

Crustacean symbiosis with Caribbean sea anemones *Bartholomea annulata*: occupancy modeling, habitat partitioning, and persistence

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ABSTRACT: Coral reef cnidarians often host crustaceans in multi-level symbioses that may cause reef-wide impacts, especially through anemoneshrimp cleaner interactions with fish clients. Despite the ubiquity and importance of these interactions, patterns of temporal and spatial variation in crustacean association with sea anemones remain largely unknown. We censused corkscrew sea anemones *Bartholomea annulata* and crustacean associates (5 shrimp and 1 crab species) every 3–6 mo for 2.5 yr at 2 reef sites in St. Thomas, US Virgin Islands. Crustacean abundance per anemone varied with species and census period. Occupancy rates increased with anemone body size, more so offshore where anemones were smaller and less abundant than inshore. Four species of obligate anemoneshrimps partitioned space among 5 microhabitat zones on the host anemone body. Dynamic multi-state models revealed that anemone persistence was enhanced primarily by anemone body size, with variable secondary effects of site and anemoneshrimp species. The presence of the snapping shrimp *Alpheus armatus* did not enhance the persistence of host anemones. However, the presence of the dedicated cleaner shrimp *Ancylomenes pedersoni* enhanced the persistence of small host anemones at the inshore site but reduced the persistence of the occasional cleaner shrimp *Periclimenes yucatanicus* on host anemones, indicating mutualistic and competitive interactions among some partners in this system. Patterns of association among anemones and crustaceans varied significantly at both temporal (seasonal and annual) and spatial scales (among anemones and reef sites), with implications for the ecology of client fishes that visit these Caribbean anemones to receive cleaning services from anemoneshrimps.

KEY WORDS: Multi-state occupancy model · Anemoneshrimp · *Alpheus armatus* · *Ancylomenes pedersoni* · *Periclimenes yucatanicus* · *Heteromysis actiniae* · Mutualism · Coral reef

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1. INTRODUCTION

Crustaceans and fishes often form shelter symbioses with sea anemones, corals, and other sessile invertebrates on coral reefs (Glynn & Enochs 2011). These associations may result in costs to the hosts in the form of predation on their tissues by mobile ectosymbionts (Fautin et al. 1995), but in most cases

these symbioses provide net benefits to both hosts and associates, and function as mutualisms (Fautin 1991, Fautin et al. 1995, Porat & Chadwick-Furman 2004). Where multiple species of associates occupy the same host species, consequences for the various partners may be complex, including a range of both positive and negative effects (Stanton 2003, Stier et al. 2012, Afkhami et al. 2014). Coral and sea anemone hosts are

less species-diverse than their fish and crustacean associates, and variation in host traits (e.g. body size, morphology, expansion behavior, toxicity) drive preferences by associates for some host species over others (Guo et al. 1996, Baeza & Stotz 2003, Khan et al. 2003, Briones-Fourzán et al. 2012, Huebner et al. 2012, Bos & Hoeksema 2015). As such, several species of associates may co-occur not only on the same host species, but frequently on the same host individual (Mahnken 1972, Chadwick et al. 2008, Briones-Fourzán et al. 2012, Bos & Hoeksema 2015).

The ecological roles and group structure of crustaceans symbiotic with benthic hosts, together with host abundance and availability, drive their patterns of association. Crustaceans that occur as 1 adult per host may competitively monopolize the host as a defended territory, but move among hosts for mating, resulting in preference for hosts with near neighbors (Baeza et al. 2001, Baeza & Thiel 2003, 2007). Crustaceans that occur as pairs competitively exclude conspecifics from sharing the host, and either do not need to move among hosts for mating, or only 1 gender moves among nearby hosts to mate with multiple partners (Knowlton 1980, Gotelli et al. 1985, Stier et al. 2012, Gilpin & Chadwick 2017). Some symbiotic crustaceans form apparently unstructured aggregations on hosts (Baeza & Thiel 2007). However, others form spatially structured social groups in which dominant individuals exhibit aggression toward smaller conspecifics and relegate them to peripheral habitats on the host (Mahnken 1972, Guo et al. 1996, Baeza et al. 2002, Colombara et al. 2017), similar to the structure of anemonefish social groups (Camp et al. 2016). Aggregations or social groups may form because some species of associated crustaceans are not able to expend the energy necessary to monopolize their hosts from use by conspecifics (Thiel & Baeza 2001, Baeza & Thiel 2007).

Conflicting patterns in the number of conspecific associates per host with relation to host body size have been reported (Stanton 1977, Nizinski 1989, Briones-Fourzán et al. 2012, Huebner & Chadwick 2012a), which may be due to various factors that affect associates, such as level of access to food resources (Mahnken 1972). Dedicated cleaner shrimps such as *Ancylomenes pedersoni*, which remove and ingest ectoparasites from client fishes (Bunkley-Williams & Williams 1998, McCammon et al. 2010), attract and clean more clients for longer durations when in large versus small groups (Huebner & Chadwick 2012b), and so may be more likely to form large groups in reef locations with high levels of access to fish clients (Mahnken 1972, Herrnkind et al. 1976). In

general, when cnidarian hosts are in high abundance or closely aggregated, the costs to ectosymbionts of moving among hosts is reduced, so symbionts may move among hosts to maximize their feeding or mating success (Knowlton 1980, Thiel & Baeza 2001, Thiel et al. 2003a, Chadwick et al. 2008). This can result in low fidelity to individual hosts, unstructured aggregations, or seemingly random distributions of associated crustaceans (Nizinski 1989, Thiel & Baeza 2001, Khan et al. 2003, Baeza & Thiel 2007). Additionally, where the available hosts occur in aggregations, mobile crustacean associates may utilize multiple hosts within a home range, instead of being confined to a single host (Stanton 1977, Thiel et al. 2003b), as do anemonefishes (Hattori 2002). Thus, the number of conspecific crustaceans per host anemone is likely to vary both temporally and spatially due to a variety of factors. However, variation in host anemone use by crustaceans among multiple time periods and reef sites, especially in relation to anemone body size and abundance, remains poorly understood.

Symbiotic crustaceans may compete for their limited habitat space, especially because their host organisms comprise discrete refuges that are small and discontinuous (Baeza & Thiel 2007). Crustacean species that cohabit on cnidarian polyps thus are likely to spatially partition the host and thereby reduce niche overlap. Microhabitat niches on host polyps potentially include the inner and outer tentacle crown, over or under fronds, along the column, near the polyp base in burrows, or on adjacent hard or soft substratum (Herrnkind et al. 1976, Stanton 1977, Khan et al. 2004, Hoeksema & Franssen 2011, Briones-Fourzán et al. 2012, Colombara et al. 2017). Spatial segregation among crustaceans on sea anemones may allow several species to co-exist on a single host, thereby enhancing biodiversity per unit area. Similar effects have been reported for terrestrial vertebrates that share a host, including warbler species that inhabit different zones on conifer trees and anole lizard species that occupy different heights on tree trunks, as described in seminal work by MacArthur (1958) and Schoener (1968), respectively. In general, when multiple species associate with host organisms, they often partition limited resources on the host, such as habitat space and diet, thereby reducing interspecific competition. Information about the extent of microhabitat partitioning and spatial competition is available for only some of the shrimp species associated with Caribbean sea anemones (Stanton 1977, Briones-Fourzán et al. 2012, Colombara et al. 2017, Gilpin & Chadwick 2019). Evidence

from coral-associated crustaceans indicates that asymmetry in competitive dominance is likely to occur among crustacean species that utilize similar microhabitat zones on hosts (Stier et al. 2012).

Some sea anemones that host symbiotic shrimps function as hubs of mutualistic networks, with ecological effects on other reef organisms through a complex interplay of benefits and costs to interacting partner species. The stinging tentacles of anemones provide shelter protection from predation to associated shrimps and crabs (Fautin et al. 1995, Ory et al. 2013). Less obviously, some shrimps also provide protection to their hosts by deterring would-be predators, which benefits not only the anemones but their other symbionts as well (Smith 1977, McCammon & Brooks 2014). For cleaning anemoneshrimps, anemones function as the center of cleaning stations and provide visual cues for fish clients to locate the shrimps (Huebner & Chadwick 2012a). Because cleaner anemoneshrimps significantly reduce parasite loads on a wide diversity of client fishes (Bunkley-Williams & Williams 1998, McCammon et al. 2010, Huebner & Chadwick 2012b), their presence may enhance reef fish abundance, similar to the known benefits of cleaner fishes to reef fish diversity, abundance, and recruitment (e.g. Waldie et al. 2011). In return, while hovering near the sea anemone hosts of some cleaner shrimps, client fishes potentially benefit the anemones by excreting nutrients that may be absorbed by endosymbiotic microalgae within the anemones (Cantrell et al. 2015). Because each clean can last up to several minutes, and many fish may be cleaned each day (Huebner & Chadwick 2012b), nutrient excretions by client fishes potentially supply >100% of the ammonia requirements of microalgae inside anemone hosts (Cantrell et al. 2015). Nutrients absorbed by anemones from ectosymbiotic cleaner shrimps or the fish clients they attract may create positive feedback loops that enhance anemone growth and provide more habitat space for symbiotic cleaners, which in turn attracts more clients (Cleveland et al. 2011, Huebner & Chadwick 2012a, Cantrell et al. 2015). However, the impacts of these types of benefits from hosting cleaner shrimp on the eventual survival and persistence of host anemones remain unknown.

Corkscrew sea anemones *Bartholomea annulata* are abundant on Caribbean coral reefs and host both obligate and facultative crustacean associates (Mahnken 1972, Herrnkind et al. 1976, Briones-Fourzán et al. 2012, Brooker et al. 2019). Preliminary observations at St. Thomas, US Virgin Islands, indicate that at least 6 species of crustaceans regularly associate with

B. annulata: the snapping shrimp *Alpheus armatus*, anemone opossum mysid *Heteromysis actiniae*, spotted anemoneshrimp *Periclimenes yucatanicus*, Pederson cleaner shrimp *Ancylomenes pedersoni*, squat anemoneshrimp *Thor amboinensis*, and arrow crabs *Stenorhynchus seticornis*. *Alpheus armatus* likely comprise a species complex (referred to here collectively as *A. armatus*) and are obligate associates of *B. annulata*, occurring either singly or as mated pairs on each host (Knowlton 1980, Hurt et al. 2013). *A. armatus* individuals use their snapping chelae to protect host anemones from predation by fireworms (Smith 1977, McCammon & Brooks 2014), so this shrimp may enhance host anemone persistence, similar to the potential effects of resident cleaner shrimps, but whether they do so in field populations is not known. *H. actiniae* are relatively small-bodied (7–8 mm body length) mysids that form obligate associations with *B. annulata*, where they may occur as swarms of numerous individuals among the host tentacles and consume material ejected from the anemone (Clarke 1955). Individuals of *P. yucatanicus* are typically found singly or in pairs on sea anemone hosts; historically they have been considered as cleaner shrimp (Limbaugh et al. 1961), but several studies indicate that they only occasionally clean reef fishes (Mahnken 1972, McCammon et al. 2010, Titus et al. 2017). *Ancylomenes pedersoni*, however, are dedicated fish cleaners (Vaughan et al. 2016) and remove more fish ectoparasites than other examined Caribbean cleaner shrimp, often occurring in large groups on each sea anemone host (Bunkley-Williams & Williams 1998, McCammon et al. 2010, Huebner & Chadwick 2012b). These 2 cleaner shrimps potentially both benefit host anemones by attracting client fishes that provide nutritional enhancement (described above), and conversely may compete for shared food resources in the form of fish ectoparasites, but their complex impacts on the persistence of hosts and each other have not been examined. Members of the *T. amboinensis* species complex are host generalists on sea anemones and may occur in large groups (Guo et al. 1996, Titus et al. 2018), but in the northern Caribbean they often comprise only 1–2 individuals per sea anemone (Baeza & Piantoni 2010). *S. seticornis* are facultative associates of sessile benthic organisms, including sea anemones (Herrnkind et al. 1976, Stanton 1977). These 6 species of crustaceans form a potentially interacting network of associates on host anemones *B. annulata*, with likely complex ecological impacts on each other and on their hosts, especially in terms of the anemoneshrimps known to provide host benefits or to share food resources.

We describe here patterns of association between *B. annulata* and the aforementioned 6 species of crustaceans (5 shrimps and 1 crab), including how they vary temporally (among seasons over 2.5 yr) and spatially (between anemone hosts at 2 coral reef sites) on St. Thomas, US Virgin Islands. Based on the above information available about their biology, we tested 4 major hypotheses concerning species interactions in this system:

(1) Crustacean group size varies temporally among seasonal census periods and spatially among sea anemone hosts with different body sizes.

(2) Crustacean associates spatially partition microhabitats on the host body.

(3) Hosts persist longer in the presence of anemone shrimps that are known to provide mutualistic benefits.

(4) Cleaner shrimps *Ancylomenes pedersoni* and *P. yucatanicus* reduce each other's persistence but enhance the persistence of hosts.

2. MATERIALS AND METHODS

2.1. Field observations

The present study was conducted on sea anemones *Bartholomea annulata* and associated crustaceans at 2 coral reef sites on St. Thomas, US Virgin Islands: Brewers Bay (BB; ~6 m depth; 18° 20' 27.95" N, 64° 58' 42.41" W) and Flat Cay (FC; ~7–9 m depth; 18° 19' 1.77" N, 64° 59' 7.49" W). We selected these sites because they were adjacent to the MacLean Marine Science Center of the University of the Virgin Islands for logistical support, they represented an inshore site (BB; 200 m from shore, inside partially enclosed Brewers Bay) versus an offshore site (FC; ~2 km offshore from Brewers Bay) for comparison between these 2 types of reef habitat, and both contained abundant individuals of *B. annulata* and associated crustaceans (O'Reilly et al. 2018, Gilpin & Chadwick 2019).

At each site in September 2006, we defined a census area that consisted of sand flats interspersed with patch reefs and contained ≥ 70 individuals of *B. annulata*, enough for study of the dynamics of host anemones and their associates (e.g. Hattori 2002): 282 m² at BB (47 × 6 m along the edge of a continuous reef margin) and 1135 m² at FC (70 × 11 m along a continuous reef margin plus 73 × 5 m extending outwards into the sand flat from the reef margin). The census area at FC was larger than at BB, because the sea anemones were more sparsely dispersed at FC than

BB. All anemones were mapped within each site, and numbered metal tags were attached to the reef adjacent to each anemone or aggregation of anemones (hereinafter referred to as 'tag location'). Anemones were considered aggregated if their tentacles contacted each other and their crustacean associates intermingled (Huebner & Chadwick 2012a). Few individuals of other sea anemone species occurred at these sites, resulting in *B. annulata* providing the primary habitat for anemone-associated crustaceans during the study period (1–9 and 0–5 individuals of giant anemones *Condylactis gigantea*, 0–3 and 0–2 branching anemones *Lebrunia neglecta*, and 0–1 and 0 knobby anemones *Laviactis lucida* observed at BB and FC, respectively; up to 12 total non-*B. annulata* anemones at both sites combined per census period).

At both sites, we conducted observations over approximately 1 wk during each 3 mo census period (representing spring [March], summer [June], fall [September], and winter [December]) for 1.5 yr (7 census periods from September 2006 to March 2008), then during each 6 mo census period for an additional year (2 census periods in September 2008 [fall] and March 2009 [spring]), for a total of 9 census periods over 2.5 yr. During each census, we visited every anemone tag location, recorded whether an anemone was present, and if so, used a plastic tape measure marked in centimeters to determine anemone tentacle crown length and width (for calculation of tentacle crown surface area [TCSA] in cm²; e.g. Hattori 2002), and identified and counted all individuals of the 6 species of observed crustacean associates (5 shrimp species: *Alpheus armatus*, *Heteromysis actiniae*, *Periclimenes yucatanicus*, *Ancylomenes pedersoni*, and *Thor amboinensis*; and 1 crab species: *Stenorhynchus seticornis*). During each census, tag locations were added where anemones newly appeared within each mapped census area. Body size measurements were taken while anemones were fully expanded; if an anemone appeared to be contracted during initial measurements, it was revisited until observed to be expanded and then measured again during the same census, or its size data were excluded from analysis for that census (after Hattori 2002). Crustaceans were considered as associates if they occurred <12 cm distant from anemones, because almost all anemone associates occur within that distance (Briones-Fourzán et al. 2012, Colombara et al. 2017). Because of significant differences in reef habitat characteristics between BB and FC (O'Reilly et al. 2018), all crustacean analyses were conducted separately for each site.

We considered each *B. annulata* tag location (individual anemone or aggregation of anemones) to be the habitat unit for associated crustaceans, because anemone associates treat aggregated anemones as a single host (Knowlton 1980, Hattori 2002). For both sites during each census period, we calculated the number of tag locations per 10 m², anemone body size at each tag location (TCSA, summed for aggregated anemones), and the number of each species of crustacean associate per tag location (crustacean group sizes). Initial assessment indicated that anemone TCSA was not normally distributed, and this was not alleviated by any transformation. Therefore, we used 3 sets of Kruskal-Wallis tests, paired with Dunn's tests for multiple comparisons, to assess between-site differences in anemone body size, with observations pooled across census periods, and among-census differences in anemone body size examined at BB and FC separately. All Kruskal-Wallis tests and post hoc comparisons were carried out using the R packages PMCMR and multcompView in R v.3.4.1 (Pohlert 2014, Graves et al. 2015, R Core Team 2017). To determine whether the aggregation patterns of host anemones affected the assemblages of associated crustaceans at BB (where enough aggregations existed for statistical comparisons), we used regression models to assess differences in species richness (Poisson regression model) and abundance (negative binomial model). In addition, we used a negative binomial model to assess whether the 2 cleaner shrimp species (*P. yucatanicus* and *Ancylomenes pedersoni*) differed in group size, because this aspect of their biology may explain in part their effects on anemone persistence (see Section 2.5). These models were fit in R v.3.4.1 using the package glmmADMB (Fournier et al. 2012).

2.2. Hypothesis 1:

Crustacean group size varies temporally among seasonal census period and spatially among sea anemone hosts with different body sizes

To test Hypothesis 1, we focused on 4 species of obligate, common anemoneshrimp (*Alpheus armatus*, *H. actiniae*, *P. yucatanicus*, *Ancylomenes pedersoni*), because members of the other 2 associated crustacean species either were too rare for statistical analysis (*T. amboinensis*) or were facultative associates for which anemones were not the only available habitat (*S. seticornis*). To determine if the group size of each of the 4 focal anemoneshrimp species varied with census period and host anemone body size at

each tag location, we first fit a global Poisson regression model with and without a random intercept associated with tag location. We used Akaike's information criterion (AIC; Akaike 1973) with a small-sample bias adjustment (AICc; Hurvich & Tsai 1989) to compare the relative fit of these models. We retained the random intercept only if its inclusion substantially improved model fit (i.e. a reduction in AICc > 2). We also assessed each Poisson regression model for evidence of overdispersion; if overdispersion was present, we instead used a negative binomial regression model. For each species and site, we fit a total of 4 Poisson or negative binomial regression models, each representing a different combination of census and anemone body size as fixed effects. For each species, we based inferences on the best-approximating model. All Poisson and negative binomial models were fit in R v.3.4.1 using the package glmmADMB (Fournier et al. 2012).

2.3. Hypothesis 2: Crustacean associates spatially partition microhabitats on the host body

To test Hypothesis 2, we defined 5 microhabitat zones on the body of host sea anemones, based on preliminary observations (modified after Khan et al. 2004; our Fig. 1). We ordered the zones by the level of potential shelter from predation that each provided to associated crustaceans, as: (1) under the tentacle crown along the anemone column, (2) inner half of the tentacle crown, (3) outer half of the tentacle crown, (4) near the tentacle crown on hard substratum, and (5) near the tentacle crown on soft substratum. During November 2009, after completion of the 9 census periods (September 2006 through March 2009), we returned to the study sites and examined every anemone tag location, identified and counted all individuals of the same 4 focal anemoneshrimp species as above (Section 2.2), and recorded how many individuals of each occurred in the 5 microhabitat zones. We then applied a Fisher's exact test for each site, to determine whether the 4 crustacean species occurred randomly across the 5 microhabitat zones. To compare the microhabitat zone use between sites for *H. actiniae*, *P. yucatanicus*, and *A. pedersoni* (*Alpheus armatus* exhibited no variation; see Section 3), we used χ^2 tests of association. For *P. yucatanicus* and *Ancylomenes pedersoni*, we combined the inner and outer tentacle crown zones (2 and 3), and the hard and soft substratum zones (4 and 5), to reduce cells with small frequencies. After these combinations, 2 main types of microhabitats were

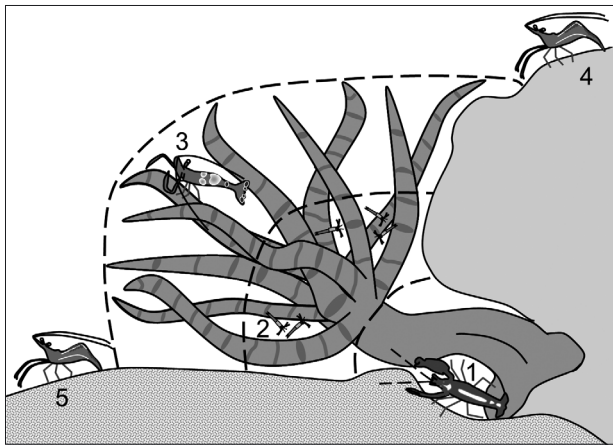


Fig. 1. Five microhabitat zones on body of corkscrew sea anemone *Bartholomea annulata*, partitioned among 4 species of obligate anemoneshrimp, as observed on coral reefs at St. Thomas, US Virgin Islands. Zones numbered in order of decreasing potential shelter from predation provided to shrimps by anemones, and occupied by the shrimp species typically observed in each zone: (1) under tentacle crown along anemone column: snapping shrimp *Alpheus armatus*, (2) inner half of tentacle crown: anemone opossum mysid *Heteromysis actiniae*, (3) outer half of tentacle crown: spotted anemoneshrimp *Periclimenes yucatanicus*, (4) near tentacle crown on hard reef substratum (gray filler): Pederson cleaner shrimp *Ancylomenes pedersoni*, and (5) near tentacle crown on soft sandy substratum (gray stippled filler): *A. pedersoni*. Anemone is shown in typical reef habitat for this species, with basal disk attached to hard substratum in a crevice near reef-sand interface

analyzed statistically for these species: on the tentacles and on substratum surrounding the host. Within each site, we also conducted a χ^2 test that compared the microhabitat use patterns of *P. yucatanicus* and *Ancylomenes pedersoni* to each other, because these 2 species use similar microhabitats on host anemones (Briones-Fourzán et al. 2012). All data were analyzed in R v.3.4.1.

2.4. Hypothesis 3: Hosts persist longer in the presence of anemoneshrimps that are known to provide mutualistic benefits

For Hypothesis 3, we focused on 2 species of anemoneshrimp (*Alpheus armatus* [AA] and *Ancylomenes pedersoni* [AP]) that are known to provide mutualistic benefits to *B. annulata* (BA), in the form of either protection from predation (AA; Smith 1977, McCammon & Brooks 2014), or nutritional enhancement via the attraction of client fishes whose excretions are absorbed by the anemone's microalgae (AP; Cantrell et al. 2015), and therefore are likely to affect

host persistence. We did not test whether anemones persist longer in the presence of the other species of associated crustaceans, because not enough evidence exists to clearly classify them as mutualists vs. non-mutualists. We developed two 3-state dynamic occupancy model sets, after MacKenzie et al. (2009). One model set was fit for each anemoneshrimp species, because AA and AP occupy different microhabitat zones on anemones (Briones-Fourzán et al. 2012; see Section 3), so we did not expect direct interactions between them. Because AA and AP are obligate associates, anemone presence was implicit for anemoneshrimp presence.

For the initial census at time t , we modeled the occupancy state of each anemone tag location as a categorical random variable with 1 of 3 possible values of z : only anemone present ($z = 1$), both anemone and AA/AP present ($z = 2$), or neither anemone nor AA/AP present (unoccupied, $z = 3$). For subsequent censuses, occupancy state transitions were defined by a transition probability matrix, to determine the probability of a tag location being in each possible state at time $t + 1$ given its state at time t . The state transition probabilities were further defined by processes of local colonization (γ) and local extinction (ϵ) events associated with each transition. For example, if a tag location transitioned from $z = 1$ to $z = 1$ from time t to time $t + 1$, this meant that the anemone(s) persisted at the tag location but AA/AP failed to colonize. This transition probability (P) is expressed mathematically as: $P[z_{t+1} = 1 | z_t = 1] = (1 - \epsilon_{BA}) * (1 - \gamma_{AA/AP})$, where ϵ_{BA} represents the probability of an anemone becoming locally extinct (therefore $1 - \epsilon_{BA}$ represents the probability of persistence), and $\gamma_{AA/AP}$ represents the probability of AA/AP colonizing the tag location. The model formulation resulted in 9 possible state transitions (Eqs. 1–9 in Text S1 in the Supplement at www.int-res.com/articles/suppl/m631p099_supp.pdf).

However, in this model, the probability of extinction of an anemone (ϵ_{BA}) was identical regardless of the occupancy status of AA/AP. We therefore constructed an additional model that allowed for the anemone extinction probability to vary depending on the occupancy status of AA/AP. Here, a new parameter, $\epsilon_{BA-AA/AP}$, was added to the transition probability matrix to represent anemone extinction probability in the presence of AA or AP (AA/AP; Eqs. 10–18 in Text S1 in the Supplement). For each site (BB, FC) and anemoneshrimp species (AA, AP), we constructed both parameterizations of the 3-state occupancy model, for a total of 8 models. Because anemone body size strongly influences anemone persistence (O'Reilly & Chadwick 2017, O'Reilly et al.

2018), we included anemone body size (TCSA) as a predictor variable in the models of anemone extinction. We modeled each state transition parameter using a logit link function, and for each parameter also included a random intercept associated with census period, to account for potential temporal dependence. All random effects were assumed to be normally distributed with a grand mean intercept and random effect-specific variance (Gelman & Hill 2006). We used Markov chain Monte Carlo (MCMC) simulation, as implemented in JAGS v.4.1.0 to fit the models (Plummer 2003), and all models were fitted by running 3 parallel chains, each with 25000 iterations, a burn-in of 12500 (i.e. the first 12500 iterations were dropped), and diffuse priors. We assessed the relative support for each pair of models using the Watanabe-Akaike information criterion (WAIC; Watanabe 2010), where lower WAIC values imply higher predictive accuracy (Gelman et al. 2014). Because of the challenges associated with Bayesian model selection (Hooten & Hobbs 2015), we chose a conservative Δ WAIC of 10 (the difference between the WAIC score for a given model and the lowest WAIC score) to identify a confidence model set. Hence, models with Δ WAIC > 10 were considered to have little support. Lastly, we conducted a posterior-predictive check to assess goodness of fit by simulating replicated data under each fitted model and comparing summary statistics from the replicated data to the observed data (Gelman & Hill 2007). Summary statistics are presented as the ratio of predicted to observed number of sites in each occupancy state, where values of <0.05 and >0.95 indicated substantial lack of fit.

2.5. Hypothesis 4:

Cleaner shrimps reduce each other's persistence but enhance the persistence of hosts

To test the final hypothesis, we focused on dedicated cleaner shrimp *Ancylomenes pedersoni* (AP) and occasional cleaner shrimp *P. yucatanicus* (PY), because they potentially reduce each other's persistence due to competition for food resources in the form of fish ectoparasites, and also for spatial resources in the form of microhabitats on host anemones (Briones-Fourzán et al. 2012). Their potential overlaps in resource use may cause them to negatively impact each other's persistence, but their cleaning behaviors may enhance the persistence of host anemones, as described under Hypothesis 3. We thus developed a 5-state dynamic occupancy model set to estimate the colonization and extinction rates

for AP, PY, and host anemones (after MacKenzie et al. 2009). PY was rare at BB, so we constructed a 5-state model set only for FC. As in the 3-state model sets above (Section 2.4), we included anemone body size (TCSA) as a predictor variable in the anemone extinction model, and anemone presence was implicit for cleaner shrimp presence.

For the initial census at time t , we modeled the occupancy state of each anemone tag location as a categorical random variable, with 1 of 5 possible values of z : anemone and only AP present ($z = 1$); anemone and only PY present ($z = 2$); anemone, AP, and PY all present ($z = 3$); only anemone present ($z = 4$); or anemone, AP, and PY all absent (tag location unoccupied, $z = 5$). For subsequent census periods, occupancy state transitions were defined by a transition probability matrix representing the probability of a tag location being in each possible state at time $t + 1$, given its state at time t . The state transition probabilities were further defined by processes of local γ and local ϵ events associated with each possible transition. For example, if a tag location transitioned from $z = 1$ to $z = 1$ from time t to time $t + 1$, this meant that AP persisted, PY failed to colonize, and the anemone(s) persisted at the tag location. This transition was expressed mathematically as: $P[z_{t+1} = 1 | z_t = 1] = (1 - \epsilon_{AP}) * (1 - \gamma_{PY}) * (1 - \epsilon_{BA})$, where ϵ_{AP} represents the probability of AP becoming locally extinct, γ_{PY} represents the probability of PY colonizing the site, and ϵ_{BA} represents the probability of the anemone(s) becoming locally extinct (therefore $1 - \epsilon_{BA}$ represents the probability of anemone persistence). This modeling framework resulted in 25 possible state transitions (Eqs. 19–43 in Text S1 in the Supplement).

In addition to enabling estimation of state transition parameters for AP, PY, and anemones, the modeling framework allowed the development of alternative models representing various scenarios of potential species interaction between AP and PY, and between AP, PY, and anemones. For each of these scenarios, we made 2 simplifying assumptions: (1) the dominant cleaner shrimp species influenced both colonization and extinction of the subordinate cleaner shrimp species, and (2) AP and PY influenced anemone persistence in the same manner (i.e. we did not assess separately the influence of AP and PY on anemone persistence). We developed 8 models, each representing different scenarios of interaction among these 3 species:

(1) Dominance of a cleaner shrimp species was established by prior residence (i.e. which species was there first), and the presence of either cleaner shrimp influenced anemone persistence.

(2) Dominance of a shrimp species was established by prior residence, and the presence of either shrimp *did not* influence anemone persistence.

(3) AP was always the dominant shrimp, and the presence of either shrimp influenced anemone persistence.

(4) AP was always the dominant shrimp, and the presence of either shrimp *did not* influence anemone persistence.

(5) PY was always the dominant shrimp, and the presence of either shrimp influenced anemone persistence.

(6) PY was always the dominant shrimp, and the presence of either shrimp *did not* influence anemone persistence.

(7) Neither shrimp species was dominant, and the presence of either shrimp influenced anemone persistence.

(8) Neither shrimp species was dominant, and the presence of either shrimp *did not* influence anemone persistence.

The example transition probability matrix (i.e. the 25 possible state transitions; Eqs. 19–43 in Text S1 in the Supplement) represented the simplest species interaction scenario, in which all state transitions occurred independently of each other (no interactions among species; scenario 8). To represent the remaining scenarios, in which species interactions were possible, we added slight changes to the transition probability matrix. For example, in models representing the scenario that the presence of either cleaner shrimp species influenced anemone persistence (scenarios 1, 3, 5, and 7), the transition probability matrix was modified such that the extinction parameter for anemones, ϵ_{BA} , was replaced by ϵ_{ba} , for transitions where anemones were present but both crustacean species were absent at time t ($z_t = 4$). In a similar manner, separate colonization and extinction parameters were used, depending on the presence or absence of cleaner shrimp species on an anemone, but which parameters were modeled separately depended on the scenario being addressed. We modeled each state transition parameter using a logit link function, and for each state transition parameter, we also included a random intercept associated with census period to account for potential temporal dependence. To facilitate the interpretation of coefficients associated with anemone body size (TCSA in cm^2) on a biologically meaningful scale, we calculated scaled odds ratios of 50 cm^2 , in that we examined the effect of each 50 cm^2 increase in TCSA, because this size class has been used previously to quantify changes in body size of *B. annu-*

lata (O'Reilly & Chadwick 2017, O'Reilly et al. 2018). All random effects were assumed to be normally distributed, with a grand mean intercept and random effect-specific variance (Gelman & Hill 2006). We used MCMC in JAGS v.4.1.0 to fit the candidate 5-state dynamic occupancy models, as implemented above (Section 2.4) for the 3-state models, and assessed their goodness of fit and relative support using WAIC.

3. RESULTS

3.1. Field observations

The abundance and body size of sea anemones *Bartholomea annulata* (or of aggregated anemones at each tag location) varied widely at the temporal and spatial scales examined here. Anemone abundance was ~5–11 fold higher at the inshore (BB) than offshore site (FC) during all 9 census periods (BB: range = 2.06–4.72 anemones 10 m^{-2} , median = 3.90 anemones 10 m^{-2} , total number of anemone tag locations = 58–133 depending on census period; FC: range = 0.28–0.71 anemones 10 m^{-2} , median = 0.61 anemones 10 m^{-2} , total number of anemone tag locations = 32–81). Aggregations were small (up to 4 individuals per aggregation), and a minority of anemones formed aggregations, with the percentage higher at BB than FC (BB: 12.4–28.5% of anemones in 5–18 aggregations depending on census period; FC: 2.4–13.5% of anemones in 1–5 aggregations). Anemone body size at each tag location for all census periods combined was significantly larger at BB than FC (Kruskal-Wallis test: $H = 10.101$, $df = 1$, $p < 0.001$; sample sizes in Fig. 2). In terms of temporal variation, at BB the anemone body sizes were significantly larger in March 2009 than in March, June, and December 2007 (Dunn's test for multiple comparisons: $z = 3.368, 3.709, 3.868$, respectively, $p < 0.05$; no significant differences between all other census periods; Fig. 2). At FC, the anemone body sizes were significantly larger in both September 2007 and March 2009 than during December 2007 (Dunn's test for multiple comparisons: $z = 3.775, 3.341$, respectively, $p < 0.05$; no significant differences between all other census periods; Fig. 2).

At both sites during most census periods, most anemones were occupied by individuals of at least 1 crustacean species (BB: 51.7–82.8% occupancy, except for 47.3% in June 2007; FC: 66.7–78.3% occupancy, except for 31.6% in March 2009). Aggregations of anemones contained a significantly higher

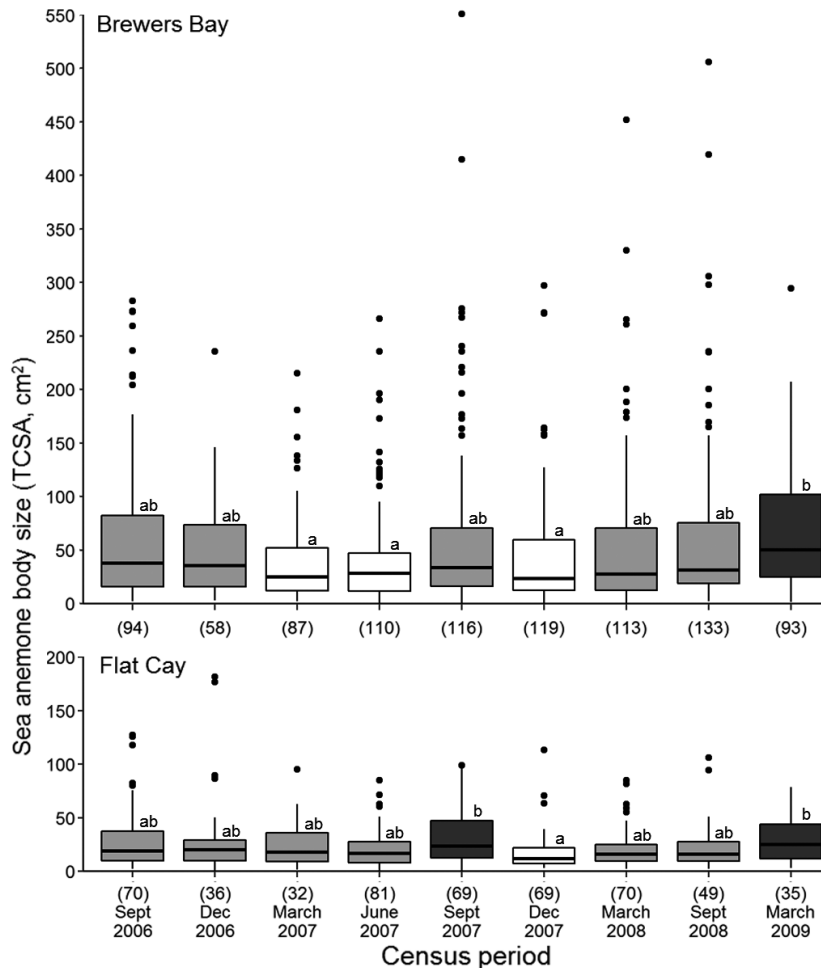


Fig. 2. Spatial and temporal variation in body size (tentacle crown surface area, TCSA) of sea anemones *Bartholomea annulata* at coral-reef tag locations (individual anemones or aggregations of 2–4 individuals), between an inshore (Brewers Bay, upper panel) and an offshore site (Flat Cay, lower panel), among 9 census periods over 2.5 yr. Each box displays first and third quartiles (interquartile range, IQR) of body size during each census, median (horizontal line), $1.5 \times$ IQR (whiskers), and outliers (black dots). Sample sizes (number of tag locations) shown in parentheses. Letters and shading indicate significant differences among census period means within each site ($p < 0.05$ in Dunn's test for multiple comparisons). Body sizes of anemones aggregated at each tag location were summed because crustaceans utilize aggregated anemones as a single host

richness of crustacean species at higher abundances than did isolated anemones (Table S1 in the Supplement). Patterns of anemone occupation varied among crustaceans (Table S2 in the Supplement): a higher percentage of anemones at BB were inhabited by *Heteromysis actiniae* and *Stenorhynchus seticornis* than at FC during all census periods, while this pattern was the opposite for *Alpheus armatus*, *Periclimenes yucatanicus*, and *Ancylomenes pedersoni* (except for *Alpheus armatus* in September 2006, and both *A. armatus* and *Ancylomenes pedersoni* in March 2009). In contrast to all other

crustacean associates, individuals of *Thor amboinensis* were absent during most census periods and were rare when they did occur.

The number of crustacean individuals per host anemone (or aggregation of anemones) varied among the 6 crustacean species examined (Table S2). The obligate anemone-shrimps *Alpheus armatus* and *T. amboinensis* occurred either singly or as a pair of individuals per host. The only exceptions were 3 individuals of *A. armatus* observed at 1 tag location during March 2008 (at BB) and 4 during June 2007 (at FC; neither at an aggregation of anemones). Similarly, individuals of *P. yucatanicus* usually occurred singly or in pairs, but occasionally formed small groups of 3–5 individuals. Individuals of *Ancylomenes pedersoni* sometimes occurred singly, but more often formed groups of up to 10 individuals. At FC (where enough *P. yucatanicus* occurred for statistical comparison), the group sizes of the dedicated cleaner shrimp *A. pedersoni* were significantly larger than those of the occasional cleaner shrimp *P. yucatanicus* (Table S3 in the Supplement). Individuals of *H. actiniae* formed the largest groups of up to 80 individuals per host. The only facultative crustacean associate, *S. seticornis*, occurred usually as 1–3 individuals, but was observed in 3 cases to form groups of 4, 5, and 10 individuals.

3.2. Hypothesis 1:

Crustacean group size varies temporally among seasonal census period and spatially among sea anemone hosts with different body sizes

The best-approximating Poisson or negative binomial models indicated that the group size (number of individuals of each shrimp species per anemone) of all 4 focal anemone-shrimps (*Alpheus armatus*, *H. actiniae*, *P. yucatanicus*, and *Ancylomenes pedersoni*) increased significantly with host anemone body size (Table 1) and did so at a more rapid rate at the

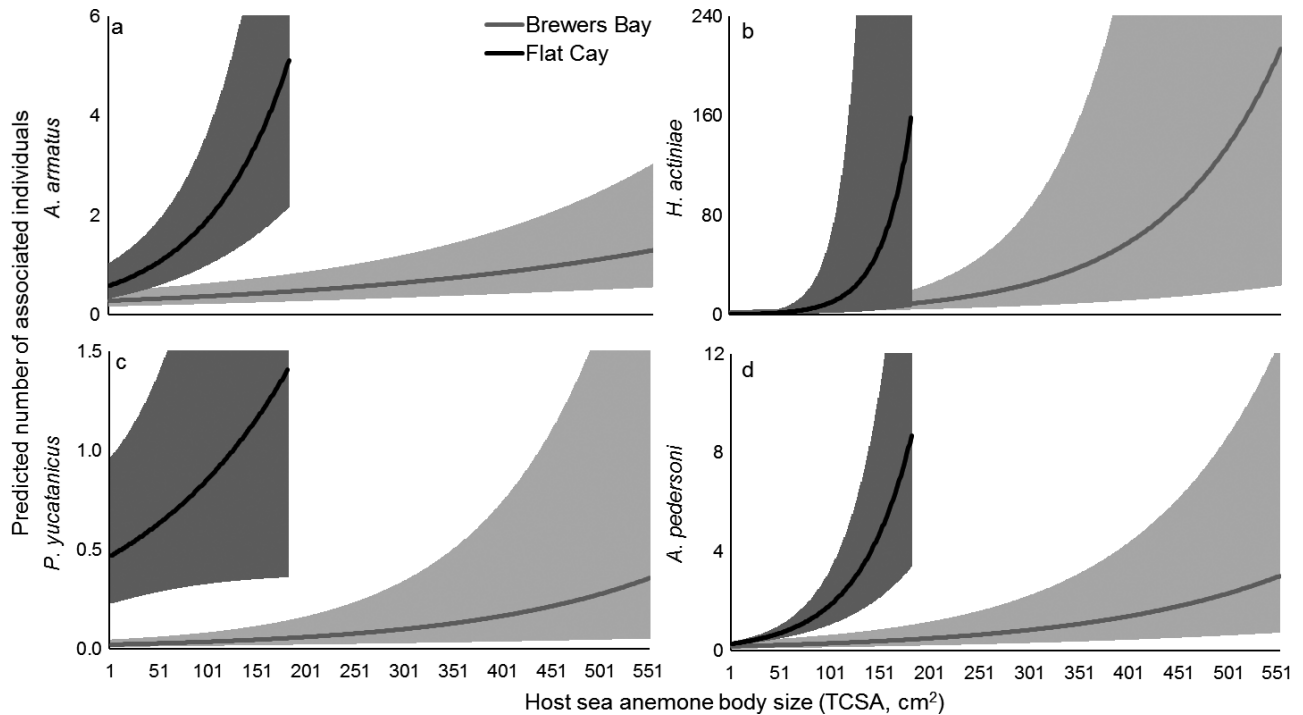


Fig. 3. Variation in predicted group size (solid lines: mean number of individuals per host; shaded regions: 95 % CI) of 4 associated anemoneshrimps (a: *Alpheus armatus*, b: *Heteromysis actiniae*, c: *Periclimenes yucatanicus*, and d: *Ancylomenes pederstoni*) with body size of sea anemones *Bartholomea annulata* (tentacle crown surface area, TCSA), at inshore (Brewers Bay) and offshore (Flat Cay) coral reef sites. Body sizes of 2–4 anemones in occasional aggregations were summed, because shrimps utilize aggregated anemones as a single host. Predictions are based on the best-approximating Poisson and negative binomial regression models for each site, as detailed in Tables S3 (*A. armatus*), S4 (*H. actiniae*), S5 (*P. yucatanicus*), and S6 (*A. pederstoni*) in the Supplement. Where models include a census effect, predictions are for Sep 2008, because this census period represents a temporal mid-point in the 2.5 yr study

crevice where the anemone was attached. In contrast, individuals of *H. actiniae* occurred in all cases among the anemone tentacles, mostly in the inner half of the tentacle crown near the anemone oral disc (zone 2), but sometimes also in the outer half of the tentacle crown (zone 3), in a pattern that did not differ significantly between the 2 reef sites ($\chi^2 = 0.968$, $df = 1$, $p = 0.32$). At BB, individuals of *P. yucatanicus* occurred only among the anemone tentacles, mostly in the outer half (zone 3) but sometimes in the inner half (zone 2) of the tentacle crown. At FC, a few individuals also occurred on the surrounding substratum near the host anemone (zones 4 and 5), but these rare occasions did not cause the microhabitat use patterns of this shrimp to differ between sites ($\chi^2 = 0.698$, $df = 1$, $p = 0.40$). Finally, individ-

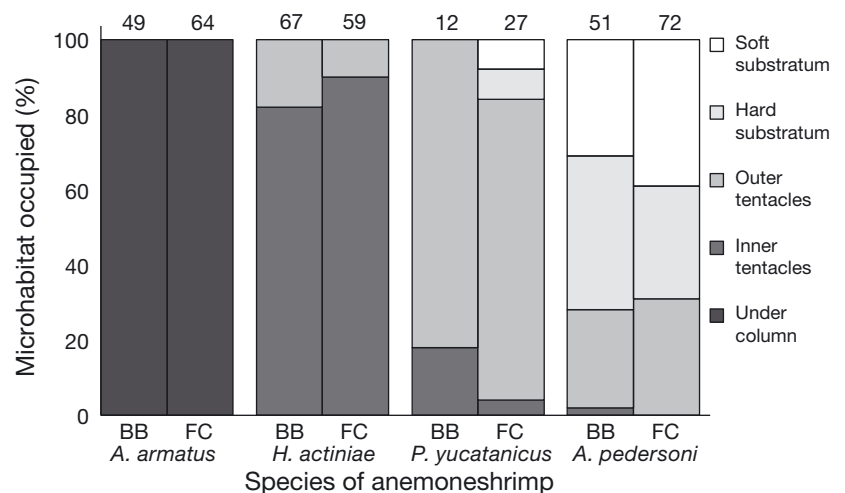


Fig. 4. Variation among 4 species of obligate anemoneshrimps (*Alpheus armatus*, *Heteromysis actiniae*, *Periclimenes yucatanicus*, and *Ancylomenes pederstoni*) in the percent of individuals that occupied each of 5 types of microhabitat zones on the bodies of sea anemones *Bartholomea annulata*, at coral reef sites Brewers Bay (BB) and Flat Cay (FC), examined in Nov 2009. Microhabitat zones ordered by level of potential shelter from predation provided to shrimp by anemones (see Fig. 1). Number of shrimps observed at each site shown at top of each bar

uals of *Ancylomenes pedersoni* usually occupied substratum near the anemone, about equally in zones 4 and 5, but occasionally perched on the anemone tentacles (in both zones 2 and 3; no significant difference between sites: $\chi^2 = 0.029$, $df = 1$, $p = 0.86$). Although their spatial patterns slightly overlapped, *P. yucatanicus* and *A. pedersoni* differed significantly in their microhabitat use at both sites: *P. yucatanicus* occurred most frequently on the tentacle crown (zones 2 and 3 combined), while *A. pedersoni* mostly occupied hard or soft substratum surrounding the anemone (zones 4 and 5 combined; BB: $\chi^2 = 18.208$, $df = 1$, $p < 0.001$; FC: $\chi^2 = 21.484$, $df = 1$, $p < 0.001$). The field data thus supported the hypothesis that crustacean associates partitioned microhabitats on the host body, at least for the 4 focal species of obligate anemoneshrimp examined here. Observations on the other 2 crustacean associates indicated that individuals of *T. amboinensis*, although rare (Table S2), occurred in zones 2 and 3, and that the facultative associate *S. seticornis* occurred in zones 3–5.

3.4. Hypothesis 3: Hosts persist longer in the presence of anemoneshrimps that are known to provide mutualistic benefits

Statistical models based on the field observations generally provided little support for the hypothesis that the presence of either snapping shrimp *Alpheus armatus* or dedicated cleaner shrimp *Ancylomenes pedersoni* influenced the persistence of host anemones ($\Delta\text{WAIC} > 10$, except for *A. pedersoni* at BB; Table 2). Instead, the models revealed that anemone persistence increased most strongly with host body size at both sites (Tables S8 & S9 in the Supplement). However, at BB, the positive effect of anemone size

on anemone persistence was much stronger in the presence of *A. pedersoni*, where parameter estimates indicated that anemones with this cleaner shrimp exhibited a lower probability of extinction (i.e. higher persistence) than did those without them (Tables 2 & S9). The assessment of posterior predictive test statistics for each model indicated that predicted observations based on the fitted models matched the observed data reasonably well at both sites.

Parameter estimates from the best-approximating 3-state models indicated a negative relationship between anemone body size (TCSA) and local extinction rate (Tables S8 & S9), with a larger effect at FC than BB. The scaled odds-ratio indicated that for every 50 cm² TCSA increase in anemone body size, anemones at BB were 1.25× less likely to disappear from a tag location, whereas anemones at FC were 3.36× less likely to disappear. As such, at FC, small anemones experienced much higher rates of disappearance from tag locations relative to large anemones than did small anemones at BB. Likewise, at FC, the probability of anemone colonization at a tag location during a given census was lower (0.17) than at BB (0.25; derived from the inverse logit transformation of the logit-scale colonization 'Intercept [BA]', Table S8), indicating that anemones were less likely to colonize previously unoccupied tag locations at FC than at BB. Conversely, the probability of *Alpheus armatus* colonization (0.38) to the relatively rare host anemones at FC was greater than their probability to colonize (0.24) the relatively abundant anemones at BB. Therefore, the hypothesis that anemone hosts persist longer in the presence of crustaceans that provide mutualistic benefits was supported only for *Ancylomenes pedersoni* at the BB site (Table 2), where presence of this cleaner shrimp enhanced the positive effect of anemone body size on anemone persistence.

Table 2. Watanabe-Akaike information criterion (WAIC), effective number of parameters (pWAIC), and ΔWAIC for candidate set of 3-state dynamic occupancy models for obligate anemoneshrimps *Alpheus armatus* (AA) and *Ancylomenes pedersoni* (AP) on sea anemones *Bartholomea annulata* (BA) at coral reef sites Brewers Bay (BB) and Flat Cay (FC). Separate model sets paired by site and anemoneshrimp species

Site	Model	WAIC	pWAIC	ΔWAIC
BB	AA does not influence BA persistence	3156.187	26.922	0.000
BB	AA influences BA persistence	3169.845	33.608	13.658
FC	AA does not influence BA persistence	1884.985	27.716	0.000
FC	AA influences BA persistence	1899.164	34.565	14.179
BB	AP does not influence BA persistence	3035.494	23.578	0.000
BB	AP influences BA persistence	3038.917	30.173	3.423
FC	AP does not influence BA persistence	1843.819	27.590	0.000
FC	AP influences BA persistence	1866.513	35.169	22.694

3.5. Hypothesis 4: Cleaner shrimps reduce each other's persistence but enhance the persistence of hosts

At FC, where enough data existed for analysis, model selection based on ΔWAIC indicated support for 3 of the eight 5-state dynamic occupancy models (Table 3). The best-approximating model indicated that dynamic rates (colonization and extinction) of dedicated cleaner shrimp *Ancylomenes pedersoni* and occasional cleaner shrimp *P. yuca-*

Table 3. Watanabe-Akaike information criterion (WAIC), effective number of parameters (pWAIC), and Δ WAIC for candidate set of 5-state dynamic occupancy models for obligate anemoneshrimps *Periclimenes yucatanicus* (PY) and *Ancylomenes pedersoni* (AP) on sea anemones *Bartholomea annulata* (BA) at Flat Cay coral reef site. Prior rights: dominance of AP or PY established by which species was there first

Model	WAIC	pWAIC	Δ WAIC
Neither shrimp dominant, shrimp presence does not influence BA persistence	2282.115	37.414	0.000
AP dominant, shrimp presence does not influence BA persistence	2287.660	43.475	5.545
Neither shrimp dominant, shrimp presence influences BA persistence	2290.428	45.662	8.313
AP dominant, shrimp presence influences BA persistence	2295.441	51.438	13.326
PY dominant, shrimp presence does not influence BA persistence	2297.002	46.410	14.887
Prior rights, shrimp presence does not influence BA persistence	2300.604	54.746	18.489
PY dominant, shrimp presence influences BA persistence	2305.635	54.860	23.520
Prior rights, shrimp presence influences BA persistence	2310.008	63.835	27.893

tanicus did not vary with their co-occurrence patterns, and that the persistence of host anemones at tag locations did not appear to be influenced by the presence of either associate (scenario 8). The second best-approximating model was consistent with the scenario that *A. pedersoni* was the dominant cleaner shrimp, *P. yucatanicus* was subordinate, and neither of them influenced *B. annulata* persistence (scenario 4). The third best-approximating model supported the scenario that although competitive interactions did not occur between *A. pedersoni* and *P. yucatanicus*, the presence of either cleaner shrimp enhanced the persistence of *B. annulata* (scenario 7; Table S10 in the Supplement).

Similar to results for the 3-state models, parameter estimates based on the 3 best-approximating 5-state models indicated a strong, negative relationship between anemone body size and local extinction (Table S10). Although there was some evidence that this relationship was weaker in the absence of either *A. pedersoni* or *P. yucatanicus* (i.e. when anemones lacked these associates), the difference was small and imprecisely estimated. Although parameter estimates were generally imprecise, those from the second-best approximating model indicated that, on average, the probability of local extinction for *P. yucatanicus* was higher (~ 0.61 vs. ~ 0.34 ; mean difference = 0.24; 95% confidence limit [CL] = -0.19 to 0.60), and the probability of local colonization was lower (~ 0.20 vs. ~ 0.32 ; mean difference = 0.12; 95% CL = -0.03 to 0.25) in the presence of *A. pedersoni* (back-transformed probabilities based on coefficients from the second-best approximating model for *P. yucatanicus* colonization and extinction; Table S10). Lastly, assessment of posterior predictive test statistics indicated that the predicted observations based on all 8 fitted models matched the observed data reasonably well. As such, the field data did not

strongly support the hypothesis that these 2 cleaner shrimp species reduce each other's persistence but enhance the persistence of hosts. There was limited model support for enhanced host persistence in the presence of these 2 cleaner shrimps, and for reduced colonization and enhanced extinction of *P. yucatanicus* when *A. pedersoni* was present, suggesting that *A. pedersoni* may be dominant during competitive interactions between the 2 cleaner shrimps.

4. DISCUSSION

We demonstrate here that on coral reefs in the US Virgin Islands, crustacean associates exhibit high overall occupancy rates on host sea anemones *Bartholomea annulata* and partition their use of microhabitats on the host body. Crustacean groups on anemones vary in size among species but remain fairly constant both temporally (among seasonal census periods) and spatially (between nearby reef sites). In contrast, most other aspects of the anemones and their associated crustaceans vary substantially over time and space at the scales examined here, with nuanced interactions among crustacean abundance, host body size, and persistence at each coral reef site. The patterns observed here of sea anemones being relatively more abundant and larger, and colonizing tag locations more frequently at an inshore than offshore site, are similar to those reported in O'Reilly et al. (2018). Here we additionally reveal that the relatively few anemones at an offshore site are not only more likely to each host crustacean associates, but also to contain larger groups of the most common associates, at smaller host sizes, than at an inshore site. Our multi-state models indicate that anemone persistence is enhanced primarily by anemone body size, with variable secondary effects of both site and the

presence of certain crustacean associates. In particular, the dedicated cleaner shrimp *Ancylomenes pedersoni* provide mutualistic benefits to their hosts, in that they enhance the persistence of anemones and allow them to achieve higher survivorship at relatively small body sizes at an inshore reef site than offshore site. Finally, *A. pedersoni* appear to be competitively dominant over the occasional cleaner shrimp *Periclimenes yucatanicus*, in that the presence of the former inhibits both the colonization and persistence of the latter shrimp species on host anemones.

The positive relationship described here between body size of host anemones and group size of anemoneshrimp is similar to that reported in some previous studies on Caribbean sea anemones (Stanton 1977, Briones-Fourzán et al. 2012), but not in others (Nizinski 1989, Huebner & Chadwick 2012a). These differences may be due in part to short-term variation in anemone body sizes that shrink and grow at rates varying widely among seasons (O'Reilly & Chadwick 2017, O'Reilly et al. 2018). Anemone body size thus may not be tracked rapidly by anemoneshrimp, and any relationship may be revealed only through repeated seasonal sampling. Further, our observation at the inshore site that aggregations of anemones host a richer and more abundant assemblage of crustaceans than do isolated anemones likely also affects this relationship, in that aggregated anemones may provide more habitat area than isolated ones. At the offshore site, relatively few aggregations of anemones existed, effectively removing this as a habitat consideration for associates. Thus, spatial variation in anemoneshrimp association with anemones, such as observed here between reef sites, also likely contributes to conflicting patterns in the literature about how associations vary with host size. In contrast, the diversity and abundance of both crustaceans and fishes consistently increase with host body size on stony corals (Gotelli & Abele 1983, Bos & Hoeksema 2015), probably in part because the calcareous skeletons of corals prevent rapid fluctuations in host body size. The pattern observed here of anemones hosting larger groups of anemoneshrimp, with higher proportions of anemones occupied at smaller host body sizes, at an offshore site where the hosts are relatively rare and small, may have occurred due to the anemones being a more limiting resource for anemoneshrimp than at the inshore site examined. Both this pattern and our modeling results indicate potential competition among anemoneshrimps for limited resource space on hosts, especially at sites where anemones are rare or small. Experimental manipulation of host anemone characteristics, including body size, relative areal

sizes of microhabitat zones, and nearest-neighbor distances, are needed to further reveal potential mechanisms of impact on anemoneshrimp.

Some of the variation observed here in spatial and temporal patterns among the 6 examined crustacean associates may be due to species-specific factors that differ among the crustaceans. Our observation that *Alpheus armatus* occurred singly more often than in mated pairs on anemones may reflect the sexual system of this shrimp, in that females are territorial but males may move among anemones to mate with multiple females (Knowlton 1980), as is known for some other crustaceans that move among symbiotic hosts (reviewed in Thiel et al. 2003a, Baeza & Thiel 2007). The observed pattern of *A. armatus* abundance being unrelated to whether this shrimp had previously occurred at an anemone tag location likewise may have been due to frequent movement by males among anemones; in general, both male and female crustaceans may move frequently among some sea anemone hosts (Thiel et al. 2003b). The microhabitat specificity of *Heteromysis actiniae* among anemone tentacles may in part explain their spatial variation among reef sites, in that swarms of this mysid are larger and occupy a higher percentage of available hosts at the inshore site where anemones are relatively large, and thus provide more among-tentacle habitat volume than at the offshore site. Variation in nematocyst abundance among anemones also could have contributed to our observation that some large anemones hosted large swarms of *H. actiniae* while others hosted none, in that *H. actiniae* prefer hosts with relatively few nematocysts near the bases of their tentacles where the mysids perch (Clarke 1955). In contrast, Indo-Pacific mysids *Idiomysis inermis* have only a facultative association with anemones; their swarms are small relative to those of *H. actiniae*, they hover over but do not reside among anemone tentacles, and they do not increase in abundance with anemone body size (Bhaduri & Crowther 2016). The low abundances of *Thor amboinensis* recorded here are similar to those known from other sites in the northern Caribbean Sea (Herrnkind et al. 1976, Stanton 1977). Given their small body size relative to most other anemoneshrimp, and their propensity to hide among anemone tentacles (in zones 2 or 3; Fig. 1) or along the host column (Herrnkind et al. 1976, Baeza & Piantoni 2010), individuals of *T. amboinensis* may be difficult to detect and thus potentially more abundant than usually recorded in field studies.

Interactions with client fishes likely cause some of the patterns reported here for dedicated cleaner shrimp *Ancylomenes pedersoni*. Occupation of more

exposed microhabitats than those used by other anemone associates (Figs. 1 & 4) allows *A. pedersoni* to quickly access visiting client fishes. The strong effect of anemone body size on *A. pedersoni* group size only at the offshore site also may be due to the relatively small maximal body sizes of anemones there, in that associating with the few large anemones available may be important for maximizing the attraction of client fishes, which visit large anemones more frequently than small ones (Huebner & Chadwick 2012a, Titus et al. 2017). The relatively large groups of ~1.5–3.0 individuals of *A. pedersoni* per anemone observed at our offshore site are similar to those reported from nearby St. John (1.1–3.3 per anemone), where Mahnken (1972) proposed they were due to high levels of fish traffic. Our observation that occasional cleaner shrimp *P. yucatanicus* occupy more sheltered microhabitat on anemones than do dedicated cleaner shrimp may relate to infrequent cleaning behavior by the former. During hundreds of hours of underwater observation on host anemones for the present and previous studies at St. Thomas (Huebner & Chadwick 2012a,b, O'Reilly et al. 2018), we did not observe any individuals of *P. yucatanicus* to clean fishes. Although our models indicated some support for competition between these 2 shrimp species, their microhabitat segregation likely reduces competitive conflicts. Experimental studies are needed to further reveal the mechanisms and outcomes of interactions between these sympatric cleaner shrimps.

Limited effects of anemoneshrimp presence on host persistence may be due in part to frequent changes in shrimp presence on the anemones examined here. In the Bahamas, individuals of *Ancylomenes pedersoni* remain present on only 37% of originally occupied anemones after 5 wk (Stanton 1977), and at St. John, US Virgin Islands, consistent shrimp abundances per anemone have been observed for only <1 mo, while anemones experimentally cleared of shrimp receive new colonists within 3.5 d (Mahnken 1972). Likewise, individuals of *Alpheus armatus*, especially males in search of mates, may often move among anemones (Knowlton 1980). As such, effects of both these mutualistic shrimp species on host anemones probably vary widely over shorter temporal scales than the 3–6 mo (seasonal) intervals examined here. Additionally, we did not model the effect of shrimp abundance on anemone persistence, only the effect of shrimp presence versus absence. Anemones that host many shrimps could accrue substantial benefits, especially from *Ancylomenes pedersoni* which form larger groups than

P. yucatanicus and clean more fishes when grouped (Huebner & Chadwick 2012b), thereby enhancing physiological benefits to anemones from client fish excretions (Cantrell et al. 2015). Between-site differences in host anemone population dynamics also likely alter anemoneshrimp impacts. Both more abundant and larger crustaceans are likely to associate with large than small anemones (Baeza & Stotz 2001), and with aggregated than single anemones (Ory et al. 2013). As well, crustaceans migrate more frequently among abundant than sparse hosts, potentially resulting in high levels of temporal variation in association (Thiel et al. 2003b). These types of factors that varied between our study sites likely impacted patterns of not only crustacean association patterns, but also their effects on hosts. Anemones at the offshore site had lower abundance, recruitment, growth, body size, and persistence than those inshore, which relate to habitat differences, including water motion level (O'Reilly et al. 2018). Offshore environmental factors thus may exert a strong limitation on anemone persistence that counteracts any benefit of hosting cleaner shrimp, causing only anemones at the inshore site to exhibit such benefits. The diverse potential effects of anemoneshrimp on host anemone fitness need to be investigated further, especially using experimental manipulations to determine mechanisms and relation to variation in host traits.

Our modeling framework indicated that anemones are more likely to persist at some tag locations than others, and colonize relatively more frequently and become larger than at other locations. This spatial variation could be due in part to some tag locations being more physically stable than others (e.g. on large reef framework vs. small piece of rubble; O'Reilly et al. 2018), allowing anemones to remain less disturbed at those locations. It is possible that anemone persistence is due primarily to these types of forces, and that only those that persist develop shrimp associations, rather than the associated shrimps strongly affecting the host anemones. However, anemone persistence also may result from some tag locations consistently containing *A. pedersoni* and *P. yucatanicus*, as suggested by the negative binomial models. Known benefits to large anemones from hosting cleaner shrimps and attracting more client fishes (Huebner & Chadwick 2012a) likely lead to positive feedback loops in which anemones that persist and grow rapidly at certain reef locations attract and support more shrimp, which in turn attract more client fishes that also may enhance anemone growth and persistence (Cantrell et al. 2015).

This type of mutualistic feedback may contribute greatly to enhancing spatial variation in host anemone size among locations on coral reefs.

The present study is the first to quantify variation in the association dynamics of crustaceans with coral-reef sea anemones across multiple years and contrasting types of reef sites. The patterns that we describe here represent baseline information for investigating changes in populations of these organisms, and a foundation for future inquiry into the dynamics of species interactions in this symbiotic network, especially in the framework of multi-state modeling. While *B. annulata* appears to be less highly co-dependent on crustacean associates than are some Indo-Pacific sea anemones that host anemonefishes (e.g. Huebner et al. 2012), our modeling framework indicates some support for the positive influence of shrimp symbionts on host anemone persistence. This type of multi-state modeling is useful for studies seeking to clarify dynamics among symbiotic partners based on their life history patterns (e.g. lifespans, diet; Gilpin & Chadwick 2017, O'Reilly & Chadwick 2017). Mutualistic networks are common in nature (Afkhani et al. 2014), including among multiple species of crustacean associates and their host organisms, such as sea anemones, urchins, molluscs, and corals (Stier et al. 2012, Ory et al. 2013). Further investigation into the particular mutualistic network that occurs among sea anemones, microalgae, crustaceans, and client fishes attracted by cleaner shrimps, especially at short time intervals, will likely yield greater understanding of the costs and benefits to partners in this system, and the resulting ecological impacts on coral reef fish assemblages.

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