The Role of Host Sea Anemones in the Cleaning Mutualism Between Anemoneshrimp and Client Fishes

by

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Abstract

Cleaning symbioses may strongly impact the diversity of coral reef fishes, but little is known about the dynamics of mutualistic networks involving cleaner shrimps that form obligate associations with sea anemones. I used field observations and experiments to determine the role of corkscrew anemones *Bartholomea annulata* in cleaning interactions between anemoneshrimp *Ancylomenes pedersoni* and a wide diversity of fish clients (>16 families) on Caribbean coral reefs. Client fishes use the large conspicuous anemones, rather than the small associated shrimp, as visual cues to locate the cleaners and as a stimulus to pose for cleaning. Visitation rates by client fishes depend on anemone size and the total number of crustacean associates (5+ species) per anemone, but cleaning rates and duration depend on the number of cleaner shrimp *A. pedersoni*. I conclude that the dependence of this fish-shrimp cleaning interaction on host anemones and their endosymbiotic zooxanthellae represents a complex mutualistic network.

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CHAPTER 1

A review of cleaning symbioses with an emphasis on cleaner shrimps

1. CLEANING SYMBIOSES

Mutualism is a type of symbiosis among species in which all partners derive a fitness benefit (Dickman 1992). Cleaning associations are mutualisms that provide nutritional benefits to one partner (the cleaner), who removes and ingests ectoparasites and damaged tissues from the other partner (the client), who benefits from reduced parasite loads and faster wound healing (Foster 1985, Losey 1987, Côté 2000). These associations occur in both terrestrial environments, where tickbirds, for example, remove parasites from ungulates (Dickman 1992), and in aquatic ecosystems, where cleaners are either fishes or crustaceans and clients are typically fishes (Côté 2000, Grutter 2002). Although this interaction occurs across habitat types, most examples of cleaning associations are from aquatic environments (Losey 1987, Côté 2000, Grutter 2002).

The diverse cleaner species that occur in marine systems demonstrate several types of convergently-evolved characteristics. Cleaners are typically small relative to their clients, feed on parasitic invertebrates, have well-developed vision for parasite recognition, and possess mouth-parts or appendages that are appropriate for picking prey off the bodies of clients (Poulin & Grutter 1996). A total of 131 species of marine cleaners were identified in a review by Côté (2000). Most of these (79%) are facultative cleaners, which derive only part of their diet from cleaning, mainly during the juvenile stage. In contrast, obligate cleaners obtain on average 85% or more of their food intake from cleaning and do so throughout their lives (Côté 2000).

Cleaners of fishes benefit through the removal and ingestion of parasites from clients, as well as the consumption of client mucus and tissues (Grutter 1997), but the universal benefit of cleaning to clients has been questioned (Losey 1987, Cheney & Côté 2003). An alternative hypothesis to cleaning as a mutualism purports that cleaners dupe clients into cleaning through tactile stimulation, and they then potentially prey upon the healthy tissues of subdued clients (Losey 1987). This would make the cleaner a behavioral parasite (Poulin & Grutter 1996). Losey (1987) suggested that tactile stimulation must be adaptive to clients because of its frequency in cleaning symbiosis and the positive response of clients to tactile stimulation; fish may seek out tactile stimulation because it is a “pleasant sensation” similar to scratching.

Client fish have innate mechanisms for recognizing cleaners. Laboratory-reared damselfish, with no previous exposure to cleaners, pose for cleaning 10% of the time on their first exposure to a cleaner. The pose posture of naïve juveniles is typical of experienced adults, suggesting that tactile stimulation is not necessary for clients to seek cleaning interactions. Naïve clients do not pose for non-cleaners that are visually similar to cleaners, suggesting a specific template for cleaner identification by clients (Losey et al. 1995). Small body size and lateral stripes denote an organism as a likely cleaner, but behavioral cues - such as cleaner dances - also are necessary to confirm to a client that the organism is indeed a cleaner (Stummer et al. 2004). Obligate cleaners demonstrate this guild coloration much more strongly than do facultative cleaners, which exhibit no similarity in coloration among species (Côté 2000).

Trivers (1971) first proposed that species, including cleaners and clients, interact altruistically to reciprocate needed benefits, but since then interactions among species have been shown to be more complex than direct reciprocal altruism. Cleaning associations can be viewed in terms of the iterated Prisoner’s Dilemma, in which interacting species either cooperate with each other for fitness benefits, or attempt to receive a benefit from cooperation without reciprocating. The concept is that two species which interact cooperatively can make choices: both cooperate, one cooperates and the other defects, or both defect. If they both defect, neither receives a benefit, and when both cooperate, they both benefit, but when the first species cooperates and the second does not, greater benefit accrues to the first species because it receives benefit without having to expend its own energy to benefit the second species. However, in such a system, there must be enough cooperation to ensure that both species maintain the interaction, otherwise it will cease. Species thus may balance between cooperating and defecting, in order to obtain maximum net benefit while still maintaining the interaction (Axelrod & Hamilton 1981).

Few experimental data support cleaning associations as an iterated Prisoner's Dilemma, however (Bshary & Grutter 2002a), so cleaning associations more recently have been viewed in terms of market theory (Noë et al. 1991). The iterated Prisoner's Dilemma does not allow for partner switching, which may occur in marine cleaning associations (Bshary & Grutter 2002a). Cleaners offer a service in demand by fishes, and fishes can choose among cleaners if they have large home ranges (choosy fish), or may have access to only one cleaner if their home range is small. Cleaners thus receive pressure to provide good service to choosy fishes, otherwise these fishes will seek service elsewhere, but cleaners may more easily provide poor service or even refuse service to resident fishes. If a cleaner cheats, as evidenced when a client jolts as a cleaner removes healthy tissue, choosy fishes can leave, but resident fishes are forced to expend energy to punish the cleaner by chasing it. Thus, fish clients can control cleaners, while cleaners can decide on the quality of service to provide. This process has been observed in cleaning associations on Indo-Pacific coral reefs (Bshary & Schäffer 2002) and in laboratory experiments with the Indo-Pacific cleaner wrasse *Labroides dimidiatus* (Bshary & Grutter 2002b). Additionally, the Red Sea cleaner shrimp *Ancylomenes longicarpus* increases its service quality for predatory clients, possibly because predatory clients are potentially more aggressive (Chapuis & Bshary 2009). However, not all cleaners and clients appear to have evolved a control-punishment system (Soares et al. 2008).

In addition to being cheated by true cleaners, clients may also be cheated by cleaner mimics. Limbaugh (1961) first suggested that some fish mimic cleaners to benefit from reduced predatory attacks, and to prey upon naïve clients. In the Indo-Pacific region, the fang-blenny *Aspidontus taeniatus* is a mimic of the obligate cleaner wrasse *L. dimidiatus*. The mimic attracts clients by adopting a dance and coloration similar to the wrasse, but then bites the client and retreats. Usually mimics fool juvenile client fishes, but more mature, experienced fishes either chase or withdraw from mimics when they recognize them (Côté 2000).

Honest participation in this association rewards both organisms the most, because the cleaner receives food and the client has its parasites and/or unhealthy tissues removed, or receives tactile stimulation, which may or may not be beneficial (Poulin & Grutter 1996). Despite the occurrence of cheating, recent studies have confirmed that cleaning associations in general are mutualisms: some cleaners do not provide tactile stimulation (Sikkel et al. 2004, Soares et al. 2008), fish with more parasites spend more time with cleaners (Grutter 1997, Sikkel et al. 2000), and fish without parasites are not attracted to cleaners (Grutter 2001). As well, client fish with wounds frequent cleaners more often than do those without, presumably to facilitate healing (Foster 1985). Just because cleaners remove parasites does not mean that some do not also provide clients with "pleasant" tactile stimulation; thus parasite removal and tactile stimulation do not have to be mutually exclusive mechanisms for the evolution of this symbiosis (Losey 1987, Poulin & Grutter 1996). One may be a proximate behavioral mechanism (clients visit cleaners because they receive tactile stimulation) and the other may be the ultimate evolutionary mechanism that drives this association (clients visit cleaners because they enhance their fitness via reduced parasite loads; Poulin & Grutter 1996).

Marine cleaning demonstrates some broad ecological trends worldwide, despite the diversity of clients, cleaners, and ecological habitats involved. There is a positive relationship between client abundance and the frequency with which clients are cleaned, with the most abundant client species more frequently cleaned than are the largest-bodied species. This is probably because larger fish are less abundant (Floeter et al. 2007). However, Grutter (1995) found that fish surface area influences cleaning rates, indicating that cleaners may use fish size as an indicator of food availability on the client. Solitary or carnivorous clients do not have any strong trends of cleaning frequency, probably because they are not abundant. Overall, cleaners are not limited by access to clients (Floeter et al. 2007), but may be limited by the abundance of suitable microhabitats for cleaning stations, such as reef holes, coral heads, or host sea anemones (Limbaugh et al. 1961).

The presence of cleaner organisms impacts reef fish diversity. In removal and addition experiments, as well as through observations of natural patterns of immigration and emigration, researchers have shown that cleaners enhance reef fish diversity on the order of weeks to months (Bshary 2003, Grutter et al. 2003). Some fishes affected by the presence of cleaners have large body sizes, and their presence in turn affects other species on the reef (Grutter et al. 2003). The cleaner effect is strongest on migratory and visiting species of reef fishes that have the ability to choose which reefs to visit, and also are often of commercial value (Bshary 2003, Grutter et al. 2003). Therefore, the growing aquarium industry should be limited in how many cleaners can be removed from each reef, so as not to negatively impact overall reef fish diversity (Grutter et al. 2003).

A direct effect of cleaning on the fitness of clients has yet to be identified (Cheney & Côté 2003), but cleaning is given a high priority in the time budgets of some fishes, prioritized over even feeding when cleaning benefits are the greatest (Sikkel et al. 2005). Cleaning activity occurs primarily at dawn, because the visually feeding cleaners are inactive at night, so parasite loads are highest due to night accumulation (Grutter 1999, Sikkel et al. 2004), and parasite-searching activity also is highest at dawn (Sikkel et al. 2006). Client fish may even wait in line at cleaning stations, postponing other activities for the opportunity to be cleaned (Limbaugh 1961, Bshary & Grutter 2002a).

Although Cheney and Côté (2003) stated that cleaners remove such an insignificant number of parasites that their beneficial impact on client fitness must be small, in a caging experiment Grutter et al. (2003) demonstrated that fish without access to cleaners experience at least a four-fold increase in parasite loads within 12 hr. Grutter (1996) estimated that the total number of parasites eaten per cleaner fish *L. dimidiatus* per day was approximately 1200, and suggested that these cleaners can strongly suppress parasite abundances on fishes. These studies demonstrate the importance of cleaning to parasite removal.

2. CLEANER SHRIMPS

Most recent studies on cleaning focus on cleaner fishes, but cleaners can also be shrimps (Côté 2000). Initially, anecdotal observations of cleaner shrimps suggested their status as cleaners (Limbaugh 1961, Limbaugh et al. 1961, Mahnken 1972), but whether cleaner shrimps actually do clean has been debated (Spotte 1998). Recently, several studies have proven that cleaner shrimps are effective in removing parasites from clients. Under laboratory conditions, *Ancylomenes holthuisi* can reduce parasite loads on surgeonfish by 74.5% within two days (Becker & Grutter 2004) and *A. pedersoni* can remove 100% of juvenile isopods *Anilocra haemuli* from client fishes (Bunkley-Williams & Williams 1998). In flow-through macrocosm experiments, *Ancylomenes pedersoni* has a significant effect on the abundance and size of monogenean parasites (McCammon et al. 2010), and in temperate waters off Sweden, two *Palaemon* shrimp species significantly reduce parasite loads on clients (Östlund-Nilsson et al. 2005). Additionally, on Indo-Pacific coral reefs, the cleaner shrimp *A. longicarpus* cleans a similar diversity of client fishes as do individuals of the cleaner wrasse *Labroides dimidiatus* (Chapuis & Bshary 2009), and on Caribbean reefs, the same pattern occurs in *A. pedersoni* versus the cleaning goby *Elacatinus evelynae* (Johnson & Ruben 1988). Conversely, some 'cleaner' shrimps do not significantly impact the abundances of some fish parasites in laboratory and macrocosm experiments (Table 1.1). For example, *Periclimenes yucatanicus* has previously been listed as an obligate cleaner, but recently researchers have failed to observe it cleaning in the field, and it has no significant effect on parasites in laboratory and macrocosm settings (Gwaltney & Brooks 1994, McCammon et al. 2010). Therefore, experimental evidence should be obtained before shrimps are described as cleaners, especially obligate cleaners.

Coral reef fish have poor visual resolving power, with their spatial and color vision potentially limited or blurred at distances beyond 5 m (Marshall 2000). Because cleaner shrimps often are cryptically-colored and potentially difficult for clients to see, they advertize their availability to clean by performing signals such as rocking dances (Becker et al. 2005), cheliped clapping (Chadwick et al. 2008, Chapuis & Bshary 2010), antennae lashing and body swaying (Limbaugh 1961, Limbaugh et al. 1961, Chadwick et al. 2008). In Pacific *Urocaridella* cleaner shrimps, a cleaning interaction followed the shrimp advertising dance 100% of the time. These shrimps dance twice as often and spend more time with clients when hungry (Becker & Grutter 2005, Becker et al. 2005). They also spend more time with parasitized clients than with those lacking parasites (Becker & Grutter 2005). These shrimp move away from clients and engage in little cleaning activity when satiated, resulting in clients spending 11x more time with hungry shrimp than with satiated shrimp (Becker & Grutter 2005, Becker et al. 2005). Thus, cleaner shrimp do not signal their presence or the presence of their cleaning station, rather they signal their readiness to clean. Researchers have observed the same pattern in the long-armed cleaner shrimp *A. longicarpus* in the Red Sea (Chapuis & Bshary 2010). These shrimp clap their chelipeds more when hungry, and clapping shrimp clean more than non-clapping shrimp. Additionally, these shrimp clap more for predatory than non-predatory clients, likely signaling identity.

Some cleaner shrimps are either facultative or obligate associates of sessile invertebrates, especially sea anemones and corals. *A. pedersoni* and *A. anthophilus* are obligate associates of sea anemones in the Caribbean Sea, while *A. longicarpus* is a facultative, diurnal associate of corals and sea anemones in the Red Sea, and is the most common shrimp on giant sea anemones there (Chadwick et al. 2008, Chapuis & Bshary 2009). *Lysmata grabhami* also facultatively associates with *Telmatactis* sp. anemones in the Canary Islands (van Tassell et al. 1994), but many species of *Lysmata* cleaner shrimps generally do not associate with cnidarians (Wicksten 2009). Caribbean cleaner shrimp *Brachycarpus biunguiculatus* associate with large sponges as the center of their nocturnal cleaning stations; however, they do not inhabit the sponge during diurnal cleaning inactivity, but instead live in crevices where they may associate with *Diadema* urchins (Corredor 1978).

Cleaning stations for anemoneshrimps are situated adjacent to host anemones (Limbaugh 1961), because the shrimp never venture far from their anemones to clean, and clients usually must come within 10 cm of the shrimp station to be cleaned (Johnson & Ruben 1988, Wicksten 1995). This in combination with the poor visual resolving power of reef fishes suggests that large host anemones may provide visual cues for fishes to identify shrimp cleaning stations (Mahnken 1972). Kulbicki and Arnal (1999) similarly suggested that anemones may serve as landmarks for fish to find cryptic *Periclimenes* cleaning stations on homogenous soft-bottom habitats and sea grass beds near the Great Barrier Reef. Host anemones also may serve as a contrasting background against which cleaner shrimps are more visible (Mahnken 1972, Gwaltney & Brooks 1994). This could potentially explain diurnal facultative associations with anemones by some shrimps like *A. longicarpus*. Cleaner shrimps display a variety of cleaning signals and lack a consistent guild coloration (Becker et al. 2005, Chapuis & Bshary 2010); the variety of benthic associations by cleaner shrimps, or lack thereof, may in part explain this variety in signals and coloration, as some shrimps may have evolved contrast with different sessile hosts.

Seven species of cleaner shrimps have been identified in the Caribbean: *A. pedersoni*, *P. yucatanicus*, *Stenopus hispidus*, *S. scutellatus*, *Lysmata grabhami*, *L. wurdemanni*, and *Brachycarpus biunguiculatus*. Only *A. pedersoni*, *P. yucatanicus*, and *B. biunguiculatus* are purported to be obligate cleaners (reviewed in Côté 2000). Whether *P. yucatanicus* is actually a cleaner is debated (Limbaugh et al. 1961, Gwaltney & Brooks 1994; Table 1.1), and recent evidence showed that this shrimp did not significantly reduce monogenean loads in captive surgeonfish in a semi-natural environment while *A. pedersoni* did (McCammon et al. 2010). Wicksten (1995) documented that *A. pedersoni* is the most common cleaner species on reefs in Bonaire, and is involved in more observations of cleaning behavior than any other cleaner, including cleaner fishes. Additionally, Bunkley-Williams and Williams (1998) used laboratory experiments to test the ability of eight Caribbean cleaners (four fish and four shrimp species) to remove juvenile isopod parasites *Anilocra haemuli* from *Haemulon flavolineatum* (French grunts), and found that *Ancylomenes pedersoni* removes these parasites 100% of the time, while all other tested cleaners do not remove this type of parasite. However, their sample sizes were small (11-12 parasites tested per cleaner). Both *A. pedersoni* and *P. yucatanicus* are obligate symbionts of sea anemones, notably the corkscrew anemone *Bartholomea annulata* and the rosetip anemone *Condylactis gigantea* (Limbaugh et al. 1961, Mahnken 1972).

Much about the ecology of cleaner shrimps is unknown, and most early studies of cleaner shrimps were anecdotal in nature (reviewed in Côté 2000). Few studies have quantified cleaner shrimp-client interactions in the field, or investigated the parasite removal capability of cleaner shrimps and subsequent effects on the parasite loads of clients. The cleaner shrimps that have been quantitatively examined for these types of effects are listed in Table 1.2, along with aspects of their biology, such as associations with sessile invertebrates, signal types, and coloration. These eight shrimp have a wide variation in signals and body coloration, as well as in hosts and microhabitats among these groups. Interestingly, cnidarian associates all display some variation of transparent bodies with white or purple markings. The differences among these species in their cleaning biology highlights a need for further investigation into the evolutionary mechanisms that select for various aspects of shrimp cleaning.

The goal of this thesis is to investigate selected aspects of the cleaning biology of the Caribbean cleaning anemoneshrimp *A. pedersoni*. In St. Thomas, U.S. Virgin Islands, *A. pedersoni* commonly associates with the host anemone *B. annulata* (Nelsen 2008). Thus, the distribution and abundance of this anemone may influence those of reef fishes because the anemone hosts *A. pedersoni* (Mahnken 1972). Further, this anemone may serve as a visual cue for reef fish to locate shrimp cleaning stations (Mahnken 1972, Gwaltney & Brooks 1994), but this role for host anemones has not been experimentally examined. As well, few data exist as to which fish species utilize *A. pedersoni* for cleaning in the Caribbean (but see Wicksten 1995, 1998 for Bonaire). Investigating these aspects of the cleaning biology of *A. pedersoni* will further our understanding of the important roles that cleaner shrimps and their host anemones play in coral reef ecology.

In this thesis research, I examine two major areas concerning the multi-level mutualism between the corkscrew anemone *B. annulata*, the cleaner shrimp *A. pedersoni*, and a wide diversity of client fishes on Caribbean coral reefs:

1) What diversity of clients visit these stations, pose for, and are cleaned by *A. pedersoni* shrimp, and how do these patterns change when the visibility of the shrimp, anemones, or both are manipulated? Further, does the anemone serve as a visual cue for client reef fishes to locate and initiate cleaning bouts at these shrimp cleaning stations? (Chapter 2)
2) What are natural patterns of client fish visitation and cleaning to these cleaning stations, and how do the characteristics of the anemones or their crustacean ectosymbionts influence the visitation and cleaning patterns of client reef fish? (Chapter 3)

Table 1.1. Evidence that some ‘cleaner’ shrimps potentially are not true cleaners. All are from coral reefs in the Caribbean Sea. Included in this table are cleaner shrimps that have been examined quantitatively for parasite removal capability, and did not demonstrate a significant effect on parasite abundances on fish hosts. These studies demonstrate that quantitative experiments on shrimps are needed before they can be termed cleaners, especially obligate cleaners.

Shrimp species	Evidence	Sources
Lysmata grabhami	did not remove juvenile isopods	Bunkley-Williams & Williams 1998
Stenopus scutellanus	did not remove juvenile isopods	Bunkley-Williams & Williams 1998
S. hispidus	did not remove juvenile isopods, no significant effect on monogeneans	Bunkley-Williams & Williams 1998; McCammon et al. 2010
Periclimenes yucatanicus	no significant effect on monogeneans, never observed cleaning in field observations	Gwaltney & Brooks 1994; McCammon et al. 2010

Table 1.2. Quantitative studies on cleaner shrimps. Included are eight cleaner shrimp species that have either been examined for parasite removal capability or had their interactions with clients quantified in the field. The region the shrimps are found (with the area in which they were studied in parentheses), microhabitat preference or sessile invertebrate hosts, time of cleaning activity, signal of cleaning availability, body coloration, whether they clean in groups or singly, and the references for this information are included.

Region	Shrimp species	Benthic associate/habitat	When cleans	Signal	Coloration	Clean in groups?	Sources
Pantropical (St. Vincent, West Indies)	*Brachycarpus biunguiculatus*	stations on large sponges; seek shelter in crevices and/or *Diadema* urchins	nocturnal	not reported	transparent to bright red orange	form pairs but do not clean together	Corredor 1978
Indo-Pacific (Australia)	*Urocaridella* sp. c	no associate, crevice-dwelling	diurnal	rocking dance	transparent, red and yellow spots, yellow chelae	yes	Becker & Grutter 2004, 2005
Indo-Pacific (Australia)	*Ancylomenes holthuisi*	*Cassiopeia andromeda* jellyfish, *Entacmaea quadricolor*, *Dofleinia armata, Heteractis* spp. anemones,	diurnal	not reported	transparent, white and purple-red spots	yes	Bruce & Svoboda 1983; Becker & Grutter 2004
Red Sea	*A. longicarpus*	facultative diurnal; *E. quadricolor*, *Heteractis aurora*, *H. crispa* anemones; *Xenia* & *Pleurogyra* corals	diurnal	cheliped clapping, body swaying	transparent, small white and violet-blue markings	yes	Bruce & Svoboda 1983; Chadwick et al. 2008; Chapuis & Bshary 2009, 2010
Caribbean (Bonaire, Virgin Islands)	*A. pedersoni*	obligate; *Bartholomea annulata*, *Condylactis gigantea* anemones	diurnal	antennae lashing, body swaying	transparent, purple lateral stripe, white antennae	yes	Chace 1958; Bunkley-Williams & Williams 1998; Wicksten 1995, 1998; McCammon et al. 2010
Bermuda	*A. anthophilus*	*C. gigantea*	diurnal	antennae lashing, rocking dance	transparent, purple lateral stripe, white banded antennae	yes	Sargent & Wagenbach 1975
Atlantic (Sweden)	*Palaemon elegans*	reside in macroalgae	not reported	not reported	transparent, red to black stripes, blue and yellow markings	not reported	Östlund-Nilsson et al. 2005
Atlantic (Sweden)	*P. adspersus*	reside in macroalgae	not reported	not reported	transparent to opaque	not reported	Östlund-Nilsson et al. 2005

CHAPTER 2

Multi-level network effects in cleaning mutualisms: Fish clients use sea anemones to locate cleaning stations of anemoneshrimp

SUMMARY

The interactions between marine cleaner organisms and their client fishes form a mutualistic network, in which a limited diversity of cleaners benefits a high diversity of clients. Some cleaners are obligate associates of coral reef cnidarians, but little is known about the role of cnidarian hosts in this complex network of species interactions. The Pederson cleaner shrimp *Ancylomenes pedersoni* is an important cleaner of fish parasites to a high diversity of fish clients (15 families observed here) on Caribbean coral reefs, and is an obligate macrosymbiont of zooxanthellate sea anemones, especially the corkscrew anemone *Bartholomea annulata*. Cleaning stations for these shrimp are centered around their host anemones, potentially causing client fishes to visually cue on anemones when they seek shrimp cleaning. We examined the importance of this anemone as a visual cue in the cleaning mutualism between *A. pedersoni* and its clients in the Caribbean Sea. We manipulated the visibility of anemones and shrimps in the field using mesh covers. Fishes continued to visit covered stations (albeit less at anemone-covered only stations), yet posed at cleaning stations where shrimps were covered and anemones visible, but not vice versa. Thus *B. annulata* incorporates a third level to the mutualistic cleaning network between *A. pedersoni* and its clients, by serving as a landmark and a visual stimulus for posing by fish. The anemone host and its shrimp guest in synergy attract more clients than does the shrimp alone. *A. pedersoni* is thus a highly connected member of the reef community, linking fishes and their parasites to host anemones and their zooxanthellae in a complex network of multi-level symbioses.

1. INTRODUCTION

Mutualistic networks are patterns of mutually beneficial interactions among species (nodes) in a community (Bascompte 2009a). Large networks have ‘hubs’, or nodes that are very well connected to other nodes in the network, much more than expected by chance alone (Bascompte 2009b). Thus, mutualistic networks are highly nested; that is, the network is organized around a few core species with a high degree of interaction (generalists) but contains many species with a few interactions (specialists). Therefore, many specialist species interact with a few generalist species, creating a heterogeneous network of asymmetric links with few to no interactions between specialists (Guimarães et al. 2007, Bascompte 2009b).

The nestedness of mutualistic networks has typically been studied in terrestrial plant-animal interactions such as plant-pollinator or plant-frugivore symbioses (Bascompte et al. 2003), but recently also has been applied to marine cleaner-client associations (Guimarães et al. 2007). Cleaning associations traditionally have been thought of as a two-species mutualism in which one type of organism, the cleaner, removes and ingests ectoparasites and damaged tissues from another type of organism, the client (reviewed in Côté 2000). These associations occur in terrestrial environments, between tickbirds and their ungulate hosts, for example (Dickman 1992), but cleaning mutualisms are most studied in marine systems, where the cleaners are either fishes or crustaceans and clients are typically fishes (Côté 2000, Grutter 2002). The utilization of cleaners by diverse client assemblages forms mutualistic networks, which display strong nestedness.

The degree to which cleaning relationships are mutualistic has been a source of some controversy (reviewed in Losey 1987, Côté 2000). However, recent work has shown not only the parasite removal ability of the cleaners, but that cleaners can strongly suppress parasite abundances on client fishes (Grutter 1996, Grutter et al. 2003, McCammon et al. 2010). Additionally, removal and translocation experiments of the cleaner fish *Labroides dimidiatus* have revealed that cleaners can impact reef fish diversity and habitat use at time scales of weeks to months, demonstrating the importance of these interactions to marine community structure (Bshary 2003, Grutter et al. 2003).

Most studies on cleaning mutualisms focus on cleaner fishes, but cleaners also may be shrimps (reviewed in Côté 2000, Becker & Grutter 2004), of which 43 species have been identified from the scientific and hobbyist literature (reviewed in Becker & Grutter 2004). Few of these species have been studied quantitatively or confirmed as true cleaners, and only recently researchers have demonstrated the parasite removal capacity of cleaner shrimps (Bunkley-Williams & Williams 1998, Becker & Grutter 2004, Östlund-Nilsson et al. 2005, McCammon et al. 2010). Not only do cleaner shrimps actually clean, but Chapuis and Bshary (2009) documented that the Red Sea cleaner shrimp *Ancylomenes longicarpus* has significant niche overlap with the cleaner wrasse *L. dimidiatus*: 35 client fish species were cleaned by both types of cleaners, and the total time that each spent cleaning clients was similar. Also, in the Caribbean, the same diversity of fish clients visited the cleaning stations both of gobies and those of nearby cleaner shrimp *A. pedersoni* (Johnson & Ruben 1988).

Coral reef fishes have poor visual resolving power, with their fine grain spatial and color vision potentially limited or blurred at distances beyond 5 m (Marshall 2000). Because cleaner shrimps are small, cryptically colored and potentially difficult for clients to detect (Corredor 1978, Chapuis & Bshary 2010), they advertize their identity (Chapuis & Bshary 2010) and hunger level to potential clients by performing signals such as rocking dances (Becker et al. 2005), antennae lashing, body swaying (Limbaugh 1961, Limbaugh et al. 1961, Chadwick et al. 2008), and cheliped clapping (Chadwick et al. 2008, Chapuis & Bshary 2010). The wide variation in coloration and signals among cleaner shrimps indicates a lack of convergence in signaling within this functional group (Becker et al. 2005, Chapuis & Bshary 2010). This diversity contrasts with the strong convergence of coloration and behavior among cleaner fish species, which all have longitudinal stripes and blue and/or yellow body color (Stummer et al. 2004, Arnal et al. 2006, Cheney et al. 2009).

Some cleaner shrimps, notably in the genus *Ancylomenes* (formerly within *Periclimenes*; Okuno & Bruce 2010), also engage in another type of mutualism: they are ectosymbionts of anthozoans (Limbaugh et al. 1961, Kulbicki & Arnal 1999). Both the Caribbean shrimps *A. pedersoni* and *P. yucatanicus* are obligate associates of sea anemones, primarily the corkscrew anemone *Bartholomea annulata* and the rosetip anemone *Condylactis gigantea* (Limbaugh et al. 1961, Mahnken 1972, Gwaltney & Brooks 1994). *A. longicarpus* is a facultative associate with a variety of sea anemones and corals (Chadwick et al. 2008, Chapuis & Bshary 2009). Additionally, van Tassell et al. (1994) noted that *Lysmata grabhami* associates with the sea anemone *Telmatactis* sp. in the Canary Islands. Benefits to cleaner shrimps from association with cnidarians primarily appear to be shelter defense from predators (Mihalik & Brooks 1995), while benefits accrue to the cnidarian hosts in the form of inorganic nutrients excreted by shrimps that fertilize endosymbiotic algae (zooxanthellae) in the host (Spotte 1996), thus adding yet another layer to this mutualistic network.

Cleaning stations for obligate anemoneshrimps are centered around their host anemones (Limbaugh 1961): the shrimp never venture far from the anemones to clean, and clients usually must come within 10 cm of the shrimp station to be cleaned (Johnson & Ruben 1988, Wicksten 1995). This association, in combination with the poor visual resolving power of reef fishes, may result in client fishes using the large host anemones (up to 36 cm diameter for *B. annulata* in this study) as visual cues to locate and identify shrimp cleaning stations, or possibly as contrasting backgrounds against which the small (up to 2.6 cm for *A. pedersoni*; Humann & DeLoach 2006) cleaners may be more visible (Mahnken 1972, Gwaltney & Brooks 1994). Kulbicki and Arnal (1999) similarly suggested that anemones may serve as landmarks for fish to locate the cleaning stations of cryptic *Periclimenes* shrimps on homogenous soft-bottom habitats and sea grass beds near the Great Barrier Reef. The abundance and distribution of host anemones in coral reef habitats may thus in part control local assemblages of client reef fishes, due to the cleaning activity of their associated anemoneshrimps (Mahnken 1972).

Ancylomenes pedersoni (Chace, 1958) is an obligate fish cleaner and anemone associate on coral reefs throughout the Caribbean Sea (Humann & DeLoach 2006). Of the seven known species of cleaner shrimps in the Caribbean (reviewed in Côté 2000), *A. pedersoni* is the only one documented to effectively reduce parasite loads on reef fishes. Bunkley-Williams and Williams (1998) used laboratory experiments to test the ability of eight Caribbean cleaners (four fish and four shrimp species) to remove juvenile isopod parasites *Anilocra haemuli* from client fish, and found that only *Ancylomenes pedersoni* removed these parasites. More recently, McCammon et al. (2010) maintained

Caribbean blue tang *Acanthurus coeruleus* with and without *Ancylomenes pedersoni* in flow-through macrocosm experiments, and revealed that *A. pedersoni* significantly reduced loads of the monogenean parasite *Neobenedenia melleni* on these fish, while other examined cleaner shrimps did not. Not only do these shrimps efficiently remove both isopod and monogenean parasites from client fishes, they also service a wide diversity of clients. Wicksten (1995) documented that *A. pedersoni* is the most common cleaner organism on coral reefs in Bonaire, and engages in cleaning behavior more frequently and with a wider variety of fish clients (22 species) than did all other cleaner species on these reefs. A representative cleaning station of *A. pedersoni* on the host anemone *B. annulata* is pictured in Figure 2.1.

Bartholomea annulata (LeSeuer, 1817) is an actinarian sea anemone common in shallow reef habitats throughout the Caribbean Sea. It hosts a variety of crustacean macrosymbionts, including *A. pedersoni* and several members of the genus *Periclimenes*, as well as pistol shrimps *Alpheus* spp., the squat anemoneshrimp *Thor amboinensis,* mysid shrimps, and the arrow crab *Stenorhynchus seticornis* (Humann & DeLoach 2006). Except for the cleaning activity of *A. pedersoni* (and possibly *P. yucatanicus*; Limbaugh et al. 1961, but see McCammon et al. 2010), the crustacean assemblage associating with this anemone represents a group of organisms that potentially interact neutrally or competitively with each other (Mahnken 1972), yet relatively little with the surrounding reef community. In terms of a mutualistic network, these associates together with the host anemone represent a module (Bascompte 2009b).

Here we describe natural patterns of client fish visitation to cleaning stations of the obligate anemoneshrimp *A. pedersoni*, and determine whether host anemones *B. annulata* serve as visual cues for fishes to locate and pose for cleaning. We predict that client fishes will both visit and pose for cleaning less frequently at stations where we have covered anemones than at those where shrimp are covered. By determining if client fishes alter their cleaning-seeking behavior based on the visual presence of anemones, we will assess the importance of anemones in this mutualistic cleaning network.

2. MATERIALS AND METHODS

(a) *Study Site*

The present study was conducted on coral reefs at St. Thomas, U.S. Virgin Islands during July 2009, November 2009, and February 2010. Field observations were conducted on coral reefs in Brewers Bay (~ 6 m depth, 18º20' N, 64º58' W), because they are close to shore and easily accessible for dawn observations, and support a high abundance of the corkscrew anemone *Bartholomea annulata* and the cleaner shrimp *Ancylomenes pedersoni* relative to nearby offshore reefs (see Nelsen 2008 for detailed site description).

(b) *Field observations*

Forty-three host anemones with *A. pedersoni* cleaning stations were selected haphazardly for field observations. Divers observed each cleaning station from ~ 1.5 m distance or more, depending on visibility. Neither the presence of scuba bubbles nor divers appeared to alter the behavior of fishes visiting these cleaning stations; after 2-3 min of the diver remaining stationary on the sea floor, reef fishes adjusted to diver presence and resumed activity around the diver, with some fish swimming <1 m from the diver. Each morning, observations began immediately after sunrise (~ 6:30 am) and concluding by mid-morning (~ 8:30 am). This corresponds with peak cleaning activity (Sikkel et al. 2004, 2005). Cleaning activity occurs primarily at dawn on coral reefs because the visually feeding cleaners are inactive at night, parasite loads are highest due to night accumulation on fishes (Grutter 1999, Sikkel et al. 2004), and parasite-searching activity also is highest at dawn (Sikkel et al. 2006).

Each cleaning station was observed for 20 min, and the following data were recorded: identity of visiting client fishes (recorded at least to family; Wicksten 1998), client posing behavior, and cleaning behavior (see Chapuis & Bshary 2009). A visit was defined as a fish approaching a cleaning station and remaining in the station vicinity for at least 3 sec (Arnal & Côté 1998), excluding other activity around the station such as grazing or territory defense. Posing behavior was defined as a fish signal to instigate a clean at a cleaning station, for example: change of body orientation (Côté et al. 1998), opening of gills and mouth, and color change (Wicksten 1998). ‘Cleans’ were defined as a client fish pose or stationary behavior near the station resulting in shrimp contact with the client body. For analyses, all cleans were counted within poses, and all poses were counted within visits. To assess natural patterns of client activity at these cleaning stations, we compared only the activity before manipulation of cleaning stations, because application of covers likely altered the activity rates.

(c) *Field Experiment*

Observed cleaning stations (N = 43) each were assigned randomly to one of four experimental treatments: a) neither anemone nor shrimp covered (control with cover nearby, N = 10 stations), b) both anemone and shrimp covered (N = 11 stations), c) anemone covered but shrimp uncovered (N = 11 stations), and d) shrimp covered but anemone uncovered (N = 11 stations). Because rates of fish visitation to cleaning stations did not vary significantly among the three months of observation (Kruskal-Wallis test: K = 2.856, p = 0.240), the results from all three months were pooled. Immediately after each 20 min observation at a cleaning station (see above), a mesh cover was placed ≤ 15 cm from the anemone, to allow fishes to acclimate to its presence on the reef. The cover was attached to a rock with nails or pushed into the sand, with the edges of the cover buried in the sand to ensure that it remained stationary.

Each cover consisted of fine mesh window screening arranged four layers thick in a hemispherical shape and affixed by zip-tie to a circle of 18 gauge aluminum wire. The covers were spray painted with Rust-oleum indoor-outdoor spray paint, satin finish, color nutmeg, to blend in with the natural coloration of the sand/reef rock environment at the field site. These malleable materials rendered the covers adjustable to fit over anemones inhabiting crevices at the reef-sand interface, where they most commonly occur (Mahnken 1972, Nelsen 2008). Covers ranged from 12-45 cm in diameter, to allow for variation in anemone and crevice size.

Two days after initial field observations, the cover was moved over the anemone, shrimps, or both, and observations of fish visitation, posing and cleaning were conducted again as described above. In the control treatment, the cover was not moved. Because shrimp could potentially escape the covers, to simulate covering the shrimp in the both- and shrimp-covered treatments, shrimp were captured by hand and held temporarily in a plastic bag hidden under the diver during the 20 min observation, and then replaced after observations concluded. In the both- and anemone-covered treatments, the anemones were disturbed by touching their tentacles, causing them to contract; then the cover was placed over the crevice or hole into which the anemone had contracted. In the anemone-covered treatment, this process did not cause associated *A. pedersoni* to vacate their anemones; the shrimp instead settled on or near the cover (< 5 cm), possibly detecting the covered anemone's location via chemical cues (Guo et al. 1996; Figure 2.2). Other macrosymbionts of these anemones, with the exception of *S. seticornis*, remained under the covers with their contracted anemones. Covers were removed immediately after the 20 min observations, and shrimps were reunited with their anemones. Later observations (1-7 d) of manipulated cleaning stations indicated that these manipulations did not affect the fidelity of the shrimps to their host anemones. All collected data were analyzed using SYSTAT 13. Repeated-measures ANOVA was used to compare the number of fish visits, poses, and cleans before and after manipulation at each anemone station. Results are presented as means ± one standard error.

3. RESULTS

(a) *Diversity of Client Fishes*

During initial observations prior to experimental manipulations, we observed members of 13 families of reef fishes visit the cleaning stations of *Ancylomenes pedersoni* shrimp associated with *Bartholomea annulata* (Figure 2.3). Members of the family Acanthuridae (surgeonfishes) represented most of the clients at shrimp cleaning stations, comprising over 50% of visits, poses, and cleans. Members of each of the other fish families represented 9% or less of visits, poses, and cleans. Some families (i.e. Labridae and Ostraciidae) visited at higher rates than at which they posed or were cleaned, whereas other families (i.e. Mullidae and Synodontidae) posed or were cleaned at relatively higher rates than at which they visited. Members of the Gerreidae and Holocentridae were observed to visit the cleaning stations during experimental observations only. The diversity and frequency of client visits, poses, and cleans before and after the covers were applied for each experimental group are listed in Table 2.1.

(b) *Visits*

In the control and both-covered treatments, rates of fish visitation did not significantly vary after covers were applied (F = 0.184, p = 0.678 for control stations, F = 0.593, p = 0.459 for both-covered stations). Rates of fish visitation significantly decreased at anemone-covered stations (F = 5.900, p = 0.036), but significantly increased at shrimp-covered stations (F = 5.800, p = 0.037; Figure 2.4A).

(c) *Poses*

The number of poses per station did not differ before versus after the covers were applied in both the control (F = 0.000, p = 1.000) and shrimp-covered treatments (F = 2.168, p = 0.172). However, the number of poses per station decreased significantly after covers were applied in the both-covered (F = 7.705, p = 0.020) and anemone-covered treatments (F = 10.811, p = 0.008; Figure 2.4B). We also analyzed the number of poses per visit at each station, and they followed the same patterns as for the total number of poses per stations.

(d) *Cleans*

In the control treatment, the number of cleans at each station did not differ before versus after the covers were applied (F = 0.043, p = 0.840). Because in the both-covered and shrimp-covered treatments shrimp were not able to clean while being held away from the anemones, these treatments were not included in repeated-measures analyses. In the anemone-covered treatment, the number of cleans per anemone decreased significantly after covers were applied (F = 9.343, p = 0.012; Figure 2.4C).

(e) *Behavioral Observations*

During initial observations, we observed fishes to remain posing at cleaning stations after shrimp had finished a clean and returned to their anemone, presumably trying to incite another cleaning bout. Similarly, some fishes swam away from cleaning stations after the shrimp finished, but traveled only a few meters or less before returning to the same cleaning station and posing again. In some cases this resulted in another clean, while in others it did not. Occasionally, some fishes lingered near stations (within 1 m) for several minutes - up to the entire 20 min observation time in this study - despite spending little time being cleaned.

We did not observe queuing behavior by client fishes for these shrimp, but we did observe some clients to either abandon clean-seeking behavior at an already occupied station, or to chase other clients from cleaning stations. These interactions were both inter- and intraspecific: *Acanthurus* spp. chased other *Acanthurus* spp. as well as mullids and tobacco fish *Serranus tabacarius* away from cleaning stations. In one such instance, *S. tabacarius* waited < 1 m from the station until the surgeonfish left, and then proceeded to pose at the station. A single ocean surgeonfish *A. bahianus* lingered < 0.5 m from a station, and chased all visiting clients from a cleaning station for the duration of our 20 min observation period, despite spending only 11 sec being cleaned. Additionally, damselfishes whose territories overlapped with the cleaning stations occasionally chased other clients away from stations.

During experimental observations at stations where anemones were covered, some fishes swam toward the station, paused, swam around the area within a few meters, returned to the station, paused, and continued this searching activity, sometimes for several minutes, before finally leaving the station area. Fishes posing at stations in the two experimental groups where shrimps were held away from the anemone maintained their pose up to 8 sec before swimming away. Some of these fishes then proceeded to another station (not included in the experiment) and posed.

4. DISCUSSION

We present here evidence that fish clients of the anemoneshrimp *Ancylomenes pedersoni* use the anemone host *Bartholomea annulata* as a cue to locate shrimp cleaning stations and pose for cleaning. Our data indicate that client fishes may use different mechanisms to locate and recognize cleaner shrimps than for interactions with cleaner fishes. In the cleaner wrasse system, the body size and striped pattern of the cleaners serve as the initial information to attract clients, while at closer range, signals of body movement by the cleaner are necessary to confirm client interest in the cleaner (Stummer et al. 2004). Similar mechanisms also regulate interactions in the Caribbean cleaner goby system (Côté 2000). In the *A. pedersoni* anemoneshrimp system, our results show that fishes rarely pose or receive cleaning at stations where anemones are covered but where shrimps are visible and available to clean. Thus, client fishes appear to use the host sea anemone as the initial visual signal at long distance to attract them to the shrimp cleaning station. After they arrive to the station, shrimp signals such as antennae lashing may be the close range cues that cause fish posing. Similarly, the crevice-inhabiting two claw shrimp *Brachycarpus biunguiculatus* maintains cleaning stations only on large (~ 50 cm opening) sponges, and are never observed cleaning away from sponges (Corredor 1978): these shrimps also may rely on a benthic invertebrate (in this case large sponges) as landmarks for client fish to locate them. Additionally, small cleaner shrimps may only clean when protected by substratum that provides cover.

When posing for cleaning at a station, fishes are close enough (< 40 cm) to likely distinguish fine grain color patterns of cleaner shrimps (Marshall 2000). That client fishes not only visited but proceeded to pose in front of anemones alone but not in front of shrimps alone is puzzling. It is possible that regardless of shrimp presence, the anemone host is the primary posing stimulus for these fishes, and that shrimps may signal after their clients pose. Sequences of cleaner-client behaviors are variable, and client posing sometimes occurs before cleaner signals in successful cleaning interactions (Sargent & Wagenbach 1975, Jonasson 1987).

Fishes may pose in front of anemones that lack visible shrimp because shrimp are not always clearly visible even if present. Cleaner shrimps sometimes hide in crevices near the anemone, or under the anemone tentacles (L. Huebner, pers. obs.), and posing by client fish could induce the shrimp to come out and clean. For parasite-loaded fishes, posing for a few seconds in front of an anemone with no shrimp, and then moving on to another anemone and posing until a cleaner shrimp is found, may be more cost effective than spending time searching around each anemone for the presence of cleaner shrimp before posing. More research is needed to investigate why fish pose in front of anemones where cleaner shrimp are not visible.

Only two fish in our study, a grouper (Serranidae) and a mojarra (Gerreidae), were cleaned at a station where the anemone was covered but the shrimp remained available to clean (out of N = 36 fishes that visited these stations). As large piscivores, groupers likely have better eyesight than do many other fishes (Wootton 1998), and thus may not need to use anemones as a cleaning cue. We also observed the same grouper to revisit a single cleaning station several days in a row; this observation supports the possibility that groupers have cleaning station fidelity and may even define their home ranges based on the availability of cleaning stations (R. Nemeth, pers. comm.). In general, fishes that are able to move among reef habitats appear to include the presence of cleaner organisms when making selections of home range (Bshary 2003). However, territorial damselfish do not appear to define their home ranges based on cleaning station locations (Whiteman et al. 2002).

Our treatment of anemones covered but shrimp visible, while a necessary experimental control in the present study, is likely a rare situation in nature to which client fish would not normally respond. *A. pedersoni* is an obligate anemoneshrimp, so those without a nearby anemone may be changing hosts (Mahnken 1972, Chadwick et al. 2008) and be unwillingly to clean, and clients may recognize this and not pose in front of such shrimps. Individuals of *A. pedersoni* without a host may even be mistaken for prey items (Mihalik & Brooks 1995), however we did not observe any predation on shrimps in the anemone-covered treatments (3.67 h observation of this treatment). During fieldwork for related population studies (Nelsen 2008), we observed that anemones sometimes contract during rough weather and their crustacean assemblages then wait near the host anemone's crevice for the anemone to expand again (L. Huebner, pers. obs.). It would be interesting to document whether the cleaning activity of shrimps is decreased under these conditions (anemone contracted) because client fishes do not then pose nearby for cleans.

Mahnken (1972) proposed that anemones serve as a contrasting background against which the white and purple body of *A. pedersoni* may visually stand out. If the anemone serves as a contrast to heighten the visibility of cleaning anemoneshrimp, this process may result from coevolution of the cleaner's signal behavior and color with the color of the host anemone. This may explain the apparent lack of convergence in cleaner shrimp coloration and signals, as some cleaner shrimp species are obligate anthozoan associates and others are not. Even among anemone-associated cleaner shrimps, there may be a lack of convergence in cleaner coloration and signals, as these shrimps each could be coevolved for contrasts with different species of host anemones. This development of visual contrast could explain in part the anemone host preference patterns of some cleaner shrimps (Gwaltney & Brooks 1994). *A. pedersoni* prefers *B. annulata* over its other main host, the rose-tip anemone *Condylactis gigantea* (Gwaltney & Brooks 1994), perhaps in part because the former has darker tentacles and potentially provides more visual contrast to its body coloration. Indeed, individuals of *C. gigantea* occur at our Brewers Bay site, but we did not observe *A. pedersoni* to associate with them (L. Huebner, pers. obs.). Recent research quantified levels of visual contrast between cleaner fishes and coral reef backgrounds (Cheney et al. 2009, Lettieri et al. 2009). Similar studies are needed on the patterns of visual contrast between cleaner shrimps and their anemone hosts, and how this affects the visitation rate of clients to these cleaning stations.

Few studies have quantified interactions between cleaner shrimps and their clients in the field (Johnson & Ruben 1988, Wicksten 1995, 1998, Chapuis & Bshary 2009), and the similarity of these processes to the interactions of cleaner fishes with clients has been investigated only recently (Chapuis & Bshary 2009). At our study site in the U.S. Virgin Islands, a variety of clients representing 15 families of reef fishes visited *A. pedersoni* cleaning stations, reinforcing the importance of *Ancylomenes* cleaner shrimps to client fishes (Chapuis & Bshary 2009). These field observations complement laboratory and macrocosm experimental work on the parasite removal ability of these shrimps (Bunkley-Williams & Williams 1998, McCammon et al 2010). The high visitation, posing, and cleaning rates of acanthurids that we observed here also support other evidence that acanthurids are major hosts of monogenean parasites on coral reefs (Grutter & Bshary 2003, Sikkel et al. 2009). Because acanthurids are mobile, important grazers of macroalgae on coral reefs (Hay 1984), the abundance of cleaning stations in a given reef area may influence grazing rates on macroalgae by attracting cleaning-seeking acanthurids.

Obligate cleaner organisms are rare relative to other organisms (Guimarães et al. 2007), yet their presence can strongly impact fish diversity, making them a key organism in coral reef ecology (Bshary 2003, Grutter et al. 2003). Further, client fishes and their cleaners exhibit preferences for certain partner species in this mutualistic network (e.g. Wicksten 1995, 1998), so this specificity of interactions may impact some species more than others if cleaners are removed from reefs. Both *B. annulata* and *A. pedersoni* are popular organisms in ornamental aquariums and are heavily collected in some reef areas for the aquarium trade (Calado et al. 2003, Rhyne et al. 2009), and large-scale removal of either from Caribbean coral reefs could result in fish client species using less-preferred cleaners, or leaving impacted reefs altogether. Thus, understanding the cascade effects of this large Caribbean sea anemone on reef fish diversity through fish client interactions with its obligate anemoneshrimp will provide important scientific information to support a sustainable management of this fishery.

5. CONCLUSIONS

The cleaner shrimp *Ancylomenes pedersoni* appears to be a highly connected and important member of Caribbean coral reef communities. Not only does it interact with other members of the crustacean ensemble that associates with its host anemone (Mahnken 1972), but it effectively removes parasites (Bunkley-Williams & Williams 1998, McCammon et al. 2010) from a diverse assemblage of client fishes (Wicksten 1995, 1998; see Results). Thus, it interacts with the host-parasite network of reef fishes, altering the dynamics of parasite infestation. By feeding on these parasites, *A. pedersoni* transfers energy and nutrients from the open water environment of some client fishes to the benthic reef community (Roopin et al. 2008), likely fertilizing the zooxanthellae of its host anemone (Spotte 1996). Because parasite emergence, and thus cleaning activity, peaks in the early morning (Sikkel et al. 2004), it would be interesting to determine if the supply of nitrogen waste from cleaner shrimps to host anemones peaks post-cleaning in the morning. As well, it would be interesting to investigate if any excretions from posing fish clients supply nitrogen to nearby host anemones.

We demonstrate here that the anemone host *Bartholomea annulata* is involved directly, albeit passively, in the cleaning symbiosis between *A. pedersoni* and its client fishes, by serving as a visual landmark for client fishes to locate *A. pedersoni* cleaning stations, and also as a stimulus for client posing. This cleaner-client mutualism appears to depend directly on the cleaner-cnidarian mutualism. While many plants produce colorful and scented flowers to attract their pollinators, *A. pedersoni* may associate with anemones for not only protective reasons (Mihalik & Brooks 1995), but also to attract clients by occurring at a visually obvious cleaning station. The anemone and shrimp in synergy attract more clients for *A. pedersoni* than the shrimp alone. Thus, we may need to modify the definition of these types of cleaning interactions to include not only the cleaner and client, but also the cleaner's sessile host (whether it be anemone, sponge, or other benthic organism), without which small cleaner shrimps may not be able to easily garner visually-oriented clients. Together these symbiotic partners constitute a network hub, enhancing the interactions of shrimps in the cleaning mutualistic network and further strengthening their positive impacts on coral reef communities.

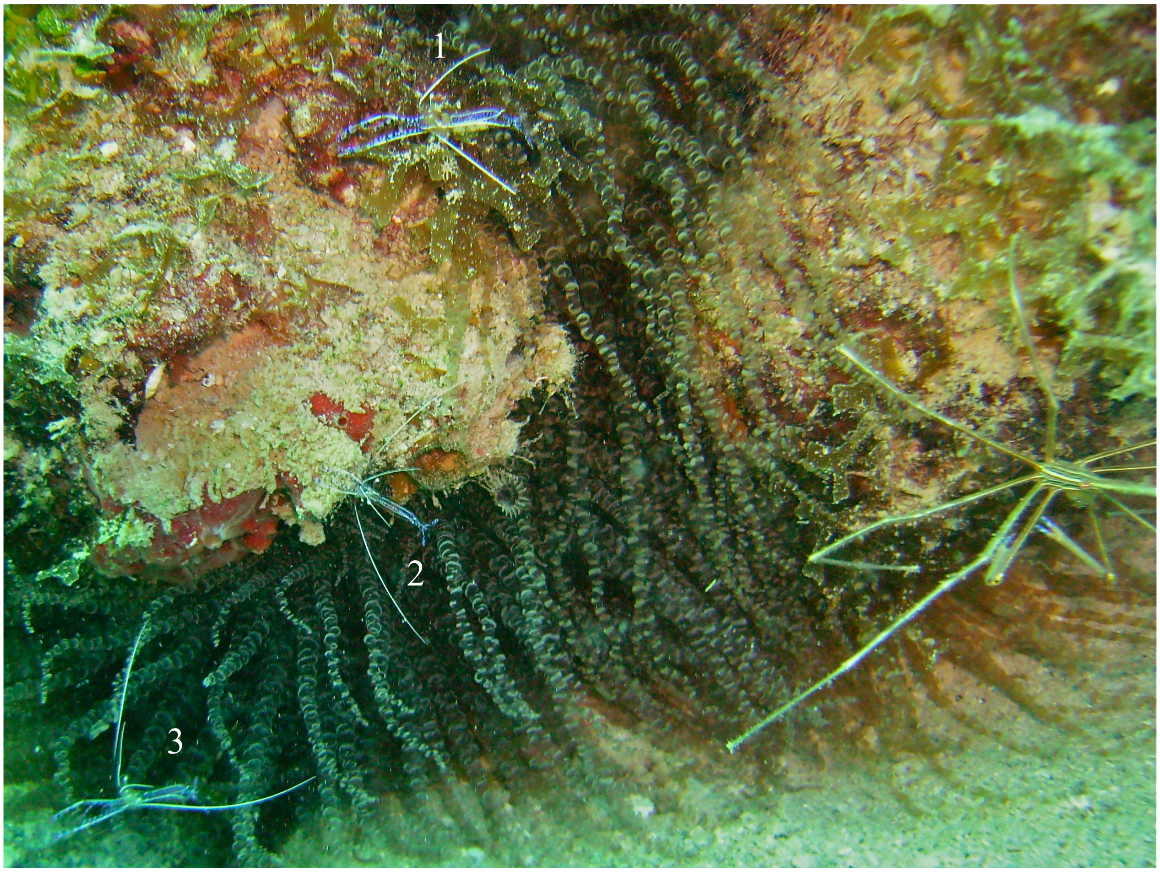

Figure 2.1. A representative cleaning station of cleaner shrimp *Ancylomenes pedersoni* and their host anemone *Bartholomea annulata* in Brewers Bay, St. Thomas, U.S. Virgin Islands. A cluster of *B. annulata* supports a large crustacean assemblage, with three *A. pedersoni* and one *Stenorhynchus seticornis* pictured. The *A. pedersoni* are numbered for reference. Note how the left antennae of *A. pedersoni* #2 are more visible in front of the dark tentacles of *B. annulata* than the right antennae in front of the light rock substrate.

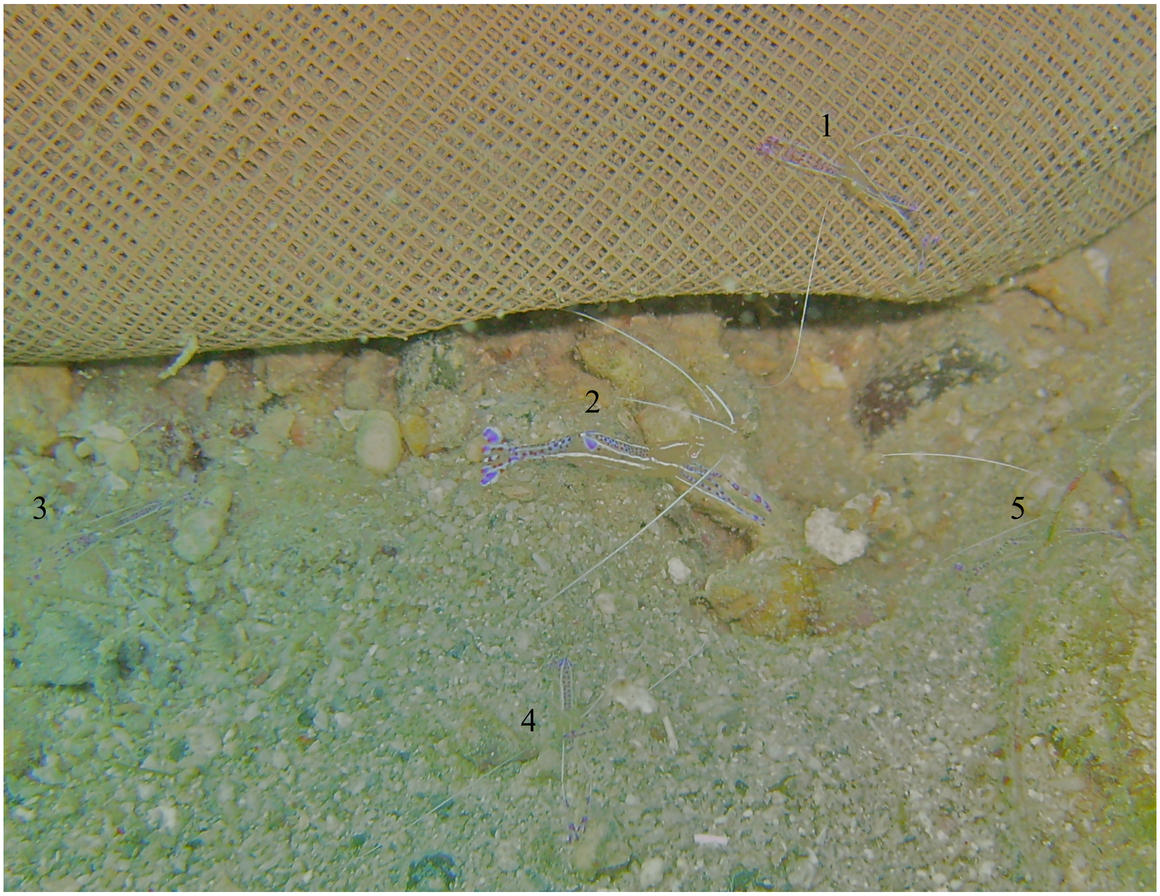

Figure 2.2. A representative anemone covered but shrimp uncovered experimental treatment on cleaner shrimp *Ancylomenes pedersoni* and their host anemone *Bartholomea annulata* in Brewers Bay, St. Thomas, U.S. Virgin Islands. The anemone is contracted into its crevice, and the mesh cover is placed over the crevice. Four associated *A. pedersoni* have settled immediately in front of the cover, and one has settled directly on the cover. The shrimp are numbered, as some are mostly transparent and potentially difficult to see.

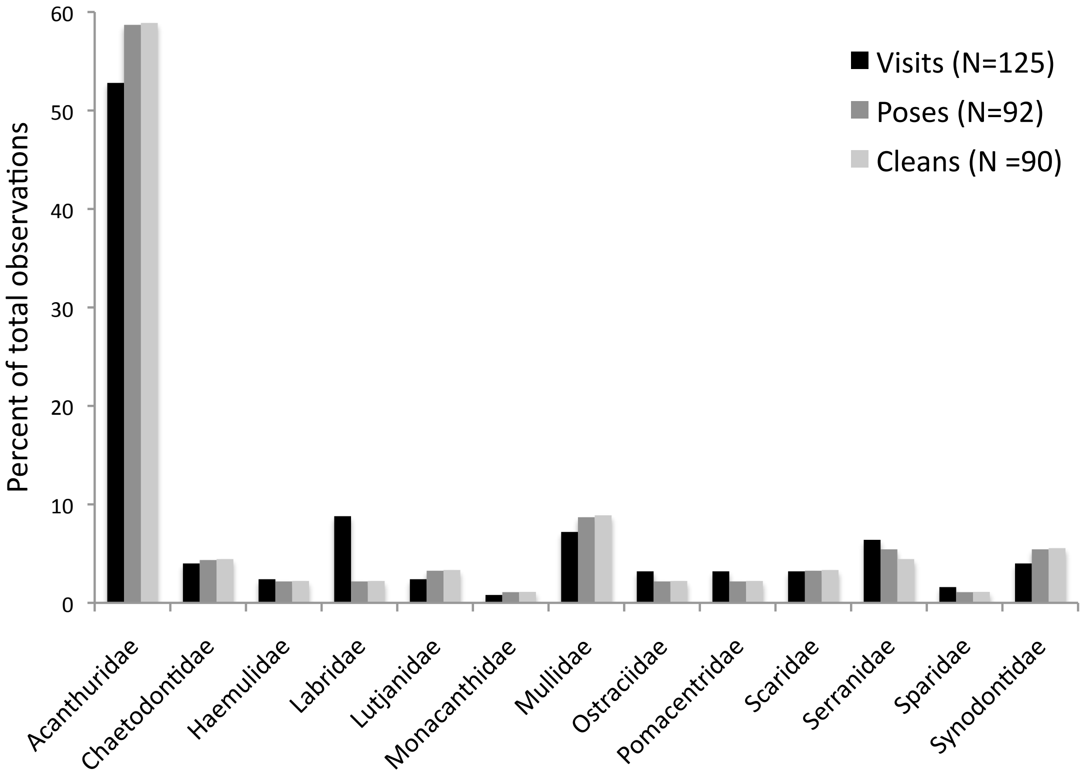

Figure 2.3. Variation in the rates of visitation, posing, and cleaning among 13 families of reef fishes that utilized cleaner shrimps *Ancylomenes pedersoni* and their host anemones *Bartholomea annulata* as cleaning stations on coral reefs at Brewers Bay, St. Thomas, U.S. Virgin Islands. Rates of each behavior were calculated from initial observations before covering experiments were performed. Note that acanthurids (surgeonfishes) were the most common clients in terms of all three types of behavior (visitation, posing, and cleaning). Members of fish families Gerreidae (mojarras) and Holocentridae (squirrelfishes) also were observed at these cleaning stations, but only during experimental observations after covers were placed over some shrimps and anemones.

Table 2.1. Diversity of client families and frequency of visits, poses, and cleans before and after the covers were applied for each experimental group in a covering experiment of *Ancylomenes pedersoni* shrimp cleaning stations on *Bartholomea annulata* anemones in Brewers Bay, St. Thomas, U.S. Virgin Islands. '-' indicates no observation, and 'x' indicates where cleaning could not have occurred because the shrimp were held away from the anemone.

		Control		Both Covered		Anemone Covered		Shrimp Covered	
		Before	After	Before	After	Before	After	Before	After
Acanthuridae	Visits	16	23	7	6	37	17	6	14
	Poses	13	18	5	-	34	2	2	5
	Cleans	12	18	5	x	34	-	2	x
Chaetodontidae	Visits	3	2	1	-	-	3	1	-
	Poses	3	2	1	-	-	-	-	-
	Cleans	3	2	1	x	-	-	-	x
Gerreidae	Visits	-	-	-	-	-	2	-	-
	Poses	-	-	-	-	-	1	-	-
	Cleans	-	-	-	x	-	1	-	x
Haemulidae	Visits	1	2	-	-	1	-	1	2
	Poses	1	1	-	-	-	-	1	-
	Cleans	1	1	-	x	-	-	1	x
Holocentridae	Visits	-	-	-	-	-	-	-	4
	Poses	-	-	-	-	-	-	-	2
	Cleans	-	-	-	x	-	-	-	x
Labridae	Visits	-	-	4	-	3	2	4	5
	Poses	-	-	1	-	-	-	1	-
	Cleans	-	-	1	x	-	-	1	x
Lutjanidae	Visits	1	-	2	3	-	3	-	1
	Poses	1	-	2	-	-	-	-	-
	Cleans	1	-	2	x	-	-	-	x
Monacanthidae	Visits	1	1	-	-	-	-	-	-
	Poses	1	1	-	-	-	-	-	-
	Cleans	1	1	-	x	-	-	-	x
Mullidae	Visits	1	1	4	3	2	2	2	3
	Poses	1	1	4	1	2	-	1	3
	Cleans	1	1	4	x	2	-	1	x
Ostraciidae	Visits	1	-	-	3	2	1	1	-
	Poses	-	-	-	-	2	-	-	-
	Cleans	-	-	-	x	2	-	-	x
Pomacentridae	Visits	-	1	1	1	3	1	-	4
	Poses	-	-	1	-	1	-	-	2
	Cleans	-	-	1	x	1	-	-	x
Scaridae	Visits	1	1	-	-	3	1	-	3
	Poses	1	-	-	-	2	-	-	1
	Cleans	1	-	-	x	2	-	-	x
Serranidae	Visits	1	1	1	1	2	3	4	6
	Poses	1	1	1	-	-	1	3	2
	Cleans	1	1	-	x	-	1	3	x
Sparidae	Visits	2	-	-	-	-	1	-	-
	Poses	1	-	-	-	-	-	-	-
	Cleans	1	-	-	x	-	-	-	x
Synodontidae	Visits	1	-	1	-	3	-	-	-
	Poses	1	-	1	-	3	-	-	-
	Cleans	1	-	1	x	3	-	-	x
Total	Visits	29	32	21	17	56	36	19	42
	Poses	24	24	16	1	44	4	8	15
	Cleans	23	24	15	0	44	2	8	0

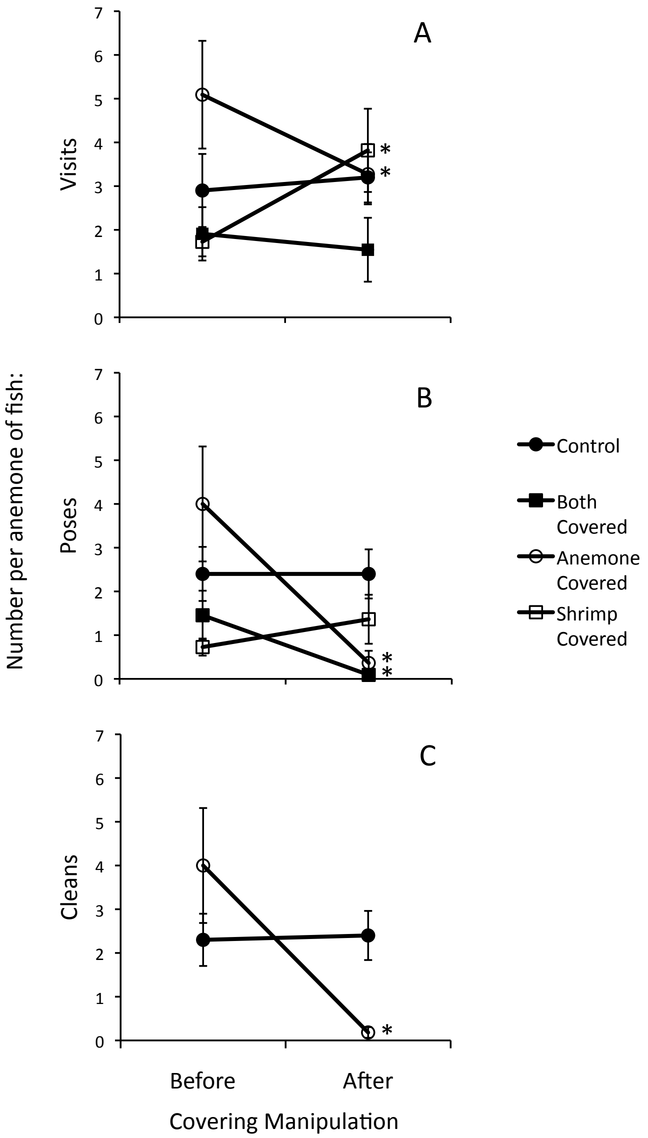

Figure 2.4. Variation in three types of behaviors of reef fish clients among four treatments in a field experiment on cleaner shrimps *Ancylomenes pedersoni* and their host anemones *Bartholomea annulata* on a coral reef at Brewers Bay, St. Thomas, U.S. Virgin Islands. Data ($\bar{x}$ ± SE) are shown for each treatment group before (immediately before covers were placed next to anemones for 2 d), and after treatments were applied (immediately after covers were moved to treatment positions). A) The number of fish visits did not vary for control and both covered treatments, but significantly (*) decreased in the anemone covered group ($p < 0.05$) and increased in the shrimp covered group ($p < 0.05$). B) The number of fish poses decreased significantly (*) at cleaning stations where anemones only ($p < 0.01$) and both anemones and shrimps ($p < 0.05$) had been covered. C) The number of fish cleaned after the cover was applied decreased significantly (*) in the anemone covered group ($p < 0.05$). In the both and shrimp only covered groups, shrimp were not available to clean.

CHAPTER 3

Client fish use patterns of shrimp cleaning stations on coral reef sea anemones

SUMMARY

Cleaner shrimps are known to effectively remove parasites from fishes on coral reefs, but are poorly studied relative to cleaner fishes, especially in terms of their diversity of fish clients. Also, many cleaner shrimps associate with benthic invertebrates, primarily cnidarians, and the complex roles of these associations in cleaning symbiosis is unknown. In St. Thomas, U.S. Virgin Islands, *Ancylomenes pedersoni* cleaner shrimps associate with *Bartholomea annulata* host anemones, which provide a visual landmark and posing stimulus for client fishes. We examined variation in aspects of fish client behavior with characteristics of host anemones and their assemblage of six species of crustacean associates, as well as describe fish client diversity and cleaning interactions. Larger anemones and those with more crustacean associates attracted significantly more fish client visits than did those with smaller bodies and less diverse associates. In addition, the number and duration of fish cleans varied significantly with the number of resident cleaner shrimp *A. pedersoni* per anemone. Fish clients included at least 30 species in 14 fish families. The complexity of this mutualistic network of anemone, shrimp, and fish, and the high diversity of fish clients affected indicates some difference in selective mechanisms and ecological effects for cleaner shrimps than for the more commonly studied symbioses between cleaner fishes and clients. Understanding how the associations of some cleaner shrimps with benthic host invertebrates affect their interactions with clients can aid in elucidating differences between cleaner shrimps and cleaner fishes.

1. INTRODUCTION

Coral reefs are home to highly evolved symbioses, including those between cleaner organisms and their client fishes who solicit cleans for parasite removal and potential wound healing (Foster 1985, reviewed in Côté 2000). Though cleaner fishes, primarily wrasses and gobies, are the most studied cleaner organisms, a variety of shrimps also are cleaners (Becker & Grutter 2004). Several cleaner shrimps have only recently been shown to remove a variety of fish parasites in tropical (Bunkley-Williams & Williams 1998, Becker & Grutter 2004, McCammon et al. 2010) and temperate waters (Östlund-Nilsson et al. 2005). Additionally, they appear to clean a similar client assemblage to that of cleaner fishes (Johnson & Ruben 1988, Chapuis & Bshary 2009). However, only a few studies have documented interactions between cleaner shrimps and their client fishes in the field (i.e. Wicksten 1995, 1998, Becker et al. 2005, Chapuis & Bshary 2009).

Cleaner organisms behaviorally signal to client fish to convey their identity, hunger, and readiness to clean (Becker et al. 2005, Chapuis & Bshary 2010). Cleaner fishes ‘dance’ to display to client fishes, and they also have a convergent blue and/or yellow striped pattern (Stummer et al. 2004, Arnal et al. 2006, Cheney et al. 2009) that client fishes innately recognize (Losey et al. 1995). Cleaner shrimps, however, neither display convergent coloration nor have a stereotypical guild signal (Becker et al. 2005, Chapuis & Bshary 2010). This lack of convergence is puzzling, because many coral reef fishes have low visual resolving power (Marshall 2000) and many cleaner shrimps are small and inconspicuous (Jonasson 1987), thus likely rendering them difficult for client fishes to locate and recognize.

The wide diversity of signals and colors of cleaner shrimps may be due in part to variation in their life histories, including their symbioses with sessile invertebrates. Some cleaner shrimps are crevice dwelling (Limbaugh et al. 1961, Wicksten 2009), others define cleaning stations around large sponges (Corredor 1978), and many are facultative (van Tassell et al. 1994, Chadwick et al. 2008) or obligate (Limbaugh et al. 1961) symbionts of corals and sea anemones. Cleaning stations for anemoneshrimps are their host anemones, as obligate anemoneshrimps usually do not venture further than about 10 cm from the anemone to clean (Johnson & Ruben 1988, Wicksten 1995). Because of this, large host anemones have been proposed to provide a visual landmark for client fishes to locate these shrimps (Mahnken 1972, Kulbicki & Arnal 1999), or as a contrasting background against which the shrimps are more visible (Mahnken 1972, Gwaltney & Brooks 1994). In St. Thomas, U.S. Virgin Islands, we determined that client fishes of the Pederson cleaner shrimp *Ancylomenes pedersoni* (Chace, 1958) use the corkscrew anemone *Bartholomea annulata* (LeSeuer, 1817), rather than the shrimp themselves, as a visual cue to locate these cleaning stations, as well as a stimulus for clients to pose for cleaning (Chapter 2).

In addition to *A. pedersoni*, the anemone *B. annulata* hosts a variety of crustacean associates in St. Thomas, U.S. Virgin Islands. These include the spotted anemoneshrimp *P. yucatanicus*, which has been purported as a cleaner (Limbaugh et al. 1961, Mahnken 1972, but see McCammon et al. 2010), as well as the sun anemoneshrimp *P. rathbunae*, red snapping shrimp *Alpheus armatus*, the squat anemoneshrimp *Thor amboinensis*, mysid shrimp *Heteromysis actinae*, the arrow crab *Stenorhynchus seticornis*, and infrequently other small crabs (identified from Humann & DeLoach 2006). The presence of these associates may have an impact on cleaning associations between *A. pedersoni* and its clients by either serving as additional visual stimuli, as additional cleaners (Limbaugh et al. 1961, Mahnken 1972) or cleaner mimics (McCammon et al. 2010), or as potential prey items (reef fishes attempt to prey on exposed *A. armatus*; L. Huebner, pers. obs.).

Based on the passive but important role of *B. annulata* in the symbiosis between *A. pedersoni* and its clients, we examine here some measures of anemone size and potential visibility, and the diverse crustacean assemblage associated with these anemones, and determine if any of these characteristics influence rates of client visitation at these cleaning stations by serving as cues or attractants. Additionally, because few field data describe interactions between *A. pedersoni* and its fish clients, we timed cleans and recorded the client diversity and the number of *A. pedersoni* engaged in the clean. These data will help to elucidate field patterns in this multilevel symbiosis.

2. MATERIALS AND METHODS

We performed this study on coral reefs at St. Thomas, U.S. Virgin Islands at two reef sites: Brewers Bay (~ 6 m depth) and Flat Cay (~ 7-9 m depth). Observations were conducted in Flay Cay in March 2009 only and in Brewers Bay in March, July, and November 2009, and February 2010. We focused our observations only in Brewers Bay during the latter periods because coral reefs in Brewers Bay are closer to shore and were logistically easier to access for dawn observations, and also they supported a higher abundance of the anemone *Bartholomea annulata* and its symbiotic cleaner shrimp *Ancylomenes pedersoni* than do those at Flat Cay (Nelsen 2008).

Observation methods are in part described in Chapter 2, and a more extensive description of these field methods is given here. Peak cleaning activity on coral reefs occurs at dawn because cleaners are diurnal, parasites accumulate at night (Grutter 1999, Sikkel et al. 2004) and parasites also search for hosts at dawn (Sikkel et al. 2006). Thus, we conducted our cleaning observations in the morning, beginning just after sunrise (~ 6:30 am) and concluding by 8:30 am. Divers observed cleaning stations from a distance of ~ 1.5 m, and observations began 2-3 min after the diver assumed a stationary observation position on the sea floor; diver presence did not appear to affect client behavior at these cleaning stations, as cleaning activity resumed after the diver became stationary.

We conducted an initial census of the site before cleaning observations to identify and map all *B. annulata* in our study area that hosted at least one *A. pedersoni*. These anemones were tagged with an engraved aluminum tag and flagging tape nailed into the substrate; tags were placed away from the anemone (i.e. on the other side of the rock from the anemone) to minimize any effect of the tag on animal behavior. Observations of fishes in this study site, which also includes a long-term census of anemone and anemoneshrimp populations requiring the same tagging method (Nelsen 2008), indicate that fishes do not respond to our tags or flagging tape (L. Huebner, pers. obs.). Of all the marked *B. annulata* that hosted *A. pedersoni*, we haphazardly selected a subset for observations, with this sample size depending on the duration of our field visit and the number of divers available to make observations (N = 7-32 stations observed per visit x four visits = 77 stations observed total). Different stations were selected for observation during each of the four field visits, because many stations from previous visits no longer could be located (shrimps and/or anemones gone) on subsequent field visits. This rapid change in cleaning stations occurs because these anemones have high rates of population turnover (Nelsen 2008).

To determine the types of cues that attract client fish to anemone cleaning stations, we defined a set of metrics of anemone size, position, visual extension beyond the substrate, and crustacean assemblage composition (hereafter ‘anemone characteristics’). Anemone characteristics consisted of: a) anemone tentacle crown surface area (TCSA), calculated from the longest and shortest diameter of the anemone tentacle crown (after Porat & Chadwick-Furman 2004), b) the maximum height the tips of the tentacles reached above the sea floor (TCASF), c) the maximum distance the tentacles reached beyond the substrate in the orientation of the anemone (TCBS), d) anemone orientation on the substrate (vertical if oral disc is perpendicular or horizontal if oral disc is parallel to sea floor), f) the number of *A. pedersoni* associates, and g) the number of other crustacean associates (including arrow crab *Stenorhynchus seticornis*, spotted anemoneshrimp *Periclimenes yucatanicus*, sun anemoneshrimp *P. rathbunae*, red snapping shrimp *Alpheus armatus*, squat anemoneshrimp *Thor amboinensis*, and unidentified crabs; identified from Humann & DeLoach 2006). Mysid shrimp *Heteromysis actinae* also occur associated with *B. annulata,* but were not included in analyses because of their small size (< 0.3 cm; Humann & DeLoach 2006) and because they inhabit the anemone deep within the tentacles (L. Huebner, pers. obs.). In some cases, individuals of *B. annulata* are present in touching clusters, the associates of which move freely between anemones. As their tentacles intertwine, these anemones are indistinguishable as separate individuals unless disturbed and contracted. In these cases, total tentacle diameters were recorded and TCSA was calculated for the whole cluster. Anemone characteristics were recorded 0-5 d before observation in March 2009, and immediately after dawn observations in the morning in July and November 2009, and February 2010.

For quantification of client visits to the station and any subsequent cleaning behavior, each anemone station was observed for 20 min and the following data were recorded: the number and identity of clients visiting the station, the number of cleans and identity of the client cleaned, and the duration (in sec) of each clean. Clients were identified to at least the family level. Visiting behavior was defined as a fish approaching and lingering near the cleaning station (Arnal & Côté 1998), excluding other fish behavior around that station (i.e. grazing, territory defense, etc.). Cleaning behavior was defined as *A. pedersoni* contact with the fish client. Any other behavior at the anemone station, such as non-*A. pedersoni* crustacean-associate interactions with clients, were noted. All data were analyzed using SYSTAT 13.

3. RESULTS

(a) *Visits*

To determine which anemone characteristics influenced client visitation rate, we conducted a stepwise backwards elimination multiple regression with the number of visits at a cleaning station as the dependent variable and TSCA, TCASF, TCBS, the number of *A. pedersoni* symbionts, and all crustacean associates (including *A. pedersoni*) as the independent variables. Anemone orientation was not included in analyses because all but one of our 77 study anemones were oriented vertically. All variables were dropped from the regression ($p = 0.003$, $R^2 = 0.148$) except TCSA ($p = 0.045$) and the total number of crustacean associates ($p = 0.011$). Neither the number of *A. pedersoni* ($R^2 = 0.002$, $p = 0.736$) nor the number of all crustacean associates ($R^2 = 0.022$, $p = 0.200$) per anemone depended on anemone body size, as measured in TCSA.

(b) *Cleans*

We observed at least 30 species representing 14 or more families of client reef fish to participate in 123 cleans at 77 cleaning stations across the four observation periods (not all clients were identified to species level, and four clients were unidentifiable to family level; Figure 3.1A). Members of Acanthuridae (surgeonfishes) accounted for nearly 50% of our observed client fishes, while the next most common client family was Mullidae (goatfishes) with 13% of observed cleans. All other families comprised less than 5% each of observed cleans. Four unidentified fishes are included in the group 'Other'. Despite being the most common clients, members of Acanthuridae did not have the lengthiest cleans (Figure 3.1B). Members of Serranidae (groupers) and Synodontidae (lizardfishes) had average cleans longer than 2 min, and members of Monacanthidae (filefishes) and Mullidae had average cleans of 74 sec. However, the variability in clean lengths within these families was high. Clean durations ranged from a few seconds up to 11 min, but most cleans lasted < 20 sec (Figure 3.2).

The number of cleans per station significantly increased with the number of *A. pedersoni* at that station ($R^2 = 0.086$, $p = 0.009$), and the total duration of all cleans per station significantly increased with the number of cleans that occurred there ($R^2 = 0.518$, $p = 0.000$), as well as the number of *A. pedersoni* present ($R^2 = 0.082$, $p = 0.007$). However, the length of individual cleans was not related to the number of shrimp cleaning at that station ($R^2 = 0.002$, $p = 0.602$). Average number of cleans and average total durations of cleans per stations with varying numbers of shrimps are displayed in Figure 3.3. A station with three shrimp at which 15 cleans occurred was not included in these analyses because it was an outlier. Out of 123 cleans, there were only three in which only some of the *A. pedersoni* present engaged in the clean; all 120 other cleans involved all the *A. pedersoni* at that station.

4. DISCUSSION

We show here that client fish appear to cue in on certain anemone characteristics to determine whether they visit an *Ancylomenes pedersoni* shrimp cleaning station on the host anemone *Bartholomea annulata*, expanding on our previous work that revealed client fish use of these anemones as a visual landmark and posing stimulus. Additionally, we examined details of cleaning interactions between *A. pedersoni* and its clients, particularly the diversity of clients that visit these stations and the durations of cleans. This study elucidates aspects of cleaner shrimp symbioses with client fishes, particularly of anemone-associated shrimps, which have been little studied in comparison to those with cleaner fishes.

Client fishes varied in their visiting behavior at these cleaning stations. Most fishes appeared attracted to cleaning stations and stopped for cleaning as they were swimming around or engaged in other activities. Some clients (notably groupers), however, swam purposefully in direct routes to these cleaning stations and solicited cleans. Based on this variation in behavior among fish types, some fishes may have a mental map of where preferred cleaning stations are, whereas other fishes may haphazardly search for cleaning stations.

Of our defined anemone characteristics, only the total crustacean assemblage size and the anemone tentacle crown surface area (TCSA) predicted the number of client fish visits at a station. That anemone TCSA is a significant predictor of client fish visits agrees with our experimental information about the anemone as a visual cue for locating stations and as a posing stimulus (Chapter 2): anemones with larger TCSAs attract more clients than smaller anemones, likely because large anemones are more highly visible, especially at a distance, than are small anemones. Because anemone TCSA does not relate to the number of *A. pedersoni* or the crustacean assemblage on the anemone, anemone size is possibly a visual attractant only, and not an indicator to fish of a cleaning station with more cleaner shrimp.

Total crustacean assemblage includes the number of *A. pedersoni*, but interestingly, the number of *A. pedersoni* alone was not a significant predictor of visits and was the second variable dropped from the model (after height the tentacle reached above the sea floor; TCASF). It may be that client fishes are attracted to anemones with large groups of crustacean symbionts, without distinguishing if those symbionts are cleaners. This may be because client fishes cannot visually distinguish if resident crustaceans are cleaners or not, until they come close and visit the anemone. This pattern supports our experimental evidence that fishes cue to anemones and not the smaller, less conspicuous shrimps, when they initiate visits. Alternately, these visits may not indicate clients seeking cleaning, but rather fishes in search of non-cleaning crustacean prey items that associate with anemones. We occasionally observed interactions between visiting client fishes and *Stenorhynchus seticornis*: individuals of *S. seticornis* waved their long chelae at visiting snapper, but not at other visiting fishes such as surgeonfish. This interference from *S. seticornis* resulted in those harassed clients abandoning poses or cleans. We did not observe this behavior from all *S. seticornis*, however, and only 27 out of 77 observed cleaning stations hosted one or two *S. seticornis,* but it would be interesting to determine how the presence of these facultative *B. annulata* symbionts may affect clean rates of *A. pedersoni* sharing an anemone.

Despite not being a significant predictor of client visits, the number of *A. pedersoni* is significantly related to the number of cleans at a station: large groups of *A. pedersoni* cleaned more clients than did small groups. The large variability and scatter of the trend we see at high shrimp numbers is likely a result of small sample sizes of anemones hosting greater than four *A. pedersoni* (Figure 3.3). This is puzzling: if the shrimp have the opportunity to engage in more cleans when associating in large groups, why are there so few anemone stations with large numbers of *A. pedersoni*? Determining the cause of this apparent contradiction might provide information about how and where these cleaning stations are formed. It may be that social interactions among the shrimp have a stronger influence on their group size than optimizing their potential number of cleans.

An anemone station with three *A. pedersoni* engaged in 15 cleans; this station was not included in analyses because it was an outlier. Biologically, it was an interesting case: all of these cleans were surgeonfish that approached the station as a school, and remained together around that station as members of the school rotated to receive cleans.

A small group of cleaner shrimp likely collectively satiates more quickly than a large group. Because the number of fish visits to a station was not related to the number of *A. pedersoni* at that station, fish may be drawn to the station by other factors (such as anemone TCSA), but if the anemone has a small group of non-signaling or already satiated shrimp, they may not signal or clean the client if it poses (Becker et al. 2005). This would result in fewer cleans at that station than a station with more shrimp, despite a similar number of visits.

Client fish may seek large groups of shrimp because their body surface area will be examined more quickly than by a single shrimp alone, even if that single shrimp is able to remove all the parasites and provide the same ‘quality’ of clean. Thus the client fish would spend less time being cleaned at a station with a large group of shrimp than with a small group. However, we found no support for this, as the number of *A. pedersoni* at a station did not relate to the duration of single cleans; thus, a higher number of shrimp engaged in the clean does not result in a shorter clean.

Fish may receive a better quality (more parasites removed) clean at stations with more shrimp, because more of their body surface area will be examined by more shrimp, increasing the chances that a greater number of parasites will be removed. Additionally, clean quality by large groups of *A. pedersoni* may be similarly better than singletons or small groups, similar to the pattern in cleaner fish that the service quality offered by pairs of cooperating *Labroides dimidiatus* is better than that by singletons (Bshary et al. 2008). It would be interesting to investigate if cleaner shrimp 'cheat', and if members of a group of cleaner shrimp control cheating by other members of the group, thus enhancing the overall benefit of cleans by the group. We did infrequently observe jolts by client fishes; however, we did not observe client fish to leave immediately or 'punish' *A. pedersoni* after a jolt, suggesting either little to no cheating by these cleaner shrimp (jolts may represent uncomfortable removal of an embedded parasite), or a lack of partner control by the client fish in this system. These cleaner shrimp may have a cleaning symbiosis with their clients without punishment, suggesting more similarity to the symbiosis of clients with Caribbean cleaner gobies than to that of the Indo-Pacific cleaner wrasse *L. dimidiatus* (Soares et al. 2008). However, there may be a cheating-control system between the cleaner shrimp *A. longicarpus* and its clients (Chapuis & Bshary 2009).

The lengths of cleans we observed in this study corroborate those observed by Johnson and Ruben (1988) of several Caribbean cleaners in St. Croix, U.S. Virgin Islands. Clean length may be < 20 sec for most cleans because parasites are generally present in low loads, resulting in little time needed for the cleaner to effectively remove parasites from each fish, or it may be that *A. pedersoni* expeditiously locate and remove even large loads of parasites. Fishes that are cleaned for these short durations may visit cleaning stations frequently, and fishes that are cleaned for longer durations (up to 11 min this study) may visit stations infrequently. Tracking of individual fish, as well as determining parasite loads on fish types (see Sikkel et al. 2000, 2009) are needed to elucidate patterns of cleans and clean durations, particularly among families of fishes.

The variability in clean length within the families of fishes with the longest average clean lengths could be due to a number of factors. Members of these four families – Monacanthidae, Mullidae, Serranidae, and Synodontidae – achieve some of the largest body sizes of the families observed at these cleaning stations. Large individuals may have more parasites, which has been shown to affect cleaning behavior in some cleaner shrimps (Becker & Grutter 2005). As well, the cleaner wrasse *L. dimidiatus* prefers large clients (Grutter et al. 2005), likely because client size correlates with parasite load (Grutter 1995). If cleaner shrimp show this preference, they may spend more time on large clients because of increased foraging success. However, *A. pedersoni* do not appear to be picky cleaners, as they also attempt to clean divers' hands and toy fish models (Wicksten 1998, L. Huebner, pers. obs.). Conversely, the average duration of cleans for these groups may be due to large body sizes requiring more time from *A. pedersoni* to scan the surface area and remove all parasites. If a large surface area requires more clean time, the large variability seen in these groups may be due to a range of sizes of individual clients within the group.

Ancylomenes pedersoni are effective cleaners of fish parasites (Bunkley-Williams & Williams 1998, McCammon et al. 2010), so it is not surprising that a large variety of clients – members of at least 14 families – visit their cleaning stations. However, the relative frequencies of client families at these stations are different in our study than in previous studies. In Bonaire, Wicksten (1995) observed *A. pedersoni* to clean clients in 13 fish families. Members of Serranidae (~ 40%) and Scaridae (parrotfishes, ~ 20%) represented the most common clients of *A. pedersoni* in Bonaire, while our most common client groups in St. Thomas, Acanthuridae and Mullidae, represented the third and fourth most common client groups in Bonaire with ~ 15% and ~ 10% (respectively) of observed cleans. Johnson and Ruben (1988) documented only five families of client fishes cleaned by these shrimp in St. Croix: Pomacentridae (damselfishes), Serranidae, Scaridae, Holocentridae (squirrelfishes), and Labridae (wrasses; listed by frequency as clients). These differences may reflect variation in the relative abundances of client fishes among regions of the Caribbean, or among reef habitats within regions. Our study and the study of Johnson and Ruben (1988) were both conducted in the U.S. Virgin Islands, but ours was in a shallow bay site and an off-shore cay, whereas Johnson and Ruben (1988) documented cleans at greater depths along a submarine canyon. It would be interesting to compare our observed cleaning rates to the abundance of client fishes at our study sites; however, collecting fish abundance data was outside the scope of this thesis due to limitations in the field.

The differences in family clean rates may also represent regional variation in cleaner species preferences among client fish groups. To determine this, clients of the same species could be collected from the field and kept in flow-through aquaria with a variety of cleaners naturally occurring in that region, and preference of cleaner could be recorded. If regional preference for cleaners varies among locations within client species, then client fishes may learn cleaner preferences socially.

The data here describe aspects of the multi-way symbiosis between the coral reef fish clients of the cleaner shrimp *A. pedersoni* and its host anemone *B. annulata*. The anemone appears to serve as a landmark cue for client fishes to locate these cleaning stations, and larger anemones (size in TCSA) are a more noticeable landmark than are smaller anemones for visiting fishes (and also are a posing stimulus; Chapter 2). Variation in cleaner shrimp signals may be due in part to the variation in their benthic lifestyles, including the coevolution of some shrimps with cnidarian hosts. The high client diversity of *A. pedersoni*, and the use of anemones as visual cues by these clients, represents a complexity of cleaner-client interactions that differ from those of the more frequently studied interactions between the cleaner fish *L. dimidiatus* and its clients. The differences between cleaner shrimp and cleaner fish symbioses are thus likely valuable areas for future research, particularly in terms of the complex interactions involving anemone-associated cleaner shrimp.

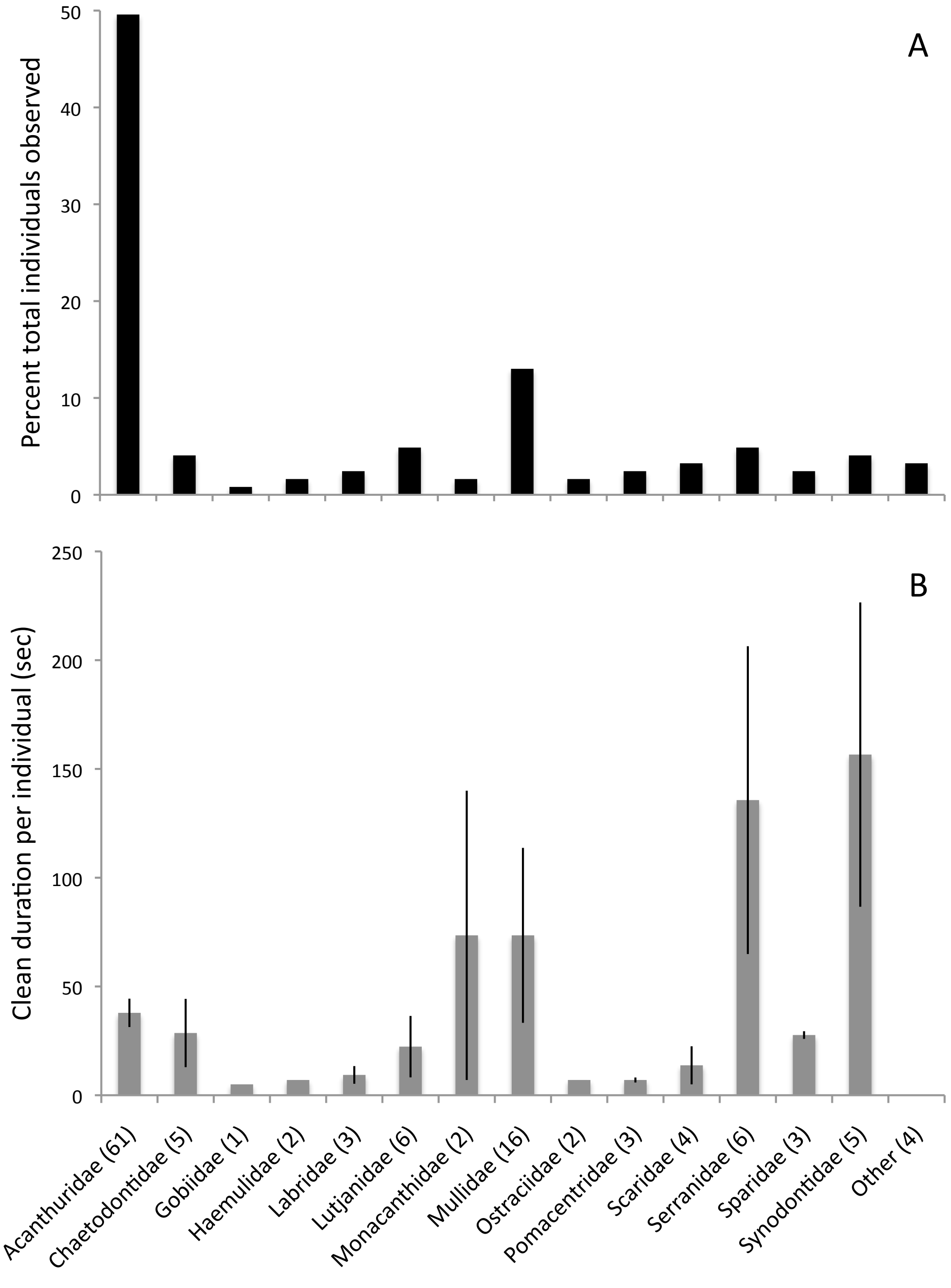

Figure 3.1. Variation among client fish families in A) number of individuals cleaned and B) duration of cleans ($\bar{x}$ ± SE) at *Ancylomenes pedersoni* shrimp cleaning stations on *Bartholomea annulata* anemones at Brewers Bay and Flat Cay, St. Thomas, U.S. Virgin Islands. The number of individuals observed in each family is included in parentheses behind the family label; a total of 123 cleans were observed in this study. 'Other' includes fish that were not identified to family level, but were observed being cleaned. Because they were not identified, we did not include a family clean time for these cleans.

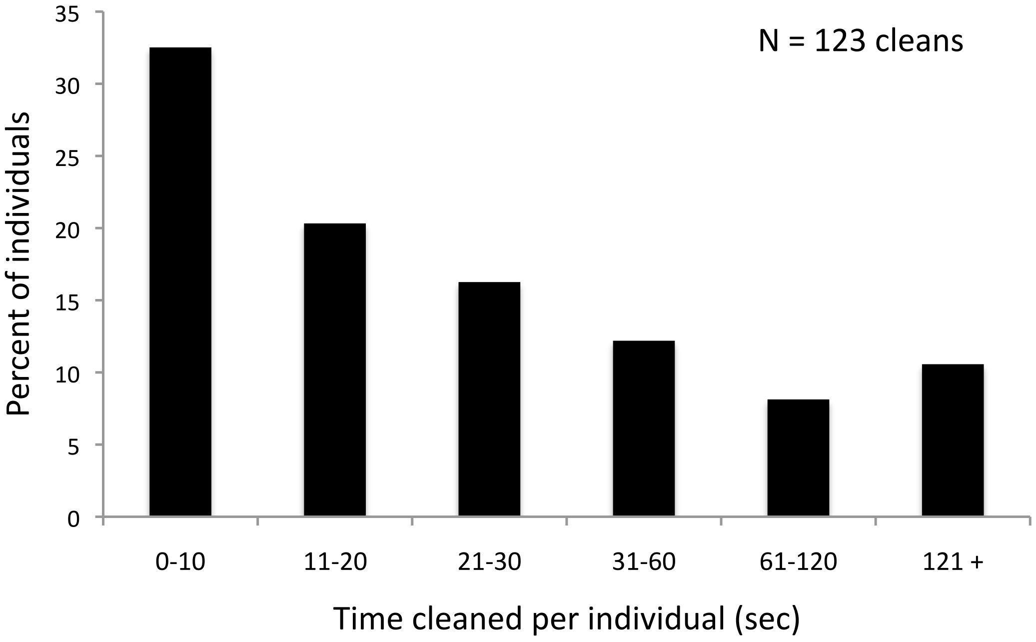

Figure 3.2. Durations of cleans of fish visitors to *Ancylomenes pedersoni* shrimp on *Bartholomea annulata* anemones at Brewers Bay and Flat Cay, St. Thomas, U.S. Virgin Islands. All clean durations were recorded in seconds.

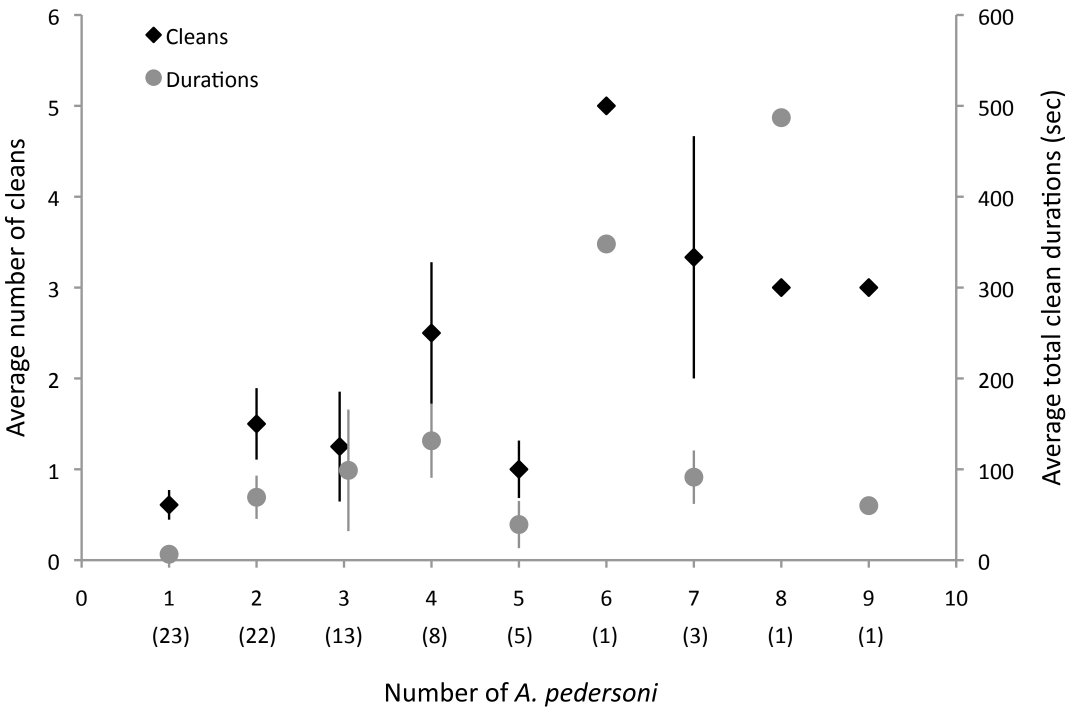

Figure 3.3. Average number and total duration of cleans ($\bar{x}$ ± SE) during 20 min observation periods at *Bartholomea annulata* anemone cleaning stations with varying numbers of *Ancylomenes pedersoni* cleaner shrimps at Brewers Bay and Flat Cay, St. Thomas, U.S. Virgin Islands. A station with three *A. pedersoni* and 15 cleans was not included in these calculations because it is an outlier; a large school of surgeonfish visited this station and together sought cleans at it. Some data points were staggered for clarity. Sample sizes of stations with each number of shrimp are listed below the shrimp number x-axis label.

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