimp among host sea anemones: Impacts of shrimp body size and social group interactions

by

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Abstract

Pederson shrimp *Ancylomenes pedersoni* are the most common cleaners of reef fishes in the Caribbean Sea. They also are obligate associates of sea anemones, especially corkscrew anemones *Bartholomea annulata*; together these shrimp and anemones form cleaning stations that are visited by client fishes for ectoparasite removal. Pederson shrimp therefore likely impact the abundance and diversity of reef fishes by enhancing fish health through reduction of parasite loads and physiological stress levels. Shrimp dispersal patterns among host anemones affect the stability and locations of cleaning stations, but the extent to which shrimp move among anemones remains unknown. Here we quantify rates and patterns of association with and dispersal among host sea anemones by these shrimp, and how they vary with characteristics of both the shrimp (body size, social rank, social group size) and the anemone host (body size, distance to nearest neighbor). Laboratory experiments revealed that shrimp level of association with anemones increases with both shrimp body size and social rank, but not with anemone size. Field observations on patch reefs at St. Thomas, USVI, indicated that shrimp social group size, and to a lesser extent, shrimp body size, significantly impact dispersal among hosts, but that other factors have little or no effect at the time scale examined. Some shrimp move frequently among anemones in the field, changing hosts on a daily basis. We conclude that large shrimp may not be affected by the presence of small conspecifics, but small shrimp may dramatically alter their anemone association behaviors when large shrimp are present. Large female shrimp remain with each host anemone for longer durations on average than do small juvenile and male shrimp, which often depart from crowded anemones and arrive at others nearby. Frequent dispersal by Pederson shrimp among anemones likely requires client fishes to search often for new cleaning stations, with consequent impacts on patterns of fish habitat use, diversity and abundance on Caribbean coral reefs.

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List of Abbreviations

TCSA………..Tentacle crown surface area

CL…………...Carapace length

M…………….Male

J……………...Juvenile

NR…….……..Non-reproductive

LP……………Large, paired shrimp

SP……………Small, paired shrimp

LS……………Large, solitary shrimp

SS……………Small, solitary shrimp

OG…………..Original anemone

AD…………..Added anemone

LFA…………Large female shrimp

LNR…………Large non-reproductive shrimp

SNR……..….Small non-reproductive shrimp

Chapter 1

Cleaning organisms, dispersal among hosts, and the relationship between sea anemones and Pederson cleaner shrimp

Introduction

Importance of animal dispersal among resource patches

Animal movement patterns, especially their dispersal among resource patches, have major impacts on the structure of natural communities. Dispersal is the movement of an organism away from one habitat patch to another, typically for reproductive purposes (Croteau 2010). Organismal dispersal can alter how predators and prey interact with each other, and consequently influence food web structure and nutrient cycles (Holdo et al. 2011). It is important to understand what variables contribute to animal dispersal, as these factors may lead to changes in biodiversity and community structure within ecosystems. Changes in social group dynamics, specifically, may cause animals to disperse to new habitats in order to maximize their fitness. Similarly, adaptive strategies for predator avoidance, such as enhanced prey locomotory capacity, are important evolutionary factors because rates and mechanism of locomotion among patches impact survival.

Habitat fragmentation, or patchiness within ecosystems, has a drastic impact on biodiversity as well as on ecological processes (Power 1996). Organismal dispersal between habitat patches is costly because risks of predation or exhaustion often are higher in the areas between than within resource patches. However, this dispersal may offer a trade-off in terms of potentially higher resource acquisition in a new patch. For example, euglossine bees are important pollinators for a wide variety of flower species. In patchy woodland areas, the number of individuals of both euglossine bees and the flower species they pollinate declines as patches become more isolated (Lovejoy et al. 1986; Powell & Powell 1987), indicating that these bees choose not to cross large areas between patches. Understory birds traverse areas between forest patches only if the intervening areas are < 100 meters in length, which may contribute to the increasing fragmentation of their populations in ever more patchy rainforests (Bierregaard and Lovejoy 1988). Therefore, understanding whether animals are willing to cross open habitat between resource patches is important, because a lack of dispersal between patches may reduce their effective population size, and consequently lead to new adaptations in the isolated populations. Knowing the frequency of this type of among-patch dispersal also may ultimately shed light on changes in the community structure of ecosystems.

Dispersal by crustaceans among symbiotic hosts

Crustaceans that form mutualistic symbioses with marine invertebrate hosts may need to disperse among their hosts in order to find mates or food. Some crustaceans remain with the same host individual throughout their lives, while others migrate between hosts (Thiel et al. 2003). For example, porcellanid crabs *Allopetrolisthes spinifrons* associate with and migrate between sea anemone hosts multiple times over the course of the crab’s lifespan. These crabs may search for ideal sea anemones to associate with, in that they receive most of their nutrition from the water flow surrounding the anemone, and may seek out hosts that receive high levels of water flow or access to planktonic food in the water column. Another possible explanation for the dispersal of these crabs is the need to search for mates because they live as a single solitary individual on each anemone. The crabs may seek locations with aggregations of anemones, which would allow them to be near other crabs for mating (Thiel et al. 2003). Other porcellanid crabs, *Liopetrolisthes mitra,* associate with sea urchins and exhibit sexually dimorphic behavior in their dispersal patterns. Males move between sea urchin hosts, whereas females are more likely to remain on one host for most of their lives. The sea urchins form aggregations, which allow the crabs to frequently contact conspecifics because dispersal between hosts is not very costly, with low risk of predation or exhaustion (Thiel et al. 2003). Individuals of a third type of symbiotic crab, *Dissodactylus mellitae*, do not leave their sand dollar hosts unless another suitable host is < 21 cm distant, indicating that they do not risk traveling large distances in order to seek out a new home. However, when a new host is available nearby (within 21 cm) and vacant, these crabs disperse to the vacant host (Bell 1984). The maximum body size of the small *L. mitra* crabs on sea urchins is only ~ 2.6 mm length, so they apparently will locomote only up to ~ 80 x their body length between hosts. This decision may indicate that the crabs shift to nearby locations based on a distance-related risk assessment. Alternately, the poor visual acuity of some symbiotic crustaceans (Caves et. al., 2016) suggests that they may simply not be able to visually detect hosts > 21 cm distant. However, crustaceans have well-developed chemosensory abilities and their larvae are known to recruit to host organisms using widely dispersed chemical cues (Arvedlund and Nielsen 1996). Symbiotic shrimp *Athanas indicus* associate with sea urchins, and may switch hosts up to 6 times in 24 hours, with males frequently switching hosts presumably to locate new females for mating, as there are rarely more than 2 shrimp per host

(Gherardi 1991). The urchin hosts examined were ~ 20 cm distant from each other, which may have facilitated frequent shrimp dispersal, and the shrimp are only up to ~ 2 mm body length (Ganapati and Kanakayya-Sastry 1972), and so their distance moved was ~ 100 x the shrimp body size, similar to the dispersal pattern of *L. mitra*. Access to reproductive partners, as well as distance among hosts relative to crustacean body size, therefore, appear to be two important factors that may contribute to controlling rates of symbiotic crustacean dispersal among hosts.

Symbiotic cleaner shrimp associations with sea anemones

Cleaning stations on coral reefs are locations where client fishes visit to receive services from cleaner organisms, in the form of removal of parasites and dead or diseased skin from the fish gills and body surfaces (Limbaugh 1961). The most common type of cleaner shrimp station on Caribbean coral reefs typically consists of a single sea anemone and up to 5 associated cleaner shrimp (Chace 1958; Mahnken 1972). The sea anemone provides protection to the shrimp from predators, because while prey organisms may be stung by nematocysts within the anemone tentacles (Fautin 1991), symbiotic crustaceans are immune to sea anemone toxins (Mebs 2009). The cleaning of an approaching client fish is typically initiated when a cleaner shrimp performs a signal indicating willingness to clean, by vibrating its antennae and/or rocking its body from side to side (Sargent and Wagenbach 1975; Cote 2000; Chadwick et al. 2008). In response, client fishes pose by remaining motionless near the cleaning station with their fins spread (Cote et al. 1998; Cote 2000), and/or positioning their bodies diagonally with the head down, but types of poses vary among fish species (Losey 1971). After the fish poses, the shrimp leaps onto the fish body and climbs over the surface (and even into the mouth and gills) to remove parasites or dead skin, often for several seconds to a few minutes before it swims away from the fish and returns to perch on its host anemone (Huebner and Chadwick 2012a). Therefore, cleaner shrimp are highly mobile both in terms of walking on fish body surfaces and swimming through the water column, during the act of fish cleaning.

The relationship between cleaner organisms and fish clients is a mutualistic symbiosis, in which cleaner organisms benefit nutritionally from material removed from the fish body surface (Cote 2000) and fishes gain reduction of their parasite infections and general stress levels (Limbaugh 1961; Bshary et al. 2007). A major benefit to sea anemones that host cleaner shrimp may be through the uptake of excreted nitrogen from the visiting fish clients, which is used for growth and reproduction of the endosymbiotic microalgae within the anemone (zooxanthellae; Cantrell et. al. 2015). Client fishes are unable to visually perceive the location of small cleaner shrimp from a distance, and instead rely on visual perception of the host anemone to locate a cleaning station (Huebner and Chadwick 2012b). Therefore, the shrimp benefit from residing on a host anemone that visually attracts client fishes, and larger anemones may attract more fishes. Consequently, some shrimp may migrate away from small anemones or those that do not regularly attract fishes, if they are able to locomote to nearby hosts that are larger or better-positioned to attract parasite-laden clients.

Distributional patterns of cleaner organisms and relation to reef biodiversity

Cleaning stations are important for contributing to biodiversity on coral reefs, in that the long-term absence of cleaner organisms results in a decrease in fish species diversity. A field experiment in the Red Sea determined that reef fish species diversity decreased by 18% when cleaner organisms (in this case cleaner fish) naturally disappeared, and by 23% when they were removed experimentally. In the same experiment, natural immigration of cleaner organisms to patch reefs resulted in an increase of 34% in local fish species diversity, and experimental addition resulted in an even larger increase of 42% (Bshary 2003). On the Great Barrier Reef in Australia, the number of fish species that visit a patch reef is 4x higher where cleaner fish are present, compared to areas where they are not (Grutter et al. 2003). In another study in Australia, Waldie et al. (2011) found that 8.5 years after removal of cleaner fish, the abundance of resident reef fishes remained 37% lower than pre-removal numbers, and that removal of cleaner organisms shifted fish size distributions toward smaller individuals. A laboratory experiment also demonstrated that juvenile damselfish are more likely to choose a habitat located near a cleaner fish than one without a cleaner fish nearby (Sun et al. 2016). Additionally, large reef groupers may center their territories around Pederson cleaner shrimp stations on Caribbean coral reefs (Sluka et al. 1999). The presence of cleaner organisms (both cleaner fish and cleaner shrimp) is important therefore not only for fish health, but also for the abundance of various types of fishes on reefs, and thus for the community structure of reefs, in that it influences fish recruitment, body size, mortality, and visitation patterns. As such, information about how cleaning stations naturally vary over space and time is critical for understanding the dynamics of fish community structure (Bshary 2003; Grutter et al. 2003).

Ecology and behavior of Pederson cleaner shrimp

On Caribbean coral reefs, both fishes and shrimps serve as cleaners. *Labroides dimidiatus* is a cleaner wrasse with bright coloration and a stereotypical dance that attracts fish clients (Potts 1973). A recently discovered cleaner, the spotted shrimp *Periclimenes yucatanicus,* is a rare, facultative cleaner that also performs a stereotypical movement when attracting clients (Titus et al. 2017). However, Pederson shrimp *Ancylomenes pedersoni* are the most abundant and effective crustacean cleaners of reef fishes in the Caribbean Sea (Mahnken 1972; Titus et al. 2017). These shrimp are obligate symbionts of corkscrew sea anemones *Bartholomea annulata*, but also associate with other hosts such as rosetip anemones *Condylactis gigantea* (Mascaro et al. 2011). To signal willingness to clean, these shrimp use a stereotypical side-to-side body swaying or rocking motion (Becker et. al. 2005), and rapid vibration or whipping of their long paired antennae (Mahnken 1972). Their behaviors that signal willingness to clean directly represent their hunger level (Chapuis and Bshary 2012), in that more signaling occurs when a shrimp is starved. Food availability may affect not only shrimp signaling rate, but also their choice of habitat; it is possible that shrimps which experience high food availability remain on their host anemones, while those experiencing low food availability (relatively few cleans) may depart from their original host to seek a different host where more cleans are possible. Field observations confirm that more cleaner shrimp occur on sea anemones in reef areas where there are relatively high levels of reef fish traffic (Mahnken 1972; N. E. Chadwick, pers. comm.).

Obligate symbionts are expected to remain with their hosts, and Pederson shrimp are defined as obligate associates, in that they occur only on or near sea anemones. However, Pederson shrimp appear to be flexible in terms of fidelity to individual hosts, in that they may occasionally leave their original hosts and migrate to new anemones (Mahnken 1972). This migratory behavior is similar to that of some Indo-Pacific cleaner shrimps which migrate among host anemones nocturnally (Chadwick et al. 2008), and of other crustaceans that migrate among their invertebrate hosts (Cowell et al. 1993; Thiel et al. 2003; see section above). Little is known about the factors that cause cleaner shrimp to disperse among anemone hosts. However, recent investigation revealed that *A. pedersoni* occur in social groups structured by size-based dominance hierarchies. Large dominant shrimp within the hierarchy tend to occupy optimal habitat in the middle of the anemone and on the tentacles, while small subordinate shrimp occupy the periphery of the tentacles or substrate near the anemone (Mahnken 1972; Gilpin and Chadwick 2019). This habitat partitioning may cause dominant shrimp to receive the most cleans, leaving subordinate shrimp with fewer cleans and lower nutritional input (Gilpin and Chadwick 2019). Pederson shrimp are rarely found in social groups larger than four individuals; the reasons for this are unclear, but social group size may be limited by food resource levels, in the form of the number of cleans that each shrimp can receive on the anemone (Huebner and Chadwick 2012a). As well, immediately after molting, crustaceans regardless of their previous social rank, move to the bottom of their social hierarchy due to the exoskeleton being temporarily soft and vulnerable (Bovbjerg 1953).

As such, juvenile and male Pederson shrimp, or newly molted individuals, may disperse among hosts to locate vacant hosts or those with smaller social groups, where they potentially can obtain more fish cleans. The number of visiting fish clients and the social group size of shrimp also increases with host anemone body size (Huebner and Chadwick 2012a), so cleaner shrimp may migrate based on the relative body sizes of neighboring host anemones. Interactions among several types of factors (relative host size, shrimp social group size and relative body size within social groups, shrimp physiological state, and distance to nearest host neighbor) likely influence the dispersal rates of shrimps among hosts.

Ecology of sea anemones that host Caribbean cleaner shrimp

Corkscrew sea anemones *Bartholomea annulata* are the most common sea anemones in the Caribbean Sea (Briones-Fourzan et al. 2012; Titus et al. 2017), and are major hosts for Pederson cleaner shrimp (Gilpin and Chadwick 2017; Huebner et al. 2019). They are typically found with their pedal discs attached underneath or between rocks, and with their tentacles extended out into the water column (Titus et al. 2017). They also tend to prefer living in the reef channels, as opposed to fore reefs or back reefs (Briones-Fourzan et al. 2012). They reproduce sexually via broadcast spawning semi-annually, and asexually via pedal laceration year-round (Jennison 1981; O'Reilly and Chadwick 2017; Titus et al. 2017). However, it has been observed that aggregations of *B. annulata* in the Florida Keys possess high genetic diversity, and therefore are most likely the product of sexual reproduction (Titus et al. 2017). Smaller individuals undergo pedal laceration more often than do larger individuals (Briones-Fourzan et al. 2012; Titus 2011), which may be a response to a lack of food or inability to remain attached to the substrate (Clayton 1985; Goldberg 2013).

Because *B. annulata* anemones serve as visual cues for fish searching for cleaning stations on Caribbean reefs, as the size of an anemone increases, the number of fish who pose for cleaning increases as well (Huebner and Chadwick 2012b; O'Reilly and Chadwick 2017). As the tentacle crown surface area (TCSA) of *B. annulata* increases, the probability that the anemone contains associated crustacean symbionts also increases, on coral reefs in both the Mexican Yucatan (Briones-Fourzan et al. 2012) and the U.S. Virgin Islands (Huebner et al. 2019). Therefore, large *B. annulata* anemones contribute to biodiversity in Caribbean reef systems by serving as hosts for up to 7 species of crustaceans (Briones-Fourzan et al. 2012; Brooker et al. 2019; Huebner et al. 2019). These crustaceans often co-occur on individual anemones, and significantly partition their use of microhabitats on the host, in that some species utilize the column or base of the anemone as a source of shelter, others occur among the tentacles, and Pederson shrimp often reside on the top of the tentacle crown (Briones-Fourzan et al. 2012) or on substrate near the anemone (Huebner et al. 2019). Thus, sea anemones represent large, physically complex habitats for cleaner shrimp, and Pederson shrimp also segregate their microhabitat use among conspecifics on each anemone, in that large females occur closer to the center of the anemone than do small individuals (Gilpin and Chadwick 2019). This spatial pattern of social group structure may influence the dispersal of shrimp among anemones, because small peripheral shrimp probably receive fewer cleans and may be more likely to disperse than the more central large shrimp. Despite information on the various potential causes of dispersal by cleaner shrimp among host anemones, no observational or experimental studies to date have quantified the patterns and causal factors for this important dispersal behavior on coral reefs.

Thesis research goals

The overall goal of this research thesis is to quantify the patterns and causes of association and dispersal behavior by Pederson cleaner shrimp among host sea anemones. In particular, I report here about: (1) impacts of shrimp body size and social rank on host association patterns, as determined through laboratory experiments (Chapter 2), and (2) patterns of shrimp dispersal and their relation to various ecological factors, as examined through field observations on coral reefs at St. Thomas, U.S. Virgin Islands (Chapter 3). These laboratory and field investigations relate to each other, in that the laboratory experiments elucidate some of the causal factors controlling host association and dispersal patterns, and the field observations quantify how those patterns vary with possible causal factors under natural field conditions.

Together, these 2 types of investigations provide important new information about aspects of the dispersal biology of cleaner shrimps on coral reefs**.**

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Chapter 2

Association patterns of Pederson cleaner shrimp *Ancylomenes pedersoni* with sea anemones: Impacts of shrimp body size and social rank

Introduction

Patterns of animal dispersal alter how predators and prey interact with each other, and consequently influence major aspects of ecosystem structure, such as food web architecture and nutrient cycling (Holdo et al. 2011). Many variables contribute to decisions by animals to disperse among habitat patches. For animals that associate symbiotically with host organisms, the host serves as a discrete habitat patch, and in order to locate a mate or escape competition for limited resources, individuals may be compelled to disperse among hosts (Baeza and Thiel 2003; Dennenmoser and Thiel 2006).

Dispersal patterns have been examined for some symbiotic crustaceans and indicate that factors such as body size of both crustaceans and their hosts, as well as sexual reproductive status may influence dispersal. For porcelain crabs *Allopetrolisthes spinifrons* that associate with sea anemones, dispersal may occur to locate hosts with relatively better access to planktonic food arriving in the water column (Baeza and Thiel 2003; Baeza and Stotz 2001). Other crustaceans such as porcelain crabs *Liopetrolisthes mitra* that associate with sea urchins, are known to move between hosts to search for mating partners (Thiel et al. 2003).

Crustaceans that provide cleaning services to fishes are essential in maintaining and enhancing biodiversity in coral reef ecosystems. Cleaning stations are reef locations that client fishes visit to receive services from cleaner organisms, in the form of removal of parasites and dead or diseased skin from fish gills and body surfaces (Limbaugh 1961). The cleaner organisms benefit nutritionally from material removed from the fish surface (Côte 2000) and fishes gain reduction in the number of parasites and general level of stress (Limbaugh 1961; Bshary et al 2007). On some coral reefs, sea anemones serve as the centers of cleaning stations, as cleaner shrimp symbiotically associate with them, and perch on the anemone tentacles to signal to passing fishes that they are willing to clean (Chadwick et al. 2008; Huebner and Chadwick 2012a). The sea anemone hosts also benefit nutritionally from this multi-level symbiosis by absorbing dissolved nitrogen excreted by fish that pose near the anemone while being cleaned; the nitrogen is used for the growth and reproduction of their associated endosymbiotic microalgae (reviewed in Cantrell et al. 2015) Therefore, dispersal patterns of cleaner shrimps among sea anemone hosts likely impacts not only the dynamics of fish cleaning on reefs, but also the health and stability of the anemones that are the centers of the cleaning stations.

Long-term absence of cleaner organisms from reefs, which may occur when cleaners disperse away from reef areas, causes decreased reef species diversity: by 18% when cleaner fish naturally disappear from a reef, and by 23% when they are experimentally removed. Conversely, natural immigration of cleaner organisms results in an increase of 34% of reef fish species diversity, and experimental addition in an increase of 42% (Bshary 2003). Additionally, the number of fish species that visit patch reefs are 4x higher in locations where cleaner fish are present, compared to areas where they are not (Grutter et al. 2003). Changes in cleaner shrimp presence on reefs also impacts reef fish diversity (work on Great barrier reef, see refs by Gruter etc.). The presence of cleaner organisms, therefore, is important not only for the health, but also the diversity and abundance of fishes on reefs. As such, information about how cleaning stations vary over space and time is critical for understanding the dynamics of fish community structure (Bshary 2003).

Pederson cleaner shrimp *Ancylomenes pedersoni* are the most prevalent and effective crustacean cleaners of reef fishes in the Caribbean Sea (Mahnken 1972; Titus et al 2017). They also are obligate associates of sea anemones; they occur with several host species but prefer corkscrew anemones *Bartholomea annulata* (Mascaro et al. 2011). Pederson shrimp have been documented to occasionally leave their hosts and disperse to new anemones (Mahnken 1972, Chapter 3). The various causes of this dispersal among anemones remain unclear, however recent investigation revealed that *A. pedersoni* occur in social groups structured by size-based dominance hierarchies (Gilpin and Chadwick 2019). Large, dominant individuals occupy the center of the anemone tentacle crown, causing small subordinate shrimp to occupy the periphery of the tentacles or the area around the anemone base (Mahnken 1972; Gilpin and Chadwick 2019). In addition, the number of fish clients and the social group size of shrimps increases with host anemone body size (Huebner and Chadwick 2012a) Therefore, these cleaner shrimp may disperse based on their relative body sizes or status in social groups, and also the relative body sizes of neighboring host anemones. Interaction among several types of factors likely influence the dispersal rate of these shrimps among hosts and are important to understand as they may affect the stability and dynamics of fish cleaning stations in the Caribbean Sea.

The goal of the present study was to experimentally investigate the effects of both body size and social rank on patterns of association by Pederson shrimp *Ancylomenes pedersoni* with host sea anemones *Bartholomea annulata* under laboratory conditions. We hypothesize that the level of association by cleaner shrimp with host sea anemones increases with both shrimp body size and social rank. We predict that as body size increases when shrimp are alone, and as relative social rank increases when they are in groups, shrimp will associate more closely with host sea anemones. Support for this hypothesis would suggest that small or subordinate shrimp disperse among host anemones more frequently than do large or dominant shrimp, thus causing a size-structured effect on cleaning station dynamics on Caribbean reefs.

Methods

Animal collection and culture

To address the hypothesis that the level of association by cleaner shrimp with host sea anemones increases with shrimp body size and social rank, we conducted laboratory experiments between September 2018 and August 2019. Pederson cleaner shrimp *Ancylomenes pedersoni* and host sea anemones *Bartholomea annulata* were obtained from a professional collector (KP Aquatics, Key Largo, Florida, USA), or collected by hand from coral reefs at St. Thomas, U.S. Virgin Islands (USVI). Organisms were shipped within a few days of collection to Auburn University, where they were cultured in closed-system tanks. Three shipments of shrimp arrived to the laboratory prior to the present study, in January 2017 (6 shrimp), January 2018 (N = 4 shrimp), and April 2018 (10 shrimp; all from Florida). Some of these shrimp were monitored for a laboratory-based life-history study (Gilpin and Chadwick 2017), prior to their use in the present study. An additional 25 shrimp were hand-collected in the USVI in May 2019 (N = 45 shrimp total). Sea anemones arrived to the laboratory in June 2018 (N = 10, from Florida), November 2018 (N = 12, Florida), May 2019 (N = 6, USVI, hand-collected), and June 2019 (N =

5, Florida; N = 33 anemones total). All arriving Pederson shrimp were allowed a 2-3 week acclimation period before use in trials.

Shrimp were cultured in small 40-L tanks (each 50 cm length x 25 cm width x 30 cm height) filled with artificial seawater (Instant Ocean, Sea Salt Mix) and equipped with external hanging filters and internal heaters. Flexible plastic mesh was wrapped around the intake tube of each filter to prevent shrimp being sucked into the filters. Lighting was provided by overhead fluorescent ceiling lights set to a 12L:12D cycle. To prevent shrimp from jumping out of tanks, plastic grating (15 mm grid size, economy grade polystyrene; Louvered Ceiling Light Panel, PLASKOLITE) covered each tank top. A thin layer (~ 2 cm thick) of course gravel covered the bottom of each tank. Tanks were maintained at ~ 25°C seawater temperature, ~ 34 ppt salinity, and low levels of dissolved nutrients to mimic natural coral reef conditions. A water change of ~50% was performed monthly to maintain low nutrient levels and buildup of microalgae and bacteria. During routine culture, shrimp were fed every 2-3 days to satiation with pellets (Formula One Pellets, Ocean Nutrition). They were cultured in groups of 3-4 individuals per tank, with at least ~1 mm body size difference (carapace length [CL]) between adjacent individuals, to mimic natural social group composition and to facilitate individual identification (after Gilpin and Chadwick 2017). Shrimp thrived under these culture conditions, as evidenced by their exhibition of normal behaviors (active feeding, signaling toward moving objects, locomotion, and social interactions with other shrimp), as well as continuous body growth (in small individuals) and sexual reproduction (in large individuals), similar to observed during previous culture of this species in the same laboratory (Gilpin and Chadwick 2017, 2019; Cantrell et al 2015). Shrimp were cultured without any sea anemones present in the culture tanks, and subsequently were selected randomly for use in laboratory experiments (see details below), to prevent any effect of shrimp residence time in the laboratory, or of their recent association patterns with anemones, on the outcomes of the shrimp behavioral experiments.

To precisely measure shrimp body sizes and determine sexual reproductive status, after they had acclimated to laboratory conditions for at least 2-3 weeks, each shrimp was removed from its culture tank using a hand net. The shrimp was placed in a shallow plastic petri dish (88 x 13 mm) with ~ 2 mm depth of seawater from its culture tank, to allow shrimp respiration but prevent locomotion, thus facilitating examination under a dissecting scope at 40x magnification. Carapace length was measured to the nearest 0.1 mm using calipers, and each shrimp was examined for the presence of oocytes in the dorsal ovaries and/or abdominal brood pouch, developing embryos in the brood pouch, and/or breeding dress (enlarged space below the abdomen, which allows for protection of brooded embryos Bauer, 2004). If any of these features were present, the shrimp was classified as female (F; body size range = 3.1 - 6.3 mm CL, N = 17 females). If lacking, the shrimp was recorded as non-reproductive (NR; see body sizes below). After measurement, the shrimp was returned to its culture tank, and was out of the tank < 2 min; shrimp recovered rapidly from this process, as indicated by their pleopod beating (respiration rate) returning to a normal slow level within 5 min (after Gilpin and Chadwick 2017).

To determine if any NR shrimp were males, those that were large enough to potentially be male (> 3.4 mm CL, Gilpin and Chadwick 2019) were moved to tanks that contained known females. If females in the tank began to brood fertilized embryos, the only NR shrimp in that tank then was classified as male (M; body size range = 3.4 - 4.0 mm CL, N = 5 males; after Gilpin and Chadwick 2017). Shrimp that did not fertilize females when cohabiting in tanks, or that were well below potential size at sexual maturity then were reclassified from NR to juveniles (J; body size range = 2.1 - 4.0 mm CL, N = 23 juveniles). The body size and sexual reproductive status of each shrimp was re-assessed every 2-3 weeks for the duration of the study. Of the 45 individuals cultured for ~ 4 - 18 months, 8 died due to accidents (sucked into filter, jumped out of tank, etc.), 1 died due to attack by a larger shrimp (C. Winn, pers. obs.), and 8 died following senescence at the end of the natural lifespan of ~ 1-2 years (Gilpin and Chadwick 2017).

Sea anemones were cultured in large tanks equipped with overhanging lights due to their hosting of endosymbiotic microalgae. Each 80-L sea anemone culture tank was equipped with an overhanging filter, heater and small protein skimmer attached to the outside of the tank. Each tank light (Light Emitting Diode (LED); Galaxyhydro, 65Watt) was set to a 12L:12D cycle, and produced ~ 100 microEinsteins m^{-2} sec^{-1} of photosynthetically active radiation (PAR), as measured near the anemone tentacles using a Biospherical Instruments Light Sensor (model QSL-2001, manufacturer Biospherical Instrument, San Diego, CA, USA) equipped with a submersible probe. This irradiance level was within the range observed in reef habitats where these anemones naturally occur (O'Reilly et al. 2018). A 50% water change was performed every 2 weeks, and up to 10 anemones were cultured per tank. They were fed weekly to satiation with small pieces of raw cocktail shrimp or thawed brine shrimp (Frozen Brine Shrimp, San Francisco Bay Brand, Inc.). Anemone body sizes were recorded occasionally during culture (~ once per month), by using a plastic tape measure to measure their tentacle crown length and width for calculation of tentacle crown surface area (TCSA, after O'Reilly and Chadwick 2017; body sizes ranged 5 - 254 cm^2 TCSA). Most appeared to exhibit healthy physiological condition, in that they attached their pedal disks to the tank bottom with their lower columns surrounded by gravel, continuously expanded their tentacles, exhibited normal brown coloration indicating abundant microalgae in their tissues, and either grew in body size or did not noticeably shrink (as observed during past long-term culture of this species in the same laboratory; Cantrell et al. 2015). Anemones were not identified individually, because they were used only in shrimp behavior experiments, and their behavior was not quantified in the present study. The few anemones that exhibited bleaching or rapid body shrinkage were not utilized as hosts during shrimp behavioral trials.

Laboratory experiments

Laboratory experiments were conducted in observation tanks that were identical to shrimp culture tanks (see above), except for the following features. The walls of each observation tank were wrapped with neoprene blackout cloth, to create blinds that prevented the shrimp from receiving visual stimuli from outside the tank, but that allowed overhanging lights to shine into the tanks. A plastic measuring tape (~ 40 cm length) was placed on the gravel along the inside edge of each tank, with a small rock at each end to hold it down, as a guide for estimating distances during behavioral observations. A small ceramic or glass bowl also was placed at each end of the tank, with the 2 bowls ~ 40 cm apart; gravel from the tank bottom was placed inside each bowl. These bowls mimicked small patch reefs surrounded by gravel, which is the most common natural habitat for corkscrew sea anemones *Bartholomea annulata* that attach their bases to hard substrate at the reef-sand interface (Briones-Fourzan et al. 2012).

Shrimp were starved for 2-3 days before their use in behavioral experiments and were not fed during experimental trials. To test for effects of body size (small vs. large, when solitary) and social rank (small vs. large, when paired in simple social groups) on association patterns of shrimp with host sea anemones, the shrimp were tested in 2 types of treatments: when solitary (only 1 shrimp in the observation tank; effect of body size alone) and when paired (simple social group of 2 shrimp in the observation tank; effect of social rank as indicated by relative body size). Social rank was defined as position in the social dominance hierarchy in relation to other shrimp within the observation tank; the larger shrimp in the tank occupied a more dominant position in the social hierarchy, and thus a higher social rank than did the smaller shrimp (after Gilpin and Chadwick 2019). These treatments thus examined separately the behavioral effects of variation in shrimp body size when alone, versus effects of relative shrimp body size (social rank) when paired. Each shrimp was observed in up to 3 replicate sessions per type of treatment trial, for both solitary and paired treatments (N = 21 solitary treatment trials and 30 paired trials, see below for details). Most shrimp were used in only one type of treatment trial (solitary or paired), but due to the limited number of shrimp available in the laboratory at any one time (see above), some shrimp were used in both solitary and paired trials (4 small shrimp, identification codes AP16, AP22, AP28, and AP37; and 8 large shrimp, identification codes AP5, AP8, AP14, AP15, AP18, AP30, AP34, and AP35).

On Day 1 of each solitary treatment trial, a haphazardly-selected anemone was removed from its culture tank by gently prying it off the substratum with a fingertip, and transferring it to a small beaker underwater. This process allowed the delicate pedal disk of each anemone to remain intact. Some of the beaker water was poured out, and the beaker then was floated in the observation tank for ~ 15 min, with a small amount of observation tank water added every few min, to acclimate the anemone to the observation tank water. Then the beaker was fully submerged, and the anemone was placed carefully in one of the bowls inside the tank. It was allowed up to ~ 1 hour to attach to the bowl surface, re-expand its tentacles, and become fully acclimated to the observation tank before a trial was begun. If the anemone remained contracted or did not attach its pedal disk to the substrate, it was removed, and the process was repeated with another haphazardly selected anemone from the culture tanks (~ 5% of trials). After the anemone had acclimated to the observation tank, a shrimp that was classified as either small (body size range = 2.6 - 4.5 mm CL; juveniles, males, and small females, see above) or large (4.6 - 6.5 mm CL; only large females) was selected randomly and then transferred from its culture tank to the observation tank using a hand net.

Initially, behavioral trials were recorded using video cameras. To video record shrimp behavior during each trial, 2 metal stands each with a clasp arm were placed in front of the observation tank, with the arms extending over the tank. A Go-Pro camera (Hero 4 Black, Go-Pro) with water-proof protective casing was secured in the clasp of each arm, pointing downward and with the camera lens just underneath the water surface inside the tank. The 2 cameras were positioned with slightly overlapping fields of view, to obtain full video frame coverage of the bottom of the tank. The cameras were set to record for the duration of behavioral observations (2 hours each day, see below) in time-lapse mode on each of the 3 days of each trial, at the standard Go-Pro video recording speed of one image every 2 seconds. Resulting digital time-lapse video files were uploaded from the microSD card in each camera to a laptop computer for analysis. To determine the location of each shrimp during each 10-min sample interval, a screenshot was taken at every 10 min interval (33 seconds of time-lapse video) when viewing the video, and the photograph was transferred to the image analysis software ImageJ.

A reference length was created in ImageJ using the ruler on the bottom of the tank, and in each image, the body size of each sea anemone was recorded as TCSA (cm^2), as well as the distance from each shrimp to the nearest anemone, and the distance between shrimp if they were paired. After 19 trials were video recorded, it was determined that the same information could be obtained more efficiently using direct behavioral observations. Therefore, for the remaining 21 trials an observer directly recorded information on shrimp behavior during each trial. Care was taken to observe shrimp from the edge of the observation tank, to prevent the shrimp from seeing the observer. Because the shrimp did not appear to react to observer presence, in that they did not approach or signal toward the observer (vibrate antennae or laterally sway the body) as done when observers (or fish models) approached tanks that did not have blinds (Caves et al. 2016), we assumed that the shrimp did not sense observers during behavior trials. During each of 3 days in each trial, we observed the shrimp every 10 min for 2 hours (120 min total / 10 min per observation = 12 observations per day), and recorded the following information during each observation period: (1) whether the shrimp associated (< 5 cm distant) with the original anemone or not (based on natural association distances of these shrimp with host anemones on coral reefs, Gilpin and Chadwick 2019), (2) distance of shrimp to anemone (measured to nearest cm using the measuring tape guide, see above), and (3) microhabitat zone occupied by the shrimp. We randomized any effects of variation in the observational methods used (analysis of video recordings vs. direct observations on the tanks), by randomly assigning shrimp to behavioral treatment trials that were interspersed temporally over the course of the study (see details above).

Six microhabitat zones were defined, based on the zones naturally occupied by shrimps associated with *B. annulata* sea anemones on coral reefs (Huebner et al. 2019): Zone 1 (under tentacle crown along column, adjacent to pedal disc), Zone 2 (inner half of tentacle crown), Zone 3 (outer half of tentacle crown), Zone 4 (near tentacle crown on hard substrate, < 5 cm distant on tank wall or bowl edge), Zone 5 (near tentacle crown on soft substrate, < 5 cm distant on gravel), and Zone 6 (not associated with anemone, > 5 cm distant; Fig. 2.1). These 6 zones were similar to those defined by Gilpin and Chadwick (2019) based on field observations of Pederson shrimps, with definitions modified here for the laboratory environment (compare Figs. 2.1 and 2.2). We also noted behaviors exhibited by the shrimp, such as locomoting across the gravel on the tank floor, climbing the tank walls, swimming in the water column, signaling (vibrating antennae, swaying body), or foraging (using chelae to pick items off substrate and moving chelae toward mouth). During the 2 hours of behavioral observations each day, the tank filter was turned off so that the small transparent shrimp could be observed easily through the still water surface, then it was turned back on after the ~2 hour trial period. This brief period of still water mimicked natural conditions on some coral reefs, in which shallow patch reefs in enclosed lagoons may experience little to no water flow during slack or low tides, and so was considered to be appropriate for behavioral observations.

On Day 2 of each behavioral trial, a second haphazardly selected anemone was added to the second bowl in the tank but was not allowed an acclimation period before behavioral recording occurred, to determine the shrimp's reaction, or lack thereof, to the added anemone. The added anemone was used in order to test the extent to which shrimp moved between anemones and associated with their initial host (original anemone) or moved to a second potential host (added anemone). The 2 anemones were located ~ 30 to 35 cm apart in the tank (i.e.: at opposite ends of the tank), to maximize the distance between them. This distance was similar to anemone nearest neighbor distances at some field sites, where these anemones occur at abundances of up to 5 individuals per m^2 (O'Reilly and Chadwick 2017) and in some cases cluster in small aggregations (Titus et al. 2017). The same behavioral observations were conducted on Day 2 as on Day 1, with minor modification; data were collected on: (1) whether the shrimp associated with the original or added anemone, (2) distance of the shrimp to the nearest anemone, and (3) microhabitat zone in relation to the nearest anemone.

On Day 3, the behavioral observations again were repeated, then the shrimp was removed from the observation tank and returned to its culture tank using a hand net. The body size (TCSA, cm^2) of each anemone also was recorded, and the anemones were returned to their culture tanks. A 50% water change was conducted in the observation tank after each 3-day behavioral trial, to reduce the presence of residual anemone mucus, which also was removed by the tank filter. Behavioral trials were repeated using a different shrimp and anemones each time, for small (N = 11) and large shrimp (N = 10), resulting in a total of 21 3-day trials on solitary shrimp. Behavioral trials on paired shrimp followed the same methods, except that 2 shrimp were selected for each trial, 1 small and 1 large, and were placed together in the observation tank (N = 13 small and 14 large shrimp, for a total of 30 paired shrimp behavioral trials; some shrimp were used more than once, see above for details). Trial order (solitary vs. paired) was randomized.

Data analysis

To determine if shrimp behavior during experimental trials varied with their date of arrival to the laboratory, a Kruskal-Wallis test was performed to compare the proportion of time that shrimp spent with each anemone (original anemone, added anemone, neither anemone, see above), between groups of shrimp that arrived on each date. Four groups were created, one for each arrival date (see details above), and the groups were compared in terms of their association behavior with each of the 3 types of anemone. Because some shrimp individuals were used in trials more than once, we averaged the values for each individual in the proportion of time with each anemone and in each microhabitat zone in order to reduce the effect of individual variation on the results. Outliers (those values outside of the 1.5*IQR range) were detected using R and removed from the data pool before the values were averaged (see Table 2.1 for frequency of outliers). To determine if there were differences between each treatment (paired and solo) and each shrimp body size class (large and small) in the percent time with each anemone (comparisons: LP vs SP; LP vs LS; SP vs SS; LS vs SS), a Kruskall-Wallis test was conducted, with a Dunn post-hoc pairwise analysis. A Kruskall-Wallis test was also conducted to determine differences between each category (paired and solo) and each size (large and small) in proportion of time in each microhabitat zone, with a Dunn post-hoc test for pairwise comparison. To determine if the size of the anemone (TCSA) had an effect on time spent with either OG or AD, a simple linear regression was done in R, and the R^2 and p-value were assessed.

Data for distance to nearest anemone and between paired shrimp was averaged similarly to the other data sets (see above), and the outliers were removed prior to averaging. To compare the distances between paired shrimp on each day of the three-day trial period, and the distances to the closest anemone by each treatment (paired and solo) and size category (large and small), a Kruskall-Wallis test was conducted in R, with a pairwise Dunn post-hoc analysis. This test compared the median values of the averaged distances on each day with those of each other day, for both data sets.

Results

Percent time associated with host anemones

The proportion of time that shrimp spent with each type of anemone did not differ significantly among the groups of shrimp that arrived to the laboratory on different dates

(Kruskal-Wallis, all p-values > 0.09). On all 3 days of each experimental trial, regardless of whether they occurred in pairs or as solitary individuals, both types of shrimp (large and small) spent most of their time either associated with the original anemone in the observation tank, or wandering around the tank and not associated with either anemone (Fig. 2.3). When they occurred as solitary individuals in the tank, the behavioral patterns of both large and small shrimp did not differ significantly from each other (Table 2.4C). When alone, by Day 3 both types of solitary shrimp spent ~ 35-45% of the time (median times) associated with the original anemone in the tank, only ~ 2-23% of the time associated with the second anemone that was added to the tank on Day 2, and ~ 23-45% moving around the tank during which they were not associated with either anemone (range of median times; Fig. 2.3). When alone, small solitary shrimp took somewhat longer to associate with the original anemone in the tank than did large shrimp, in that small shrimp spent a relatively high median percent of their time not associated on Day 1, but then by Day 3 spent as much time as did large shrimp in association with the original anemone. Interestingly, neither shrimp spent much time initially with the second anemone after it was added on Day 2. They both spent an increasing but still relatively small proportion of time with the added anemone, by Day 3.

These behavioral responses changed when shrimp were placed in pairs together in the observational tanks, but only for small shrimp. Large shrimp when paired did not significantly alter their percent time allocations from the behavioral pattern that they exhibited when alone (Fig. 2.3; Table 2.4A). In contrast, when paired the small shrimp spent significantly less time associated with the original anemone than did large shrimp, during all 3 days of observation (Fig. 2.3, Table 2.4D). Small shrimp spent only ~ 5 to 17 % of their time (range of median times) with the original anemone when a large shrimp was present, while large shrimp continued to occur with the original anemone ~ 31 to 62% of the time (Table 2.2). Conversely, small shrimp also spent significantly more time moving around the tank and not associated with an anemone, than did large shrimp when both were together, during all 3 observation days (Fig. 2.3, Table 2.4D). Similar to their behavior when solitary, both types of paired shrimp spent little time with the second anemone when it was added to the tank on Day 2, but this percent increased to ~ 0 to 5% by Day 3 (range of median times, Fig. 2.3).

The percent time spent with each anemone (original or added) did not vary significantly with anemone body size, on any of the 3 observation days per trial (variation with body size of original anemone on Day 1, $R^2 = 0.0247$; Day 2, $R^2 = 0.0039$; Day 3, $R^2 = 0.0004$; $p > 0.1$ for all 3 days; with body size of added anemone on Day 2, $R^2 = 0.0010$; Day 3, $R^2 = 0.0013$; $p > 0.1$).

Percent time in each microhabitat zone

In terms of their occupation of microhabitat zones on the anemones, during most observation days both types of shrimp, regardless of whether they were paired or solitary, occurred most frequently in Zones 4 & 5 (on substrate < 5 cm from an anemone) or in Zone 6 (not associated with an anemone; Fig. 2.4, see Table 2.6A-D). Overall, the shrimp divided their time roughly equally between Zones 4/5 and Zone 6. Shrimp rarely occupied Zones 1 and 2 (along the anemone column or in the inner tentacle crown, respectively), and because of their rarity, all instances of Zone 1 Zone 2 were considered outliers and removed from the data pool. The shrimp also rarely occurred in Zone 3 (outer half of the anemone tentacle crown, Fig. 2.4). These results indicate that within 72 hours of introduction to a new host anemone, *A. pedersoni* shrimp do not utilize these areas of sea anemones. However, a lack of stimuli for shrimp in this laboratory experiment (i.e. moving fish stimulus), may have prevented shrimp from utilizing these zones.

When the shrimp interacted as solitary individuals with anemones in the observational tanks, they did not differ significantly in the percent time that large vs. small shrimp spent in each zone, except for slightly (but significantly) more time spent by small than large shrimp in Zone 3 on Day 2, and in Zone 4 on Day 3 (Fig. 2.4, Table 2.7D). However, when paired, the large vs. small shrimp differed significantly in their use of specific microhabitats on the anemones (Fig. 2.4, Table 2.7C). Large shrimp spent significantly more time in Zones 3-5 (on or near anemones) than did small shrimp, during most observation days, as the small shrimp rarely occurred in the outer tentacle ring or tended to perch on substrate near an anemone. Conversely, when paired the small shrimp shifted their microhabitat use to spend significantly more time than did large shrimp in Zone 6, not associated with an anemone (Fig. 2.4, Table 2.7C). Few significant differences were seen in microhabitat zone utilization in either type of shrimp when paired versus when solo. No significant differences were found for the large shrimp (Table 2.7A), but differences were found for small shrimp in Zone 4 on Day 1 and D3, and Zone 3 on Day 2 (Table 2.7B).

Distance to nearest anemone and between shrimp

Overall, the shrimp were observed to occur ~ 3-22 cm from the nearest anemone (range of median distances; Fig. 2.5). Their minimum and maximum observed distances to the nearest anemone ranged widely from 0 to 45 cm for small shrimp, and 0 to 39 cm for large shrimp. Small shrimp moved slightly closer to anemones as the experiment progressed, from 22 and 14 cm distant on Day 1 (median distances, Table 2.8) to only 7 and 5 cm distant by Day 3, when they were both solitary and paired, respectively, but this trend was not significant (Table 2.9B). Large shrimp also moved slightly closer to the anemone from Day 1 to Day 2, but only the large, paired shrimp displayed a significant decrease in distance, possibly suggesting territoriality of the sea anemone, when a smaller conspecific is present (Table 2.9A).

The observed pattern of distances between paired shrimp did not differ significantly among the 3 observation days of each experimental trial (KW, χ^2 =0.141, df = 1, p = 0.93, Fig. 2.6). However, an increase was observed in the number of trials in each distance category, between Day 1 and Day 2 from 0-10 cm up to 10.2-20 cm, indicating active avoidance between shrimp after 24 hours (Fig. 2.6.). This increase did not continue into Day 3. We observed social interactions between the small and large shrimp, in the form of aggressive behavior, specifically chasing, by large shrimp toward small ones (observed 13 times), and submissive behaviors, specifically retreating, exhibited by small shrimp in response to large ones (observed 12 times). No instances of attack by the large shrimp on the small shrimp was observed in experimental trials, but was seen in culture tanks, with one instance resulting in death of the smaller shrimp. The large shrimp were often observed grabbing the tentacles of the sea anemone with their chelae (observed 13 times), whereas the small shrimp were rarely seen exhibiting this behavior (observed 2 times).

Discussion

General comments

We experimentally demonstrate here that position in the social dominance hierarchy strongly impacts the anemone association behaviors of Pederson cleaner shrimp *Ancylomenes pedersoni* under laboratory conditions. Our results show that when solitary shrimp (i.e., not in social groups) encounter sea anemones, both small and large individuals behave similarly toward potential host anemones. However, when they are placed into simple social groups (one large and one small individual), the responses of these shrimp differ strikingly depending on their relative body size, and hence their social rank in the dominance hierarchy known for this species (Gilpin and Chadwick 2019). In the presence of large shrimp, small ones spent less time associated with anemones and occupy microhabitat zones further away from the center of anemones. In contrast, large shrimp do not alter their anemone association behaviors based on the presence of small conspecifics. It is notable that these shrimp tend to avoid each other under laboratory conditions, in that they occur farther away from each other in laboratory tanks than predicted by chance alone. Large shrimp exhibit dominant behaviors toward small ones (approach and chasing behaviors) that cause small ones to retreat away from host anemones, and thus to spend time further away from anemones than when the small shrimp are alone. Therefore, the combination of complex association behaviors exhibited by these shrimp toward host anemones (percent time with host, microhabitat use, distance from host, spacing among shrimp on hosts) all likely are strongly affected by aggressive behaviors on the part of large shrimp toward small conspecifics, which react with responses that indicate subordinate social rank, such as retreat (Gilpin and Chadwick 2019). The various responses of shrimp to experimental manipulation of their social rank in the laboratory all indicate that in the field, small shrimp are likely to disperse more frequently among host anemones than are large shrimp, especially when they occupy relatively low social rank on a given anemone.

Percent time associated with host anemones

Our results indicate large *A. pedersoni* shrimp exhibit behavior related to their position in the social dominance hierarchy, even in the absence of conspecifics. Large shrimp may spend significantly more time associated with an anemone than do small shrimp due to their territorial defense of the host, a pattern which has been demonstrated for other species of symbiotic crustaceans (Thiel et al. 2003).

The significant increase in time that large solitary shrimp spent associated with the added anemone on the final day of observation (Day 3) indicates possible environmental exploration by large shrimp in the absence of another shrimp. Further research is needed to determine the extent to which the host fidelity of large, sexually mature shrimp is affected by various additional types of conspecifics in social groups (presence of mates, of other similarly sized individuals, etc.). Based on our field observations, some large shrimp move among anemones among days within a given week (Chapter 3). However, the factors that motivate this dispersal remain unknown. Possible factors include potentially searching for nearby anemones that may be more beneficial to them based on the presence of conspecifics for mating purposes, relatively high visibility to roaming client fishes, etc.

Percent time in each microhabitat zone

The microhabitat-use patterns of Pederson shrimp observed here under laboratory conditions are strikingly similar to those observed on coral reefs, in which individuals most frequently occur on substrate near anemones, or sometimes on the tips of anemone tentacles, but not in more sheltered microhabitats on the anemones (Fig. 2), which are occupied by other anemoneshrimp species (Huebner et al. 2019). The tendency of Pederson shrimp to occur in peripheral habitats on anemones likely relates to the fish cleaning behavior of this species, in that perching on the tentacle tips or around the anemone may allow them easy access to approaching clients (Huebner et al. 2019). These shrimp also are largely immune to predation by fishes due to their cleaner status, so they do not need to occupy more sheltered habitats on anemones as a form of shelter from predation (Stuart 2016). Interestingly, in the presence of native grouper fish, these shrimp occur in peripheral habitats around host anemones as observed here, but when non-native lionfish are introduced to the reef area, the lionfish prey on some of the shrimp, and the latter shift their habitat use to occur closer to anemone hosts (Ellis and Faletti 2016).

In terms of effects of social rank on microhabitat zone occupation, *Ancylomenes pedersoni* shrimp occur in a social dominance hierarchy, in which large females occupy the preferred location on a host anemone (on the tentacle crown) as this allows them to be more visible to visiting client fishes. Smaller females, males and juveniles occupy the periphery of the anemone tentacle crown, and the area surrounding the sea anemone (Gilpin and Chadwick 2019). We show here that both social dominance status and absolute body size influence how individuals of different size classes interact with host sea anemones.

Previous studies have found that crustaceans associated with sea anemones have a resistance to the anemone toxins (Giese et al. 1996; Levine and Blanchard 1980), and that they require an acclimation period to associate closely with them (up to 5 hours; Levine and Blanchard 1980). It is therefore possible that on Day 1 of each experimental trial, the shrimp were not able to fully interact with the anemones due not being fully acclimated, and not yet being immune to the anemone nematocyst toxins. Our observation that even on Day 3 very few shrimp were seen in Zones 1-3 shows that avoidance of these microhabitats by *A. pedersoni* on host anemones occurs even when the other anemoneshrimp species who normally use these zones are not present. Presence of client fishes, or simply movement by human observers around the outside of observation tanks, may cause shrimp to more closely associate with anemones and thus to more frequently occupy sheltered microhabitat zones (Stuart 2016). However, even under field conditions when many reef fishes are in the area, these shrimp still do not occupy inner microhabitat zones on sea anemones (Huebner et al. 2019). The impacts of fish presence and other types of disturbance on shrimp association behaviors under both laboratory and field conditions needs to be investigated further.

Distance to nearest anemone and between shrimp

Our results also clearly show that these shrimp maintain a minimum distance among individuals of at least ~ 10.0 cm, likely maintained by small shrimp avoiding and retreating from large shrimp when they encounter them and reinforced by large shrimp chasing small ones who approach. The regular spacing of conspecifics on anemones, and avoidance among individuals, also has been observed in field populations, in which these shrimp arrange themselves to maintain maximum distance from each other in the limited habitat space on anemones (Gilpin and Chadwick 2019).

The increase in distance between the paired shrimp from Day 1 to Day 2 indicates that small shrimp may require up to 24 hours to detect the presence of other nearby shrimp and/or to assess their social position and then avoid large shrimp. Conversely, we conclude that large shrimp do not appear to adjust their location based on the presence of small shrimp, either because they do not easily detect their presence, or they simply ignore them, in that large shrimp spend most of their time with the original anemone on Day 1 regardless of whether small shrimp are present or not. The lack of change in distance between the paired shrimp from Day 2 to Day 3 reinforces this idea, as the small shrimp continue to avoid the large shrimp. The observed instances of attack by large shrimp toward small shrimp, and retreat by small shrimp, confirm that these shrimp behaviors are driven largely by their positions in the social dominance hierarchy (Gilpin and Chadwick, 2019).

Predictions and conclusions

We predict that in natural settings, juveniles and possibly small sexually mature males and females of *A. pedersoni* disperse to other host anemones more frequently than do large, sexually mature females. This prediction is supported by the size- and gender-dependent dispersal patterns known for other symbiotic crustaceans (Bovbjerg 1953; Courchesne and Barlow 1971). The wide variation in aspects of behavior that we observed among individual shrimp and trial days also shows that the overall patterns may not be exhibited by all *A. pedersoni* individuals. Some of this variation may have been due to our experiments being run in the absence of complex field factors such as the presence of client fishes or potential shrimp predators approaching the anemones.

We conclude that because small shrimp spent relatively more time with the original anemone when alone, they have an increased potential for association with anemones in the absence of large shrimp. However, more experimental trials may need to be run to determine if this pattern is consistent over many days. The behaviors of small, subordinate shrimp observed here suggest that they may not remain associated for long with host anemones in the field. Their high incidence of occurrence in Zone 6 (not associated with an anemone) indicates the potential tendency of small individuals to repeatedly or even continually explore the reef environment and move among anemones. The relative body size of Pederson cleaner shrimp is heavily driven, therefore by position in the social dominance hierarchy, even in the absence of conspecifics.

The host association patterns of *A. pedersoni* shrimp likely affect the establishment and stability of fish cleaning stations on coral reefs. Because Pederson shrimp take at least 6 months to grow from newly settled juveniles to large sexually mature individuals (Gilpin and Chadwick 2017), there is a long period for potential dispersal of small juveniles among anemones. This dispersal period is important for the colonization of anemones that also are dynamic and exhibit rapid recruitment to new habitats (O’Reilly and Chadwick 2017). Therefore, frequent juvenile shrimp dispersal among hosts likely enhances the establishment of new cleaning stations and the disappearance of old ones, which then alter the searching and attraction patterns of diverse client fishes toward dynamic cleaning station locations on reefs (Huebner and Chadwick 2012a; Titus et al. 2017)

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Table 2.1. Frequency of outliers in the experimental data. Variation in the number of behavioral trials removed from datasets for analysis, based on 1.5*IQR criteria. Shown are only datasets with outliers. Note that there were 6 microhabitat data sets (Z1-Z6) and 3 association datasets (OG, AD, NO) for each of 4 treatment types. Thus, using these criteria 37.0-70.4% of all datasets contained outlier data (i.e.: ~30-63% of datasets had no outliers), and up to 24% of the trials within some datasets were removed from analysis as outliers. For those datasets with outliers, the percent of outlier trials within each dataset ranged ~ 3-24%. The mean percent outlier trials per treatment (N = 4 treatments) ranged 10.3 to 11.4. The percent of outlier trials did not vary significantly among the 4 treatments (ANOVA test, F = 0.08, p < 0.9). See text and other figures for abbreviation details.

Treatment type (shrimp size and social rank)	Day of trial	Type of dataset	# Trials removed	# Trials total	Percent outlier trials removed
Large paired (N = 14/27 datasets with outliers)	D1	Z3	4	30	13.3
		Z4	4		13.3
		Z5	1		3.3
		AD	5		16.7
	D2	Z1	1	30	3.3
		Z2	3		10.0
		Z3	3		10.0
		Z4	5		16.7
		AD	5		16.7
	D3	Z1	1	30	3.3
		Z2	4		13.3
		Z3	4		13.3
		Z4	5		16.7
		AD	2		6.7
Mean + SE					10.77 + 1.42
Large solitary (N = 15/27 datasets with outliers)	D1	Z2	2	26	7.7
		Z3	3		11.5
		Z4	5		19.2
		Z5	3		11.5
	D2	Z1	1	26	3.8
		Z2	1		3.8
		Z3	4		15.4
		Z4	1		3.8
		Z5	3		11.5
		AD	2		7.7
	D3	Z1	3	26	11.5
		Z2	5		19.2
		Z3	5		19.2
		Z4	2		7.7
		Z5	3		11.5
Mean + SE					11.0 + 1.4

Table 2.1 continued

Small paired (N = 19/27 datasets with outliers)	D1	Z2	1	30	3.0
		Z3	4		13.3
		Z4	6		20.0
		Z5	4		13.3
		Z6	3		10.0
		OG	3		10.0
		NO	3		10.0
	D2	Z1	1	30	3.3
		Z2	2		6.7
		Z3	4		13.3
		Z4	4		13.3
		Z5	4		13.3
		OG	4		13.3
		AD	4		13.3
	D3	Z2	1	30	3.3
		Z3	5		20.0
		Z4	1		3.3
		Z5	2		6.7
		AD	3		10.0
Mean + SE					10.3 + 1.2
Small solitary (N = 10/27 datasets with outliers)	D1	Z3	2	21	9.5
		Z4	1		4.8
		Z5	2		9.5
	D2	Z3	1	21	4.8
		Z4	4		19.1
		Z5	2		9.5
		AD	3		14.3
	D3	Z2	1	21	4.8
		Z3	5		23.8
		Z5	3		14.3
Mean + SE					11.4 + 2.0

Table 2.2. Variation in the percent time spent by Pederson shrimp *Ancylomenes pedersoni* in association with each type of sea anemone during behavioral trials. Shown are the median percent time and the range of percent times spent with each type of anemone (N = 3 types) during each trial day (N = 3 days per trial), under each type of treatment condition (N = 4 conditions).

Treatment type (shrimp size and social rank)	**Anemone**	**Day of trial**	**Median percent time**	**Range of percent time**
Large/Paired	Original	D1	30.8%	0%-69.2%
		D2	61.9%	10.3%-100%
		D3	48.4%	0%-100%
	Added	D2	0.0%	0%-18.2%
		D3	0.0%	0%-27.3%
	Neither	D1	69.2%	30.8%-100%
		D2	31.8%	0%-57.1%
		D3	34.8%	0%-72.7%
Small/Paired	Original	D1	4.6%	0%-28.6%
		D2	9.4%	0%-42.9%
		D3	13.6%	0%-57.1%
	Added	D2	0.0%	0%-7.3%
		D3	4.8%	0%-23.1%
	Neither	D1	95.5%	71.4%-100%
		D2	75.5%	0%-100%
		D3	60.4%	35.2%-91.7%
Large/Solo	Original	D1	45.8%	18.0-61.5%
		D2	45.7%	5.1%-93.9%
		D3	17.5%	0%-69.9%
	Added	D2	2.6%	0%-13.6%
		D3	35.6%	0%-100%
	Neither	D1	58.6%	38.5%-82.1%
		D2	44.7%	6.1%-84.6%
		D3	34.7%	0%-90.9%
Small/Solo	Original	D1	21.0%	0%-81.8%
		D2	45.5%	9.1%-100%
		D3	38.6%	0%-100%
	Added	D2	2.6%	0%-9.1%
		D3	15.4%	0%-45.1%
	Neither	D1	79.0%	18.2%-100%
		D2	45.5%	0%-75.5%
		D3	45.5%	0%-78.8%

Table 2.3. Results of statistical tests (Kruskal-Wallis and Dunn Tests) of pair-wise comparisons in the percent time spent by Pederson shrimp *Ancylomenes pedersoni* in association with each of 3 types of sea anemone (OG = original anemone, AD = added anemone, NO = neither anemone), on each of 3 trial days under 4 treatment conditions. Note that AD was added to the experimental tank on Day 2 (D2) of each trial, so pairwise comparisons were only between OG and NO on Day 1.

Treatment (shrimp size and social rank)	Day of trial	χ^2	Type of anemone comparison	Z-score	P-Value
Large, paired	D1	10.45	OG-NO	3.23	0.0012
	D2	23.66	OG-AD	-4.85	0.0000
			OG-NO	-2.06	0.0398
			AD-NO	-2.70	0.0070
	D3	18.18	OG-AD	-4.18	0.0000
			OG-NO	-1.39	0.1650
			AD-NO	-2.86	0.0042
Small, paired	D1	19.24	OG-NO	4.39	0.0000
	D2	20.71	OG-AD	-1.39	0.1656
			OG-NO	3.02	0.0026
			AD-NO	-4.44	0.0000
	D3	23.11	OG-AD	-1.66	0.0976
			OG-NO	3.08	0.0020
			AD-NO	-4.74	0.0000
Large, solitary	D1	8.71	OG-NO	2.95	0.0032
	D2	14.08	OG-AD	-3.34	0.0008
			OG-NO	-0.18	0.8586
			AD-NO	-3.16	0.0016
	D3	2.74	OG-NO	1.41	0.1574
			OG-AD	1.45	0.1466
			AD-NO	0.04	0.9696
Small, solitary	D1	8.35	OG-NO	2.89	0.0038
	D2	15.85	OG-AD	-3.64	0.0002
			OG-NO	-0.31	0.7600
			AD-NO	-3.35	0.0008
	D3	6.59	OG-AD	-1.79	0.0732
			OG-NO	0.70	0.4860
			AD-NO	-2.49	0.0128

Table 2.4. Results of statistical tests of pair-wise comparisons in the percent time spent by Pederson shrimp *Ancylomenes pedersoni* in association with each type of sea anemone (OG = original anemone, AD = added anemone, NO = neither anemone), depending on whether they were large or small in body size, and in paired vs. solitary treatments. Note that the largest number of significant differences occurred between large and small shrimp when they occurred in pairs (Table D), and that in contrast, they exhibited no significant differences in association when they each occurred as solitary individuals with anemones (Table C). Significant p-values are lightly shaded.

A. Large shrimp in solitary vs paired trails

Type of sea anemone	**Day of trial**	χ^2	**Df**	**Z-score**	**Dunn test p-value**
OG	D1	1.54	1	-1.24	0.2146
	D2	2.04	1	1.43	0.1534
	D3	4.21	1	2.05	0.0402
AD	D2	2.54	1	-1.59	0.1108
	D3	5.95	1	-2.44	0.0148
NO	D1	0.29	1	0.53	0.5978
	D2	0.75	1	-0.87	0.3852
	D3	0.10	1	0.31	0.7562

B. Small shrimp in solitary vs. paired trials

Anemone State	**Day**	χ^2	**Df**	**Z-score**	**Dunn test p - value**
OG	D1	1.01	1	-1.01	0.3148
	D2	9.38	1	-3.06	0.0022
	D3	1.78	1	-1.33	0.1822
AD	D2	0.30	1	-0.55	0.5812
	D3	1.49	1	-1.22	0.2222
NO	D1	1.01	1	1.01	0.3148
	D2	4.35	1	2.09	0.0370
	D3	2.54	1	1.59	0.1110

C. Large vs. small shrimp in solitary trials

Anemone State	**Day**	**χ^2**	**Df**	**Z-score**	**Dunn test p-value**
OG	D1	2.22	1	1.49	0.1364
	D2	0.01	1	-0.07	0.9438
	D3	1.81	1	-1.35	0.1780
AD	D2	0.48	1	0.69	0.4898
	D3	2.76	1	1.66	0.0968
NO	D1	2.22	1	-1.49	0.1364
	D2	0.001	1	-0.04	0.9720
	D3	0.01	1	-0.11	0.9158

D. Large vs. small shrimp in paired trials

Anemone State	**Day**	**χ^2**	**Df**	**Z-score**	**Dunn test p-value**
OG	D1	10.47	1	3.24	0.0012
	D2	13.39	1	3.66	0.0002
	D3	6.32	1	2.51	0.0120
AD	D2	0.17	1	-0.41	0.6802
	D3	0.04	1	-0.19	0.8478
NO	D1	10.47	1	-3.24	0.0012
	D2	6.55	1	-2.56	0.0104
	D3	6.62	1	-2.57	0.0102

Table 2.5. Results of statistical tests of pair-wise comparisons in the percent time spent by Pederson shrimp *Ancylomenes pedersoni* in association with each type of sea anemone (OG = original anemone, AD = added anemone, NO = neither anemone), depending on the trial day. Note that percent time spent with each anemone type varied between some pairs of the trial days, under all 4 treatment conditions.

Treatment (shrimp size and social rank)	Anemone	χ^2	Type of day comparison	Z-score	P-Value
Large/Paired	OG	6.11	D1-D2	-2.35	0.0188
			D1-D3	-1.83	0.0668
			D2-D3	0.52	0.6056
	AD	2.19	D2-D3	-1.48	0.1390
	NO	12.39	D1-D2	3.06	0.0220
			D1-D3	3.31	0.0024
			D2-D3	-0.14	0.8892
Small/Paired	OG	4.67	D1-D2	-0.69	0.4882
			D1-D3	-2.12	0.0336
			D2-D3	-1.39	0.1650
	AD	1.98	D2-D3	-1.41	0.1590
	NO	13.60	D1-D2	2.57	0.0102
			D1-D3	3.58	0.0004
			D2-D3	1.10	0.3122
Large/Solo	OG	5.27	D1-D2	-0.15	0.8788
			D1-D3	1.91	0.0566
			D2-D3	2.06	0.0394
	AD	8.25	D2-D3	-2.87	0.0040
	NO	2.82	D1-D2	1.52	0.1274
			D1-D3	1.37	0.1702
			D2-D3	-0.15	0.8788
Small/Solo	OG	3.12	D1-D2	-1.75	0.0800
			D1-D3	-1.07	0.2824
			D2-D3	0.68	0.4990
	AD	2.92	D2-D3	-1.71	0.0876
	NO	7.29	D1-D2	2.30	0.0216
			D1-D3	2.36	0.0176
			D2-D3	0.08	0.9384

Table 2.6. Results of statistical tests (Dunn Tests) of pair-wise comparisons in the percent time spent by Pederson shrimp *Ancylomenes pedersoni* in 6 types of microhabitat zones (Z1-Z6) on host sea anemones, on each of 3 trial days (D1-D3). Upper value within each cell = Z-score, lower value = p-value. Shown are results for 4 types of treatment conditions: A. Large paired shrimp, B. Large solitary shrimp, C. Small paired shrimp, and D. Small solitary shrimp. Note that there were significant differences in microhabitat use between pairs of zones, for all 4 types of shrimp treatments on all 3 trial days.

A. Large, paired shrimp

Day of trial	Zone	Z1	Z2	Z3	Z4	Z5
D1	Z2	0.00 1.0000				
	Z3	0.00 1.0000	0.00 1.0000			
	Z4	-1.76 0.0778	-1.76 0.0778	-1.76 0.0778		
	Z5	-3.76 0.0002	-3.76 0.0002	-3.76 0.0002	-1.99 0.0460	
	Z6	-6.038088 0.0000	-6.04 0.0000	-6.04 0.0000	-4.27 0.0000	-2.28 0.0228
D2	Z2	0.00 1.0000				
	Z3	-1.74 0.0818	-1.74 0.0818			
	Z4	-4.08 0.0000	-4.07 0.0000	-2.34 0.0198		
	Z5	-3.61 0.0004	-3.61 0.0004	-1.87 0.0614	0.47 0.6450	
	Z6	-4.37 0.0000	-4.37 0.0000	-2.63 0.0086	-0.30 0.7658	-0.76 0.4482
D3	Z2	0.00 1.0000				
	Z3	-1.72 0.0850	-1.72 0.0850			
	Z4	-2.78 0.0054	-2.78 0.0054	-1.09 0.2758		
	Z5	-3.82 0.0002	-3.82 0.0002	-2.10 0.0360	-0.97 0.3942	
	Z6	-5.03 0.0000	-5.03 0.0000	-3.31 0.0010	-2.15 0.0316	-1.21 0.2270

B. Large, solitary shrimp

Day of trial	**Zone**	**Z1**	**Z2**	**Z3**	**Z4**	**Z5**
D1	Z2	0.00 1.0000				
	Z3	0.00 1.0000	0.00 1.0000			
	Z4	-1.67 0.0948	-1.67 0.0940	-1.67 0.0940		
	Z5	-3.39 0.0006	-3.39 0.0006	-3.39 0.0006	-1.72 0.0852	
	Z6	-5.35 0.0000	-5.35 0.0000	-5.35 0.0000	-3.68 0.0002	-1.96 0.0500
D2	Z2	0.00 1.0000				
	Z3	0.00 1.0000	0.00 1.0000			
	Z4	-3.55 0.0004	-3.55 0.0004	-3.55 0.0004		
	Z5	-3.23 0.0012	-3.23 0.0012	-3.23 0.0012	0.32 0.7492	
	Z6	-4.89 0.0000	-4.89 0.0000	-4.89 0.0000	-1.34 0.1798	-1.66 0.0966
D3	Z2	0.00 1.0000				
	Z3	-1.71 0.0872	-1.71 0.0872			
	Z4	-3.64 0.0002	-3.64 0.0002	-1.83 0.0666		
	Z5	-3.32 0.0008	-3.32 0.0008	-1.52 0.1282	-1.06 0.2886	
	Z6	-4.38 0.0000	-4.38 0.0000	-2.55 0.0106	-0.74 0.4596	-1.06 0.2886

C. Small, solitary shrimp

Day of trial	**Zone**	**Z1**	**Z2**	**Z3**	**Z4**	**Z5**
D1	Z2	0.00 1.0000				
	Z3	0.00 1.0000	0.00 1.0000			
	Z4	-1.92 0.0546	-1.92 0.0546	-1.87 0.0614		
	Z5	-1.61 0.1078	-1.61 0.1078	-1.57 0.1174	0.31 0.7536	
	Z6	-5.27 0.0000	-5.27 0.0000	-5.12 0.0000	-3.34 0.0008	-3.66 0.0002
D2	Z2	0.00 1.000				
	Z3	-1.48 0.1396	-1.48 0.1396			
	Z4	-1.88 0.0598	-1.88 0.0598	-0.40 0.6924		
	Z5	-2.60 0.0094	-2.60 0.0940	-1.09 0.2750	-0.70 0.4864	
	Z6	-4.76 0.0000	-4.76 0.0000	-3.15 0.0016	-2.75 0.0060	-2.03 0.0420
D3	Z2	0.00 1.0000				
	Z3	-0.46 0.6480	-0.46 0.6480			
	Z4	-4.10 0.0000	-4.12 0.0000	-3.54 0.0004		
	Z5	-1.29 0.1976	-1.29 0.1976	-0.80 0.4252	2.81 0.0048	
	Z6	-4.24 0.0000	-4.24 0.0000	-3.67 0.0002	-0.13 0.8940	-2.95 0.0032

D. Small, paired shrimp

Day of trial	**Zone**	**Z1**	**Z2**	**Z3**	**Z4**	**Z5**
D1	Z2	0.00 1.0000				
	Z3	0.00 1.0000	0.00 1.0000			
	Z4	0.00 1.0000	0.00 1.0000	0.00 1.0000		
	Z5	-2.23 0.0260	-2.23 0.0260	-2.25 0.0260	-2.18 0.0292	
	Z6	-6.25 0.0000	-6.25 0.0000	-6.25 0.0000	-6.13 0.0000	-4.03 0.0000
D2	Z2	0.00 1.0000				
	Z3	-0.33 0.7424	-0.32 0.7472			
	Z4	-2.24 0.0252	-2.19 0.0282	-1.19 0.0612		
	Z5	-1.77 0.0804	-1.73 0.0836	-1.40 0.1610	0.51 0.6118	
	Z6	-5.60 0.0000	-5.48 0.0000	-5.15 0.0000	-3.25 0.0012	-3.83 0.0002
D3	Z2	0.490 0.623				
	Z3	-0.28 0.7796	-0.22 0.8280			
	Z4	-2.95 0.0032	-3.38 0.0008	-3.23 0.0012		
	Z5	-2.20 0.0278	-2.65 0.0082	-2.48 0.0132	0.75 0.4518	
	Z6	-5.25 0.000	-5.63 0.0000	-5.53 0.0000	-2.30 0.0216	-3.05 0.0240

Table 2.7. Results of statistical tests (Kruskal-Wallis and Dunn Tests) of pair-wise comparisons in the percent time spent by Pederson shrimp *Ancylomenes pedersoni* in 6 types of microhabitat zones (Z1-6) on host sea anemones, during each of 3 trial days (D1-D3). Shown are results for 4 types of pairwise comparisons: A. Large shrimp when solitary vs. paired, B. Small shrimp when solitary vs. paired, C. Paired shrimp when small vs. large, and D. Solitary shrimp when small vs. large. Note that large shrimp did not alter their zone use when solitary vs. paired, but that small shrimp did (Tables A vs. B). Also note that large vs. small shrimp occupied significantly different zones when they were paired, but less so when they were solitary on anemones (Tables C vs. D). NA = pairwise comparison not applicable because none of the shrimp occurred in this type of zone.

A. Large shrimp: solitary vs. paired

Day of trial	Zone	χ^2	df	Z-score	P-Value
D1	Z1	NA	1	0.00	1.00
	Z2	NA	1	0.00	1.00
	Z3	NA	1	0.00	1.00
	Z4	0.09	1	0.30	0.76
	Z5	0.31	1	0.56	0.58
	Z6	1.70	1	1.30	0.19
D2	Z1	NA	1	0.00	1.00
	Z2	NA	1	0.00	1.00
	Z3	7.10	1	2.66	0.10
	Z4	0.56	1	0.75	0.46
	Z5	0.71	1	0.84	0.40
	Z6	0.76	1	-0.87	0.38
D3	Z1	NA	1	0.00	1.00
	Z2	NA	1	0.00	1.00
	Z3	0.03	1	-0.18	0.86
	Z4	0.09	1	-0.30	0.77
	Z5	0.47	1	0.69	0.49
	Z6	0.31	1	0.56	0.58

B. Small shrimp: Solitary vs. paired

Day of trial	Zone	χ^2	df	Z-score	P-Value
D1	Z1	NA	1	0.00	1.00
	Z2	NA	1	0.00	1.00
	Z3	NA	1	0.00	1.00
	Z4	7.26	1	-2.69	0.01
	Z5	0.30	1	-0.55	0.58
	Z6	1.68	1	1.29	0.20
D2	Z1	NA	1	0.00	1.00
	Z2	NA	1	0.00	1.00
	Z3	4.19	1	-2.05	0.04
	Z4	0.38	1	-0.61	0.54
	Z5	3.33	1	-1.82	0.07
	Z6	1.78	1	1.33	0.18
D3	Z1	NA	1	0.77	0.38
	Z2	NA	1	0.00	1.00
	Z3	0.11	1	-0.33	0.74
	Z4	3.84	1	-1.96	0.05
	Z5	0.38	1	0.62	0.54
	Z6	3.02	1	1.74	0.08

C. Paired shrimp: Large vs. small

Day of trial	**Zone**	**χ^2**	**df**	**Z-score**	**P-Value**
D1	Z1	NA	1	0.00	1.00
	Z2	NA	1	0.00	1.00
	Z3	NA	1	0.00	1.00
	Z4	6.83	1	2.61	0.01
	Z5	9.23	1	3.04	0.00
	Z6	10.31	1	-3.21	0.00
D2	Z1	NA	1	0.00	1.00
	Z2	NA	1	0.00	1.00
	Z3	5.91	1	2.43	0.02
	Z4	8.92	1	2.99	0.00
	Z5	6.70	1	2.59	0.01
	Z6	3.81	1	-1.95	0.05
D3	Z1	1.00	1	-1.00	0.32
	Z2	NA	1	0.00	1.00
	Z3	5.43	1	2.33	0.02
	Z4	1.45	1	-1.21	0.23
	Z5	3.20	1	1.79	0.07
	Z6	5.21	1	-2.28	0.02

D. Solitary shrimp: Large vs. small

Day of trial	**Zone**	**χ^2**	**df**	**Z-score**	**P-Value**
D1	Z1	NA	1	0.00	1.00
	Z2	NA	1	0.00	1.00
	Z3	NA	1	0.00	1.00
	Z4	0.24	1	-0.48	0.63
	Z5	1.53	1	1.24	0.22
	Z6	1.48	1	-1.22	0.22
D2	Z1	NA	1	0.00	1.00
	Z2	NA	1	0.00	1.00
	Z3	5.25	1	-2.29	0.02
	Z4	1.88	1	1.37	0.17
	Z5	1.05	1	1.02	0.31
	Z6	0.07	1	-0.26	0.79
D3	Z1	NA	1	0.00	1.00
	Z2	NA	1	0.00	1.00
	Z3	2.66	1	1.63	0.10
	Z4	5.15	1	-2.27	0.02
	Z5	3.37	1	1.84	0.07
	Z6	0.41	1	-0.64	0.52

Table 2.8. Variation in distances to the nearest sea anemone exhibited by Pederson shrimp *Ancylomenes pedersoni* during behavioral trials. Shown are the median distance and range of distances for each trial day (N = 3 days per trial), under each type of treatment condition.

Treatment type (shrimp size and social rank)	Day of trial	Median distance (cm)	Range of distances (cm)
Large, paired	D1	8.1	1.4-39.3
	D2	3.6	0.0-12.5
	D3	4.3	0.0-16.1
Large, solitary	D1	11.0	1.9-28.8
	D2	4.1	0.0-18.0
	D3	4.3	0.2-15.8
Small, paired	D1	11.7	1.5-45.5
	D2	7.4	0.0-14.4
	D3	5.4	0.7-21.8
Small, solitary	D1	22.3	5.0-40.0
	D2	10.6	0.8-14.5
	D3	7.4	2.3-14.2

Table 2.9. Statistical test results of variation in the distances of Pederson shrimp *Ancylomenes pedersoni* to the nearest sea anemone in behavioral trials. A. Pairwise comparison of the median distances between trial days (N = 3 days in each trial) within each type of treatment condition. B. Pairwise comparisons between solitary vs. solitary treatments for both large and small shrimp, on each trial day. Note that there were significant differences in shrimp distances to anemones, between some of the trial days in most treatments, but no differences between the solitary vs. paired treatments for either type of shrimp body size. Significant results are lightly shaded.

A. Pairwise comparisons between trial days

Treatment (shrimp size and social rank)	**Type of trial day comparison**	**χ^2**	**P-value**	**Df**	**Z-score**	**P-value**
Large paired shrimp	D1-D2	12.43	0.0000	1	3.35	0.0008
	D1-D3				2.78	0.0540
	D2-D3				-0.61	0.5476
Small paired shrimp	D1-D2	4.88	0.0900	1	1.81	0.0704
	D1-D3				2.43	0.0410
	D2-D3				0.22	0.8222
Large solitary shrimp	D1-D2	4.101	0.1300	1	1.87	0.0618
	D1-D3				1.58	0.1130
	D2-D3				-0.18	0.8904
Small solitary shrimp	D1-D2	7.86	0.0200	1	1.80	0.0712
	D1-D3				2.76	0.0058
	D2-D3				0.96	0.3384

B. Pairwise comparisons between paired vs. solitary treatments

Size	Day	χ^2	Df	P	Z-score	P-Value
Large	D1	0.13	1	0.72	0.35	0.7237
	D2	0.42	1	0.52	0.65	0.5152
	D3	0.24	1	0.62	0.49	0.6242
Small	D1	0.23	1	0.63	-0.48	0.6338
	D2	0.05	1	0.83	0.22	0.8345
	D3	0.01	1	0.933	-0.08	0.4665

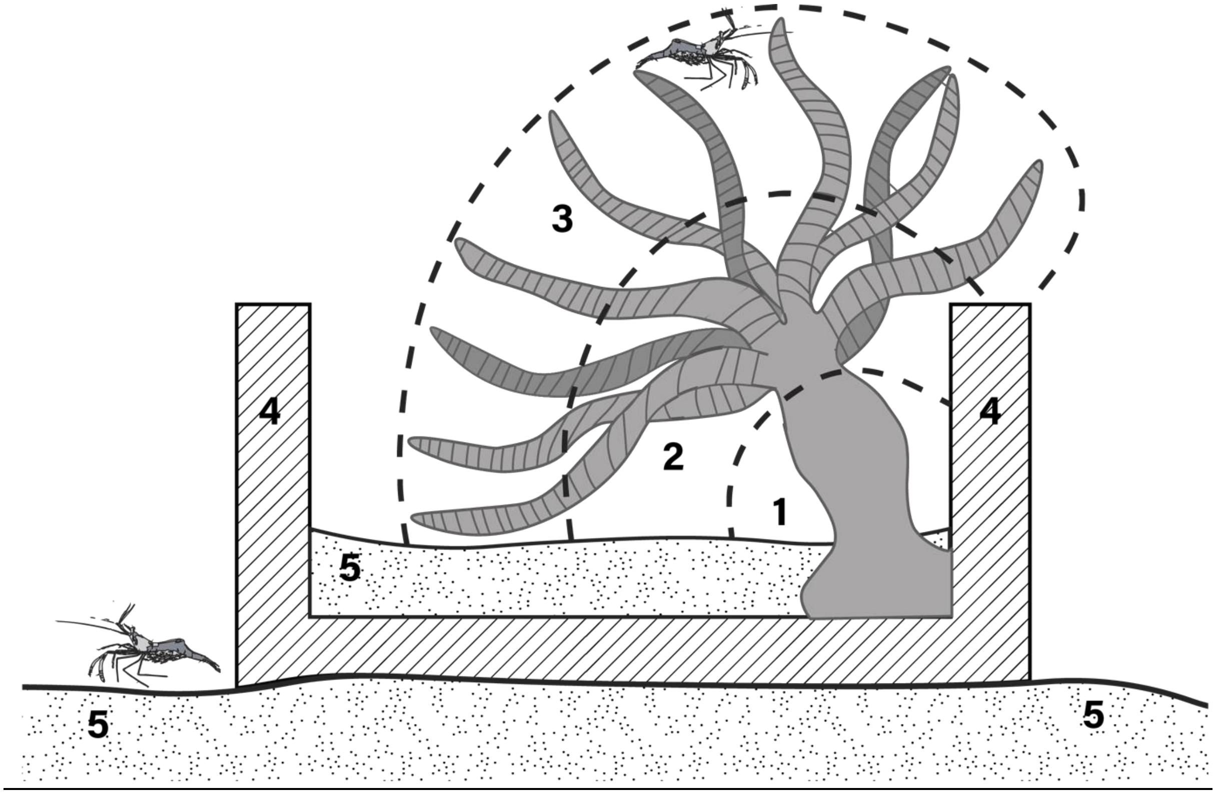

Figure 2.1. Diagram of microhabitat zones occupied by *Ancylomenes pedersoni* shrimp in observational tanks, in relation to host sea anemones *Bartholomea annulata*. Zone 1 (under tentacle crown along column, adjacent to pedal disc), Zone 2 (inner half of tentacle crown), Zone 3 (outer half of tentacle crown), Zone 4 (near tentacle crown on hard substrate, < 5 cm distant on tank wall or bowl edge), Zone 5 (near tentacle crown on soft substrate, < 5 cm distant on gravel), and Zone 6 (not associated with anemone, > 5 cm distant; Fig. 1). These 6 zones were similar to those defined by Gilpin and Chadwick (2019, see Fig. 2.2) based on field observations of Pederson shrimps, with definitions modified here for the laboratory environment (compare Figs. 1 and 2). Figure design by Craig Barker.

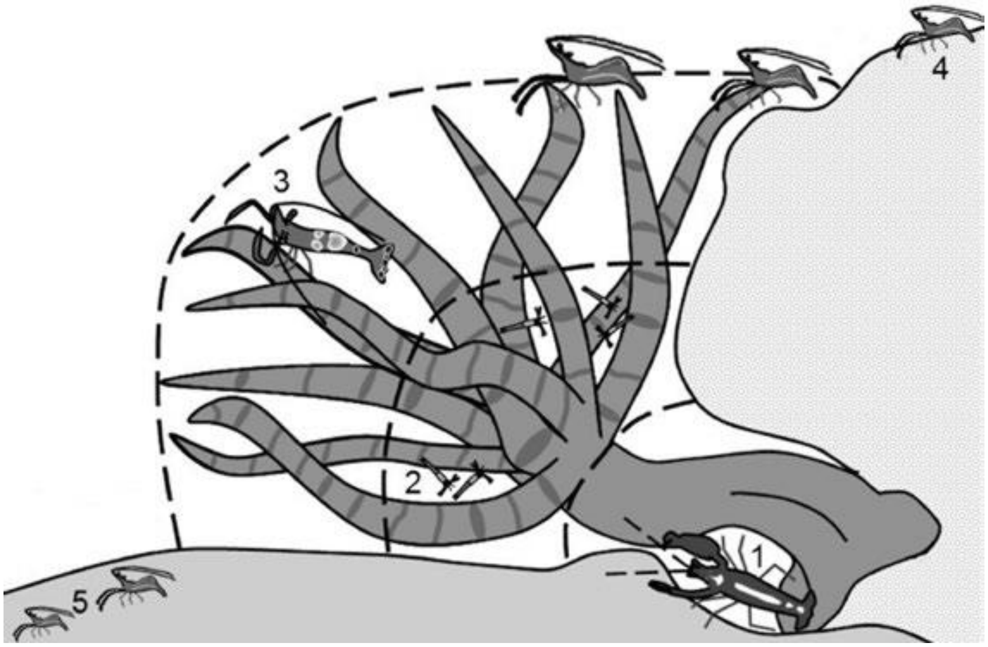

Figure 2.2. Microhabitat zones occupied by *Ancylomenes pedersoni* shrimp on *Bartholomea annulata* sea anemones in natural coral reef environments. Microhabitats were classified into 5 zones on or near the host anemone body; 1: anemone column, 2: inner tentacle crown, 3: outer tentacle crown, 4: hard 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 reproduced from Gilpin and Chadwick (2019) with permission of the authors.

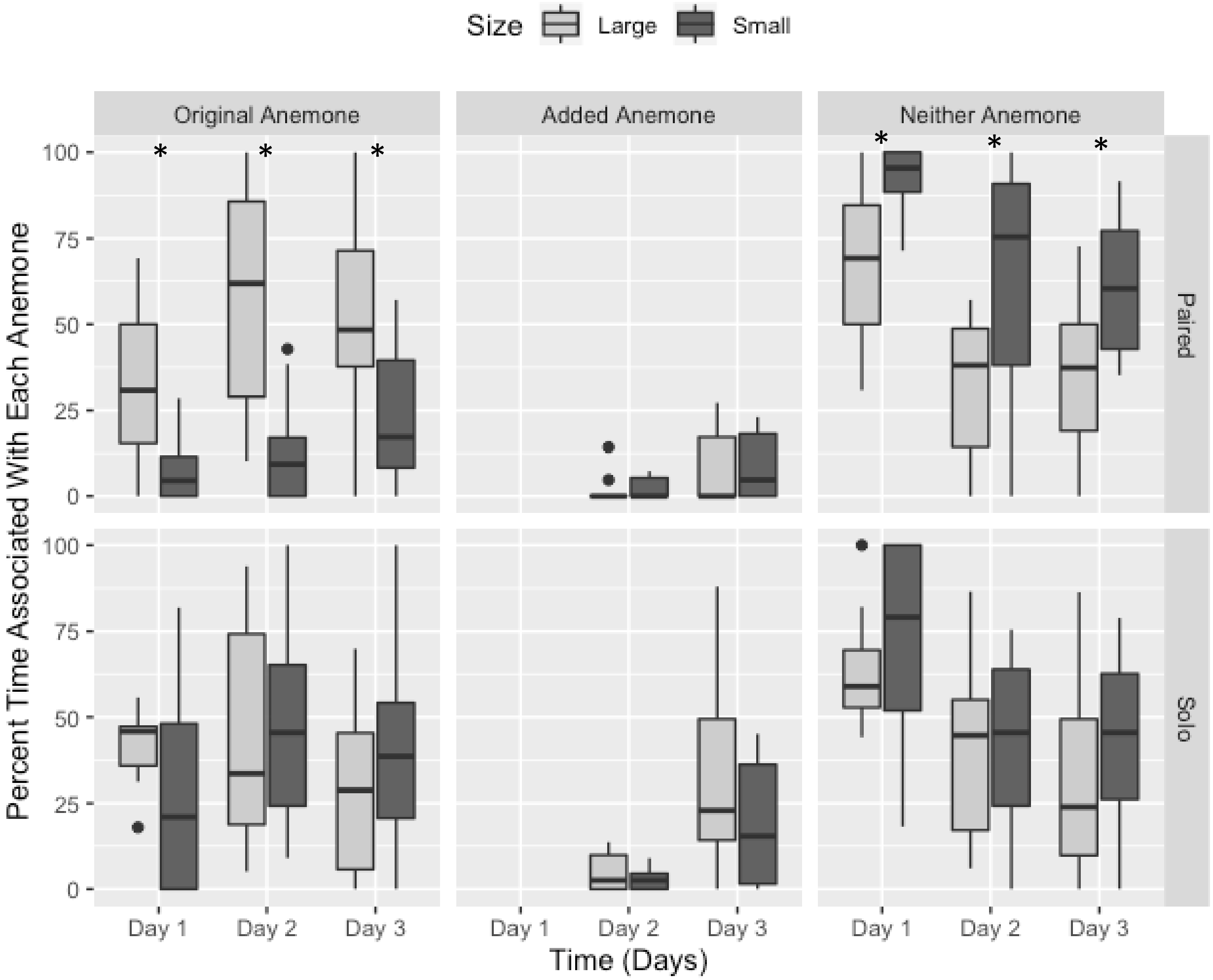

Figure 2.3. Percent time of Pederson cleaner shrimp *Ancylomenes pedersoni* in association with each anemone type when in different treatment states (Paired and Solo) and between different size categories (large and small). Asterisks denote significant differences between size categories. Note the lack of significant differences in behavior of large and small shrimp when solo.

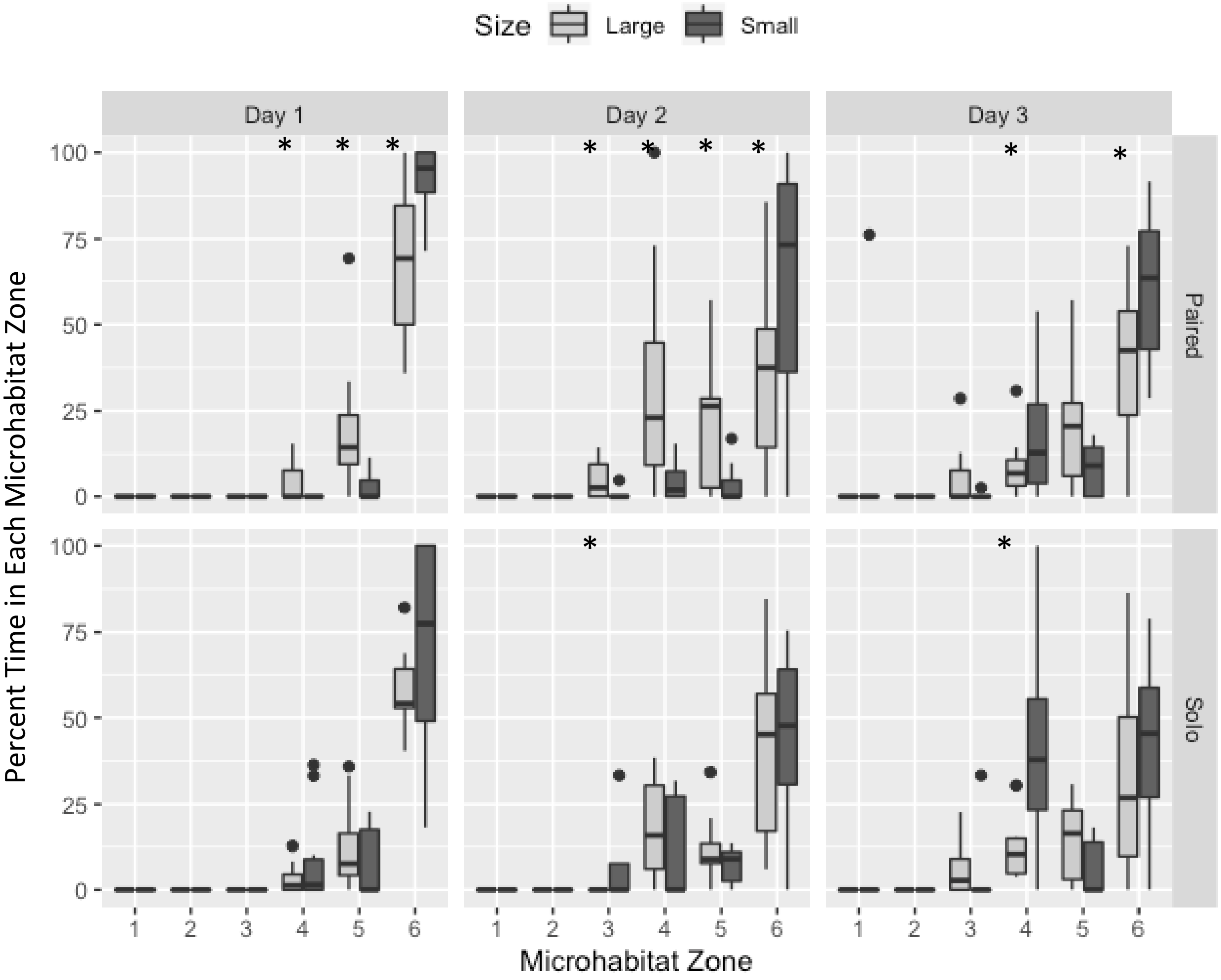

Figure 2.4. Microhabitat zones occupied by Pederson shrimp *Ancylomenes pedersoni* associated with *Bartholomea annulata* sea anemones when in different treatment states (paired and solo) over a three-day trial period. Note asterisks depicting significance between size classes (large and small).

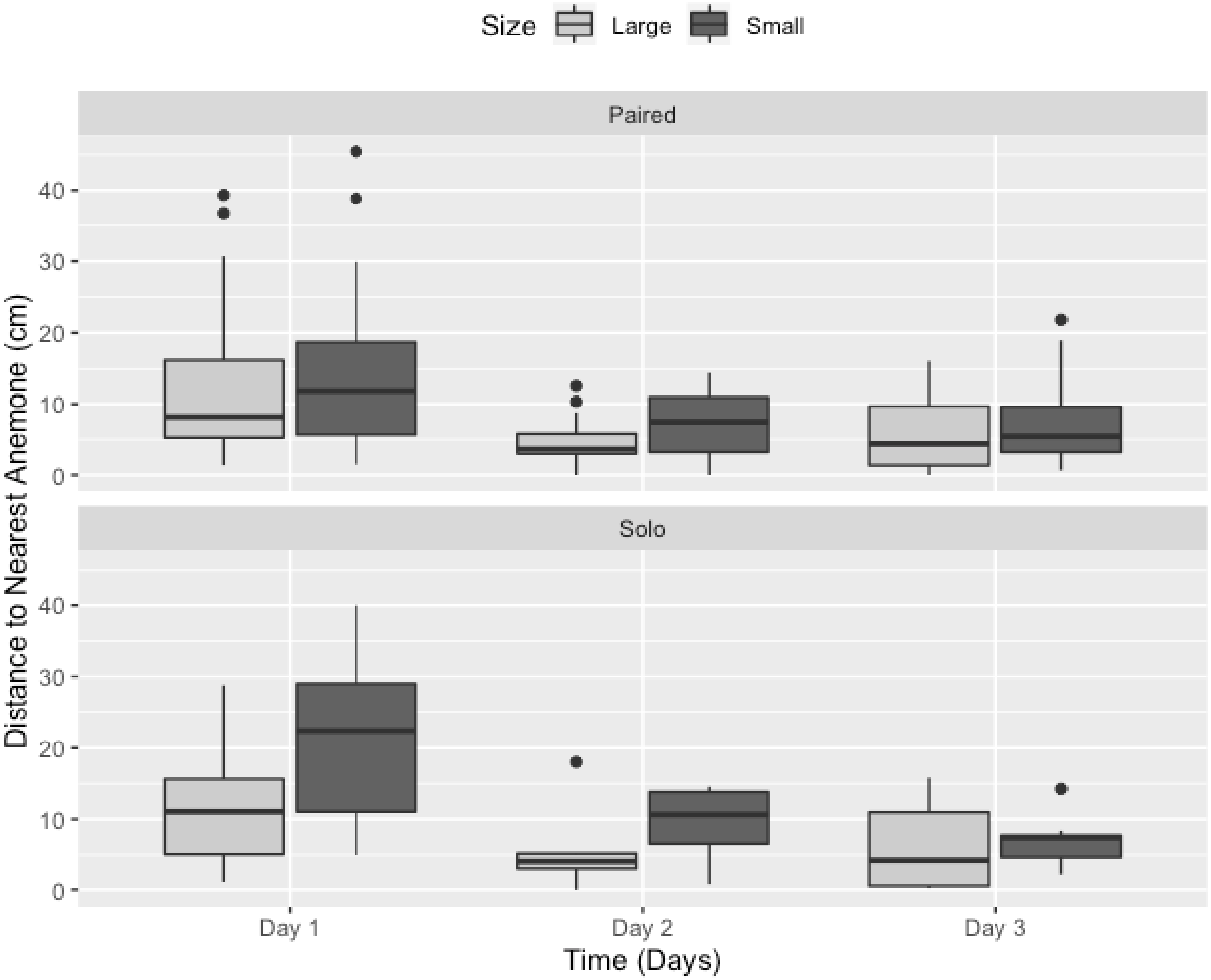

Figure 2.5. Distances of Pederson shrimp *Ancylomenes pedersoni* to the nearest sea anemone in behavioral trials for large and small shrimp when in different treatment states (paired and solo). Note the lack of significant differences in each state on each day.

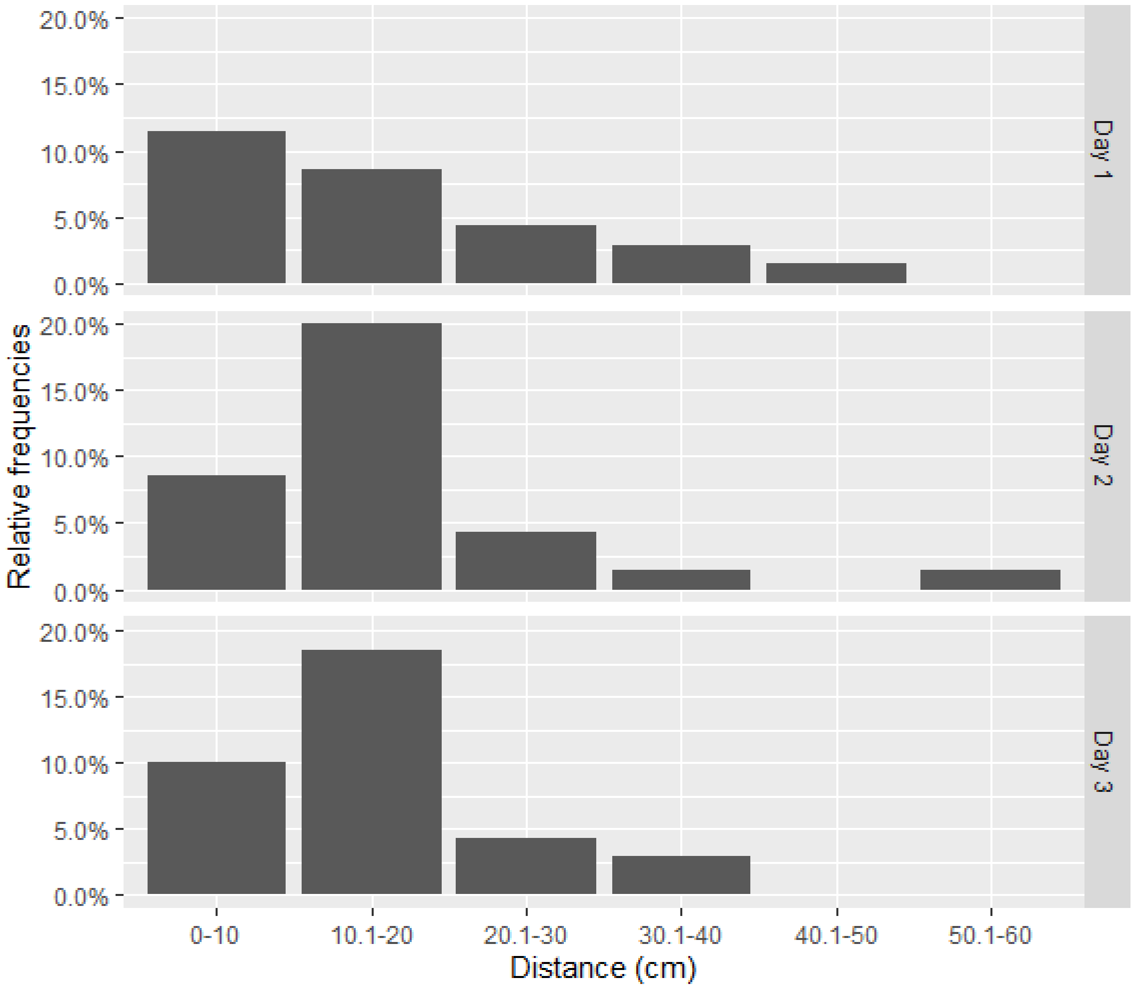

Fig. 2.6. Percentage of trials with average distance between paired *Ancylomenes pedersoni* shrimp individuals in each distance category on each trial day (N = 20, Day 1; N = 23, Day 2; N = 25 Day 3). Note the increase of percentage of trials in distance category 10.1-20 from day 1 to day 2.

Chapter 3

Dispersal patterns of Pederson cleaner shrimp *Ancylomenes pedersoni* among host sea anemones on a Caribbean coral reef

Introduction

Pederson shrimp *Ancylomenes pedersoni* are the most prevalent and effective crustacean cleaners of fishes on Caribbean coral reefs (Mahnken 1972; Titus et al. 2017), and are obligate symbionts of corkscrew sea anemones *Bartholomea annulata,* which are the most common anemones on many Caribbean reefs (Briones-Fourzan et al. 2012; Titus et al. 2017), as well as on some other anemone hosts (Mascaro et al. 2011). The extent to which individual shrimp remain with a given host sea anemone remains unknown but may have important effects on the stability of anemone-centered cleaning stations in the Caribbean. In order to signal willingness to clean, Pederson shrimp use a stereotypical side-to-side body swaying or rocking motion (Becker et. al. 2005), and rapid vibration or whipping of their long paired antennae (Mahnken 1972). Their behaviors that signal willingness to clean directly represent their hunger level (Chapuis and Bshary 2012), in that more signaling occurs when a shrimp is starved. Food availability may affect not only shrimp signaling rate, but also their choice of habitat; it is possible that shrimps which experience high food availability remain on their host anemones, while those experiencing low food availability (relatively few cleans) may depart from the host to seek a host where more cleans are possible. Field observations confirm that relatively more cleaner shrimp occur on sea anemones in reef areas where there are high levels of reef fish traffic (Mahnken 1972; N. E.

Chadwick, pers. comm.), however the underlying mechanisms which trigger shrimp dispersal and causes of this pattern are not understood.

Some obligate symbionts remain with their hosts throughout the lifespan of the symbiont; in contrast, Pederson shrimp appear to occasionally leave their hosts and migrate to new anemones (Mahnken 1972). This migratory behavior is similar to that of some Indo-Pacific cleaner shrimps which may migrate among host anemones nocturnally (Chadwick et al. 2008), and of other crustaceans that are known to migrate among their invertebrate hosts (Cowell et al. 1993; Thiel et al. 2003). Frequency of Pederson shrimp dispersal among hosts is likely to vary with shrimp body size and social rank, in that these shrimp occur in social groups structured by size-based dominance hierarchies, with small juveniles and medium-size males signaling less frequently and potentially receiving fewer fish cleans than do large dominant females (Gilpin and Chadwick 2019).

Microhabitat partitioning also occurs in this species, in which the large dominant females tend to occupy the most prominent positions in the middle of the anemone tentacle crown, while smaller subordinate shrimp (males and juveniles) occur around the periphery of the tentacles or on adjacent reef substrate (Mahnken 1972; Gilpin and Chadwick 2019). This habitat segregation may contribute to dominant shrimp receiving the most fish cleans, leaving subordinate shrimp with relatively fewer cleans and lower fitness (Gilpin and Chadwick 2019). Another related pattern is that Pederson shrimp rarely occur in social groups of larger than four individuals, which could be caused by food resource limitation in the form of the number of cleans that each can shrimp receive on a given anemone (Huebner and Chadwick 2012a). Immediately after molting, crustaceans regardless of their previous social rank, move to the bottom of their social hierarchy due to the exoskeleton being temporarily soft and vulnerable, so occasionally even large shrimp may become socially subordinate and lose access to fish clients (Bovbjerg 1953). As cleaner organisms, these shrimp provide important services to fishes and thus have no known native predators among the fishes on Caribbean reefs, which instead recognize them and signal to them to receive cleaning services (Huebner and Chadwick 2012a). In contrast, when non-native Indo-Pacific lionfish are introduced to their reef areas, the lionfish prey on the shrimp, and the shrimp adjust their microhabitat use to move in closer to their host anemones (Ellis and Faletti 2016).

All of these social and microhabitat use patterns suggest that especially on reefs not yet impacted by non-native predators, juvenile and male shrimp, or newly molted individuals, could disperse frequently among hosts to locate vacant hosts or those with smaller social groups where they may obtain more fish cleans. The frequency of visits by fish clients, as well as the social group size of shrimp, both increase with host anemone body size (Huebner and Chadwick 2012a), so another factor influencing cleaner shrimp migration rates is likely to be the relative body sizes of neighboring host anemones. All the above factors, in particular shrimp physiological state (hunger level), social rank and group size, combined with distances between and relative body sizes of adjacent host anemones, may interact to determine dispersal rates of shrimp among hosts.

Cleaning stations are important contributors to biodiversity on coral reefs, in that changes in the abundance of cleaner organisms can have major impacts on the health and diversity of reef fishes (Bshary 2003, Grutter et al. 2003, Waldie et al. 2011). The presence of sea anemones that host Pederson shrimp may even cause large groupers to center their feeding territories around the anemones (Sluka et al. 1999), so shrimp movement among anemones could cause these piscivores to alter their patterns of predation on the reef. Large individuals of corkscrew anemones *B. annulata* also enhance reef biodiversity by serving as hosts to 6 other species of crustacean associates (Briones-Fourzan et al. 2012; Brooker et al. 2019; Huebner et al. 2019), some of which occasionally function as cleaners (Briones-Fourzan et al. 2012, Limbaugh et al. 1961). As such, information about how cleaner shrimp association patterns with these anemones (e.g. cleaning station locations) vary over space and time is critical for understanding the dynamics of fish community structure (Bshary 2003; Grutter et al. 2003).

Here we quantify for the first time patterns of dispersal by Pederson shrimp *A. pedersoni* among host sea anemones *B. annulata* on a Caribbean coral reef. Based on the above known aspects of cleaner shrimp biology, including the outcomes of our laboratory experiments (Chapter 2), we hypothesized that shrimp dispersal among hosts varies with shrimp social rank and social group size (number of shrimp per anemone). We therefore examined how shrimp dispersal patterns vary with these 2 factors, as well as with 2 other types of shrimp characteristics (body size and gender), and 2 types of host anemone characteristics (body size and nearest neighbor distance).

Methods

To address our hypothesis that shrimp dispersal among host anemones varies with shrimp social rank and social group size, we conducted field observations during 5 successive days (May 13-17, 2019) on patch reefs in Brewers Bay, St. Thomas, US Virgin Islands. This time scale was used because related studies (Chadwick et al. 2008) and preliminary observations at the study site (S. Ratchford, pers. comm.) indicated that cleaner shrimp numbers may change substantially on some anemones among days in < 1 week. The study site (18°20’27.5” N, 64°58’43” W) was at 6-8 m depth, ~ 220 m from shore near the MacLean Marine Science Center of the University of the Virgin Islands (UVI), which provided logistical support for boating and diving operations. This site consisted of small patch reefs interspersed with sand and rubble, and contained corkscrew sea anemones *Bartholomea annulata* and associated Pederson’s cleaner shrimp *Ancylomenes pederson*i which have been the subject of several previous field studies on this symbiotic system (for detailed site descriptions, see Huebner and Chadwick 2012a,b; O’Reilly et al. 2018; Titus et al. 2017; Gilpin and Chadwick 2019).

To delineate the study site, we selected an area of ~ 1,000 m^2 in the shape of an irregular polygon which contained 49 *B. annulata* anemones with associated shrimp. The exact location and dimensions of the study site differed somewhat from those examined in our past studies in this bay (O’Reilly and Chadwick 2017), because Hurricane Irma struck the area in September 2017 and greatly reduced the abundance of the anemones present. We therefore scanned the area to locate a site with enough anemones and shrimp for examination, then mapped and tagged all *B. annulata* anemones within the site by attaching a numbered metal tag to hard substrate adjacent to each anemone (after Dixon et al. 2017). There were no other species of reef anemones visible within the site, so individuals of *B. annulata* comprised the only host organisms available to *A. pedersoni* shrimp, which are obligate symbionts of sea anemones (Bauer, 2004). Thin string was attached to the reef surface and strung as guidelines between adjacent anemones, to aid in quickly relocating each anemone during successive days of the 5-day study. Initially we surveyed the site to record the number of *A. pedersoni* shrimp on each anemone. Then in order to focus on anemones that were occupied by shrimp (in contrast to those with no shrimp), we selected all anemones that contained at least 1 individual of *A. pedersoni* (N = 22 out of 57 anemones present; 38.59 % occupied). Only 1 other type of associated crustacean (spotted anemoneshrimp *Pereclimenes yucatanicus*) was present within the site, on 2 anemones. Effects of the presence of these other associates (much less common than reported about a decade ago during 2006-2009 in the same bay; Huebner et al. 2019), were not considered in the present study, because their impacts were considered to be minimal due to their rarity.

We used a random number generator to assign each occupied anemone to one of 2 treatments: (1) Handled Shrimp (all shrimp on N = 10 sea anemones), and (2) Not Handled Shrimp (all shrimp on N = 12 sea anemones). Shrimp in the Handled Shrimp were the main focus of our field observations and were removed briefly from their anemones each day to measure their body sizes and determine gender (see details below). Shrimp in the Not Handled Shrimp group were included to obtain similar but less precise observations on shrimp that were not removed each day from their anemones, as a control for effects of shrimp handling on their natural behavior in the field. On May 13 (Day 1), SCUBA divers used hand nets to collect all associated Pederson shrimp from each anemone in the Handled Shrimp group. Shrimp were considered to be associated with an anemone if they were < 8.5 cm from the anemone, which is the maximum distance that these shrimp usually occur around host anemones in the field (Gilpin and Chadwick 2019). Each collected shrimp was transferred underwater to a separate slide lock plastic bag (Ziplock, S.C. Johnson & Son, Racine, Wisconsin) filled with seawater, and all bags containing the shrimp from a single anemone were placed inside a drawstring mesh bag. The mesh bag also contained a plastic bag filled with air for buoyancy, a small piece of plastic paper (Waterproof & Tear Resistant Copier paper, Duracopy, Tacoma, Washington) with the anemone tag number and total number of shrimp on that anemone written in pencil, and a small lead weight.

The drawstring on the mesh bag was closed and clipped onto a rope (using an aluminum 8-cm carabiner clip, Kong-USA LLC, Bristol, Rhode Island) that was attached at one end to the body of the diver on the sea floor, and at the other end to a floating dive flag on the sea surface. The mesh bag floated along the rope up to the sea surface, where it was unclipped from the dive flag rope by a snorkeler, and transferred to a support boat with a shade canopy (UVI research vessel Lana June, 10-m length), which was moored at a buoy near the study site. Onboard the boat, the plastic bags were opened and the following data were collected for each: host anemone identification number, shrimp body size (total length [TL, to nearest mm] = anterior tip of rostrum to posterior tip of telson), and shrimp gender (female or non-female; after Gilpin and Chadwick 2017). To measure each shrimp, most of the water was emptied from the bag to trap the shrimp in a thin layer of water between the two sides of the bag; this reduced the ability of shrimp to locomote, and allowed fairly accurate body size measurement (see below for accuracy levels). This method has been used previously to measure TL for this species underwater, and did not appear to cause excessive stress to shrimp, in that they resumed swimming normally afterwards (Gilpin and Chadwick 2019). Calipers were placed against the outside of the bag to take measurements, and care was taken to not injure the shrimp.

Reproductive status was recorded initially as either female [F] (presence of oocytes, embryos or breeding dress; see Chapter 2 for details), or non-female [NF], because definite determination of male vs. juvenile status was not possible in the field (Gilpin and Chadwick 2017). The bag then was refilled with seawater, and all plastic bags each containing a shrimp from the same anemone were replaced into the mesh bag. Air was removed from the airbag, and the mesh bag assembly was returned to the floating dive flag, hooked onto the flag rope line using the carabiner clip, and allowed to sink back down to the diver at the bottom, weighted by the lead in the bag. The diver released all shrimp back onto their original host anemone and proceeded to the next selected anemone in the Handled shrimp group, to collect shrimp. Each shrimp was away from its host anemone for < 20 min, and all were observed to re-associate with the host after this process, similar to our previous studies that also involved brief removal of shrimp from host anemones at the same field site (Gilpin and Chadwick 2017, 2019).

In addition to collecting and returning shrimp from anemones in the Handled Shrimp group, divers also observed all shrimp on each anemone in the Not Handled Shrimp group, without touching or otherwise disturbing the anemone or its resident shrimp. Using underwater slates, they recorded the number of Pederson shrimp per anemone, the gender of each shrimp (F, NF), and the approximate size category (SNF: small non-female; LNF: large non-female; LFA: large female). We then repeated this process each day for 5 days, to collect daily information for all Pederson shrimp that occurred on all host anemones in both treatment groups (Handled and Not Handled Shrimp groups).

Characteristics of most of the *B. annulata* sea anemones in the study site (N = 48 total, including some of those without resident Pederson shrimp) were recorded at the end of the 5-day study. The tentacle crown length and width of each anemone was recorded in cm using a short plastic measuring tape, for calculation of body size (tentacle crown surface area [TCSA], after Hirose 1985; Gilpin and Chadwick 2019). Nearest neighbor distance (NND) also was measured for each anemone, as the linear distance to the closest individual of *B. annulata* (whether it contained shrimp or not), using a 25-m long plastic transect tape marked in cm, on a plastic reel (Lufkin).

Data analysis

To determine the number of days that each shrimp remained on each host anemone over the 5 days of the study, we analyzed the collected data on shrimp body sizes and genders. To obtain a conservative estimate of the minimal potential rates of shrimp dispersal among anemones, we assumed that shrimp with the same body size and gender on each anemone during successive days were the same individuals. If an observed shrimp was clearly different in body size and gender than all resident shrimp recorded during the previous day's census, it was assumed to be a new arrival. Likewise, if a shrimp of given body size and gender, which had been present on the previous day, was no longer present the next day, we assumed that it had left the anemone (either dispersed or died). To account for observer error (N = 2 observers) in the precision of measuring shrimp body sizes through the plastic bags onboard ship, we classified large shrimp (10 - 20 mm TL) as the same individuals among successive days, if they varied by < 4 mm in measured TL throughout the study, and small shrimp (2 - 10 mm TL) if they varied by < 2.5 mm in measured TL. To account for diver error (N = 2 divers) in detecting and collecting all shrimp on each anemone underwater, we also classified shrimp of each size and gender as remaining present on anemone (i.e., not dispersing) if they were recorded as missing for up to 1 day between 2 days on which they were recorded as present. We interpolated the presence, body size and gender of these shrimp between the 2 surrounding days on which they were observed, in order to be even more conservative in our estimate of shrimp dispersal rates among anemones. Shrimp body sizes were interpolated as the mean of TL measurements on the 2 days surrounding each 1 day of missing data. For the Not Handled Shrimp, presence was interpolated if there was a missing day between two shrimp of the same estimated size class (LFA, LNR, SNR). Using these criteria, size data were interpolated for 15.1% of shrimp in the Handled group (10 interpolated / 66 total Handled individuals) and presence data were interpolated for16.7% of shrimp in the Not Handled group (12 interpolated / 72 total Not Handled individuals).

Two sea anemones whose shrimp had been randomly assigned to the Handled group (see above) were not examined in the field on Day 1, and 1 anemone whose shrimp were in the Not Handled group was examined only on Day 1, due to underwater dive time limitations. These 3 anemones were excluded from the analyses of shrimp dispersal rates over 5 days, leaving 10 anemones with shrimp in the Handled group and 11 anemones with shrimp in the Not Handled group with complete datasets (Tables 3.2 and 3.3).

The TL of each shrimp was standardized for data analyses, as the mean TL calculated from all days that the shrimp was classified as present on an anemone. Shrimp TL data were available only in the Handled group, so we used linear regression analysis to determine how shrimp dispersal rate (number and percent of days on the same anemone host) varied with shrimp body size (TL), for all shrimp in the Handled group. We then assigned each shrimp to a body size class based on its mean TL (4 size classes: 0.1- 5.0, 5.1-10.0, 10.1-15.0, and 15.1-20.0 mm TL). We further assigned each Handled shrimp to 1 of 3 gender categories: female (oocytes or embryos visible in the abdominal brood pouch, see above), male (body size at least 10.0 mm TL body size, but no oocytes or embryos present), or juvenile (< 10 mm TL body size). Slightly different cutoff limits of body size for each gender were used here than in our previous study, because these shrimp become much larger in the laboratory than they do in the field (Gilpin and Chadwick 2017, 2019).

We applied a generalized linear model with a binomial distribution to determine which of 3 major factors (2 shrimp characteristics: body size and social group size; 1 temporal characteristic: day of the study) contributed most to the presence vs. absence of a given shrimp individual on a given host sea anemone during each day. Therefore, the 3 factors included in the model were: day (1-5), shrimp body size (mean TL of shrimp size over the 5-day study, continuous variable; see Table 3.1), and shrimp social group size (total number of shrimp on a given anemone during a given day), with individual shrimp identification code as a random intercept.

To analyze variation in shrimp behavior with gender, for shrimp in the Not Handled group, we assumed that all shrimp belonging to each major size and gender category (SNF, LNF, LFA) comprised the same individuals among successive days, again in order to be conservative in our estimates of the level of shrimp dispersal among anemones. We assigned each of the Not Handled shrimp to gender categories: female (see above), male (large non-females at least ~ 10 mm TL), and juvenile (small non-females < 10 mm TL), similar to gender categories for the Handled shrimp. We then applied non-parametric Kruskal-Wallis tests to assess variation in dispersal rate among the 4 body size classes of shrimp (in the Handled group), and among the 3 gender / relative size categories (in both groups), followed by Dunn post-hoc pairwise tests to determine variation between each pair of size classes or categories.

Variation in shrimp dispersal rate with shrimp social rank was analyzed for the Handled group only. Social rank in this experiment is defined as the assumed position of the shrimp in a social group based on its body size (TL) relative to those of all other shrimp on the same anemone on the same day (after Gilpin and Chadwick 2019). The shrimp with the largest TL was assigned the highest social rank (most dominant), and the smallest shrimp was assigned the lowest rank (most subordinate). Shrimp with the same calculated mean TL as other shrimp within a social group were assigned a social rank randomly among all same-sized individuals. Then we applied a chi-square test to analyze variation in the percent of events in which shrimp dispersed from vs. remained on an anemone during a given day, with the shrimp's social rank the previous day. Variation in the percent change in social group size on a given day, with the size of the social group on the previous day, was used as a measure of how shrimp social group size impacted dispersal rates. Linear regressions of percent change with social group size were applied to the Handled and Not Handled groups separately.

We used the percent time spent on the same anemone (number of days observed on the same anemone out of the 5 days of observation) as another measure of shrimp dispersal rates among anemones. We applied a Kruskall-Wallis test followed by post-hoc pairwise comparisons, to assess variation in shrimp percent time on the same anemone with both treatment type (Handled vs. Not Handled) and shrimp gender (juvenile, male, or female status).

Linear regressions were used to determine variation in shrimp percent time on the same anemone, with the 2 major anemone characteristics (anemone body size and distance to the next nearest anemone [NND]).

Results

Overall patterns

Of the 49 tagged individuals of corkscrew sea anemones *Bartholomea annulata* within the study site, we collected complete data on only 21 individuals (42.86 % of individuals) that contained Pederson shrimp (see Methods and below, for details). Total anemone abundance therefore was 49 individuals / 1,000 m^2 (0.49 individuals/ 10 m^2). Anemone body sizes ranged widely from 7.07 to 117.75 cm^2 TCSA and only 3 of the 38 total measured individuals reaching in the largest size class (> 100.0 cm^2 TCSA (7.89% of individuals). Of the 49 sea anemones that occurred within the study site, about half hosted Pederson shrimp (23/49 = 46.9%). Pederson shrimp occupancy rate varied somewhat among anemone size classes, from 25 to 100 % (Fig. 3.1). Of the 23 anemones which contained Pederson shrimp, we included 21 in our 5-day monitoring study, due to time constraints underwater. On these 21 anemones, there were 138 Pederson shrimp total (66 shrimp in 10 anemones in the Handled Shrimp group, and 72 shrimp on 11 anemones in the Not Handled Shrimp group, Tables 3.2 and 3.3). This shrimp population contained more small (< 10.0 mm TL; N = 44) than large individuals (> 10.0 mm TL; N = 22, Fig. 3.2), indicating active recruitment of shrimp to the population. Very small individuals (< 5.0mm TL) appeared to be recently metamorphosed juveniles, that had newly recruited to host sea anemones from the plankton, with the smallest shrimp only 2.9 mm TL. The population contained a low percentage of mature females that were brooding oocytes or embryos, only 17/138 individuals (12.32%), but more females may have been present that were not brooding.

The number of Pederson shrimp observed on each host anemone varied widely among anemones that were similar in body size and did not correlate significantly with anemone body size during any of the 5 days examined ($p > 0.3$ on all 5 days; Fig. 3.3). The smallest anemone at the study site (only 7.07 cm^2 TCSA) harbored up to 8 Pederson shrimp, while the largest one (117.75 cm^2 TCSA) harbored up to only 2 shrimp (Fig 3.8). Large shrimp (> 15mm TL) were not only rare (see above) but also fairly evenly dispersed among host anemones (i.e., they did not aggregate on anemones). Four of the anemones whose shrimp were assigned to the Handled group contained only small to medium-sized shrimp (e.g. no large ones), while 4 anemones each contained only 1 large shrimp, and only 2 anemones harbored 2 shrimp that were large.

Variation in shrimp dispersal with shrimp body size and gender

The generalized linear model indicated that both shrimp body size and shrimp social group size (number of shrimp per anemone) contributed significantly to the predicting the presence of an individual shrimp on a given sea anemone during each day of the study, with social group size contributing the larger effect (Table 3.1) As the social group size increased, the likelihood of absence on the next day increased. In contrast, the factor of "Day" did not contribute significantly to whether a shrimp was present or not, indicating that none of the examined days contributed more to shrimp presence than did any of the other days (Table 3.1).

The percent time that each shrimp remained on the same host anemone varied widely among individuals from 20-100% and did not vary significantly with shrimp body size as an isolated factor ($p = 0.27$, Fig. 3.4). The largest (> 15.0 mm TL, $N = 8$) and smallest individuals (< 5.0 mm TL, $N = 6$) exhibited bimodal distributions in terms of their dispersal behavior, in which they spent either 100% of their time over 5 days on the same anemone ($N = 4$ and 2 individuals, respectively), or they spent only ≤ 40% time on the same anemone ($N = 4$ for both groups). Additionally, the 2 smallest shrimp remained only 20% time while the 2 largest shrimp remained 100% of the time (all 5 days) on the same anemone. In contrast, medium-sized shrimp (5.0 - 15.0 mm TL) exhibited the full range of variation in percent time spent with the same host anemone.

Percent time on the same anemone also did not vary significantly when the shrimp were grouped into size classes (Kruskall-Wallis test, $\chi^2 = 1.28$, $df = 3$, $p = 0.733$, fig. 3.5). However, the median percent time increased with body size class, from juveniles (50.0%) to males (80.0%) and females (100.0%), but only in the Handled group, and this difference was not significant

(Table 3.4B). In the Not Handled group, this median increased from juveniles (80.0%) to males (100.0%) but then decreased in females (60%), such that males spent significantly more percent time with one anemone than did females in this group (Dunn test, p = 0.011, Table 3.4B). Percent time spent with the same sea anemone varied widely among individuals within each gender (Fig. 3.6). In contrast, this behavior did not vary significantly between shrimp in the Handled vs. Not Handled treatment groups, indicating no overall effect of handling on shrimp dispersal rates (Table 3.4A).

Variation in shrimp dispersal with shrimp social rank and social group size

The number of occasions on which a shrimp abandoned vs. remained on an anemone did not vary significantly with social rank (Table 3.5) or between 2 major social rank classes (high rank [1-3] vs. low rank [4-6+]; Chi-square test, $\chi^2 = 3.42$, df = 1, p-value = 0.064, Table 3.6). In most cases shrimp remained on the same host anemone between consecutive days (105 occurrences) rather than dispersing from the anemone (14 occurrences; includes shrimp that potentially died between days). Shrimp with low social rank (5+) exhibited a lower retention rate (only 74% of occurrences of remaining on an anemone between days vs. dispersing) when compared with all other social ranks which had higher retention (range of % retention = 86 to 96%).

Due to our classification of shrimp social rank by relative body size within each social group, the body size of shrimp that migrated into anemones (newly appeared on anemones during the study) correlated significantly with their rank in the newly joined social group (Fig. 3.7). Based on the sizes of other shrimp already present on a given anemone, relatively large shrimp (> 15.0 mm TL) always attained high social rank upon joining a new group (ranks 1-3), whereas very small shrimp (< 5.0 mm) always entered at low social rank (5-10). The shrimp that attained the lowest social rank upon recruiting into a sea anemone (rank 10; the smallest individual in a group of 10 residents) was the smallest individual observed during the entire study (2.9 mm TL).

The number of shrimp per sea anemone host (shrimp social group size varied widely not only on a spatial scale (among anemones during a given day, see above) but also on a temporal scale (among days of the study). Due to daily arrivals and departures of shrimp to/from anemones, shrimp social group sizes on each anemone varied somewhat between consecutive days, with a loss or gain of up to 3 individuals per social group. This relatively high level of change in social group size between days was observed on only 7 occasions during the study. In about half of comparisons between consecutive days (58%), shrimp social group size remained the same or changed by only 1 individual on a given host. The number of occasions on which a shrimp was observed to arrive to a given anemone did not vary significantly with the size of the social group that the shrimp joined (Fig 3.8A and B). In contrast, the number of observed shrimp departures increased significantly with size of the social group that the shrimp abandoned (Fig 3.8C). This relationship was significant for shrimp in both the Handled and Not Handled groups ($p < 0.02$ for both), indicating that both when shrimp were handled or only observed by researchers, they tended to disperse away from (or die in) large vs. small social groups on anemones. The same pattern was indicated when analyzing effects of shrimp body size and social group size together, in the generalized linear model described above (Table 3.1).

Variation in shrimp dispersal with host anemone characteristics

The number of occasions of change in shrimp social group size did not vary significantly with host sea anemone body size (Fig 3.9). Changes in shrimp social group size varied widely among anemones of similar body size, from a minimum of 0 changes in an anemone of 7.07 size TCSA, to a maximum of 3 changes in an anemone of 117.75 TCSA size.

Most sea anemones occurred near each other at the study site, with the majority < 7 m distant (nearest neighbor distance, NND), but a few occurring > 10 m distant from the nearest anemone (Fig. 3.10). The number of occasions of shrimp arrival to or departure from a given anemone on consecutive days (changes in shrimp social group size) varied widely among anemones that were similar distance from each other and did not vary significantly with nearest neighbor distance (Fig. 3.10). Shrimp that resided on host anemones which were located < 7 m distant from the next anemone appeared to move fairly frequently among hosts. Two of these nearby anemones experienced 8 changes in the social group sizes of their resident shrimp within only 5 days, and most of them (12 / 17 anemones with < 7 m NND, 70.58%) incurred 4 or more such changes. In contrast, the 2 most isolated anemones (> 7 m NND) both experienced < 3 changes in the social group sizes of their resident shrimp.

Discussion

Overall patterns

We show here that patterns of association by Pederson shrimp *Ancylomenes pedersoni* with host sea anemones *Bartholomea annulata* vary widely over a short period on a Caribbean coral reef. Shrimp recruited to and dispersed away from individual anemone hosts on a daily basis, leading to highly dynamic numbers of shrimp per host among the observed days. The most important contributor to shrimp dispersal away from a host, among the various factors examined here, was shrimp social group size on the host. Shrimp tended to leave hosts that contained a large number of conspecific shrimp, and to remain on hosts where there were few conspecific residents. A smaller but significant effect of shrimp body size was evident in the generalized linear model analysis, with small shrimp potentially dispersing more than large ones, although dispersal rate varied widely within each shrimp body size class examined here. Some of the shrimp that disappeared from anemones between days at our study site may have died rather than dispersed. However, our observations of many shrimp also arriving to anemones each day indicate that at least some departing individuals moved to other nearby anemones rather than leaving the site or dying. The estimates presented here of shrimp dispersal rates among host anemones are conservative and represent potentially minimal rates of change, due to the stringent criteria that we applied when assigning individual shrimp identities.

The population patterns documented here for corkscrew anemones *B. annulata* indicate that they may be in the process of recovering from damage incurred by Hurricane Irma, which struck St. Thomas in 2017 and reduced anemone abundances to near zero at this site (S. Ratchford, pers. comm.). The anemone abundance observed here was almost 10-fold lower than at the same site prior to Hurricane Irma, when estimates varied from ~ 4 anemones / 10 m^2 (O’Reilly and Chadwick 2017) to ~ 5 anemones / 10 m^2 (Titus et al. 2017). These anemones also previously had formed small aggregations at this site (two or more anemones per aggregation; 43% of the population, Titus et al. 2017), but no aggregations were detected during in the present study, only solitary individuals. Because rates of clonal reproduction and aggregation formation may indicate enhanced anemone energetic state (A. Colombara and N. Chadwick, unpublished data), the lack of aggregations at this site currently suggest that these anemones have not yet recovered physiologically from the hurricane disturbance. The low observed abundance of anemones (0.049/m^2) also may in part result from a lack of crevices and shelter spaces for these organisms, as they prefer convoluted reef habitat in which they can attach their pedal disks deep inside holes or crevices (Jennison 1981, Sebens 1976). The large patch reefs inside this bay which existed prior to the hurricane have now been reduced to rubble and small, low-rugosity patch reefs which likely provide less habitat space for this host species. Gradual recovery of this population is indicated by ~ 50% of the observed Individuals belonging to the smallest size classes (TCSA < 50 cm^2), which are classified as juveniles (O’Reilly and Chadwick 2017) and are assumed to be recent recruits to the reef (O’Reilly et al. 2018). Recruitment rates do not appear to be as high as in 2016, when a major proportion of individuals were very small recruits (<25.0 cm^2, Gilpin and Chadwick, 2019). Because this anemone has the most rapid rate of population turnover known for a tropical reef anemone, at only 1 - 2 years (O’Reilly and Chadwick, 2017), it is not surprising that this species appears to be recovering on St. Thomas, more rapidly than for all other reef cnidarians including soft and hard corals (N. Chadwick and S. Ratchford, pers. obs.).

The population structure of *A. pedersoni* shrimps found here is consistent with that documented in previous studies at the same site (Gilpin and Chadwick 2017). The presence of many small (< 10 mm TL) and relatively few large individuals (> 10 mm TL) indicates recruitment and survival of juveniles, with a potentially stable or growing population. The social group sizes (1-10 shrimp per occupied anemone) observed here also are similar to those documented previously (Gilpin and Chadwick, 2017). The relatively large numbers of Pederson shrimp associated with even the smallest anemones at the site (e.g. 8 shrimp on an anemone only 7.07 cm^2 TCSA) suggest crowding caused by a shrimp population limited in size by the low abundance of anemone hosts. This pattern may have contributed to the strong effect of shrimp social group size on dispersal rates, as revealed in the generalized linear model. Some anemones at the site also were observed to not harbor any Pederson shrimp, due to unknown factors possibly related to some aspect of anemone quality or detectability by symbiotic shrimp. The occupancy rate of *Bartholomea annulata* anemones by Pederson shrimp observed here was slightly lower than that reported for this site in 2016 (54.29%; Gilpin and Chadwick 2019). Notably, the abundance and diversity of other types of crustacean symbionts of these anemones (including species of crabs and mysids) were very low relative to that quantified during intensive surveys in 2006-2009 (Huebner et al. 2019), indicating that Pederson shrimp may be among the first species of symbiotic crustaceans to recover on corkscrew anemones following hurricanes.

Variation in shrimp dispersal with shrimp and anemone characteristics

To our knowledge, this is the first study to quantify rates of movement of symbiotic shrimp among host sea anemones, and the first to document rates of location change on coral reefs by individual cleaner shrimp. Most of the shrimp (gender, relative body size as indicated by social rank) and anemone characteristics (body size, nearest neighbor distance) examined here did not appear to drive strong patterns of dispersal behavior by Pederson shrimp. However, the lack of observed patterns may have been due in part to the limited sample sizes and duration of the present study. The trend revealed here of large shrimp social group size appearing to cause shrimp of all body sizes to depart from anemones has not been observed before in symbiotic shrimps that occupy invertebrate hosts. Potential mechanisms driving this pattern may include competition for food among social group members, which would be more intense in large than small social groups and is expressed via aggressive interactions among conspecifics in Pederson shrimp (Gilpin and Chadwick 2019). Previous research on patterns of departure by symbiotic crustaceans from hosts indicates that causal factors include the need to search for a mate for sexual reproduction (Thiel et al. 2003) as well as overcrowding that negatively impacts individuals due to aggression between conspecifics (Bell, 1984). In terms of reproductive strategy, Pederson shrimp exhibit pure search polygynandry (Gilpin and Chadwick, 2017) in which both males and females may switch hosts for reproductive purposes, suggesting that a search for additional mates may motivate the dispersal of at least some sexually mature individuals among hosts.

The observed trend of median percent time on the same host increasing with shrimp body size (from juvenile to male to female), as well as the shrimp body size effect detected in the generalized linear model analysis both suggest that some large shrimp may remain on host anemones for longer periods than do small shrimp. Because the largest shrimp on a host anemone are often female, and are socially dominant in that they chase away smaller shrimp and signal most frequently to potential fish clients, females could remain on the same anemone longer because they have priority access to food and mates within a given social group. Other symbiotic crustaceans exhibit similar behavioral patterns, in which large females remain on a single host organism for long periods (Thiel et al. 2003, Van der Meij 2014), remaining stationary as they wait for males to arrive and fertilize them. In the present study, about half the large females remained on the same anemone for all 5 days of observation, so this pattern does not apply to all females, who may disperse among hosts for a variety of reasons.

Future research is needed to individually mark and follow the movement patterns of these small delicate shrimp over longer periods, however technical barriers are likely to limit this possibility. Shrimp tagging methods, such as those utilized by Gilpin and Chadwick (2017) under laboratory conditions, allow identification of individual shrimp only until they next molt and shed their carapace (< 2 weeks), and may not be conspicuous enough for use in field studies. Subcutaneous markers and other methods are likely to damage or kill the small transparent individuals. Application of unique patterns of antenna and/or leg clipping on each shrimp may be technically possible, but also would cause body damage and could last only for a single molt period. The methods used here of distinguishing shrimp by relative body size and gender in each social group worked well for making conservative estimates about shrimp movement and could be applied in future field studies.

The lack of relationship observed here between host anemone body size and resident shrimp social group size differed from the pattern known from previous research, in which Pederson shrimp number per host increased significantly with *B. annulata* host size (Briones-Fourzan et al. 2012, Gilpin and Chadwick 2019). The lack of pattern detected here may have been due in part to the current recovery process of both shrimp and anemone populations at this site, in which both populations are not yet in stable state. The degree of correlation between these

2 characteristics varies among years, as shown by Gilpin and Chadwick (2019) who detected a significant correlation during 2016, but none in 2015. Various factors such as the low abundance of anemones (0.049/m^2), absence of aggregations, the distances between anemones currently at the site may have contributed to the lack of pattern during our observations in 2019. Finally, the absence of significant effect of anemone nearest neighbor distance (NND) on the number of days that shrimp remained with each anemone, indicates that anemones at this site, even though relatively rare, were still close together enough to frequently receive new shrimp arrivals (of all body sizes) and to experience shrimp departures. It appears that < 7 m distance to the nearest anemone, and < 5 m in most cases, is close enough for these shrimp to disperse among hosts, mostly likely at night when anemoneshrimps are known to disperse (Chadwick et al. 2008).

Conclusions

We conclude that Pederson shrimp association patterns with host anemones are highly dynamic over a period of only 5 days on a coral reef in the U.S. Virgin Islands, and that a major factor causing shrimp to disappear from anemones (disperse or die) is the social group size of shrimp on the same anemone. Individuals of all sizes and genders both arrive to and depart from hosts on a daily basis. Our hypothesis was supported that shrimp dispersal patterns vary with the shrimp characteristics of both social group size and shrimp body size. Therefore, a major factor driving an individual to disperse away from a host anemone may be shrimp overcrowding leading to resource limitation, possibly both in terms of access to mates and food resources. Future research is needed to individually mark and track these small delicate shrimp, and especially to observe their behaviors nocturnally when most dispersal may occur. Frequent dispersal by Pederson shrimp among anemones likely requires reef fishes to search often, even on a daily basis, for new cleaning stations on reefs, as old ones disappear or change substantially in cleaner shrimp composition.

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Table 3.1. Results of generalized linear model to determine which of 3 major factors contributed most to the presence vs. absence of individual Pederson shrimp *Ancylomenes pedersoni* on host sea anemones *Bartholomea annulata*, as observed on patch reefs at St. Thomas, USVI during each of 5 days in May 2019. The model used a binomial distribution to test the contribution of 3 major factors (2 shrimp characteristics: body size and social group size; 1 temporal characteristic: day of the study) to the presence vs. absence of a given shrimp individual on a given host sea anemone during each day. Specifically, the 3 factors included in the model were: day (1-5), shrimp body size (mean TL of individual shrimp size over the 5-day study), and shrimp social group size (total number of shrimp on a given anemone during a given day), with individual shrimp identification code as a random intercept (details in Table 3.2). N = 312 presence/absence observations on 66 shrimp. Note that both shrimp social group size and shrimp individual identity were highly significant contributors ($p < 0.001$), shrimp body size was a lesser but still significant contributor ($p < 0.05$), and that day of the study did not significantly contribute to the overall pattern.

Factor	**Z-value**	**P-value**
Random intercept (Shrimp identification code)	-3.184	0.00014
Day	0.065	0.948
Shrimp body size	2.285	0.022
Shrimp social group size	4.707	2.51e-6

Table 3.2. Variation in the presence and body size (total length [TL] in mm) of Pederson shrimp *Ancylomenes pedersoni* (N = 66 shrimp total) on host sea anemones *Bartholomea annulata* (N = 10 tagged anemones) among 5 consecutive days of observation on patch reefs at St. Thomas, U.S. Virgin Islands. Shown are data for shrimp in the Handled group (see Methods for details), in terms of the identification number of each tagged sea anemone (Anemone ID#), identification number of each resident shrimp observed on that anemone (Shrimp ID#), recorded body size of each shrimp on all days that it was observed to be present, mean body size of each shrimp calculated from all days that it was present, and total number of shrimp per anemone (shrimp social group size) on each day. On the first day of appearance of each shrimp, it was ordered by body size relative to other new shrimp on that day. A shrimp was assumed to be the same individual among days, if its recorded body size varied by < 2.5 mm if it was a small individual (1.0 to 10.0 mm TL) or by < 4.0 mm if large (> 10.0 mm TL), due to observer error in precisely measuring shrimp onboard a support vessel moored above the study site. Gray cells represent days that each shrimp was not observed to be present; black cells are shrimp sizes extrapolated from surrounding days, for up to 1 day due to assumed diver error in detecting a shrimp on a given anemone during a given day, if a similar-sized individual was observed on the days immediately before and after. Blue cells represent missing data for a given anemone (anemone was not examined that day). Note that the number of shrimp per anemone (shrimp social group size) varied widely both spatially (among anemones, 0-10 shrimp) and temporally (among days, 2 – 7 shrimp on a given anemone).

Anemone ID#	**Shrimp ID#**	**Recorded body size of shrimp (TL, mm) on Day:**					**Mean body size of shrimp (TL, mm)**
		1	**2**	**3**	**4**	**5**	
BA33	AP01	20.0	17.6	15.9	16.0	18.0	17.5
	AP02	10.0	10.2	8.7	9.3	10.5	9.7
	AP03	9.0	8.1	6.8	8.9	9.0	8.4
	AP04	9.0	8.0				8.5
	AP05		5.8				5.8
	AP06					8.0	8.0
	Total # Shrimp	4	5	3	3	4	
BA19	AP07	7.3	5.0				6.2
	Total # Shrimp	1	1	0	0	0	
BA42	AP08	11.2	10.9	11.0	10.0	10.0	10.6
	AP09	9.0	7.6	9.0	9.0	9.5	8.8
	AP10	6.4	6.5	6.5	6.25	6	6.3
	AP11		6.6	7.0	8.0		7.2
	AP12				18.0		18.0
	AP13				13.5	13.3	13.4
	AP14					12.0	12.0
	Total # Shrimp	3	4	4	6	5	
BA8	AP15	21.0	18.5	19.0	22.5	19.1	20.0
	AP16	17.0	18.0	17.0	15.5	15.0	16.5
	AP17	10.0	11.0	11.0			10.7
	AP18		11.0	13.5	14.5		13.0
	AP19				5.5	5.0	5.25
	AP20				5.0	6.3	5.65
	Total # Shrimp	3	4	4	5	4	

Table 3.2 Continued

Anemone ID#	**Shrimp ID#**	**Recorded body size of shrimp (TL, mm) on Day:**					**Mean body size of shrimp (TL, mm)**
		1	**2**	**3**	**4**	**5**	
BA74	AP21	19.0	18.7	18.0	18.0	19.0	18.5
	AP22	11.0	11.3	10.5	14.8	10.4	11.6
	AP23				7.7	6.8	7.3
	AP24				5.8	6.5	6.2
	AP25				3.9	4.2	4.05
	AP26					15.5	15.5
	AP27					4.6	4.6
	AP28	6.0					6.0
	AP29	6.0					6.0
	Total # Shrimp	4	2	2	5	7	
BA79	AP30	16.2	17.5	18.8	17.0	19.5	17.8
	AP31	12.5	11.9	11.3	13.5	9.9	11.8
	AP32			9.0	8.2	7.4	8.2
	Total # Shrimp	2	2	3	3	3	
BA84	AP33	9.3	10.3	10.0	10.8	11.6	10.4
	AP34	8.8	7.3	9.7			8.6
	AP35	6.0	5.0	5.2	7.0		5.8
	AP36		11.2	12.2	13.5	13.0	12.48
	AP37			3.9			3.9
	AP38				17.0		17.0
	Total # Shrimp	3	4	5	4	2	
BA59	AP39	5.9	7.6	6.5	7.6	8.0	7.12
	AP40	6.1	5.5	5.6	7.0	7.0	6.24
	AP41	6.0	6.3	6.6	5.0		5.9
	AP42	5.1	3.9	3.5	4.2	5.0	4.34
	AP43	4.7	4.9	3.0	3.4	4.0	4.0
	AP44		13.5	13.5	13.2	13.0	13.3
	AP45		9.0	7.6	9.0	10.0	8.9
	AP46		9.0	11.4	7.1	11.0	9.63
	AP47			13.0			13.0
	AP48			2.9			2.9
	Total # Shrimp	5	8	10	8	7	
BA70	AP49		10.9	11.4	11.5	10.9	11.2
	AP50		7.1	7.3	7.7	8.0	7.5
	AP51		6.3	6.2	6.8	6.1	6.4
	AP52			9.9	12.7	9.9	10.8
	AP53			8.5	9.0	9.2	8.9
	AP54			7.7	8.8	8.3	8.3
	AP55				5.0	4.7	4.9
	AP56					6.0	6.0
	Total # Shrimp		3	6	7	8	

Table 3.2 continued

Anemone ID#	**Shrimp ID#**	**Recorded body size of shrimp (TL, mm) on Day:**					**Mean body size of shrimp (TL, mm)**
		1	**2**	**3**	**4**	**5**	
BA58	AP57		11.5	15	10.5	12.5	12.38
	AP58		11.4	10.5	11.0	11.0	10.98
	AP59		8.3	8.5	9.0	8.0	8.45
	AP60		7.9	8.0	9.0	7.0	7.98
	AP61		7.5	7.5	8.5	8.0	7.88
	AP62		6.6	8.5	6.5	7.0	7.15
	AP63		6.7				6.7
	AP64		6.5				6.5
	AP65				10.0	10.0	10.0
	AP66				9.0	9.0	9.0
	Total # Shrimp		8	6	8	8	

Table 3.3. Variation in the presence and body size categories of Pederson shrimp *Ancylomenes pedersoni* (N = 48 shrimp total) on host sea anemones *Bartholomea annulata* (N = 11 tagged anemones, plus one that was observed for only 1 day) among 5 consecutive days of observation on patch reefs at St. Thomas, U.S. Virgin Islands. Shown are data for shrimp in the Not Handled group (see Methods for details), in terms of the identification number of each tagged sea anemone (Anemone ID#), identification number of each resident shrimp observed on that anemone (Shrimp ID#), body size category of each shrimp, and total number of shrimp per anemone (shrimp social group size) on each day. A shrimp was assumed to be the same individual among days, if its recorded body size category was the same (SNF = small non-female [juvenile]; LNF = large non-female [male]; LFA = large female, assumed to be the largest size category, after Gilpin and Chadwick 2017). On the first day of appearance of each shrimp, it was ordered by body size category relative to other new shrimp on that day. Gray cells represent days that each shrimp was not observed to be present; black cells are shrimp presence extrapolated from surrounding days, for up to 1 day due to assumed diver error in detecting a shrimp on a given anemone during a given day, if an individual in the same size category was observed on the days immediately before and after. Blue cells represent missing data for a given anemone (anemone was not examined that day). Note that the number of shrimp per anemone (shrimp social group size) varied widely both spatially (among anemones, 2-9 shrimp) and temporally (among days, 3 – 7 shrimp on a given anemone).

Anemone ID#	**Shrimp ID#**	**Presence of Shrimp on Day:**					**Body size category of shrimp**
		1	**2**	**3**	**4**	**5**	
BA40	NH01	Present	Present	Present	Present	Present	SNF
	NH02	Present	Present	Present	Present	Present	SNF
	NH03	Present	Present	Present	Present	Present	SNF
	NH04	▨	▨	Present	Present	Present	LNF
	NH05	▨	▨	▨	Present	Present	LNF
	NH06	▨	▨	▨	▨	Present	LNF
	Total # Shrimp	3	3	4	5	6	
BA7	NH07	Present	Present	Present	Present	Present	LNF
	NH082	Present	Present	Present	Present	Present	SNF
	NH09	▨	▨	Present	▨	▨	SNF
	NH10	▨	▨	▨	Present	Present	LNF
	Total # Shrimp	2	2	3	3	3	
BA21	NH11	Present	Present	▨	▨	▨	LFA
	NH12	Present	Present	Present	Present	Present	LNF
	NH13	Present	Present	Present	▨	▨	SNF
	NH14	▨	Present	Present	Present	Present	LNF
	NH15	▨	▨	Present	Present		LNF
	Total # Shrimp	3	4	4	3	2	
BA41	NH16	Present	Present	Present	Present	Present	LNF
	NH17	Present	Present	Present	Present	Present	LNF
	NH18	Present	Present	Present	Present	Present	LNF
	NH19	Present	Present	Present	Present	▨	SNF
	NH20	▨	▨	Present	Present	Present	LFA
	Total # Shrimp	4	4	5	5	4	

Table 3.3 continued

Anemone ID#	**Shrimp ID#**	**Presence of Shrimp on Day:**					**Body size category of shrimp**
		1	**2**	**3**	**4**	**5**	
BA44	NH21	Present	Present	Present	Present	Present	LFA
	NH22	Present	Present	Present			LFA
	NH23	Present	Present	Present	Present	Present	LNF
	NH24	Present	Present	Present	Present	Present	LNF
	NH25	Present	Present	Present	Present	Present	LNF
	NH26			Present	Present	Present	SNF
	NH27				Present	Present	LNF
	NH28				Present	Present	LNF
	NH29					Present	SNF
	Total # Shrimp	5	5	6	7	8	
BA45	NH30	Present	Present	Present	Present	Present	LFA
	NH31	Present	Present	Present	Present	Present	LNF
	NH32	Present	Present	Present	Present	Present	LNF
	NH33	Present					LNF
	NH34		Present	Present	Present	Present	SNF
	NH35			Present			LFA
	NH36			Present			SNF
	Total # Shrimp	4	4	6	4	4	
BA52	NH37	Present	Present	Present	Present	Present	LFA
	NH38	Present	Present	Present	Present	Present	LNF
	NH39	Present	Present	Present	Present	Present	LNF
	NH40	Present	Present	Present	Present	Present	LNF
	NH41	Present					LNF
	NH42		Present	Present	Present	Present	SNF
	NH43		Present	Present	Present	Presetn	SNF
	NH44		Present				SNF
	NH45			Present			LFA
	Total # Shrimp	5	7	7	6	6	
BA54	NH46	Present	Present	Present	Present		LFA
	NH47	Present	Present	Present	Present	Present	LNF
	NH48	Present	Present	Present	Present	Present	LNF
	NH59		Present	Present	Present	Present	SNF
	NH60			Present	Present	Present	LNF
	NH61			Present	Present	Present	LNF
	NH62				Present	Present	SNF
	NH63				Present	Present	SNF
	NH64				Present		SNF
	Total # Shrimp	3	4	6	9	7	

Table 3.3 Continued

Anemone ID#	**Shrimp ID#**	**Presence of Shrimp on Day:**					**Body size category of shrimp**
		1	**2**	**3**	**4**	**5**	
BA55	NH65	Present	Present	Present	Present	Present	LNF
	NH66	Present	Present	Present	Present	Present	LNF
	NH67	Present	Present	Present	Present	Present	SNF
	NH68	Present	Present	Present	Present	Present	SNF
	NH69	Present	Present	Present	Present		SNF
	NH70		Present	Present	Present	Present	LNF
	NH71				Present		LFA
	NH72				Present		SNF
	Total # Shrimp	5	6	6	8	5	
BA57	NH73	Present	Present	Present			LFA
	NH74	Present	Present	Present	Present		SNF
	NH75		Present	Present	Present	Present	LNF
	NH76		Present	Present	Present	Present	LNF
	NH77		Present	Present	Present		SNF
	NH78				Present		LNF
	Total # Shrimp	2	5	5	5	2	
BA56	NH79	Present					SNF
	NH80	Present	Present	Present	Present	Present	LNF
	NH81		Present				LFA
	NH82		Present	Present	Present	Present	LNF
	Total # Shrimp	2	3	2	2	2	
BA37	AP137	Present					SNF
	Total # Shrimp	1	NA	NA	NA	NA	

Table 3.4. Results of statistical tests on variation in the percent of days that each Pederson shrimp *Ancylomenes pedersoni* spent on each host sea anemone *Bartholomea annulata*, over 5 consecutive days of observation on patch reefs at St. Thomas, USVI. A. Chi-square test of variation between shrimp of each gender that were in the Handled versus Not Handled groups. B. Chi-square tests of variation among the 3 gender categories, separately for the Handled and Not Handled groups, followed by Dunn post-hoc pairwise tests on variation between each pair of gender categories. Dunn test statistic values within each cell [Z-score] are shown above p-values, which are in parentheses. Gender categories were assigned based on shrimp body size, as Female (> 15 mm total length [TL]), Male (10.0-15.0 mm TL), or Juvenile (< 10 mm TL). Note that there were no significant differences for any comparisons, except for between males and females in the Not Handled group (bolded b-value). See text for details.

A.

Handled vs. Non-Handled shrimp			
Shrimp gender	X^2	df	P
Female	1.49	1	0.222
Male	0.39	1	0.531
Juvenile	0.62	1	0.432

B.

Shrimp group	X^2	df	p-value	Gender	Female	Juvenile
Handled	1.87	2	0.392	Juvenile	1.08 (0.141)	
				Male	0.27 (0.395)	-1.08 (0.140)
Not Handled	5.81	2	0.055	Juvenile	-1.06 (0.143)	
				Male	-2.29 **(0.011)**	-1.45 (0.073)

Table 3.5. Number of times that an individual Pederson shrimp *Ancylomenes pedersoni* remained on versus abandoned (left) a given host sea anemone *Bartholomea annulata* betweeen pairs of observation days: Variation with shrimp social rank (body size relative to all other shrimp on the anemone) during the previous day, based on 5 consecutive days of observation on tagged sea anemones on patch reets at St. Thomas, U.S. Virgin Islands. Note that shrimp with a rank of 5+ had a lower retention rate than all other groups, but that due to high retention between pairs of observation days, there was no significant variation in percent retention the next day with social rank on the previous day.

Number of times shrimp observed to:	Social rank (relative body size) of shrimp during the previous day:					All social ranks
	Rank 1	Rank 2	Rank 3	Rank 4	Rank 5+	
Remain on anemone	27	25	23	13	17	105
Abandon anemone	3	1	2	2	6	14
All observations	30	26	25	15	23	119
Percent of observations in which shrimp remained	90%	96%	92%	86%	74%	88.2%

Table 3.6. Table of values used for chi-square analysis of number of individuals of Pederson shrimp *Anyclomenes pedersoni* in each rank group (ranks 1-3 and 4-6+) and the number of instances that individuals within groups either remained on the *Bartholomea annulata* sea anemone host or abandoned the host.

	Rank 1-3	Rank 4-6+	Total
Remained on sea anemone	75	30	105
Abandoned sea anemone	6	8	14
Total	81	38	119
Percent retention (%)	92.59	78.95	88.24

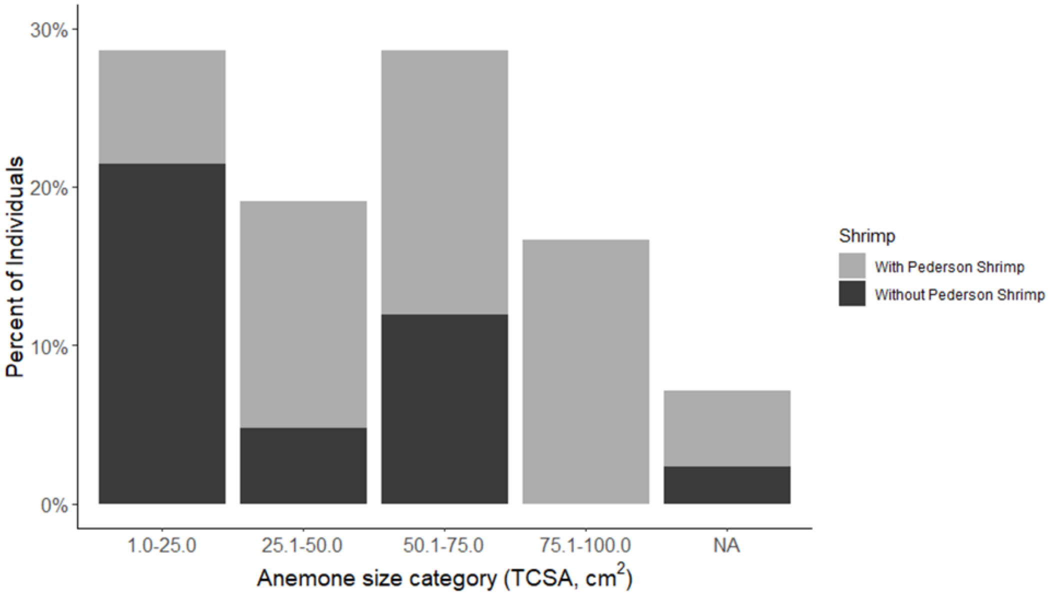


Fig. 3.1. Population size structure of corkscrew sea anemones Bartholomea annulata sea anemones on patch reefs examined during May 2019 at St. Thomas, U.S. Virgin Islands (N = 41 anemones), and proportion of anemones in each size class that contained Pederson shrimp *Ancylomenes pedersoni*. Note that most anemones were small, and that anemones in all size classes harbored Pederson shrimp, but small anemones contained the lowest proportion of associated shrimp.

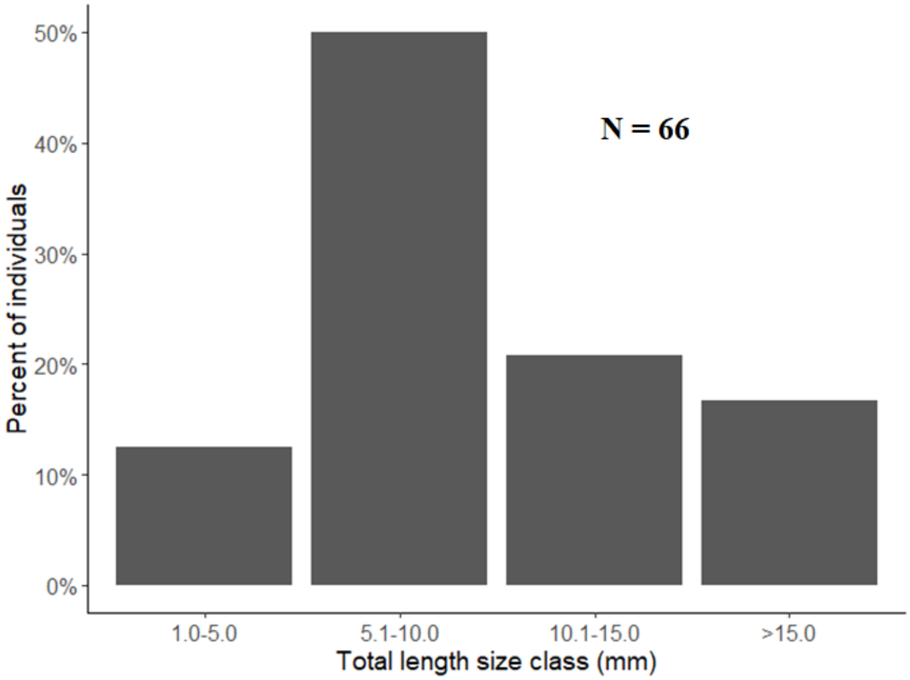


Fig. 3.2. Population size structure of Pederson shrimp *Ancylomenes pedersoni* on corkscrew sea anemones *Bartholomea annulata* examined during May 2019 on patch reefs at St. Thomas, U.S. Virgin Islands. Shown is the percent of individuals in each size class, measured as total length (TL; range = 2.9 to 20.0 mm). Note that most individuals were small to medium in body size. Individuals in the smallest size class were assumed to be post-larval individuals that had recently metamorphosed and recruited to host anemones from the plankton.

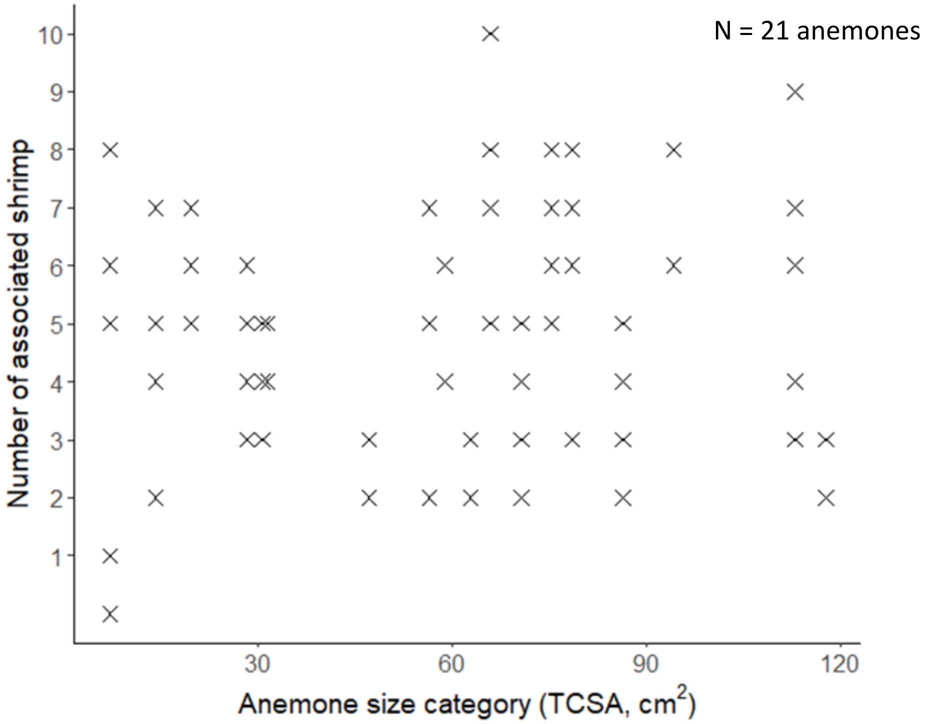


Fig. 3.3. Variation in the number of Pederson shrimp *Ancylomenes pedersoni* associated with each individual host sea anemone *Bartholomea annulata*, among anemones of different body sizes (tentacle crown surface area [TCSA] in cm^2) and among 5 consecutive days of observation on each anemone (represented by 2-5 data points [x's] for each anemone size) on patch reefs at St. Thomas, U.S. Virgin Islands. Note the high variability in both the minimum and maximum number of shrimp observed per anemone among days, and the lack of significant variation among anemone sizes.

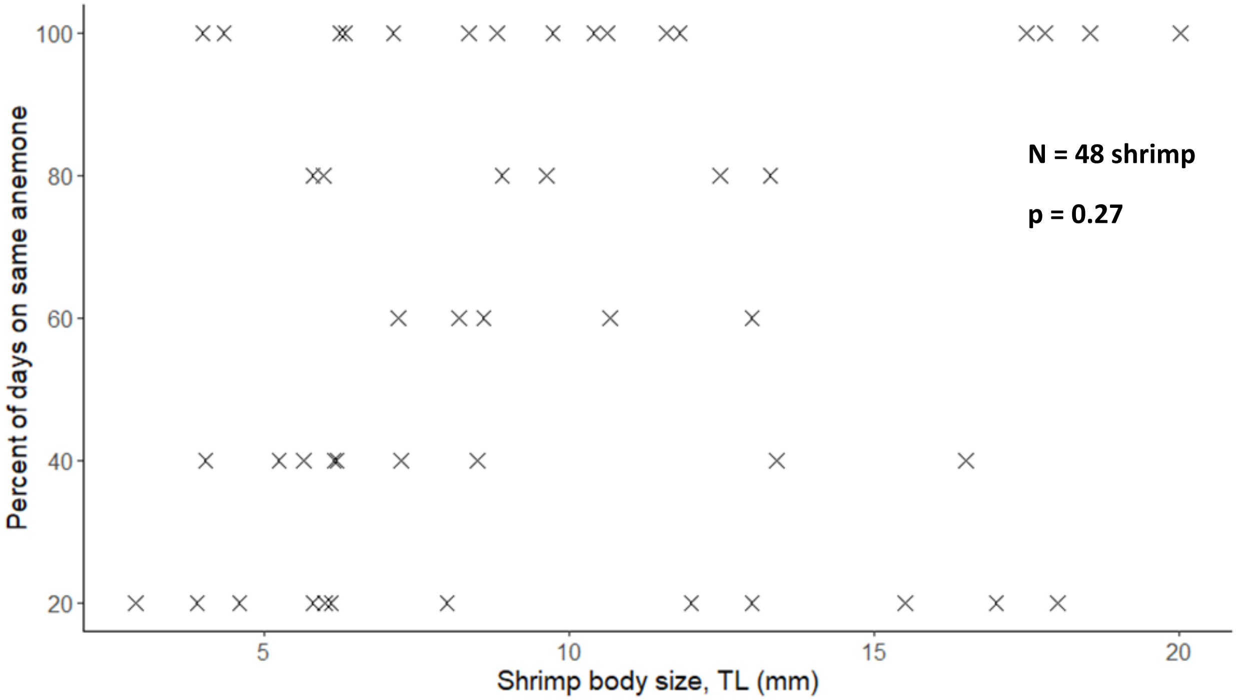


Fig. 3.4. Variation in the percent of days that Pederson shrimp *Ancylomenes pedersoni* shrimp remained on the same host sea anemone *Bartholomea annulata*, with shrimp body size over 5 consecutive days of observation on patch reefs at St. Thomas, U.S. Virgin Islands. Note that the percent days on each anemone varied widely and did not correlate significantly with body size, and that very small (< 5 mm total length {TL]) and large shrimp (> 15.0 mm) both were bimodal in their behavior. The 2 smallest shrimp remained only 20% of the 5 observation days (1 day each) on the same anemone, and in contrast the 2 largest shrimp remained all 5 days.

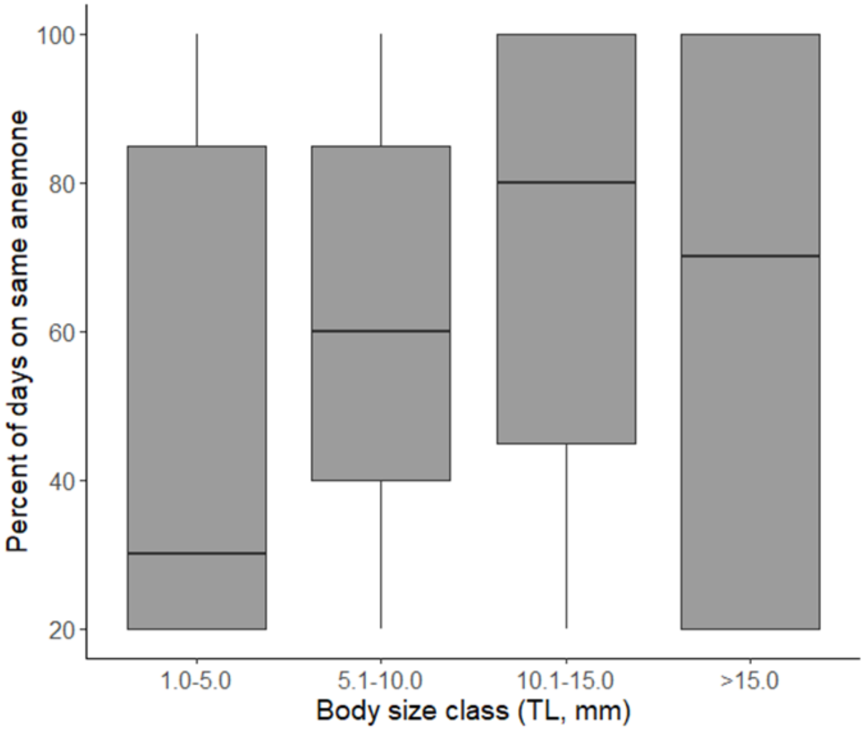


Fig. 3.5. Variation in the percent of days that Pederson shrimp *Ancylomenes pedersoni* shrimp remained on the same host sea anemone *Bartholomea annulata*, among 4 body size classes of shrimp, over 5 consecutive days of observation on patch reefs at St. Thomas, U.S. Virgin Islands. Note the trend of increasing median percent days on each anemone with increasing body size class, but wide variation within each size class leading to a lack of significant differences among the 4 size classes. The highest variability in behavior was exhibited by the smallest (1.0-5.0 mm total length [TL]) and largest shrimp (> 15.0 mm TL).

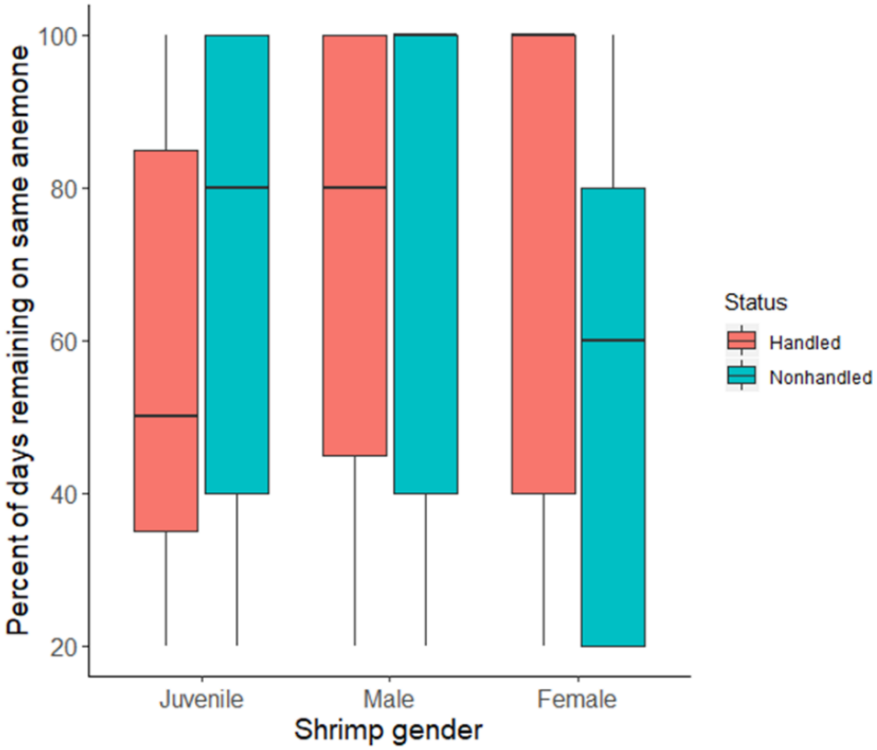


Fig. 3.6. Variation in the percent of days that Pederson shrimp *Ancylomenes pedersoni* shrimp remained on the same host sea anemone *Bartholomea annulata*, among 3 gender categories and between Handled vs. Not Handled Shrimp groups, over 5 consecutive days of observation on patch reefs at St. Thomas, U.S. Virgin Islands. Note that the median percent days remaining on an anemone increased from the small juveniles to medium-sized males to large females, in the Handled but not in the Not Handled group. Note also the wide variation in behavior within each group, leading to no significant difference among genders, or between the Handled and Not Handled groups within each gender. See Table 3.3 and text for details.

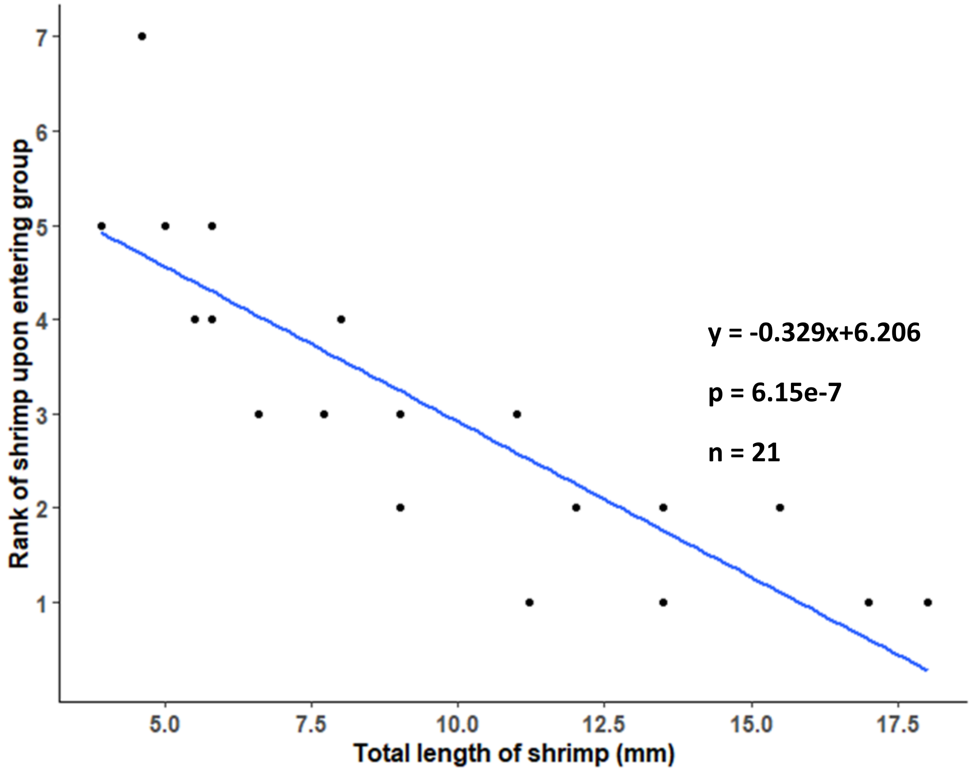
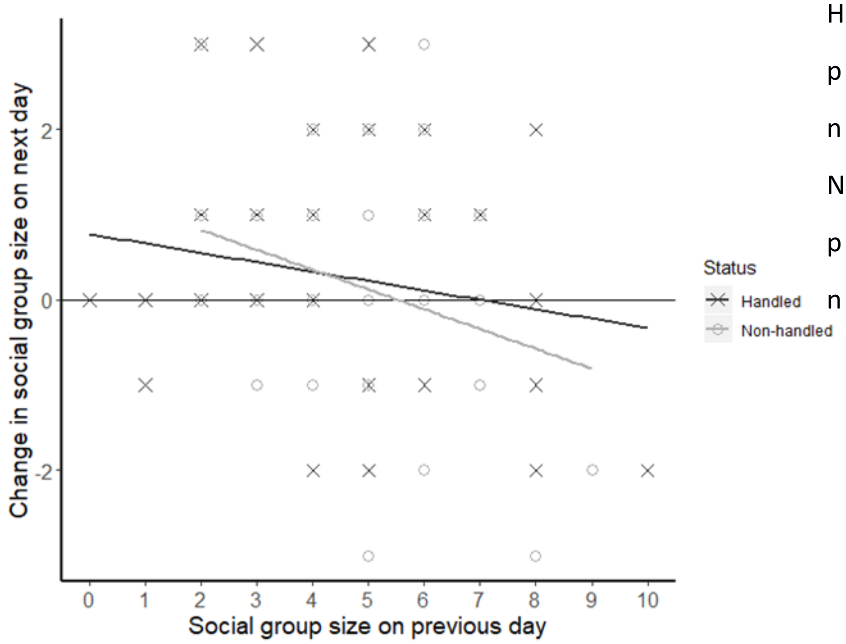


Fig. 3.7. Effect of the total length (TL, mm) of Ancylomenes pedersoni shrimp on their rank when first entering a new social group over 5 consecutive days of observation on coral reefs at St. Thomas, U.S. Virgin Islands. Note that as the y-axis increases, the rank in the social group decreases (becomes more subordinate, i.e. rank 1 is the most dominant shrimp within the social group). There is a significant correlation in which the rank decreases (the shrimp is more dominant) as the TL of the shrimp increases. Note also that the highest rank a shrimp attained upon entering a group was rank 7, even though anemones were found with social groups of up to 10 shrimp.

A.



Handled:

p = 0.035

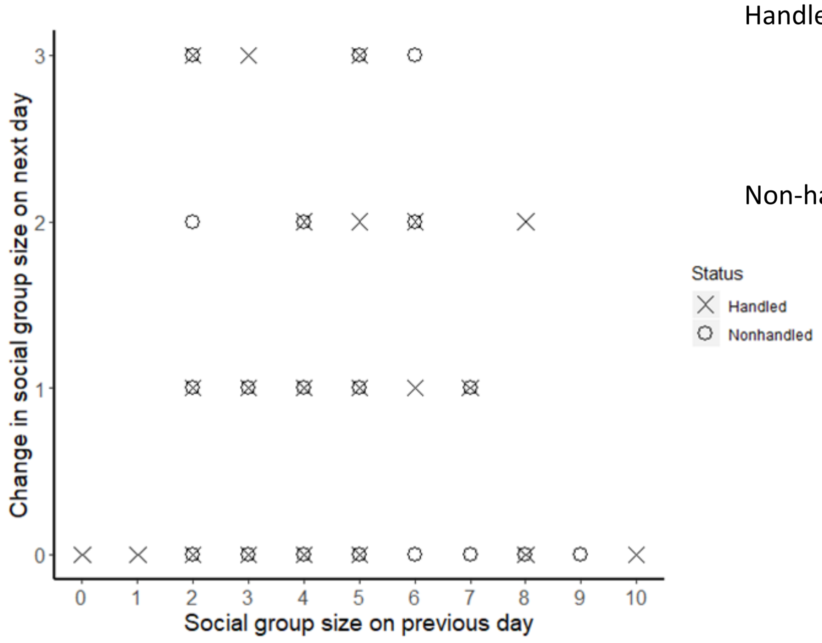
n = 38

Non-Handled:

p = 0.047

n = 44

B.



Handled:

p = 0.7041

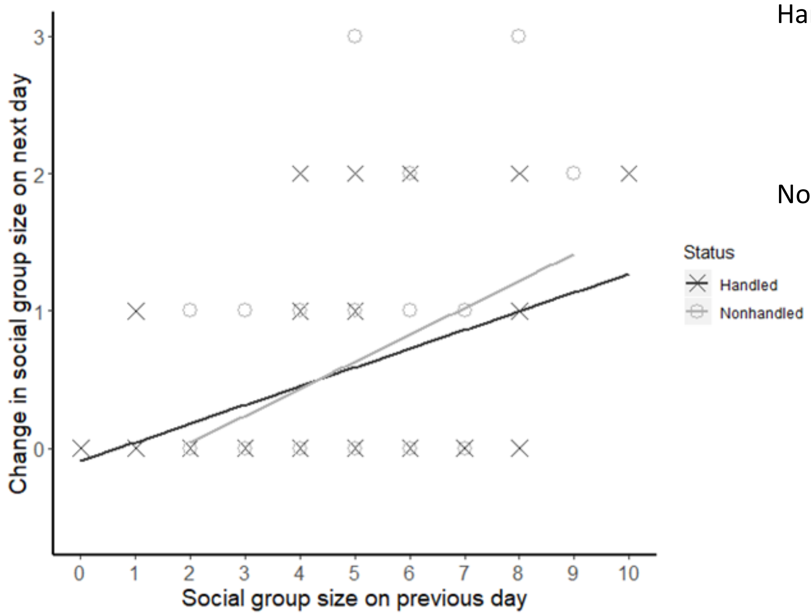
n = 38

Non-handled

p = 0.0675

n = 44

C.



Handled:

p = 0.014

N = 38

Non-Handled:

Fig. 3.8. Variation in aspects of the behavior of Pederson shrimp *Ancylomenes pedersoni* with their social group size on host sea anemones *Bartholomea annulata*, as observed during 5 consecutive days on tagged anemones on patch reefs at St. Thomas, USVI. Shown is the shrimp social group size (number of shrimp on each host sea anemone) during the day immediately before (previous to) the day of the shrimp behavior examined.
A. Variation in the amount of change in shrimp social group size on a given day (number of shrimp the previous day minus the number that day, with the social group size on the previous day. Note that there were no instances of social groups changing by more than 3 individuals between consecutive days, and no significant trends. Most changes (n = 58) involved no change in social group size, or a change of only 1 shrimp, and did not vary with social group size.
B. Variation in the number of shrimp that arrived to an anemone with the social group size immediately before their arrival (the previous day). Note that most (64/78) observations were of either zero or one arrivals of new shrimp to a group, and few (18/78) were of two or more shrimp arriving to a group. The number of arrivals did not vary significantly with the size of the social group.
C. Variation in the number of shrimp that departed from an anemone with the social group size of the group they departed from (group size the day before departure). Note that in both the handled and non-handled shrimp groups, the number of shrimp departures on a given day increased significantly with shrimp social group size during the previous day.

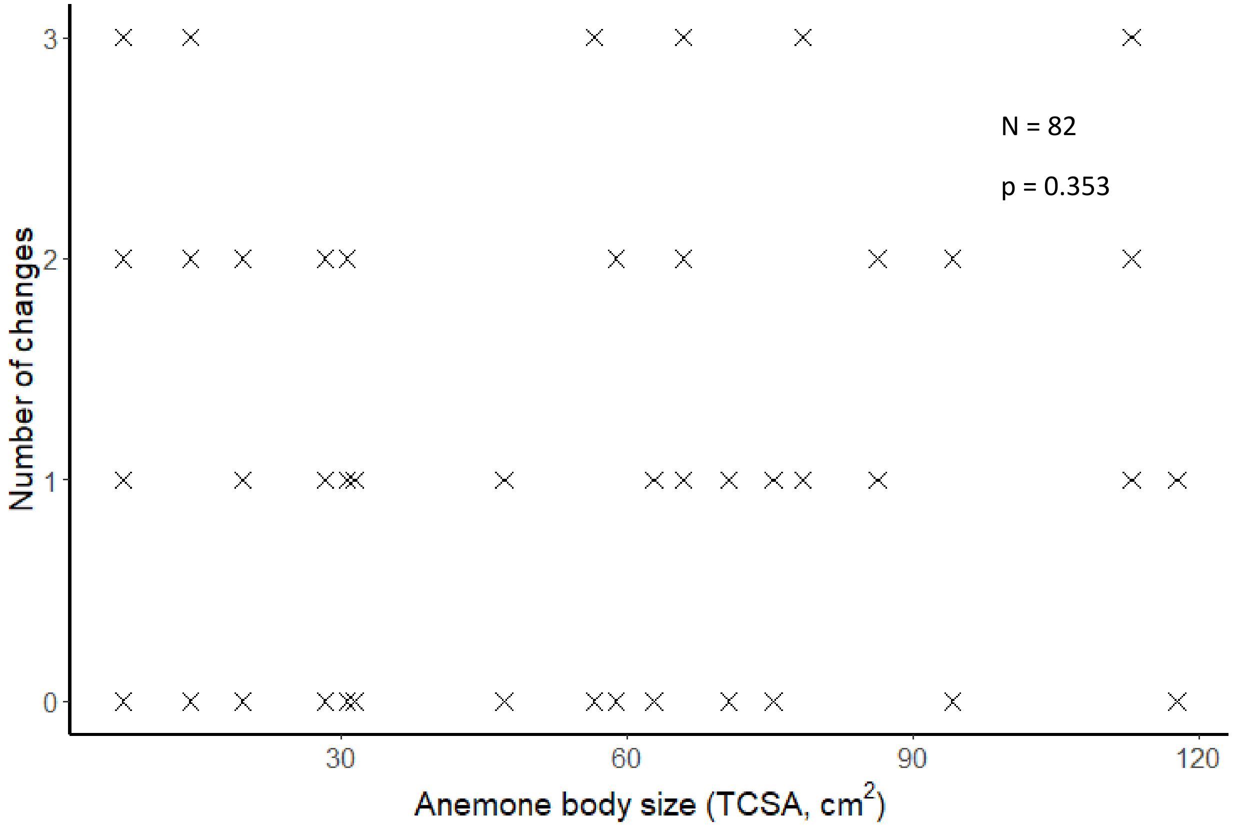


Fig. 3.9. Variation in the number of changes (arrivals and departures) in the social group size for each host Bartholomea annulata sea anemone among anemones of different body sizes (tentacle crown surface area [TCSA] in cm^2) and among 5 consecutive days of observation (represent by 2-4 data points [x's] for each anemone on patch reefs at St. Thomas, U.S. Virgin Islands. Note the high variability among sea anemones of all sizes and the lack of significant variation.

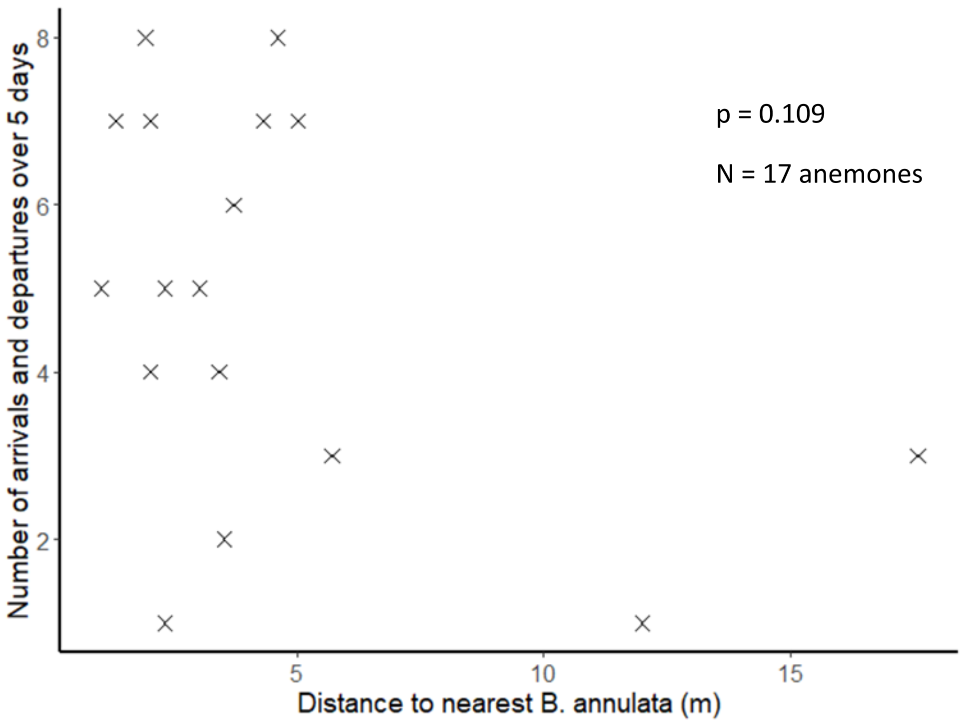


Fig. 3.10. Variation in the number of occasions on which Pederson shrimp *Ancylomenes pedersoni* shrimp changed host sea anemones *Bartholomea annulata* (arrival + departure events), with the distance to the nearest neighboring anemone (nearest neighbor distance [NND] to the next individual of B. annulata), during 5 consecutive days of observation on tagged sea anemones on patch reefs at St. Thomas, U.S. Virgin Islands. Note that most anemones were < 5 m distant from the nearest neighbor, and that shrimp arrivals plus departures varied widely for nearby anemones but was low for the few anemones that were > 5 m distant from each other.