Reef fishes use sea anemones as visual cues for cleaning interactions with shrimp

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Abstract

Marine cleaners benefit diverse fish clients via removal of ectoparasites, yet little is known about how fishes locate small, inconspicuous cleaner shrimps on coral reefs. Pederson shrimp Ancylomenes pedersoni are effective cleaners in the Caribbean Sea, and additionally form obligate associations with corals. Sea anemones Bartholomea annulata, which also serve as hosts to a variety of other crustacean symbionts. We examined the visual role of B. annulata to reef fishes during cleaning interactions with A. pedersoni by comparing anemone characteristics with fish visitation rates, and by manipulating the visibility of anemones and cleaner shrimp in field experiments using mesh covers. Rates of visitation by fishes to cleaning stations increased primarily with anemone body size and the total number of crustacean symbionts, but did not change consistently in response to covers. Fishes posed for cleaning at stations only where anemones remained visible, regardless of whether shrimp were visible. Shrimp at stations where anemones were covered performed fewer cleaning interactions with fishes, as fishes did not continue to pose when anemones were not visible. We conclude that anemones serve as visual cues to client fishes prior to cleaning by shrimp. These visual cues facilitate fish cleaning interactions with shrimp, and provide a previously unknown symbiotic benefit to cleaner shrimp from association with sea anemones.

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1. Introduction

Marine cleaning mutualisms traditionally have been regarded as two-species systems in which one type of organism, the cleaner, removes and ingests ectoparasites and damaged tissues from another type of organism, the client (Côté, 2000). Most studies on cleaning mutualisms have focused on cleaner fishes, however shrimps (Order Decapoda) also serve as important cleaners in marine environments. Of the 43 species of shrimps proposed to be cleaners (Becker and Grutter, 2004), few have been studied quantitatively or comprehensively as true cleaners, and only recently have their parasite removal capacities and niche overlap with cleaner fishes, and among species of obligate cleaner fishes (Cheney et al., 2009). In addition to this variation, cleaning mutualisms with shrimps may be more complex than those with cleaner fishes because some cleaner shrimps also form mutualisms with sea anemones and corals (Chapuis and Bshary, 2009; Kulbicki and Arnal, 1999; Mahnken, 1972). Cleaner shrimps that are also obligate anemoneshrimps center their cleaning stations around the anemone body, and client fishes must approach within ~10 cm of the anemone to be cleaned (Johnson and Ruben, 1988; Wicksten, 1995). Thus fishes may use the relatively large anemone hosts as landmarks to locate symbiotic cleaner shrimp, which appear to be more visible against the contrasting background of the anemone tentacles than against other reef substrata (Kulbicki and Arnal, 1999; Mahnken, 1972; Fig. 1a). Similarly, client fishes may use other types of landmarks to locate cleaner fish Labroides dimidiatus and shrimp Brachycarpus biunguiculatus, which both position themselves on conspicuous reef features, such as coral heads and sponges (Corredor, 1978; Potts, 1973). However, no experimental studies have been performed yet to determine whether host sea anemones perform a similar function, or play any type of direct role, in cleaner shrimp–client symbioses.

Individuals of the Pederson shrimp Ancylomenes pedersoni (Chace) (formerly within Periclimenes; Okuno and Bruce, 2010) are obligate symbionts of sea anemones and also serve as major cleaners on Caribbean coral reefs. These shrimps can significantly reduce parasite loads on client fishes (Bunkley-Williams and Williams, 1998; McCammon et al., 2010), and on some reefs they engage in cleaning more frequently and with a wider variety of fish clients than do all other cleaners, including...
The purpose of the present study is to determine experimentally whether the persistence of the cleaning station (McCammon, 2010). The purpose of cleaning activity of associates (Humann and DeLoach, 2006). Little is known about the ecological roles of these crustaceans (Mahnken, 1972), apart from the fact that they appear to affect the behavior of visiting fishes: after 2–3 min of the diver remaining stationary on the sea floor, fishes adjusted to diver presence and resumed their normal activities; 20 min observations began after this initial 2–3 min. A visit was defined as a fish approaching a cleaning station and remaining in the station vicinity for at least 3 s (similar to Arnal and Côté, 1998), excluding other activity such as grazing or territory defense.

To determine the types of cues that attract client fishes to cleaning stations, we defined five characteristics of host sea anemones: a) body size (measured as tentacle crown surface area [TCSA], calculated from the long and short diameters of the anemone tentacle crown; after Hattori, 2002), b) height of tentacle tips above the sand plain surrounding the reef, c) extension distance of tentacle tips beyond the immediately surrounding substratum, d) number of symbiotic crustaceans (including A. pedersoni, spotted anemone shrimp Periclimenes yucatanicus, sun anemone shrimp P. rathbunae, A. armatus, squat anemone shrimp Thor amboinensis, and arrow crab associates Stenorhynchus seticornis; identified from Humann and DeLoach, 2006). Within aggregations of B. annulata (up to four individuals), the tentacles and crustacean symbionts of anemones intermingle and appear indistinguishable as separate anemones (Fig. 1a), so these aggregations were treated as single cleaning stations and TCSA was calculated for the entire aggregation. Anemone characteristics were recorded for each cleaning station before (March 2009) or immediately after (July 2009, November 2009, and February 2010) observations on fish clients. Observational data on fish visitation rates were pooled among the four sampling periods because they did not differ significantly (Kruskal–Wallis test: $H_k = 3.567, P = 0.312$).

2.2. Anemone characteristics

At both reef sites during all four sampling periods, a total of 77 cleaning stations (anemones with symbiotic cleaner shrimp) were selected haphazardly for observations of fish visits ($N = 15$ stations at FC and 17 at BB in March 2009, and 16, 22, and 7 stations at BB in July 2009, November 2009, and February 2010 respectively). Anemones observed during each period were identified and mapped, and then subsequently excluded from haphazard sampling during later periods. Observations on each cleaning station began immediately after sunrise (~06:30 h) and concluded by mid-morning (~08:30 h), to correspond closely with peak activity of parasitic gnathiid isopods and fish cleaning on the reef (Sikkel et al., 2004, 2005, 2006). Scuba divers observed each station for 20 min from a distance of ≥1.5 m, depending on visibility, and recorded the number of visiting client fishes. The presence of divers did not appear to affect the behavior of visiting fishes: after 2–3 min of the diver remaining stationary on the sea floor, fishes adjusted to diver presence and resumed their normal activities; 20 min observations began after this initial 2–3 min. A visit was defined as a fish approaching a cleaning station and remaining in the station vicinity for at least 3 s (similar to Arnal and Côté, 1998), excluding other activity such as grazing or territory defense.

2.3. Field experiment

In addition to visits, we also recorded posing behaviors of client fishes and cleaning interactions at BB reef stations during the latter three periods only (July 2009, November 2009, and February 2010; $N = 43$ stations). A pose was defined as a fish signal to instigate a cleaning interaction, for example change of body orientation, opening of gills and/or mouth, or color change (Côté et al., 1998; Mahnken, 1972). A cleaning interaction was defined as a client fish pose that resulted in cleaner shrimp contact with the client body. The 43 cleaning stations then were each assigned randomly to one of four experimental treatments: a) neither anemone nor shrimp covered (control, $N = 10$), b) anemone with shrimp present (control, $N = 10$), c) only anemone present (control, $N = 10$), d) only shrimp present (control, $N = 10$).
b) both anemone and shrimp covered (N = 11), c) anemone covered but shrimp uncovered (N = 11; Fig. 1b), and d) shrimp covered but anemone uncovered (N = 11). Based on the length of the field period, only a few stations were assigned to each treatment during each experimental period, then the data from all periods were pooled because rates of fish visitation did not differ significantly among the three periods (Kruskal–Wallis test: H₂₋₄ = 2.856, P = 0.240). Immediately after each 20 min observation of fish behavior at a cleaning station, a mesh cover was placed ≤ 15 cm from the anemone, to allow fishes to adjust to its presence on the reef. Each cover consisted of fine mesh window screening arranged four layers thick in a hemispherical shape and affixed by zip-tie to a circle of 18 gauge aluminum wire, then painted with Rust-oleum indoor–outdoor spray paint, color neutral, to blend in with the natural color of the reef–sand environment at the field site. These malleable materials rendered the cover shapes adjustable to fit over anemones inhabiting crevices at the reef–sand interface, where most anemones occurred (Mahken, 1972; Nelsen, 2008; Fig. 1).

Two days after initial observations, adjacent covers were moved over the anemones (both- and anemone-covered treatments) or left next to the station (control and shrimp-covered treatments), and observations of fish behavior were conducted again as described above. Because shrimp could potentially escape from under the covers in the both- and shrimp-covered treatments, the shrimp in these two treatments were captured by hand and held in a plastic bag hidden under the diver during observations. In the both- and anemone-covered treatments, we manually contracted anemones by touching their tentacles, then placed the cover over the crevice into which the anemone had contracted. In the anemone-covered treatment, this process did not cause symbiotic A. pedersoni to vacate their anemones; the shrimp instead settled on or near the cover (< 5 cm; Fig. 1b), possibly detecting the anemone’s presence via olfaction (Guo et al., 1996). Other crustacean symbionts of these anemones remained under the covers with their contracted anemones, except for associates S. seticornis, which remained outside. Immediately after experimental observations, all covers were removed and shrimps were reunited with their anemones.

2.4. Data analysis

A stepwise multiple-regression with backward elimination was used to determine how the number of visits by client fishes varied with the five anemone characteristics. Neither the number of A. pedersoni (simple linear regression [SLR]: F₁,₇₅ = 0.114, P = 0.736, R² = 0.002) nor the number of all crustacean symbionts (including A. pedersoni; SLR: F₁,₇₅ = 1.673, P = 0.200, R² = 0.022) per anemone depended on anemone body size, so crustacean abundance and anemone body size were excluded as confounding variables, and thus appropriate to include in the multiple regression model as separate factors. Additionally, Akaike’s Information Criterion (AICc) scores were used to assess the relative values of each multiple regression model in explaining fish visits (Burnham and Anderson, 1998). AICc simultaneously considers all models and objectively selects the model that best fits the data; the model with the lowest AICc score is the most parsimonious. We used AICc scores for small sample sizes (AICc), which also reduce the chances of model over-fitting. We adjusted the scores by subtracting the smallest AICc score from the others, creating ΔAICc values, with the smallest score standardized as zero. Adjusted scores with values < 4 are competitive with the top model (Burnham and Anderson, 1998). One-way repeated-measures analyses of variance were used to compare the numbers of fish visits, fish poses, and cleaning interactions within treatment before and after manipulation at each cleaning station (after McCammon et al., 2010). All client fish species were pooled for all analyses, because the abundance of individuals in most species was low (Huebner and Chadwick, in press). All data were analyzed using SYSTAT 13.

3. Results

3.1. Anemone characteristics

Of the five anemone characteristics analyzed for relationships to client fish visitation, three were dropped from the top model (lowest AICc score: F₂,₇₄ = 6.403, P = 0.003, R² = 0.148); anemone body size in TCSA and the total number of crustacean symbionts (including A. pedersoni) per anemone were both retained and showed positive relationships with fish visits (Table 1). Though not significant regressions, two other models in the backward selection both had ΔAICc < 4, and thus may be competitive models in explaining fish visits to A. pedersoni stations (Table 1). These models showed negative relationships with fish visits by the extension distance of tentacle tips beyond the immediately surrounding substratum and the number of symbiotic A. pedersoni only. The only characteristic not included in a potentially competitive model was the height of tentacle tips above the sand plain surrounding the reef.

Resident individuals of S. seticornis occasionally waved their chelae at posing clients that were invertebrate predators such as snappers (Lutjanidae), thereby causing the clients to leave and interfering with the cleaning interaction. These crab associates did not wave their chelae at herbivorous clients such as surgeonfishes (Acanthuridae). Other non-cleaner crustacean symbionts appeared to have no interaction with client fishes during poses or cleaning interactions. Common client fishes to these stations were surgeonfishes, goatfishes (Mulidae), groupers (Serranidae), and snappers; overall, clients were members of 16 fish families (Huebner and Chadwick, in press).

3.2. Field experiment

When we left cleaning stations uncovered (control treatment) or covered both the anemone and shrimp, fish visitation rates did not change significantly (F₁,₉ = 1.814, P = 0.678 control, F₁,₀ = 0.593, P = 0.459 both-covered; Fig. 2a). In contrast, fish visitation rates decreased significantly at stations where we covered anemones only (F₁,₀ = 5.900, P = 0.036), and increased significantly at those where we covered shrimp only (F₁,₀ = 5.800, P = 0.037). Some fishes swam toward both-covered and anemone-covered stations, paused, swam around the area within a few meter radius of the covered station, and then returned to the station vicinity and paused again. In some cases, they repeated this behavior for several minutes before

Table 1

<table>
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<th>Model ΔAICc</th>
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<th>Coefficient β</th>
<th>SE of coefficient</th>
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leaving the station without posing. At anemone-covered stations, some cleaner shrimp commenced antennae signaling (Mahnken, 1972) in response to approaching fishes, but the fishes swam away without posing.

The presence of covers alone did not influence rates of fish posing per station (control treatment, $F_{1,9} = 0.000, P = 1.000$; Fig. 2b). Posing rates also did not change when shrimp were covered ($F_{1,10} = 2.168, P = 0.172$), revealing that fishes posed in front of anemones alone. In contrast, rates of posing per station decreased significantly after covers were applied to both anemones and shrimp ($F_{1,10} = 7.705, P = 0.020$) and to anemones only ($F_{1,10} = 10.811, P = 0.008$), indicating that fishes did not pose without anemones visible. At shrimp-covered cleaning stations, fishes maintained their poses in front of anemones for up to 8 s before swimming away. Some of these fishes then proceeded to visit and pose at other nearby cleaning stations that were not part of our experiment.

Finally, the rates at which shrimp cleaned fishes per station did not change after covers were left near anemones in the control treatment ($F_{1,9} = 0.043, P = 0.840$; Fig. 2c). However, cleaning interactions decreased significantly after anemones were covered, even though shrimp were still visible and available to clean ($F_{1,10} = 9.343, P = 0.012$).

4. Discussion

Here we show that sea anemones *B. annulata* are an integral part of fish cleaning interactions with anemone-shrimp *A. pedersoni*, in that they appear both to attract visiting client fishes and act as visual cues for the initiation of cleaning postures by clients. Large anemones appear to attract more fish clients than do small anemones, probably because they are more visually conspicuous to fishes. Because neither the number of *A. pedersoni* nor the total number of crustacean symbionts vary significantly with anemone size, the latter appears to serve as a visual attractant only, and not as an indicator to fishes of stations with more cleaner shrimp. Interestingly, client fishes were attracted to anemones with abundant crustacean symbionts in general, perhaps as prey items (fishes such as wrasses [Labridae] and snappers attempt to prey upon *P. yucatanicus* and *A. armatus* when they are removed from anemones; L.K. Huebner, pers. obs.). The chela-waving response that we observed by *S. seticornis* toward clients that were invertebrate predators appears to support this idea, although the exact intent of this behavior is not understood. More research is needed on the ecological roles of the diverse crustaceans that associate with *B. annulata*, and how they influence the cleaning interactions of *A. pedersoni* beyond potentially serving as visual attractants to fishes. As indicated by the close AIC, scores of the two models in the backward selection previous to the final, significant regression, the extension distance of tentacle tips beyond the immediately surrounding substratum and the number of *A. pedersoni* only may also have effects on fish visits, although it is not clear why these relationships were negative. Some *A. pedersoni* that occur in large social groups are small in body size, not very colorful (Fig. 1b), or hidden in crevices near the anemone (L.K. Huebner, pers. obs.), thus possibly not serving as visual attractants and skewing the model. Determination of which station characteristics attract clients merits further investigation, and can include other factors we did not examine, such as station location on the reef relative to the open sand plain versus reef rugosity (Mahnken, 1972).

Many fishes use mental maps or landmark sequences to navigate underwater environments (Braithwaite and de Perera, 2006). Our observations that some fishes searched repeatedly for anemones after they had been covered indicate that these fishes may use mental maps to relocate anemone-shrimp cleaning stations which they visited previously, as do clients of cleaner wrasse *L. dimidiatus* (Potts, 1973). The decrease in fish visits to covered anemones also suggests that anemones potentially serve as local landmarks for some fishes to pinpoint the location of cleaner shrimp after arrival to the station area. In the both anemone and shrimp covered group, however, the decrease in visits was unexpectedly not significant; this may be because the stations haphazardly assigned to this group had lower visits overall than the stations in the anemone-covered group, thus making a significant difference in visits after the cover was applied difficult to observe. The observed increase in fish visits to stations after only shrimp were covered was also unexpected. At five out of the 11 stations observed, the same fish species visited multiple (two-four) times after the shrimps were covered. Thus, it is possible that the same fishes returned repeatedly to an anemone station seeking a clean after an initial visit in which they did not receive one. We did not mark fishes and thus were not able to determine if the same fish visited our stations multiple times (unless they did so within
our field of view, in which case they were counted only once). Overall, given the mixed results of fish visitation behavior, it is unclear whether host anemones of A. pedersoni serve as landmarks to visiting fishes. Future studies which track individual fish movement among cleaning stations are needed to elucidate whether fishes use anemones as landmarks.

Our experimental treatment of anemones covered but shrimp visible likely is not a situation to which client fishes normally are exposed. A. pedersoni are obligate anemone symbionts, thus shrimp encountered without an anemone nearby may be in the process of moving among hosts (Mahnken, 1972) and be unwilling to clean. Individuals of A. pedersoni without an anemone host may even be regarded as prey by some fishes (Mihalik and Brooks, 1995), but we did not observe predation on shrimp during observations on anemone-covered stations. Indeed, obligate cleaners are generally immune to predation (Côté, 2000), so A. pedersoni may associate with anemones not only for protection, but also because the anemones attract potential fish clients. We observed antennae signaling toward visiting clients by shrimp on covered anemones, indicating that shrimp may attempt to initiate cleaning interactions even when they do not have access to the shelter of their host anemone. However, because the anemone was covered, cleaning interactions decreased for these shrimp, emphasizing the importance of the anemone to fish initiation of cleaning interactions.

When posing at cleaning stations, fishes appear to be close enough (<30 cm distant) to visually detect the presence of resident shrimp. Thus, it is puzzling that client fishes posed in front of anemones with shrimp covered. Regardless of shrimp presence, anemones may be the primary posing stimulus for these fishes, and some cleaner shrimps may use body movements to signal interest only after clients pose. Sequences of cleaner–client behaviors are variable, and client posing can occur before cleaner signals during successful cleaning interactions (Potts, 1973). Additionally, fishes may regularly pose in front of anemones that lack shrimp in part because the shrimp are not always clearly visible on cleaning stations. Cleaner shrimp sometimes hide in crevices near anemones, or under anemone tentacles (L.K. Huebner, pers. obs.), so the act of posing by client fishes could induce hidden shrimp to emerge and clean. By providing a visual cue of cleaner shrimp presence, the anemone itself may be regarded as a signal. Known signals used by cleaner shrimps have included mainly shrimp behaviors (Becker et al., 2005; Chapuis and Bshary, 2010; Mahnken, 1972), but in general the body size, color combinations or stripes of cleaners may also serve as signals to clients (Cheney et al., 2009; Stummer et al., 2004). As an additional signal, the size and/or coloration of host anemones may advertise the presence of anemonehirmp stations and potential cleaning services there, thus inciting reef fishes to pose. However, determination of the specific signaling benefits to cleaner shrimps of associating with anemones, in comparison with their potential benefits of associating with other types of conspicuous reef features (such as corals or sponges; Corredor, 1978; Lettieri et al., 2009; Potts, 1973), remains an area for further investigation.

Obligate cleaning organisms are not highly abundant on coral reefs (Sazima et al., 2010), yet their presence can strongly impact fish health and diversity, making them key organisms (Bshary, 2003; Grutter et al., 2003; Waldie et al., 2011) that play central roles in structuring fish assemblages (Floeter et al., 2007; Sazima et al., 2010). Cleaner shrimps are now recognized to effectively remove ectoparasites on the surface of the client (Côté, 2000). Alpheid shrimp defend anemones from fireworm predators, potentially allowing for the persistence of cleaning stations (McCammon, 2010).

collected from coral reefs for the ornamental aquarium trade (Calado et al., 2003; Rhyne et al., 2009), and over-collecting could have detrimental effects on reef fishes that utilize these cleaners. Further understanding of the indirect effects of sessile invertebrate hosts on reef fishes via their obligate shrimp symbionts is important to provide a more scientific basis for the sustainable management of the ornamental fishery trade.

We conclude that the sea anemone B. annulata is a direct, albeit passive participant in the cleaning symbiosis between cleaner shrimp A. pedersoni and client fishes. Reef features such as corals and sponges in the territory of a cleaner may serve as contrasting backgrounds to heighten cleaner visibility (Lettieri et al., 2009), and for cleaning anemone shrimps, sea anemones may provide this function (Mahnken, 1972). We hypothesize that potential coevolution of contrast between some shrimps and their obligate cnidarian hosts may explain the apparent lack of convergence among cleaner shrimp species in body coloration and signals, because some species are obligate associates of cnidarians and others are not. However, we demonstrate here that anemones serve not only as potential contrasts for client fishes to locate and identify cleaner shrimp, but that clients respond to sea anemones alone as visual cues for cleaning postures (Fig. 3). This reveals a previously unknown symbiotic benefit that cleaning anemone shrimps gain from associating with anemones. Thus, the cleaner–client mutualism appears to depend in part on the cleaner–anemone mutualism. The interactions between A. pedersoni and client fishes, coupled with fish use of sea anemones as visual cues, create linkages between two major types of mutualisms on coral reefs: those among cleaners and clients (Côté, 2000), and those involved in the three-way symbiosis among host cnidarians, their macro symbionts (usually fishes and crustaceans), and their endosymbiotic microalgae (Cleveland et al., 2011; Spotte, 1996). Future work is needed to determine additional types of direct and indirect benefits among these partners (such as nutrient flow; e.g. Cleveland et al., 2011; Spotte, 1996), and is likely to reveal added complexity in this multi-level symbiotic system.

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