Protection of host anemones by snapping shrimps: a case for symbiotic mutualism?

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Abstract The sea anemone *Bartholomea annulata* is an eco-logically important member of Caribbean coral reefs which host a variety of symbiotic crustacean associates. Crustacean exosymbionts typically gain protection from predation by dwell-ing with anemones. Concurrently, some symbionts may provide protection to their host by defending against anemone predators such as the predatory fireworm, *Hermodice carunculata*, which can severely damage or completely devour prey anemones. Herein we show through both field and laboratory studies that anemones hosting the symbiotic alpheid shrimp *Alpheus armatus* are significantly less likely to sustain damage by *H. carunculata* than anemones without this shrimp. Our results suggest that the association between *A. armatus* and *B. annulata*, although com-plex because of the numerous symbionts involved, may be closer to mutualism on the symbiotic continuum.

Keywords *Alpheus* · Anemone · *Bartholomea* · Fireworm · *Hermodice* · Shrimp symbionts

1 Introduction

Symbiotic interactions have been reported from a wide range of taxa (Dickman 1992; Poulin and Vickery 1995) and are

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especially common in marine environments (Roughgarden 1975; Poulin and Grutter 1996; Côté 2000). Mutualism; a type of symbiotic relationship in which both partners derive some benefit from the association, are also widespread across taxa (Boucher et al. 1982). The benefit(s) of symbiont-mediated protection of host species from microbial disease, parasites, and predators is increasingly evident (Haine 2008). Protection mechanisms are diverse and include various sym-biont derived chemical defenses (Haine 2008) as well as maintenance behaviors (Heil and McKey 2003; Stier et al. 2012) and defensive social interactions (Glynn 1980; Brooks and Gwaltney 1993; Heil and McKey 2003; McKeon et al. 2012). Previous studies have demonstrated that some crusta-ceans will actively defend host cnidarians in their natural marine (Smith 1977; Glynn 1980) and laboratory settings (Brooks and Gwaltney 1993; McKeon et al. 2012).

Some Caribbean cnidarians are well studied due to their importance as focal locale for marine cleaning symbiosis, though concentration has been on the interactions between visiting client fish that has ectoparasites removed by an unre-lated cleaner organism which generally associates with a cnidarian host (Losey 1972; Van Tassell et al. 1994; Côté 2000; Floeter et al. 2007). Some anemone-dwelling shrimps have been reported to engage in marine cleaning symbiosis, eating parasites from fish in captivity (Bunkley-Williams and Williams 1998; Becker and Grutter 2004; Östlund-Nilsson et al. 2005; McCammon et al. 2010) and appear to remove ectoparasites from free-living reef fishes (Côté 2000; Sikkel et al. 2004; Huebner and Chadwick 2012a). There is evidence that client fish and cleaner organisms benefit from the cleaning station association, however, whether the anemone host benefits from the anemone-crustacean symbioses is un-clear. Despite the potential importance of anemones as con-spicuous base stations for fish cleaning activity on coral reefs (Côté 2000; Humann and Deloach 2003; Huebner and Chadwick 2012b) few quantitative research studies exist on

patterns of abundance, symbiont diversity, or importance of crustacean macrosymbionts for the host sea anemone.

One of the largest and most common sea anemones on Caribbean coral reefs is the corkscrew anemone *Bartholomea annulata* (LeSueur, 1817), which hosts a variety of crustacean exosymbionts (Criales 1984) the majority of which are from multiple families within Order Decapoda, that can inhabit a host anemone as solitary or mated pairs and as aggregations of individuals of mixed species (Calado et al. 2007). Derived host-symbiont recognition and acclimation mechanisms seem to be species specific (Crawford 1992) and are currently unknown for many *B. annulata* associates. Decapod symbionts such as the purple Pederson shrimp, *Ancylomenes pedersoni* (Chace, 1958) (formerly within genus *Periclimenes* (Okuno and Bruce 2010)), the spotted cleaner shrimp, *Periclimenes yucatanicus* (Ives, 1891), and the red snapping shrimp, *Alpheus armatus* (Rathbun, 1901), are obligate associates that remain with the same host anemone for life and preferentially choose their natural host over other anemone species (Knowlton and Keller 1985, 1986; Gwaltney and Brooks 1994; Silbiger and Childress 2008). *A. pedersoni* is generally a non-aggressive, anemone-shrimp that is an effective cleaner organism for reef fishes (Bunkley-Williams and Williams 1998; McCammon et al. 2010). *P. yucatanicus* may be a cleaner mimic (McCammon et al. 2010) with similar morphological and behavioral characteristics as *A. pedersoni. A. armatus* is an anemone-shrimp known for its aggressive territoriality, especially with non-mated conspecifics (Knowlton and Keller 1982; Hughes 1996). Other crustaceans symbionts of *B. annulata* are facultative associates which do not rely on a single anemone host throughout their life (Guo et al. 1996). The arrow crab, *Stenorhynchus seticornis* (Herbst, 1788), is common on Caribbean reefs (Humann and Deloach 2002) and has been reported to associate with anemones (Herrnkind et al. 1976; Wirtz et al. 2009). *S. seticornis* may be a facultative symbiont of *B. annulata* (Chadwick pers. Comm.) and is known anecdotally in the aquarium trade to eat polychaetes, such as fireworms.

The anemone-crustacean association imparts some benefit(s) to both the host and its symbionts. Crustaceans that dwell with anemones can provide their host with nitrogenous waste that fertilize endosymbiotic zooxanthellae, increasing the number of zooxanthellae cells (Spotte 1996) and potentially increasing algal photosynthesis derived food supply for the anemone (Spotte 1996; Porat and Chadwick-Furman 2005). Shrimp may gain a food source while maintaining the host anemone by removing excess mucus, necrotic tissue, and inorganic debris (Herrnkind et al. 1976; Nizinski 1989; Crawford 1992). Due in part to its large polyp size, fleshy tentacles and relatively toxic nematocysts, *B. annulata* may serve as protective host for facultative and obligate crustacean symbionts (Mihalik and Brooks 1995). Is the role of protector reciprocal?

Although defense by shrimp and crab associates of cnidarians has been demonstrated (Glynn 1980; Brooks and Gwaltney 1993), only one study has reported that a crustacean associated with *B. annulata* will display defensive behavior in the presence of an anemone predator (Smith 1977). The amphinomid polychaete, bearded fireworm, *Hermodice carunculata* (Pallas, 1766), is a known cnidarian predator (e.g., Witman 1988; Vreeland and Lasker 1989 ; Souza et al. 2007) that has been documented to eat solitary anemones (Lizama and Blanquet 1975) including *B. annulata* (Smith 1977). *H. carunculata* has been reported to forage diurnally (Witman 1988) and nocturnally (Fine et al. 2002; Genovese and Witman 2004) feeding on *B. annulata* by everting its buccal mass over the anemone tentacles partially or completely eating the anemone down to the pedal disk (Pers. Obs.). Damaged anemones exude defensive white threadlike acontia with cnidae (Smith 1977, Pers. Obs.).

Most of *B. annulata*'s crustacean associates are relatively small and cryptic making it difficult to investigate interactions between these benthic invertebrates in the field without potentially influencing behaviors. Setting up assemblages similar to those documented in the natural community facilitated observation and documentation of interspecific interactions. In situ and laboratory microcosm experiments were conducted to test predictions of the hypothesis that anemone associates play a deterrent or predator protection role for their host anemone.

2 Materials and methods

2.1 Study localities

Field experiments were carried out between July, 2008 and March, 2010 in three shallow water (1–5 m) coral reef field localities in two south shore bays of the U.S. Virgin Islands; Donkey Bite Reef (DB) in Great Lameshur Bay on St. John (18°18′51″N, 64°43′16″W), Black Point Reef (BP), (18°20′ 37″N, 64°58′54″W), and Ratchford Reef (RR), (18°20′24″N, 64°58′44″W), in Brewers Bay on St. Thomas. Multiple study localities were used for field experiments to investigate broad patterns across multiple bays. Donkey Bite and Black Point Reef are predominately *Orbicella annularis* complex coral beds and Ratchford Reef is a small patch reef. Laboratory experiments were conducted within 25×30×33 cm, aquaria set in an outdoor wet table with open seawater flow at The University of the Virgin Islands, McLean Marine Science Center, on St. Thomas.

2.2 Organism collection

Species identification for all organisms was made using reference materials (Fauchald 1973; Knowlton and Keller 1985;

Spotte 1997; Camp et al. 1998; Humann and Deloach 2002; Barroso and Paiva 2007; Meinkoth 2007; Budd et al. 2012).

Bartholomea annulata anemones crown length ranged from 2 to 40 cm with an average of 9.3 cm (±5.8 SD, *n*=327). The average tentacle crown surface area (TCSA=π × 0.5 L × 0.5 W; Hattori 2002) was 61.5 cm² (±77.9 SD, *n*=327). *B. annulata* attached to small pieces of non-coral live rock rubble were collected from Brewers Bay, and transported in collection buckets. The carapace length (CL) (from the tip of the rostrum to the posterior edge of the carapace) of all decapod associates was approximated and large size class individuals were collected with small aquarium nets and transported in hermetically sealed plastic bags; *Ancylomenes pedersoni* (1.5 to 2 cm CL, *n*=30), *Periclimenes yucatanicus* (1 to 1.5 cm CL, *n*=8), *Alpheus armatus* (3 to 4 cm CL, *n*=20), and *Stenorhynchus seticornis* (4 to 5 cm CL, *n*=10). Rocks with anemones attached were placed in 40 l aquaria with live sand and crustacean associates (when applicable), and allowed to acclimate for at least 24 h, before each trial began. *Hermodice carunculata* were collected in traps set at reef sites and in intertidal zones within the research bays, and transported in hermetically sealed plastic bags. Each of three fireworm traps consisted of a large (8 cm diameter) PVC pipe T-connector with funnels attached to the two opposite ends and a removable plastic and rubber band lid, placed on the T section. Traps were baited with commercial squid bait and set for 1 to 24 h. The total body lengths of each fireworm specimen collected in traps were measured with a metric ruler placed alongside worms outstretched and crawling along the perimeter of a small (4 l) holding tank with seawater to ensure predators of similar size class were introduced to appropriate treatments for each experimental trial. The capture rate of *H. carunculata* was 0 to 3 individuals per trap. Fireworm lengths ranged from 3 to 18 cm long, with a mean length of 9.9 cm (=/−3.4 SD, *n*=80). *H. carunculata* used for all in situ and laboratory experiments ranged in size from 5 to 10 cm. Fireworms were housed in 19 l aquaria for 24 h before trials began.

2.3 Experiments

Experimental trial duration was 24 h. Water temperatures for field and laboratory experiments were consistently 29–30 ° C. All organisms were used for one experimental trial only, and most were released to their respective home reef (collection localities) within 3 days after experimentation. Anemones were tested for nematocyst firing activity before trials for randomly chosen anemones (*n*=5) per reef site and the laboratory using a method similar to that of Brooks and Mariscal (1984). Briefly, a glass microscope slide was put into contact with anemone tentacles and transported in hermetically sealed bags with seawater to the laboratory for examination under compound microscope to confirm nematocyst discharge.

Microscopic examination of the stomach contents of *H. carunculata* was performed (*n*=4; 1 from each site and laboratory), from treatment groups/trials in which anemones were damaged, to confirm ingestion of anemone (i.e., cnidae in gut).

2.4 Field trials

In situ experimental areas were enclosed with large plastic buckets with the bottoms cut out. The result was a double open-ended tapered plastic arena with a height of approximately 50 cm, a topside diameter of 75 cm and a bottom resting on the benthos of 39 cm diameter. We used eight arenas throughout this study with treatment types assigned randomly to arenas used for each trial.

The following experimental treatments were used: 1) Solitary anemone; 2) Anemone with a fireworm (FW), *Hermodice carunculata*; 3) Anemone with a FW and alpheid shrimp, *Alpheus armatus* and; 4) Anemone with a FW and suite of common crustacean symbionts; 1 or 2 *A. armatus*, 1 to 5 *Ancylomenes pedersoni*, 0, 1 or 2 *Periclimenes yucanicus*, 0, 1 or 2 *Stenorhynchus seticornis* (Fig. 1). The suite of associates in treatment type 4 emulates the most common natural associate group dynamic documented on the reefs studied (Nelsen 2008; McCammon 2010). Arenas were placed over the anemone, and a twisting action was used to embed the bottom edge of the arena into the sand floor; gaps along the perimeter were filled with sand. Arena walls were hand wiped before trials to dislodge fouling debris that could facilitate fireworm traction and escape. Arenas were weighted with local volcanic rock in hermetically sealed bags attached to the sides with cable ties. Arena tops were covered with plastic mesh hardware cloth to curb loss of fireworms due to predation by sparid fishes (Pers. Obs.). We used the following protocol for data collection and trials: 1) located suitable small non-coral live rock with attached *B. annulata* along the sand-reef interface, 2) measured crown length and width of anemone with metric ruler (before and after trial), 3) documented associate species and quantities (before and after trial), 4) relocated associates not used in treatment (collected with plastic bag and introduced to suitable anemone host not used in trial), 5) mimicked the removal of crustaceans that remained in treatment (to mimic disturbance other treatment anemones and crustaceans experience in preparation of trial; crustaceans were collected in plastic bag and immediately released near natural host), 6) set arena with treatment type randomly assigned, 7) introduced a randomly selected *H. carunculata* predator (keeping fireworm size distribution between treatments/trials as similar as possible), 8) counted predators before and after trial when applicable, 9) documented damage to anemone as 0 = Undamaged, 1 = Damaged (anemones partially or completely eaten were noted but not distinguished
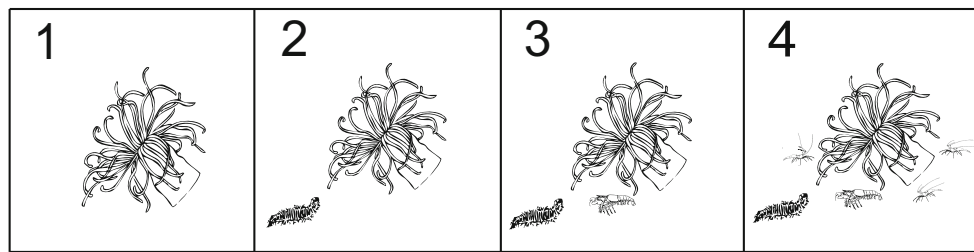
Fig. 1 Experimental treatment types used to test for anemone host defense by crustacean(s) against a fireworm predator in field and laboratory trials; *1* = solitary anemone, *2* = anemone with fireworm predator, *3* = anemone with alpheid shrimp and fireworm predator, *4* = anemone with a suite of decapod associates and fireworm predator. Interspecific behavioral interactions observed within the first hour of laboratory trials were recorded. Body condition and size were noted for all associates in each treatment before and after the 24 h trials

in this dichotomous investigation), 10) removed arenas and released organisms on home reef when trials were completed.

2.5 Laboratory trials

Microcosm experiments were conducted to facilitate close observation and video documentation of interactions between a benthic anemone predator and the relatively small crustaceans dwelling among anemone tentacles. Due to the cryptic coloration and behavior of some of the partners as well as the complex habitat and depth of the cleaning station assemblage under investigation, field observations were deemed unsuitable for meaningful behavioral data gathering. Laboratory treatment types were the same as those used in field trials (Fig. 1). Four 25 l plastic aquaria were prepared with natural diffused light, flowing seawater, and live sand benthos. Treatment types were randomly assigned to aquaria used for each trial. Interspecific behavioral interactions observed within the first hour of each trial were categorized, quantified, and when possible, recorded with a Sony® DCR-HC52 digital video camera. During each trial we recorded the following: 1) number of times the fireworm approached the anemone (moves toward and within 1 cm of anemone), 2) which crustacean species actively engaged the fireworm, 3) number of times the defender rushed the predator (quickly moved toward the fireworm, chelipeds first, in a pushing stance), 4) number of times the defender snapped its chelipeds, 5) number of times the defender pinched the predator with its chelipeds. After the 24 h trial time, each participant organism (e.g., anemone, crustaceans, fireworm) was inspected for damage and anemone crown dimensions were re-measured.

2.6 Data analysis

Sample sizes for trials on each reef location were $n=11$ on BP, $n=18$ on DB, $n=18$ on RR, and $n=10$ in the laboratory. Chi-square tests were used to analyze the data from each set of trials. The null hypothesis was that damage to anemones would occur with equal frequency, regardless of the type of experimental treatment. All statistical analyses were performed in JMP® (Version *11*. SAS Institute Inc., Cary, NC,

1989–2007). Figures were created in Adobe® Illustrator® (Version CS3 *13*. Adobe Systems Inc., San Jose, CA, 1987–2013).

3 Results

3.1 Experiments—field and laboratory trials

Data analyses for field trials and pooled field and laboratory trials (Table 1) indicate there was no difference between treatments 1, 3 and 4; all resulted in 0 % anemones damaged. Anemone damage was seen in treatment 2 (solitary anemone with the fireworm *H. carunculata*). The chi-square analysis for the field treatments alone is slightly suspect because the expected outcomes are less than 5 (4.5 for each treatment), chi-square 63.4, p value <0.001. However, we remain very confident in the results because only treatment 2 had any observed positive outcomes and the corroborating chi-square analysis for the combined lab and field treatments is not suspect, chi-square 88.0, p value <0.001. For treatment type 2: 72.7 % of anemones were damaged on BP, 33.3 % anemones were damaged on DB, 27.8 % anemones were damaged on RR, and 70 % anemones were damaged in laboratory trials. All anemones tested actively discharged tentacular cnidae ($n=20$). Microscopic examination of the stomach contents of fireworms from experimental treatments where anemones were damaged confirmed the presence of cnidae in the anterior intestine of all fireworms dissected ($n=4$).

3.2 Laboratory observations

When *B. annulata* tentacles contacted *H. carunculata* setae, anemone tentacles retracted and became dark and shriveled within a few minutes. Fireworms reacted by drawing away but not retreating upon initial contact with anemone tentacles. If a fireworm ingested any portion of *B. annulata*, the anemone responded with heavy acontia discharge from pores in the pedal column. However, there was no lasting retreat by or

Table 1 The number of *Bartholomea annulata* anemones damaged in field and laboratory trials

Location	Experimental Treatment				Chi square	*p* value
	1 Solitary anemone	2 Anemone with FW	3 Anemone with FW & shrimp	4 Anemone with FW & crustacean suite		
Field						
Three sites						
n=47 per treatment	0	19	0	0	63.4	<0.001
Field & laboratory data pooled						
Three sites & laboratory						
n=57 per treatment	0	26	0	0	88.0	<0.001

Experimental treatments: 1) Solitary anemone, 2) Anemone with a fireworm (FW), *Hermodice carunculata*, 3) Anemone with a FW and alpheid shrimp, *Alpheus armatus* and, 4) Anemone with a FW and suite of common crustacean symbionts; *A. armatus*, *Ancylomenes pedersoni*, *Periclimenes yucanicus* and *Stenorhynchus seticornis*

discernible damage to the fireworms after contact with the anemones' tentacles, acontia or cnidae.

When *H. carunculata* was introduced to experimental aquaria it would typically explore the enclosure for 0 to 45 min. The worms assumed a search posture with the prostomium (head region) elevated and swinging laterally, likely using their olfactory caruncle to detect directional chemosensory stimuli. In all cases fireworms ceased exploration of the tank before the end of the 1 h observation period. Further explorations likely took place intermittently during the remaining 23 h of the trial.

H. carunculata was documented to approach solitary anemones 0 to 2 times, evert buccal mass over anemone tentacles or pedal disk in 2 trials, and ultimately consume some portion of the *B. annulata* in 7 out of 10 trials, eating down to the basal disk in 3 cases. Anemones whose tentacles and/or pedal disk were not completely eaten survived for at least 3 days after the trial. Long term recovery and survival rate for the 4 anemones damaged but not killed, are unknown because they were returned to reefs they were collected from after the 3 day observation period. In treatment groups in which an anemone was host to a single alpheid shrimp or a suite of associates, fireworms approached the anemone 0 to 8 times. Alpheid shrimp responded to fireworm approaches by: rushing the intruder 1 to 2 times in 20 % of the trials, snapping chelipeds 1 to 13 times in 75 % of trials, pinching with chelipeds 1 to 18 times in 70 % of trials. *H. carunculata* suffered damage in 35 % of laboratory trials and 21 % of field trials when anemone associates were present. Damaged fireworms exhibited lesions on all portions of the body; dorsal, ventral, anterior prostomium, posterior pygidium, and mid-body trunk. A total of 4 fireworms from laboratory and 1 from field experiments, with severe lesions (more than 3 lesions and/or internal tissues exuded) were found dead at the end of their respective trials. The effect of direct contact with the
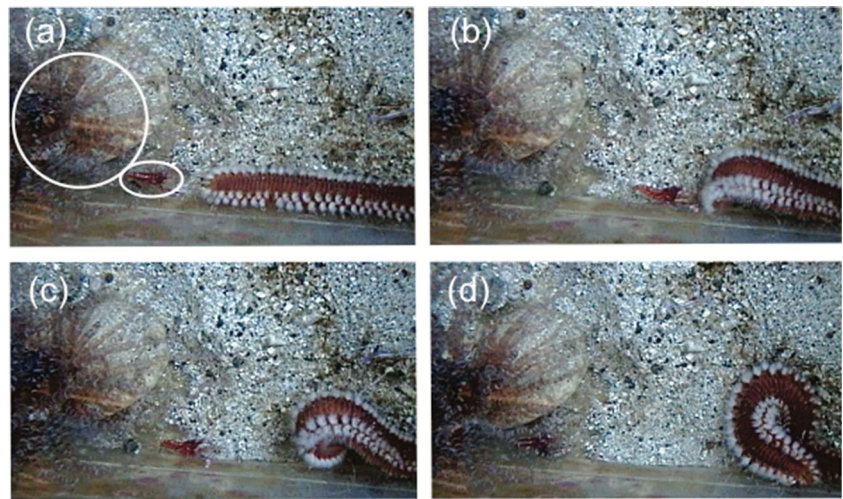
toxic setae of the fireworm by the decapod crustaceans is unknown but appeared to be benign. *A. armatus* observed to engage in fighting bouts with *H. carunculata* were inspected under a dissecting microscope for damage but no discernible damage was detected; fireworm setae did not penetrate or imbed in the carapace of the snapping shrimp.

A. pedersoni and *P. yucatanicus* were typically seen a few cm away from the anemone, displaying their characteristic rocking dance, shifting laterally without moving the walking legs, which is used to signal their willingness to clean potential fish clients (e.g., Limbaugh 1961; Becker et al. 2005; Chapuis and Bshary 2010). *A. pedersoni*, *P. yucatanicus* and *S. seticornis* individuals were observed to move away from fireworms, move nearer to anemone, or did not discernibly respond when *H. carunculata* was introduced to aquaria. However, *A. pedersoni* and *S. seticornis* were occasionally seen touching and picking at resting or wounded fireworms with their chelipeds. *A. armatus* were the only associates documented to actively engage fireworms. The alpheid deterred polychaete predators by non-damaging rushes and cheliped snapping bouts that redirected predator movements away from the anemone and by direct conflict fighting bouts in which shrimp pinched invading fireworms (Fig. 2, Supplemental video). Contact was usually via shrimp antennae and chelipeds, and all portions of the worm body including parapodia with calcareous setae, and resulted in damage to fireworms only.

4 Discussion

This study provides evidence that anemones with crustacean associates, specifically *A. armatus*, are less likely to be preyed upon than lone anemones. Alpheids will actively defend their home territory against potential anemone predators such as

Fig. 2 Image sequence captured from video of interaction between *Hermodice carunculata* fireworm and *Alpheus armatus* snapping shrimp. **a** Fireworm approaches *Bartholomea annulata* (anemone indicated with *large circle*) with alpheid symbiont (shrimp indicated with *small oval*), **b** Alpheid engages and pinches fireworm with celiped, **c** Alpheid retreats to host anemone, **d** Fireworm retreats away from anemone-alpheid complex

H. carunculata, and can fatally damage persistent intruders. It is difficult to accurately conduct a cost-benefit analysis and assign a category of symbiotic relationship to such ecologically complex interspecific associations. Much of the evolutionary theory and research on mutualism focuses on two partners in a multi-species or multi-trophic level association (Roopin et al. 2008), likely due to the difficulty in evaluating the network of complex interactions involved. If relationships between anemones and their crustacean associates were mutualistic, we would expect a significant increase in anemone fitness when associated with particular partners, and adaptations that facilitate the symbiosis (Boucher et al. 1982; Côté 2000; Herre et al. 1999). Long term monitoring of anemones with and without crustacean associates is needed to investigate possible fitness benefits.

Crustaceans may receive protection, a direct or indirect food source, a mate, or combinations thereof by living in association with anemones. Conversely, there is mounting evidence that the anemone benefits from the association with some decapod species. Nitrogenous wastes from associates can be utilized by the anemones endosymbiont, which may subsequently increase the internal food source for the anemone (Spotte 1996; Porat and Chadwick-Furman 2005). Maintenance behaviors have been observed in which symbionts remove excess mucus, necrotic tissue and inorganic debris from the host anemone (Herrnkind et al. 1976; Nizinski 1989; Crawford 1992). Here we show that anemones in the presence of alpheid shrimp sustain significantly less damage by predators than solitary anemones exposed to predators.

Agonistic interactions may be deemed guarding behaviors employed to monopolize resources from competitors or territoriality when an individual or group actively excludes intruders with aggressive displays (Baeza and Thiel 2003). When a finger is placed near the tentacles of an anemone with an alpheid shrimp, the decapod responds by rushing toward the finger and snapping its chelae (Knowlton and Moulton 1963)

until the intruding finger is removed. Smith (1977) witnessed this territorial behavior by alpheids during his fireworm predator trials, but was unable to determine if the shrimp actually contacted the worm. By conducting close observations of predator–prey–protector interactions in the lab we document interspecific interactions and direct contact bouts. Smith's pioneering paper investigated the nature of the association between snapping shrimp and anemones, however, his in situ anemone monitoring efforts led to presumed predation by fireworms with little empirical support. The results of our study provide both lab and field experimental evidence supporting this interpretation and confirm the role of *A. armatus* as a protector of territory and thus, the anemone host, *B. annulata*.

Male and female alpheids possess an enlarged major chela, or claw, which when rapidly closed, propels a jet of water and creates an audible snap, used for offense and defense (Knowlton and Moulton 1963; Knowlton and Keller 1982). The water jet can stun or frighten enemies (Knowlton and Moulton 1963), and damage has been reported in fighting bouts between conspecifics (Knowlton and Keller 1982). To our knowledge this is the first report of direct contact via alpheid chelae, where resultant pinch wounds on host predators are confirmed. Five fireworms died during this study, likely due to the severe lesions inflicted by Alpheid shrimp snapping bouts.

By including *A. armatus* in treatments with anemones, and a suite of associates, we emulated the most common natural associate group dynamic documented on the reefs studied and created a secondary control group. The alpheid-anemone treatment results indicate that the snapping shrimp provide protection to their host anemone. The results of the treatment with a suite of associates including *Alpheus*, indicate that alpheids continue to provide protection to their host anemone in the presence of other crustacean associates. Future research should include investigation of the protective ability of the less common anemone-crustacean assemblage, which does not include *A. armatus*.

Alpheids were the only decapods documented to agonistically engage *H. carunculata* anemone predators in this study. Perhaps in the absence of snapping shrimp, other anemone associates take on the role of protector. The smaller decapod associates may be more suited to defend against smaller potential anemone predators, such as pycnogonids (Mercier and Hamel 1994). *A. pedersoni* and *S. seticornis* seen touching resting or wounded *H. carunculata* with their chelipeds may have been removing tissue or fireworm copepod parasites (Yáñez-Rivera and Suárez-Morales 2008). Cleaner shrimp have been documented to clean resting fish (Jonasson 1987) and may treat stationary invertebrates as they would any other substratum removing potential food items from the body surface. Although *S. seticornis* is used as a biological control of *H. carunculata* in the aquarium trade (Stanton 2003), no predatory behavior by arrow crabs towards fireworms was observed during the present trials. Perhaps arrow crabs feed on the smallest polychaete individuals or opportunistically inspect and forage on resting or wounded fireworms.

If *H. carunculata* feeds on *B. annulata* the result can be fatal despite the anemones' defensive acontia and tentacle cnidae. The fireworm presumably has some immunity to cnidarian nematocysts, given their voracious appetite for animals in this phylum. The fireworm capillary notosetae are filled with an uncharacterized toxin that is a powerful irritant, documented here to damage anemones. The toxin is likely a neurotoxin that disrupts the ion gradient of cells, common in the marine environment (Walker and Masuda 1990), leading to the shriveled or desiccated look of anemone tentacles. Thus, anemones that contact fireworms risk damage by toxic stress and predation. Other predators of anemones include highly mobile fish such as Chaetodontids, which rush in, bite tentacles and swim away. Whether *A. armatus* could deter such a vertebrate predator with loud snaps of the chelae is unknown and warrants further investigation.

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