A Tale of Two Anemone Shrimps:
Predation Pressure and Mimicry in a Marine Cleaning Mutualism

by

Mark A. Stuart

A thesis submitted to the Graduate Faculty of Auburn University
in partial fulfillment of the
Requirements for the Degree of
Master of Science

Auburn, Alabama
December 10, 2016

Keywords: mimicry, cleaner shrimp, mutualism, predation, visual signals, microhabitat

Approved by

Nanette E. Chadwick, Chair, Associate Professor in the Department of Biological Sciences, Auburn University
Raymond P. Henry, Professor and Associate Dean for Research in the College of Mathematics and Sciences, Auburn University
Paul Sikkel, Associate Professor of Biology, Arkansas State University

Abstract

For mutualistic relationships between different organisms, coloration and behavior can be used to indicate the ability to provide a service. These visual signals allow other organisms to mimic them to gain their benefits without providing the same negative or positive reinforcement for the signals as the model organism. This has been previously demonstrated for the fangblenny (*Plagiotremus rhinorhynchos*), an aggressive mimic of a major cleaner fish (*Labroides dimidiatus*). For my Master's thesis I examined the possible mimicry of the Pederson cleaner shrimp *Ancylomenes pedersoni* by the spotted anemoneshrimp *Periclimenes yucatanicus* in the Caribbean. I first quantified the coloration of *A. pedersoni*, *P. yucatanicus*, and their host anemones *Bartholomea annulata* and *Condylactis gigantea* using spectrographic methods. Overall, *A. pedersoni* were significantly more contrasting against all anemone backgrounds than *P. yucatanicus*. Additionally, I measured the predation pressure of *A. pedersoni*, *P. yucatanicus* and a non-cleaner *Alpheus armatus* in-situ on a coral reef, and found that potential client fish were significantly more likely to orient towards and attack the non-cleaner more than the other treatments. I also measured the average distance *A. pedersoni* and *P. yucatanicus* reside away from their two host anemones species in a laboratory setting, and the propensity of the shrimps to physically signal to potential client fish in-situ on a coral reef. There was a significant effect for the duration of the trial on the distance from the anemone for all shrimp treatments, and overall *A. pedersoni* stayed significantly farther away from *C. gigantea* than *P. yucatanicus,* but not for *B. annulata*. Lastly, the distribution of responses to potential client fish were significantly

different, with *A. pedersoni* more likely to signal, and *P. yucatanicus* more likely to retreat. These data indicate that *P. yucatanicus* is receiving some benefit (reduced predation) from physically looking like and behaviorally acting like *A. pedersoni*, but is not a perfect mimic.

Acknowledgements

First of all, I would like to thank my advisor Dr. Nanette Chadwick for her guidance and support through these last few years, and the many different stages of my project. With a different advisor, I am not sure this thesis would have been possible. I would also like to thank my committee members Dr. Raymond Henry and Dr. Paul Sikkel for their helpful comments on my thesis, and Dr. Geoffrey Hill for his insight during the earlier stages of my project. Next, I would like to thank Dr. David Clark, my former advisor at Alma College, for inspiring me to go to graduate school and for the use of his spectrometer, without which my shrimp color study would not have been completed. Additionally, I would like to thank my fellow graduate students and lab mates Erin O'Reilly, Matt McVay, Channing Cantrell, Nick Par, Alex Columbara, and Bobbie Renfro for their help in the lab and field, and caring for and maintaining my shrimps, fishes, and anemones, as well as the multitude of undergraduates that have been in our lab during the last couple years. I would also like to extend a special thank you to Jessica Gilpin for being supper supportive my whole time at Auburn, and for being the best dive buddy (without her I am pretty sure my dive flag would have killed me), and sounding board anyone could ask for. Lastly, I would like to thank my wife Heather and my father Duncan and the rest of my family for their unfailing love, understanding, and support through all of the ups and downs during my time at Auburn.

Table of Contents

List of Tables

List of Figures

Chapter 1

Visual and Behavioral Mimicry in Marine Environments: Implications for Mutualistic Networks

Conspecific and heterospecific communication is a complicated subject because organisms utilize a wide array of mechanisms to send and receive signals, which in turn convey a multitude of meanings. Signals can be delivered through chemical, tactile, auditory, or visual means, with the message indicating anything from an opponent's size to a potential mate's reproductive status (Vickery et al. 2012). In the marine environment, especially for crustaceans, the main forms of communication are through chemical and tactile signals. Chemical signals in marine or aqueous environments can be either long-range (dispersed chemicals) or short-range (cell surface-attached chemicals; Diaz et al. 2004; Vickery et al. 2012; Pelosi et al. 2014). Crustaceans are equipped with two sets of antennae for detecting chemical and tactile information. The first pair (short antennules) typically have specialized chemo-receptors for detecting dispersed chemicals, whereas the longer antennae are used typically for detection of tactile cues or surfaced-attached chemicals (Bauer 2004; Vickery et al. 2012). For example, the snapping shrimp *Alpheus heterochaelis* uses its antennules to detect dispersal chemicals indicating the sexual status, dominance status, or individual identification of nearby conspecifics, and then uses its long antennae to gather additional, tactile information during subsequent aggressive or sexual interactions (Vickery et al. 2012).

In addition to chemical and tactile signals, crustaceans also use auditory signals, typically created by clapping their claws. The most prolific and well-studied crustacean acoustic signalers are snapping shrimps in the family *Alpheidae,* whose snap is formed by a large claw that

produces a cavitation bubble when closed (Versluis et al. 2000; Bauer et al. 2004; Bohnenstiehl et al. 2016). These snaps are used by shrimps in the genus *Alpheus* during aggressive interactions with conspecifics, usually over territory (burrow) or a mate, with the loser usually becoming injured in some fashion (Bohnestiehl et al. 2016). Additionally, it has been suggested that snapping could be used for prey capture or to deter predation from heterospecifics (McCammon and Brooks 2014; Brohnestiehl et al. 2016; Stuart personal observations).

Lastly, visual signals (for example: color patterns or behavioral movement of body parts) can be used in the marine environment to indicate a variety of different messages. On coral reefs, poisonous or venomous organisms may be brightly colored (aposematic) effectively relaying their unpalatable state to potential predators, such as occurs in nudibranchs which are small soft-bodied and brightly-colored gastropods that accumulate toxic compounds from their food sources (Cheney et al. 2014). Other organisms may display cryptic coloration to avoid detection and predation, such as in the seagrass shrimp *Tozeuma carolinense*, which has two color morphs (green and brown) to camouflage them against live or dead sea-grass respectively. In addition to background matching for camouflage, many invertebrates including shrimps have evolved to completely lack pigments, i.e. in glass shrimp which are transparent to avoid detection and predation in the marine environment (Johnsen 2001). Coloration also can be used by organisms to indicate that they are able to provide services, such as in cleaner fishes. Cleaner fishes have evolved a blue, black, and yellow stripe coloration scheme that is consistent among most species (Stummer et al. 2004; Cheney et al. 2009; Lettieri et al. 2009). This coloration is optimized to be a highly conspicuous color signal against the coral reef background environment, so that cleaner fishes can clearly display their status as cleaners and not prey (Stummer et al. 2004; Cheney et al. 2009; Lettieri et al. 2009). In addition to color, body movements can also be used as a visual

signal to indicate a message. Many crab species stand up and open their claws in a threatening manner to indicate aggression (Booksmythe et al. 2010), which could deter negative interactions with conspecifics or predation by heterospecifics. Interestingly, during interactions between crabs *Trapezia ferruginea* and snapping shrimp *Alpheus lottini*, the shrimp is required to perform "appeasement" rituals from the crabs' own behavioral repertoire (a behavior that the shrimp does not perform toward conspecifics) in order to associate with the crabs in coral host habitats (Vannini 1985). Also, the Indo-Pacific cleaner shrimp *P. longicarpus* performs a stereotypical clapping of claws that apparently is not used to attract potential clients from a distance, but to signal cleaner identity (similar to the coloration of cleaner fishes) after clients have arrived at the station. Similarly, the cleaner shrimp *Ancylomenes pedersoni* performs a rocking motion with its body, and waves its antennae in anticipation of interacting with a client fish (Wickstein 1998, Huebner and Chadwick 2012).

When organisms develop color or behavioral signals that provide a benefit to the sender, there is the possibility of mimicry by other organisms to obtain that benefit without incurring the same cost. Typically, mimicry is thought of in terms of batesian, mullerian, camouflage, or aggressive mimicry, all of which occur in coral reef environments. In mullerian mimicry, all species are similarly colored and are equipped with the same deterrent to predation (co-mimicry), such the white, blue, and yellow coloration of chromodorid nudibranchs, whom all contain chemical defenses derived from their food sources (Haber et al. 2010). In this system, predators generalize the un-palatability of the white, blue, and yellow coloration, which leads to reduced predation for all of the species of nudibranchs involved. In contrast, batesian mimicry occurs when a harmless and palatable species (the mimic) has similar coloration to a harmful or unpalatable species (the model), resulting in reduced predation on the mimic (Rainey and

Grether 2007). An example of batesian mimicry in the marine environment is the morphological structure of many larval teleost fishes, whose appendages resemble harmful and unpalatable jellyfish (scyphozoans; Greer et al. 2016). Another form of mimicry is camouflage, in which the mimic resembles an inanimate object in order to avoid detection by predators, such as the seagrass shrimp *T. carolinense* described previously. Lastly, aggressive mimicry occurs when the mimic takes advantage of the model's coloration or behavior in order to gain resources (the mimic is the predator and not the prey), such as the aggressive mimic (fangblenny *Plagiotremus rhinorhynchos*) of a major cleaner fish (*Labroides dimidiatus*). The fangblenny poses as a cleaner fish, but instead of cleaning client fishes, it bites off scales and mucus, thus both obtaining food and injuring the client fish (Cheney and Cote 2007).

In addition to the four major types of mimicry mentioned above, a unique potential type of mimicry may occur when a mimic copies the color patterns and/or behaviors of a model species that provides beneficial services to other community members, in order to gain the protective benefits enjoyed by the model species. In this potentially fifth major type of mimicry, the mimic gains protection from predation by appearing to be a beneficial species, rather than a toxic or harmful one. This type of system could function similarly to batesian mimicry, but with a twist in which predators avoid consuming the mimic not because they perceive it to be harmful, but because they perceive it to be beneficial. This type of mimicry system could occur in cleaning symbioses, in which a small non-cleaner mimics a cleaner to avoid predation by large clients attracted cleaning stations to be cleaned. This type of protective mimicry has been observed in some populations of cleaner fish mimic, the saber-toothed blenny *Aspidontus taeniatus* in the indo-pacific. In these populations, fish rarely prey on client fish, but rather obtain

food from other food sources (Cheney et al. 2014). Other populations of *A. taeniatus* on the other hand do frequently prey upon client fishes as aggressive cleaner fish mimics.

The purpose of my thesis is to investigate the relationship between the cleaner shrimp *Ancylomenes pedersoni* as a model species in a potential exclusively protective mimicry system (no aggression), and its mimic the spotted "cleaner" shrimp *Periclimenes yucatanicus* in the Caribbean Sea. Both shrimp species associate with two sea anemone species *Bartholomea annulata*, and *Condylactis gigantea,* which serve as long-range cues to client fishes who use them to locate cleaning stations (Huebner and Chadwick 2012). Both shrimps also have similar coloration patterns, and display similar behaviors (rocking body and waving of antennae) toward client fishes. Individuals of *A. pedersoni* are the only cleaner shrimp in the Caribbean that have been shown to significantly reduce client fish parasite loads (Bunkley-Williams and Williams 1998; McCammon et al. 2010). In contrast, *P. yucatanicus* does not appear to perform any substantial cleaning of fishes, although its color patterns and behavioral signals suggest cleaner status. As such, the latter shrimp may be a beneficial mimic of the cleaner shrimps that co-occur with it on host anemones. For my Master's thesis, I examined the extent of this potential beneficial mimicry through comparison of the body coloration patterns, predation pressure, utilization of anemone microhabitats, and propensity to behaviorally signal towards client fishes in each of these 2 anemoneshrimp species.

In Chapter 2, I measured the coloration of *A. pedersoni*, *P. yucatanicus*, and their host anemones *Bartholomea annulata* and *Condylactis gigantea* to assess how contrasting the shrimps were against their host anemone backgrounds. Additionally I measured the predation pressure of *A. pedersoni* and *P. yucatanicus* as well as a non-cleaner *Alpheus armatus* in-situ on a coral reef as a function of their coloration. In Chapter 3, I measured the average distance *A.*

pedersoni and *P. yucatanicus* reside away from their host anemones *B. annulata* and *C. gigantea* in order to assess their dependence on the anemone host for protection. I also measured the propensity of the shrimps to physically signal to potential client fish, as an indicator cleaner status, or willingness to clean. Together these two chapters provide different facets to the potential mimicry of *A. pedersoni* by *P. yucatanicus*, and complement each other in that Chapter 2 analyzes the color mimicry and the degree of protection it provides, whereas Chapter 3 analyzes the shrimps dependence on the host anemone for protection as an indicator of the shrimps confidence in their protected cleaner status, along with behavioral mimicry of physical signaling (waving of antennae) to client fishes as an indicator of willingness to clean as complement to the color mimicry.

References

Bauer, R. T. (2004). Remarkable shrimps: Adaptations and natural history of the carideans. Norman: University of Oklahoma Press.

Bohnenstiehl, D. R., Lillis, A., & Eggleston, D. B. (2016). The curious acoustic behavior of estuarine snapping shrimp: Temporal patterns of snapping shrimp sound in sub-tidal oyster reef habitat. *PLOS ONE, 11*(1), e0143691. http://dx.doi.org/10.1371/journal.pone.0143691

Booksmythe, I., Milner, R. N. C., Jennions, M. D., & Blackwell, P. R. Y. (2010). How do weaponless male fiddler crabs avoid aggression? *Behavioral Ecology and Sociobiology*, *64*(3), 485-491. doi: 10.1007/s00265-009-0864-9

Bunkley-Williams, L., & Williams, E. H. (1998). Ability of Pederson cleaner shrimp to remove juveniles of the parasitic cymothoid isopod, *Anilocra haemuli*, from the host. *Crustaceana*, *71*(8), 862-869. http://www.jstor.org/stable/20106067

Chapuis, L., & Bshary, R. (2010). Signalling by the cleaner shrimp *Periclimenes longicarpus*. *Animal Behaviour*, *79*(3), 645-647. doi: 10.1016/j.anbehav.2009.12.012

Cheney, K. L., Cortesi, F., How, M. J., Wilson, N. G., Blomberg, S. P., Winters, A. E., Umanzor, S., & Marshall, N. J. (2014). Conspicuous visual signals do not coevolve with increased body size in marine sea slugs. *Journal of Evolutionary Biology*, *27*(4), 676-687. doi: 10.1111/jeb.12348

Cheney, K. L., & Côté, I. M. (2007). Aggressive mimics profit from a model-sign receiver mutualism. *Proceedings of the Royal Society B*, *274*(1622), 2087-2091. doi: 10.1098/rspb.2007.0543

Cheney, K. L., Grutter, A. S., Blomberg, S. P., & Marshall, N. J. (2009). Blue and yellow signal cleaning behavior in coral reef fishes. *Current Biology*, *19*(15), 1283-1287. doi: 10.1016/j.cub.2009.06.028

Cournoyer, B. L., & Cohen, J. H. (2011). Cryptic coloration as a predator avoidance strategy in seagrass arrow shrimp colormorphs. *Journal of Experimental Marine Biology and Ecology*, *402*(1), 27-34. doi: 10.1016/j.jembe.2011.03.011

Diaz, E. R., & Thiel, M. (2004). Chemical and visual communication during mate searching in rock shrimp. *The Biological Bulletin, 206*(3), 134-143. doi: 10.2307/1543637

Greer, A. T., Woodson, C. B., Guigand, C. M., & Cowen, R. K. (2016). Larval fishes utilize Batesian mimicry as a survival strategy in the plankton. *Marine Ecology Progress Series*, 551, 1-12. doi: 10.3354/meps11751

Haber, M., Cerfeda, S., Carbone, M., Calado, G., Gaspar, H., Neves, R., Maharajan, V., Cimino, G., Gavagnin, M., Ghiselin, M. T., & Mollo, E. (2010). Coloration and defense in the nudibranch gastropod *Hypselodoris fontandraui*. *The Biological Bulletin, 218*(2), 181-188. doi: 10.1086/BBLv218n2p181

Huebner, L. K., & Chadwick, N. E. (2012). Patterns of cleaning behavior on coral reef fish by the anemoneshrimp *Ancylomenes pedersoni. Journal of the Marine Biological Association of the United Kingdom, 92(*7) 1557-1562. http://dx.doi.org/10.1017/S0025315411001822

Johnsen, S. (2001). Hidden in plain sight: The ecology and physiology of organismal transparency. *The Biological Bulletin, 201*(3), 301-338. doi: 10.2307/1543609

Lettieri, L., Cheney, K. L., Mazel, C. H., Boothe, D., Marshall, N. J., & Streelman, J. T. (2009). Cleaner gobies evolve advertising stripes of higher contrast. *Journal of Experimental Biology*, *212*, 2194-2203. doi: 10.1242/jeb.025478

McCammon, A. M., & Brooks, W. R. (2014). Protection of host anemones by snapping shrimps: A case for symbiotic mutualism? *Symbiosis*, *63*(2), 71-78. doi: 10.1007/s13199-014-0289-8

McCammon, A., Sikkel, P. C., & Nemeth, D. (2010). Effects of three Caribbean cleaner shrimps on ectoparasitic monogeneans in a semi-natural environment. *Coral Reefs*, *29*(2), 419-426. doi: 10.1007/s00338-009-0583-8

Pelosi, P., Iovinella, I., Felicioli, A., & Dani, F. R. (2014). Soluble proteins of chemical communication: An overview across arthropods. *Frontiers in Physiology, 5*(320), 1-13. http://dx.doi.org/10.3389/fphys.2014.00320

Rainey, M. M., & Grether, G. F. (2007). Competitive mimicry: Synthesis of a neglected class of mimetic relationships. *Ecology, 88*(10), 2440-2448. doi: 10.1890/06-1717.1

Stummer, L. E., Weller, J. A., Johnson, M. L., & Côté, I. M. (2004). Size and stripes: How fish clients recognize cleaners. *Animal Behaviour*, *68*(1), 145-150. doi: 10.1016/j.anbehav.2003.10.018

Vannini, M. (1985). A shrimp that speaks crab-ease. *Journal of Crustacean Biology*, *5*(1), 160-167. Retrieved from http://www.brill.com/journal-crustacean-biology

Versluis, M., Schmitz, B., von der Heydt, A., & Lohse, D. (2000). How snapping shrimp snap: Through cavitating bubbles. *SCIENCE*, 289(5487), 2114-2117. doi: 10.1126/science.289.5487.2114

Vickery, R., Hollowell, K., & Hughes, M. (2012). Why have long antennae? Exploring the function of antennal contact in snapping shrimp. *Marine and Freshwater Behaviour and Physiology*, 45(3), 161-176. http://dx.doi.org/10.1080/10236244.2012.699644

Wicksten, M. K. (1998). Behaviour of cleaners and their client fishes at Bonaire, Netherlands Antilles. *Journal of Natural History*, *32*(1), 13-30. http://dx.doi.org/10.1080/00222939800770021

Chapter 2

Color Mimicry, Cleaning Interactions, and Predation Pressure in a Cleanershrimp Mutualistic Network

Introduction

Many types of organisms use visual signals to attract symbiotic partners and facilitate the transfer of mutually beneficial services. For example, flowering plants may attract visually-oriented pollinators such as insects and birds by displaying distinctive color patterns, including those that reflect ultraviolet wavelengths (Horth et al. 2014). Visual signals between partners also are important in cleaning mutualisms, in which cleaners attract clients and remove external parasites from them (Grutter 1997). In all known cases of cleaning mutualisms, the clients are relatively large, mobile, visually-oriented vertebrates such as ungulate mammals, reptiles, or fishes, and the cleaners are relatively small, also visually-oriented organisms such as birds, fishes, or crustaceans (Becker and Grutter 2004; Bradshaw and White 2006; McCammon et al. 2010). Benefits to cleaners who visually signal their status to clients, which allows the clients to distinguish them clearly from prey or micropredators, have led to the evolution of unique patterns of body coloration and behavior in cleaner organisms, especially among cleaner fishes (Stummer et al. 2004; Cheney et al. 2009; Lettieri et al. 2009). Cheating also has evolved in cleaner fish systems, in which some small fishes mimic the visual signals of cleaners to attract clients, but consume scales or mucus from client fishes instead of removing their ectoparasites (Oates et al. 2012). Mimicry in cleaner shrimp mutualisms has not yet been explored, including the functions and evolution of color patterns versus behavioral signals by cleaner shrimps and their potential mimics.

Cleaning mutualisms are important for coral reef fishes, because cleaners effectively remove common ectoparasites such as gnathid isopods and monogenean flatworms that may infest their skin and gills (Grutter and Poulin 1998; Grutter et al. 2002), resulting in reduced parasite loads (Cheney et al. 2009; Huebner and Chadwick 2012b). This parasite removal improves the overall health of client fishes and enhances their diversity on reefs (Bshary 2003; Grutter et al. 2003; Waldie et al. 2011; Sun et al. 2012). Most studies of cleaning symbioses on coral reefs have focused on the small fishes that clean larger client fishes (reviewed in McCammon et al. 2010), but crustaceans such as shrimps also potentially can serve as effective fish cleaners (Becker and Grutter 2004; Ostlund-Nilsson et al. 2005; Chapuis and Bshary 2009; McCammon et al. 2010). The ability to consume fish parasites may vary widely among potential cleaner shrimps; a recent study revealed that among three species of reported cleaner shrimps in the Caribbean Sea (*Ancylomenes pedersoni*, *Periclimenes yucatanicus*, and *Stenopus hispidus*), only *A. pedersoni* significantly reduced the size and abundance of monogenean ectoparasites *Neobenednia melleni* from blue tang fish *Acanthurus coeruleus* (McCammon et al. 2010). Laboratory trials also indicated that when four reported species of cleaner fishes plus four cleaner shrimps were examined for their ability to remove cymothoid isopods *Anilocra haemuli* from French grunts *Haemulon flavolineatum*, only individuals of *A. pedersoni* significantly reduced the parasitic isopods loads on fish (Bunkley-Williams and Williams 1998). Thus, some shrimps (and even fishes) that have been described as cleaners may not effectively clean fishes at an ecological scale on Caribbean coral reefs. Some of these species may instead function as cleaner mimics. On Indo-Pacific reefs, an aggressive mimic (the fangblenny *Plagiotremus rhinorhynchos*) of a major cleaner fish (*Labroides dimidiatus*) has evolved to take advantage of client fish trust in the cleaner-client relationship (Cheney and Côté 2007). The visual similarity

of this mimic to the cleaner varies with the perceptive abilities of the client fishes; members of some client species are better able than others to distinguish between the mimics and the true cleaner (Cheney and Marshall 2009). Based on both their visual and behavioral signaling similarity, as well as their obligate symbiosis with the same sea anemone hosts, two species of anemoneshrimps in the Caribbean Sea may form a cleaner-mimic system, in which *A. pedersoni* is the true cleaner, and *P. yucatanicus* is the mimic (Fig. 1). Only one instance of cleaning has been reported of *P. yucatanicus* actually removing parasites, from a single Nassau grouper in Pine Cay, off the Caicos Bank in the Caribbean (Spotte et al. 1991). Subsequent replicated trials have indicated no substantial cleaning by this shrimp, in contrast to *A. pedersoni*, which was observed to consume large amounts of fish parasites (McCammon et al. 2010). I hypothesize that individuals of *P. yucatanicus* may receive benefits by visually and behaviorally mimicking *A. pedersoni*, including avoidance of predation by fishes, without providing any regular services to client fishes that approach sea anemone cleaning stations on Caribbean coral reefs.

In addition to mimicry of cleaners by non-cleaning species, some true cleanerfishes also cheat by occasionally consuming the body tissues or mucus of clients rather than their parasites (Grutter and Bshary 2003). Punishment of cheaters by client fishes reduces this occurrence (Bshary and Bronstein 2004; Soares et al. 2010), but variation in the home range size of cleanerfishes mediates the ability of clients to recognize and punish repeat-offenders (Oates et al. 2012). For example, individuals of the cleanerfish *L. dimidiatus* have small home ranges; when they cheat resident fishes, the latter punish them by chasing the offender (Bshary and Grutter 2002) or visiting other cleaning stations, a control mechanism known as partner switching (Bshary and Schäffer 2002; Bshary and Grutter 2005; Oates et al. 2012). Individuals of congeneric cleanerfish *L. bicolor* on the other hand have large home ranges, and thus are able to

avoid repeat interactions and punishment by resident fishes, allowing them to eat their preferred meal of fish mucus without repercussions (Oates et al. 2012). Some cleaner shrimps also are fairly mobile and can move among cleaning stations (Mahnken 1972; Chadwick et al. 2008), but probably not on a scale large enough to avoid repeated interactions with resident fishes. Some anemoneshrimps such as *Lysmata grabhami* that stray from their host sea anemones *Telmatactis crioides* are consumed by nearby fishes (Wirtz 1997; Bauer 2004). These factors (small body size, low mobility relative to cleaner fishes, and dependence on anemones for shelter from predation) may pressure cleanershrimps such as *A. periclimenes* to provide higher quality cleaning services with less cheating than is present in cleanerfish systems. This would allow *P. yucatanicus* to persist as a cheating mimic of *A. periclimenes,* especially if client fishes do not visually (or behaviorally) distinguish the difference between these two shrimps. It is possible that *P. yucatanicus* uses a form of Batesian mimicry to passively avoid predation rather than to attack incoming fish clients (in contrast to the cleanerfish mimic system), given the evidence that *P. yucatanicus* rarely approaches fishes (Stuart personal observations).

Visual cues used by cleaner shrimps to communicate with fish clients are similar to those used by cleaner fishes, but differ from them in several important aspects. Most cleaner shrimps are substantially smaller (2–5 cm total length, Chockley and St. Mary 2003) than cleaner fishes (4–15 cm, Côté 2000), but both tend to be brightly colored (Marshall 2000; Losey et al. 2003; Humann and DeLoach 2006; Siebeck and Marshall 2007; Cheney et al. 2009), and to advertise their services by perching near or on large symbiotic hosts such as sea anemones, sponges, or massive corals, or on prominent physical features of coral reefs (knolls or large crevices, Soares et al. 2008a; Huebner and Chadwick 2012b; Mascaro et al. 2012). Association with a large reef organism or prominent physical feature may enhance the visibility of small cleaners to passing

clients (Huebner and Chadwick 2012a), as well as the ability of clients to relocate them for repeat visits (Potts 1973; and Huebner and Chadwick 2012a). However, patterns of body coloration differ markedly between cleanerfish and shrimps. A cleaning coloration clade has evolved among the 15 obligate and 84 facultative species of coral reef cleaner fishes (Côté 2000), in which they all exhibit a variation of black, blue and/or yellow lateral stripes that clearly advertise their status (Stummer et al. 2004; Lettieri et al. 2009; Cheney et al. 2009). In contrast, cleaner shrimps (6 obligate and 18 facultative species, Côté 2000) do not all possess uniform coloration patterns (Humann and DeLoach 2006; Stuart, personal observations); Caribbean cleaner shrimps may display either blue and white lateral stripes (*A. pedersoni*), blue-violet and white spots (*P. yucatanicus*), red and white lateral stripes (*L. grabhami*), red and white bands (*S. hispidus*), or a yellow body with red and white bands (*S. scutellus*, Figure 2a-d). This color variation may be due in part to the low visual resolution abilities of reef fishes which could prevent them from clearly discerning the color patterns of small cleaner shrimps at a distance, and cause them to utilize more obvious visual cues such as their large symbiotic hosts (Marshall 2000; Huebner and Chadwick 2012a,b). The host organisms upon which cleaner shrimps perch (sponges, corals, and sea anemones) also vary in body coloration, shape, and size, and thus in the visual contrast that they offer as backgrounds against which cleaner shrimps display. The coloration clade of cleaner shrimps thus may need to be broader and more varied than that of cleaner fishes, due to their smaller body size and more varied distinct background habitats than in cleaner fishes. The cleaner shrimp color clade thus usually consists of a transparent body with some variation of blue or purple stripes or spots, and long white antennae (Huebner and Chadwick 2012b, Fig. 2). These color patterns allow them to stand out visually, because both

blue/violet and white (in contrast to red or orange) offer high contrast against typical coral reef backgrounds (Letteri 2009).

Individuals of *A. pedersoni* clean members of at least 16 fish families (Huebner and Chadwick 2012b). In the U.S. Virgin Islands, individuals of *A. pedersoni* spend most of their time cleaning surgeonfishes (Acanthuridae), goatfishes (Mullidae) groupers (Serranidae), and parrotfishes (Scaridae, Huebner and Chadwick 2012b), and in Bonaire their main clients also include groupers (Serranidae) and parrotfishes (Scaridae), as well as damselfishes (Pomacentridae, Wicksten 1998), with many of their less frequent visitors overlapping between both locations. Because *A. pedersoni* shrimp clean the members of many reef fish families, their color signals may have evolved to be visible to a wide range of fish viewers that differ in their visual systems.

To obtain a biologically-relevant perspective on how organisms appear visually to signal receivers in their natural environments, several factors must be taken into consideration (Fig. 4). Firstly, what is the spectral reflectance of the focal object (Endler et al. 2005; Lettieri et al. 2009; Cournoyer and Cohen 2011)? The focal objects for client fishes in cleanershrimp systems are likely to be the stripes and spots on the bodies of various species of cleanershrimps. Secondly, the spectral reflectance of the shrimp's background habitat needs to be characterized (Endler et al. 2005; Lettieri et al. 2009; Cournoyer and Cohen 2011). Depending on the background, the body colors of organisms vary in their visual contrast to viewers, a factor that is widely used by organisms in developing camouflage (Cournoyer and Cohen 2011). A receiver independent analysis of anemoneshrimp and host sea anemone backgrounds can be used to analyze how they appear visually to fishes, due to the wide array of visual systems employed by client fishes on reefs. Additionally, the light environment on coral reefs typically is characterized by clear blue

water and intense solar radiation (McFarland and Munz 1975; Barry 1996; Barry and Hawaryshyn 1999), but varies widely with depth, turbidity, and whether or not the sky is clear or overcast, resulting in a high degree of spectral complexity (Stambler and Shashar 2007). Though there is a high degree spectral complexity in the marine environment, the cleaner shrimp *A. pedersoni* and potential mimic *P. yucatanicus* usually are located at depths shallower than 20 m, where the water is clear and visibility is high (Humann and DeLoach 2006). As such, the perception of visual signals by client fishes may be examined without taking into account variation in the ambient light environment or in visual systems among the different species of potential client fishes similar to the complex light environment and multiple receiver visual systems wolf spiders encounter described in Clark et al. (2011).

Understanding how cleaner shrimps effectively attract their clients, clean them, and avoid predation can contribute to revealing some of the mechanisms that contribute to high fish biodiversity on coral reefs, as aspects of effective mutualisms that control fish parasites. This type of information also may provide a more a scientific basis to support conservation management on coral reefs, by revealing the importance of visual communication in cleaner shrimp – client interactions. Understanding of the role of cleaner shrimp coloration and its ecological importance will be especially helpful in conservation efforts if it can be used to monitor and assess overall reef health. This type of information can reveal potentially negative consequences of anthropogenic changes to water quality and transparency on coral reefs, such as changes due to nutrient pollution and sediment runoff (Partridge and Cummings 1999), in terms of their impacts on the transmission and receipt of visual signals crucial to the success of cleaning interactions. Extensive evidence exists concerning the detrimental impacts of low water clarity on the visual foraging abilities of fishes (Vineyard and O'Brien 1976; Eggers 1977;

Aksnes and Utne 1997; Utne 1997; Sornes and Aksnes 2004; Aksnes 2007). Reef fishes likely use similar visual mechanisms to locate cleaning stations; thus, their abilities to use parasite cleaning services may be severely impacted by anthropogenically-reduced water clarity on reefs.

In the present study, I addressed two major hypotheses concerning body coloration and visual signaling in cleaning interactions between client fishes and known cleaner shrimp *A. pedersoni* versus potentially evolving cleaner mimics *P. yucatanicus*, which are obligate crustacean associates of sea anemones on Caribbean coral reefs:

1. Caribbean cleaner shrimp *A. pedersoni* exhibit higher visual contrast against typical background habitats, and thus transmit more robust color signals to client fishes, than do potential mimics *P. yucatanicus.*
2. Visual characteristics of *A. pedersoni* deter predation by approaching client fishes, more so than do those of potential mimics *P. yucatanicus*, which deter more than those of other non-mimic anemoneshrimps.

To address these hypotheses, I quantified cleaner shrimp and host anemone coloration patterns through reflectance measurements and assessed how they contrast against each other. I then conducted a field experiment to quantify predation pressure on both cleaner and non-cleaner shrimps, which revealed the types of shrimp visual characteristics that are most effective in attracting client fishes and in causing them to pose for cleaning, and also to deter their predation on anemoneshrimps.

Methods

Animal Sources and Laboratory Culture

Sea anemones (*Bartholomea annulata* and *Condylactis gigantea*) and anemoneshrimps (*Ancylomenes pedersoni* and *Periclimenes yucatanicus*) were obtained from commercial sources: KP Aquatics Inc, (Key Largo, Florida, USA), Preuss Pets (Lansing, Michigan, USA), and Dynasty Marine (Marathon Key, Florida, USA), or were collected by hand from shallow nearshore habitats in the Florida Keys, Florida, USA. Field-collected animals were placed in 1 L Ziploc bags, transferred to 19 L buckets of seawater aerated with battery-powered bubblers, and transported by auto to Auburn University (AU) within 24 hours of collection. Upon arrival to AU, all organisms were housed in closed-system 80 L tanks connected to 80 L sump tanks supplied with artificial seawater and kept on a 12:12 h light:dark photoperiod using high-output fluorescent lighting (Sunlight Supply, Pompano, FL, USA; for culture details see Roopin and Chadwick 2009; Huebner et al. 2012; Cantrell et al. 2015). Anemones and shrimps were cultured together in each tank, in natural groupings that reflected their associations in the field as members of a mutualistic network (Cantrell et al. 2015). Shrimp were fed every other day with Formula One Marine Pellets (Ocean Nutrition, San Diego CA, USA), and anemones were fed once a week with pieces of raw cocktail shrimp (after Szczebak et al. 2013). Shrimps were weighed regularly to obtain wet mass, and anemone tentacle crown surface area were measured to monitor health and growth. All animals maintained body size or grew, and many of the shrimps produced larvae, indicating that they were healthy, similar to our previous observations on these organisms in the same culture system (Cantrell et al. 2015).

Measurement of Color Characteristics

Color characteristics of the anemones and shrimps were quantified using reflectance measurements (after Lettieri et al. 2009; Clark et al. 2011; and Pike et al. 2011) obtained with an Ocean Optics JAZ Spectrophotometer and Ocean Optics SpectraSuite 1.0(1.0) Software (Ocean Optics, Inc., Dunedin, FL, USA). Reflectance readings were taken at a 45° angle using the bare end of a 200mm fiber optic cable. A PX-2 Xenon illuminator (Ocean Optics, Inc., Dunedin, FL, USA), capable of emitting a spectral range of 300-700 nm was used as the light source for the reflectance readings, which included the biologically-important UV portion of the spectrum (Endler 1993; Barry and Hawryshyn 1999; Marshall 2000; Siebeck and Marshall 2001; Endler et al. 2005; Cheney et al. 2009) as well as the visible spectrum that matches the visual capabilities of fishes (Vorobyev and Osorio 1998; Vorobyev et al. 2001; Lettieri et al. 2009; Cournoyer and Cohen 2011). Two types of color patches were sampled on the shrimps, the dorsal abdomen spot and the uropod spot (Fig. 1), because these were the largest and most brightly-colored patches on their bodies, and thus potentially the most-used characteristics of the shrimps as color signals for reef fishes, similar to the blue, black, and yellow lateral stripes of cleaner fishes (Stummer et al. 2004; Lettieri et al. 2009; Cheney et al. 2009). On the sea anemones, the tentacles and oral disc were sampled as the background areas against which the shrimp color patterns were visible to reef fishes, because these were the areas of their bodies that protruded from reef holes and were visible to passing fishes, and comprised the microhabitats where the obligate anemoneshrimps normally resided under natural conditions (Briones-Fourzan et al. 2012; Huebner et al. 2012 a, b; Stuart personal observations).

Three spectral readings were taken from each of the two selected areas on the body of each shrimp and anemone, and averaged to obtain a mean reading for that color patch/area on

that individual (after Clark et al. 2011). Reflectance readings for the shrimp and anemones were obtained by removing animals from their culture tanks or containers (field collected shrimps) and placing them on a matt black piece of felt cloth in a darkened room (similar to Lettieri et al. 2009). The reflectance probe was placed on each animal for about 60 seconds total (2 locations x 3 scans), and then the animals were returned to their tanks or host anemones in the field. Shrimps and anemones fully recovered from this process within a couple minutes, as evidenced by calm behavior (shrimps) and by re-expansion of tentacles (anemones). The reflectances of 13 *A. pedersoni*, and 5 *P. yucatanicus* were measured in the field at Marathon and Long Key, Florida, where they were collected and sampled within 20 minutes after collection during March 2014. In addition, 11 *A. pedersoni*, and 21 *P. yucatanicus* were obtained from suppliers and their reflectances measured at Auburn University. Reflectance patterns did not differ between the field- and lab-measured shrimps (ie: the distribution of their color-scores were similar) so the reflectance results were presented as a group for 24 *A. pedersoni* and 26 *P. yucatanicus* total. Additionally 21 individuals each of *Bartholomea annulata* and *Condylacis gigantea were* obtained from suppliers and measured at Auburn University.

Statistical Analysis of Spectrophotography Data

The spectrophotometric data included information on brightness (intensity, or the total area under the spectral curve), hue (the shape of the curve, revealing the types of colors emitted), and chroma (a measure of the dominant wavelength of the spectra; Endler 1990; Cuthill et al. 1999; Clark et al. 2011). For the purposes of the present study, I defined color contrast as the difference in spectral shape (hue) between the shrimp body parts (abdomen and uropod spots) and the host anemone background body parts (oral disc and tentacles), expressed independently of intensity (after Cuthill et al. 1999). I also defined brightness (intensity) contrast as the

difference in the total area underneath the spectral curve of the shrimp body parts versus the background anemone body parts (after Clark et al. 2011).

I then used two common methods to statistically assess variation in the reflectance data: principal component analysis (PCA; Cuthill et al. 1999; Grill and Rush 2000; Macedonia 2001; Clark et al. 2011), and color segment classification analyses for graphical representation of spectral variation (Endler 1990; Clark et al. 2011). Because the spectral data were complex (for each sample there are 400 values, one for each wavelength measured, 300-700nm), PCA allowed the 400 data points per sample to be summarized into a couple orthogonal values. Before PCA was conducted, the raw data were condensed to 20nm increments using the median value in each bandwidths to avoid artificial spikes in the spectra associated with spectra means (after Cuthill et al. 1999). I then subtracted mean reflectance from all 400 wavelengths to standardize the condensed spectra, because most of the variation in spectral measurements is due to brightness differences; by standardizing the spectra, I was able to analyze the spectral shape (hue) independent of intensity (Cuthill et al. 1999). Included in the spectral analysis were data from 13 *A. pedersoni* abdomen spots, 24 *A. pedersoni* uropod spots, 25 *P. yucatanicus* abdomen spots, 26 *P. yucatanicus* uropod spots, 13 *B. annulata* oral discs, 21 *B. annulata* tentacles, 20 *C. gigantea* oral discs, and 21 *C. gigantea* tentacles, for a total of 163 different spectra. Differences in sample sizes among the groups were due to some shrimps lacking abdomen or uropod spots, and the anemones retracting (specifically *B. annulata*) preventing the measurement of the oral disc. To test the differences among the PC scores, a general linear model with a random effect for individuals was used to examine pairwise differences between shrimp and anemone body parts for the PC1 and PC2 scores. For detailed methods on the color score differences in chroma, and

color contrast analyses (euclidean distance, and brightness differences), see Clark et al. 2011 (substitute shrimps and anemones for spiders and leaf litter).

Field experiment

A field experiment was conducted during July 13 – 19, 2015, on shallow coral reefs (6 to 30 m depth) in Brewer's Bay, St. Thomas, U. S. Virgin Islands (see Huebner and Chadwick 2012 a, b for detailed study site description). The experiment was conducted using SCUBA, with the MacLean Marine Science Center (MMSC) of the University of the Virgin Islands as a base for operations, in accordance with AAUS (American Academy of Underwater Sciences) scientific diving standards.

The experiment focused on determining how reef fish behavior varied toward three species of obligate anemoneshrimps when they were physically separated from their anemone hosts *B. annulata*, and thus not physically protected by the host. For purposes of the experiment, I classified the shrimps as: "cleaner" (*A. pedersoni*), "cleaner mimic" (*P. yucatanicus*), and "non-cleaner" (*Alpheus armatus*, after Huebner and Chadwick 2012a,b; Briones-Fourzan et al. 2012). Individuals of *B. annulata* (N = 20) that contained *A. pedersoni* shrimps were selected haphazardly at the study site, and marked with a numbered small orange flag. The largest cleaner shrimp *A. pedersoni* associated with each marked sea anemone was collected using a hand net, and later returned to its home anemone immediately after each experiment (N = 20 individuals of *A. pedersoni*). Due to their relatively low abundances at the study site, only three individuals each of *P. yucatanicus* and *A. armatus* were collected from different anemones other than the marked anemones. These latter 6 shrimps then were transferred to a flow-through seawater tank at the MMSC and placed individually in a 0.5 L plastic container with holes cut in it to allow

water flow to maintain them overnight between the consecutive days during which the field experiment was conducted (3-4 trials per day for 6 days). These shrimp were re-used in the experiments, because the trials examined the behavioral reactions of fishes, and the shrimps were only visual cues, so their species identity (ie: coloration patterns) but not their individual identity was expected to affect the experimental outcomes.

To conduct each experimental trial, 1 individual of each shrimp species was transferred separately into a 0.5 L Ziploc plastic bag, and the bag was fully inflated with seawater using a turkey baster to ensure that there were no wrinkles in the bag and to allow the shrimp to move about freely, and the bag was sealed shut. Each shrimp thus was surrounded by enough water for normal respiration during each trial, which lasted 1 hour (similar to the 250 ml beaker of seawater for each shrimp in excretion measurements for 2 hours, after Cantrell et al. 2015). Additionally a small quantity of air was added to each bag using a SCUBA regulator to keep the bag buoyant, and then the bag was tethered to a small weight (~100g steel nut) with clear fishing line about 20 cm long. To begin each trial, the 3 bags each containing 1 shrimp (either *P. yucatanicus*, *A. pedersoni*, or *A. armatus*), plus 1 control bag that contained only seawater and air (N = 4 bags total) each were placed either near to (adjacent to and touching) or far from (1 m distant) one of the 20 marked sea anemones at the field site. The order of near versus far trials conducted at each of the 20 cleaning stations (sea anemones) examined was randomized. A GoPro Hero4 Black® edition set to 1080p wide view at 30 fsp was mounted on a weighted tripod, and placed 1 m distant from the bags and left running for the duration of each trial. Additionally, during each trial the behavioral reactions of fish clients were quantified via direct observation by a diver who remained motionless on the bottom at 3m distance from the bags, which is far enough away to not impact normal reef fish behavior (after Huebner and Chadwick 2012). The

behaviors of passing fishes toward the bags (both those containing shrimps and the empty control bag) were quantified as: frequency and duration of orientation toward the bag (fish motionless and oriented so that it faced the bag), frequency and duration of posing (fish with body motionless in head-down position near bag, used by fish to signal readiness for parasite cleaning (Limbaugh et al. 1961), frequency of attack (one or more instances of fish striking or biting the bag in rapid succession; used to indicate attempted predation on the shrimp or the empty bag), the number of strikes per attack (number of repeated instances of biting the bag in rapid sequence during an attack), and number of bubble attacks (fish clearly biting bubbles in the bag, and not the shrimps or empty control bag; after Arvedlund and Neilsen 1996; Huebner and Chadwick 2012). The recorded video footage and the direct observational notes by divers were analyzed together to quantify fish behaviors during the trials. After the first trial was completed on each focal anemone, the bags were moved to the other position (either near or far), and the process was repeated (25 minutes for each near and far position, 50 minutes total observed at each cleaning station per dive; 1 station per dive x ~ 3 dives per day x 7 days = 20 stations observed total). Trials were conducted about every 3 hours between 06:00 and 18:00 each day, to assess the effect of time of day on fish behavior, because at some anemone sites fish visitation and cleaning interactions may occur mostly in the morning when parasite abundances are high (Sikkel et al. 2006; Huebner and Chadwick 2012,), but at other sites the time of day may not be a significant factor for anemone-hosted cleaner shrimp cleaning services in the Caribbean (Titus et al. 2015). Additionally N = 5, 50m^2 video transects were conducted randomly through the study site to measure fish family abundance for the purposes of comparing general fish abundance to fish behaviors towards the treatments. A 50m transect tape was carefully laid out across the reef, and a GoPro Hero3 silver edition set to 1080p wide view at 30 fsp was hand held by a diver

pointed along the transect tape for each of the 1m x 50m transects. Mean fish family abundance / $50m^2$ was then calculated for the study site (abundance data used with permission from Jessica Gilpin).

Statistical Analysis of the Field Experiment

The total number of orients, duration oriented, number of attacks, and number of bites/attack towards the 4 types of bag treatments (with each shrimp *A. pedersoni*, *P. yucatanicus*, *A. armatus*, and the control) were statistically analyzed using pairwise comparisons of a general linear model with a random effect for trial site (anemone). The variables of distance of the bags from the anemone (near vs. far), time of day, near vs. far order, type of fish family interacting, individual identity of fish interacting, and feeding group (herbivore, invertivore, omnivore) of fish interacting did not significantly improve the fit of the model, so were not included. Additionally, the percent of all interactions (orients, attacks, bubble attacks, and no-interaction) were calculated to compare to mean fish abundances at the trial site.

All statistical analyses were conducted using R vs.3.1.1, R Core Team (2013; R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/).

Results

Spectral Color Analysis

The body color spots of cleanershrimp *A. pedersoni* exhibited a single large reflectance peak at 380-400nm, for spots on both the abdomen and uropod (Fig. 2a), indicative of bright blue coloration at both spot locations. The mimic shrimp *P. yucatanicus* exhibited multiple peaks at

340, 480, and 560nm on abdominal spots, and peaks at 360, 480, and 580nm on uropod spots (Fig. 2b), both of which indicated purple/pink coloration. The host sea anemone *B. annulata* produced peaks at 560 and 600nm on both the oral disc and tentacles, revealing yellow/tan coloration (Fig. 2c). In contrast, the host sea anemone *C. gigantea* peaked at 380, 600, and 640nm on the oral disc, corresponding to pink/purple coloration, as well as at 360, 500, and 580nm on the tentacles which characterized purple coloration (Fig. 2d).

The overall brightness (area under the reflectance curve) of the uropod was greater than the carapace for *A. pedersoni* for all wavelengths, and both of the *A. pedersoni* spots were less bright than both of the *P. yucatanicus* spots for all wavelengths (Fig. 2a,b). Conversely, the overall brightness of abdominal spots on *P. yucatanicus* was greater than for uropod spots, for all wavelengths. The overall brightness of the *B. annulata* oral disc was greater than for all other locations examined (on both shrimps and anemones), for all wavelengths (Fig. 2a-d). For *C. gigantea,* overall brightness of the tentacles was lower than on the oral disc and *P. yucatanicus* abdominal spots, but similar to the brightness of the *P. yucatanicus* uropod for all wavelengths (Fig. 2b,d). When compared to the *A. pedersoni* uropod spots, the overall brightness of both the *C. gigantea* tentacles and oral disc was lower between 300-450nm, but higher between the wavelengths of 450-700nm (Fig. 2a,d).

The spectral values of body spots on both the *A. pedersoni* and *P. yucatanicus* uropods (Figs. 3a and 4a) and abdomens (Figs. 3b and 4b) revealed contrasting patterns when plotted in color space (according to the segment classification method: Endler 1990; Cuthill et al. 1999; Clark et al. 2011) together with those of the anemone *B. annulata* and *C. gigantea* oral discs and tentacles (Figs. 3a,b and 4 a,b, respectively). The anemone oral discs and tentacles exhibited considerable overlap within species, but occupied distinct color space regions between the two

anemone host species. The *A. pedersoni* uropod spots also showed some overlap with abdominal spots on the same shrimp species, but occupied distinctly different color space locations than did the *P. yucatanicus* uropod and abdominal spots. They also exhibited the greatest distance of all shrimp measurements from the color spaces of the host anemones, for both *B. annulata*, and *C. gigantea* on both their oral discs and tentacles. The color space of *A. pedersoni* overlapped somewhat with that of *P. yucatanicus* for both uropod and abdominal spots, but had no overlap with any of the anemone locations. In contrast, the color space exhibited by *P. yucatanicus* uropod spots overlapped considerably with both the abdomen spots on the same shrimp species, as well as with the oral disc and tentacles of one of the anemone hosts (*C. gigantea*) but not the other (*B. annulata*).

A general linear model analysis of Euclidean distances (color contrast) revealed a significant effect of the spectral locations of shrimp uropod (Fig. 5a), and abdominal spots (Fig. 5b) against the host anemone backgrounds ($F = 28.89$, d.f. = 284, $p < 0.0001$). The *A. pedersoni* uropod and abdomen spots both contrasted significantly more against all anemone backgrounds than did the corresponding *P. yucatanicus* spots ($t = -10.58$, d.f. = 284, $p < 0.0001$ (uropod against *B. annulata* oral disc); $t = -10.28$, d.f. = 284, $p < 0.0001$ (uropods against *B. annulata* tentacles); $t = -10.12$, d.f. = 284, $p < 0.0001$ (uropods against *C. gigantea* oral disc); $t = -9.61$, d.f. = 284, $p < 0.001$ (uropods against *C. gigantea* tentacles); $t = -6.55$, d.f. = 284, $p < 0.001$ (abdomens against *B. annulata* oral disc); $t = -6.56$, d.f. = 284, $p < 0.001$ (abdomens against *B. annulata* tentacles); $t = -5.61$, d.f. = 284, $p < 0.001$ (abdomens against *C. gigantea* oral disc); $t = -5.24$, d.f. = 284, $p < 0.001$ (abdomens against *C. gigantea* tentacles)). Additionally, *A. pedersoni* uropod and abdomen spots contrasted significantly more against *B. annulata* tentacles than they did against *C. gigantea* tentacles ($t = -4.26$, d.f. = 284, $p < 0.001$; $t = -3.04$, d.f. = 284, $p =$

0.0026 respectively), but there was no difference against the oral discs of the two anemone species. The *P. yucatanicus* uropod spots were significantly more contrasting against *B. annulata* tentacles than *C. gigantea* tentacles ($t = -3.38$, d.f. = 284, $p = 0.0008$), but not against the anemone oral discs. Conversely, *P. yucatanicus* abdominal spots contrasted significantly more against the oral discs of *C. gigantea* than of *B. annulata* ($t = 2.87$, d.f. = 284, $p = 0.0044$), but did not differ significantly against the anemone tentacle backgrounds.

In terms of color intensity (brightness), there was a significant effect of spectral location for both shrimp uropods (Fig. 6a) and abdomens (Fig. 6b) against the anemone backgrounds ($F = 21.91$, d.f. = 284, $p < 0.0001$). The mean brightness of all shrimp color spot locations was less than the mean brightness of all anemone body locations, resulting in all-negative values for intensity contrast. The *A. pedersoni* uropod and abdomen spot intensities were significantly more contrasting against all anemone backgrounds than the corresponding *P. yucatanicus* spot locations ($t = 1.259$, d.f. = 284, $p < 0.0001$ (uropod against *B. annulata* oral disc); $t = 1.890$, d.f. = 284, $p < 0.0001$ (uropod against *B. annulata* tentacle); $t = 2.153$, d.f. = 284, $p = 0.0323$ (uropod against *C. gigantea* oral disc); $t = 2.654$, d.f. = 284, $p = 0.0084$ (uropod against *C. gigantea* tentacle); $t = 2.797$, d.f. = 284, $p = 0.0055$ (abdomen against *B. annulata* oral disc); $t = 3.986$, d.f. = 284, $p = 0.0001$ (abdomen against *B. annulata* tentacle); $t = 4.475$, d.f. = 284, $p < 0.001$ (abdomen against *C. gigantea* oral disc); $t = 5.414$, d.f. = 284, $p < 0.001$ (abdomen against *C. gigantea* tentacle; Fig. 6a,b). Within species, the *A. pedersoni* uropod exhibited significantly higher intensity contrast against *B. annulata* than *C. gigantea* for both the oral discs and tentacles ($t = 3.588$, d.f. = 284, $p = 0004$; $t = 3.221$, d.f. = 284, $p = 0.0014$, respectively, Ffig. 6a). The *A. pedersoni* abdomen also displayed significantly higher intensity contrast against the *B. annulata* oral disc than the *C. gigantea* oral disc ($t = 2.010$, d.f. = 284, $p = 0.0454$), but no significant

difference against the tentacles (Fig. 6b). For *P. yucatanicus*, the uropod and abdomen had significantly higher intensity contrast against *B. annulata* than *C. gigantea* for oral discs and tentacles ($t = 5.980$, d.f. = 284, $p < 0.0001$ (uropod against oral disc); $t = 6.967$, d.f. = 284, $p < 0.0001$ (abdomen against oral disc); $t = 5.277$, d.f. = 284, $p = 0.0472$ (uropod against tentacles); $t = 6.070$, d.f. = 284, $p < 0.0001$ (abdomen against tentacles; Fig. 6a,b).

Most of the variation in raw spectral data (~90.0%) is due to differences in mean reflectance (Cuthill et al. 1999). Normalization of the data for reflectance allowed analysis of only the shape (hue) of the spectral curves; PC1 and PC2 of the normalized spectra thus described 79.4% (58.8% and 20.6% respectively) of variation in the shape of the spectral curves (Fig. 7). PC1 represented the relative amount of reflectance in long (> 500nm) to short wavelengths (< 500nm). For example, the *A. pedersoni* uropod displayed almost no reflectance in long wavelengths, but high amounts in short wavelengths (Fig. 2a), resulting in negative PC1 scores, whereas the majority of the *B. annulata* oral disc reflectance (Fig. 2c) was between 500-650nm, resulting in positive values. The PC2 score represented the slope of the curve at long wavelengths compared to short wavelengths; for example, the *A. pedersoni* uropod had a steep slope from 380-500nm (Fig. 2a), after which it leveled off resulting in negative PC2 values (Fig. 7a). Similarly, the *P. yucatanicus* uropod had a steep slope from 360-450nm, and a secondary peak from 500-650nm (Fig. 2b), which resulted in PC2 scores less negative than the *A. pedersoni* uropod PC2 scores (Fig. 7a).

For PC1 comparisons, the *A. pedersoni* uropods were significantly different than all other locations (on both shrimps and anemones; Table 1, Figs. 7a, 8a), and the abdomens were significantly different from all anemone locations, but not from the color locations of *P. yucatanicus* uropods and abdomens (Table 1, Figs. 7b, 8b). The *P. yucatanicus* uropods also

differed significantly from than all anemone locations, but not from the *P. yucatanicus* abdomens or the *A. pedersoni* abdomens (Table 1). Similarly, the *P. yucatanicus* abdomens were significantly different than all anemone locations, but not the *P. yucatanicus* uropods or *A. pedersoni* abdomens (Table 1). On the host anemones *B. annulata* and *C. gigantea*, the oral discs were not significantly different from each other, but both sets of tentacles were significantly different from all other locations (on both the shrimps and anemones, Table 1). For PC2 comparisons, the *A. pedersoni* uropods were significantly different than all other locations except the *B. annulata* oral discs and tentacles (Table 2, Figs. 7a, 8a). The *A. pedersoni* abdomen was significantly different than its uropod, and the *P. yucatanicus* abdomen, but not from other locations (Table 2, Fig. 7b, 8b). Additionally, the *P. yucatanicus* uropods were significantly different than the *P. yucatanicus* abdomen, but not from the other locations examined (Table 2). The anemones *B. annulata* and *C. gigantea* also did not differ significantly from each other, for all comparisons among their oral discs and tentacles.

Field experiment

Surveys at the field site on St. Thomas, USVI, revealed an overall abundance 213 fishes, or 0.852 total fish per square meter on the reef. Members of the family Gobiidae (gobies) occurred at the highest abundance (9.63 fish / m^2), followed by Pomacentridae (damselfishes, 7.62 fish / m^2), and Labridae (wrasses, 6.05 fish / m^2, Fig.9).

During the 40 experimental trials (1 near and 1 far at each of 20 sea anemone cleaning station sites), I observed 84 reef fishes in 13 families perform 148 interactions with the 4 bagged shrimp treatments (*Ancylomenes pedersoni*, *Periclimenes yucatanicus*, *Alpheus armatus*, and control, Table 3, Fig. 9). The most common fish families to interact with the experimental

treatments were members of the Pomacentridae (38.51% of interactions), followed by Serranidae (29.05% of interactions), and Labridae (27.70% of interactions).

During 8 of the trials (4 anemone locations), no fish entered the vicinity of the cleaning station for the duration of the trials, so these 8 were excluded from analysis of fish reactions, leaving 36 trials for analysis. In addition to the 84 fish who interacted with the treatments, an equal number of fishes (N = 84) swam near the treatment bags (within 50 cm) but did not stop to interact with them (Fig. 8). Most of the swim-by fishes belonged to the families Labridae (wrasses, 22.6%, N = 84), followed by Scaridae (parrotfishes, 16.7 %), Serranidae (11.9 %), and Pomacentridae (damselfishes, 11.9%), with the remaining 9 families making up the rest (39.6 %, Fig. 9). Thus, the total number of fish observed near the 16 cleaning stations was 168 (84 that interacted with the bags and 84 that did not).

During 6 of the trials (16.7 % of 36 trials where fish were present), a total of 10 cleaning interactions occurred, in which fishes visited and were cleaned by the free-living (not bagged) *A. pedersoni* shrimps that remained on the anemones near the experimental bag treatments. These cleaning interactions involved 9 fish from the family Serranidae (groupers) and 1 from the Synodontidae (inshore lizardfish). All 9 fish also interacted with the experimental bags, and thus were included in the 84 fish who engaged in a total of 148 behavioral interactions with the shrimps in the experimental bag treatments at the cleaning stations.

Of the 148 behavioral interactions observed between fishes and the experimental bag treatments, most (62.8%) involved fishes orienting toward the bags (ie: facing the bags). Some fish also attacked shrimp in the bags (23.6%), or attacked bubbles visible in the bags (13.5%, almost evenly distributed between the 4 types of treatment bags). Most of the fishes that oriented

toward the bags belonged to the families Labridae (wrasses, 31.2% of N = 93), Pomacentridae (damselfishes, 31.2% of N = 93) and Serranidae (groupers, 31.2% of 9 = 93), with 10 other fish families making up the remaining 6.4% (Fig. 10a). Even though the families Labridae, Pomacentridae, and Serranidae comprised equal percentages of all orient interactions, the composition of their responses to the four treatments differed (Fig. 10a). Most attacks were exhibited by Labrids (40% of N = 35), followed by Serranids (31.4% of N = 35) and Pomacentrids (28.6% of N = 35) (Fig. 10b). Both Serranids and Pomacentrids attacked the bags which contained *A. armatus* shrimps more than they attacked all other treatments (20.0% and 25.7% of all attacks), whereas the Labrids attacked the *P. yucatanicus* shrimp (25.7% of all attacks) more than they attacked the other bag treatments (Fig. 10b).

Treatment type significantly affected the number of fish that oriented toward the experimental bags ($F = 20.78$, d.f. = 317, $p < 0.001$, Fig. 11a). Pairwise comparisons revealed that fishes oriented significantly more frequently toward bags that contained *A. armatus* than toward any other treatment (*P. yucatanicus, A. pedersoni*, or the empty control bag; $t = 6.29$, d.f.=317, $p<0.0001$; $t = 4.60$, d.f. = 317, $p<0.0001$; $t = -7.28$, d.f. = 317, $p = 0.0005$, respectively), and also more toward *P. yucatanicus* than toward the empty control bag ($t = -2.68$, d.f. = 317, $p = 0.0319$). The duration of fish orientations toward the bags also depended on treatment type ($F = 7.40$, d.f. = 317, $p = 0.0001$, Fig. 11b), with *A. armatus* eliciting significantly longer orientation times from fishes than did either *A. pedersoni*, *P. yucatanicus*, or the control ($t = 3.76$, d.f. = 317, $p = 0.0002$; $t = 3.56$, d.f. = 317, $p = 0.0004$; $t = -4.14$, d.f. = 317, $p < 0.0001$ respectively). There was no significant difference in total orient time for the other pair-wise comparisons.

The number of fish attacks also varied significantly with treatment type ($F = 7.92$, d.f. = 317, $p = 0.0001$, Fig. 12a). Bags containing *A. armatus* received significantly more attacks than

did those containing *A. pedersoni* or containing no shrimp (control bags, t=3.85, d.f.=317, t=3.85, p=0.0001; t = -4.38, d.f. = 317, p < 0.0001 respectively). Bags with the cleaner mimic shrimp *P. yucatanicus* received significantly more attacks than did the empty control bags (F = -2.39, d.f. = 317, p = 0.0174).

In terms of the number of strikes per attack bout, the effect of treatment on the total number of strikes was significant (F = 4.75, d.f. = 317, p=0.003 fig. 12 b), where *A. armatus* received significantly more strikes than did *A. pedersoni* or the control, (t = 2.87, d.f. = 317, p = 0.0044; t = 3.25, d.f. = 317, p = 0.0013 respectively), and *P. yucatanicus* elicited significantly more strikes than did the control (t = -2.25, d.f. = 317, p = 0.0249). There was no significant difference in the number of attacks or strikes for all the other pair-wise comparisons. Additionally, there was no significant effect of treatment on the number of fish who attacked bubbles in the bags in each treatment.

Discussion

Our analysis of spectral coloration indicates that cleaner shrimp *A. pedersoni* exhibit high contrast against the background habitat of their host anemones, and therefore may be highly visible to client fishes, at least at close range. This pattern is expected for a cleaner organism, and confirms that this cleaner shrimp utilizes high-contrast body color spots, likely as signals to potential clients for cleaning interactions, as known from cleaner fish systems (Stummer et al. 2004; Cheney et al. 2009; Lettieri et al. 2009). We also confirmed that anemone shrimp *P. yucatanicus* exhibit blue body spots with spectral coloration similar to those of cleaner shrimp that co-occur in the same anemone host habitat, and thus may mimic the cleaner shrimp to some extent. However, this mimicry appears to be imprecise and to project a significantly lower-

contrast than exhibited by cleaner shrimp, which exhibit higher visual contrast against their typical background habitats on both host anemones, than do mimic shrimp *P. yucatanicus,* indicating that while *P. yucatanicus* appear to partially mimic the body coloration of cleaner shrimp, they are not perfect mimics, in that they transmit less robust color signals to reef fishes. The mimic shrimp's reflectance overlapped somewhat with anemone background reflectance patterns, especially on *C. gigantea* anemones, suggesting that this mimic shrimp may engage in "bet-hedging" in which they balance cleaner mimicry with partial camouflage on the host. Thus, the mimic may employ dual signaling strategies in which their limited camouflage allows them to avoid detection by some approaching fishes, and their cleaner-similar coloration also allows them to appear to be a beneficial cleaner to any fishes that visually detect them.

Cleaner shrimp exhibited more pure blue color on their uropod and abdomen spots than the purple/pink color of similar spots on mimic shrimp, indicating greater similarity to the blue colorations of both cleaner wrasses *Labroides bicolor*, *L. dimidiatus*, *L. phthirophagus*, and *L. pectoralis* and cleaner gobies *Elactinus oceanops* and *E. evelynae*. All six of these cleaner fish species are conspicuous to a variety of client fish visual systems *(*described in Cheney et al. 2009), suggesting that pure blue is a highly effective signaling color to client fishes. Additionally, of the six cleaner shrimp species that have demonstrated parasite removal abilities, three of them exhibit blue and white coloration (*A. pedersoni*, *A. longicarpus*, and *A. anthophilus*, reviewed in Huebner and Chadwick 2012b). Conversely the cleaner shrimps *Brachycarpus biunguiculatus*, *Urocaridella* sp. c, and *A. holthuisi* display variations of red, yellow, and white stripes and spots. This variety may indicate that even though blue coloration is more conspicuous to client fishes, it is not the only major color indicator of cleaner status for cleaner shrimps, in contrast to blue signal uniformity among cleaner fishes (Huebner and Chadwick 2012b).

The color score analysis revealed that *A. pedersoni* uropods and abdomens occupied a distinct region of color space which was more dissimilar than the color space of *P. yucatanicus* uropod and abdomens, as well as from both the oral discs and tentacles of host anemones *B. annulata* and *C. gigantea*. Although the *P. yucatanicus* color scores were relatively closer to the anemone scores (ie: more similar to anemones than were *A. pedersoni*), they still occupied relatively discrete regions in color space from the body regions of all 3 other species, indicating some color contrast with the hosts. These data also were supported by the PCA results on spectral shapes, which revealed that the uropods and abdomens of both shrimps were significantly different for PC1 (58.8% of variation) than the oral discs and tentacles of both anemone species. However, only the *A. pedersoni* uropods differed significantly in spectral shape from the *P. yucatanicus* uropods and abdomens, indicating that abdomen color spots on the latter species may fairly closely mimic those of the cleaner.

In terms of signal brightness, *A. pedersoni* uropods and abdomens were significantly more contrasting against all anemone backgrounds for both color contrast (Euclidean distance) and intensity (brightness contrast) than *P. yucatanicus* uropods and abdomens, further revealing that brightness in addition to hue differed between the shrimps. Variation in contrast patterns between background habitats offered by the two host species also showed that *A. pedersoni* contrasted significantly more against *B. annulata* than *C. gigantea.* Thus*, B. annulata* appears to be the optimal anemone habitat background for *A. pedersoni* color signals to client fishes. Similarly, *P. yucatanicus* uropods were significantly more visible against the tentacles of *B. annulata* than those of *C. gigantea* (for both color contrast and intensity), but their abdomens contrasted more with the oral discs of *C. gigantea* than of *B. annulata*. These data indicate that

though *P. yucatanicus* are generally more contrasting against *B. annulata* than *C. gigantea* for most color comparisons, their visibility to fishes generally was less than that of *A. pedersoni.*

Results of the field experiment supported our hypothesis that visual characteristics of cleaner shrimp *A. pedersoni* deter predation by approaching client fishes, more so than do those of mimic shrimp *P. yucatanicus*, which deter more than those of other non-mimic anemoneshrimps such as *A. armatus*. A high diversity of fishes occurred at our reef site (15 families), with damselfish exhibiting the highest overall abundance (35.0% of all fish observed), followed by gobies (26.8%) and wrasses (14.6%), similar to their relative abundances on other reef areas in the Caribbean (Wicksten 1998; Huebner and Chadwick 2012a). Interestingly, the fish families that interacted with our shrimp treatments at cleaning stations also comprised mainly damselfishes (38.5%) and wrasses (27.7%), but even though gobies were common at our site, very few of them visited the stations. The dearth of goby visitors may have been due to their small size and site-attachment, ie: low mobility, and association with corals or sponges and not anemones. Additionally, some gobies serve as cleaners rather than clients, due in part to their small body size (Soares et al. 2008; Cheney et al. 2009; Soares et al. 2012). In contrast, many groupers and hamlets (serranids) visited shrimps at our cleaning station (29.1% of all fish visitors), even though they made up < 1% of fish present at this reef site. The disproportionate amount of interactions relative to abundance for serranids was similar to the pattern observed previously, in which they were frequent visitors to *A. pedersoni* cleaning stations in the US Virgin Islands (Huebner and Chadwick 2012b), and is not surprising given that groupers may even structure their territories around cleaning stations (Sluka et al. 1999). Overall, the relative abundances of fish families that visited shrimps at our cleaning stations were similar (an abundance of serranids) to those observe previously (Huebner and Chadwick 2012b), with some

differences in that surgeon fishes (Acanthuridae) and goatfishes (Mullidae) were the most frequent clients, as opposed to damselfishes and wrasses in our study. This variation in pattern between 2011 and 2016 may have been due to the presence of shrimps in bags in our study, which may have attracted more invertivore type fishes instead of herbivores to the cleaning stations.

Reduced predation relative to non-cleaners has been shown experimentally for cleaner fishes (Cote 2000; Franchihi-Filho et al. 2000), but has not yet been documented for cleaner shrimp. Our experimental field results demonstrate for the first time reduced predation pressure on a cleaner shrimp in-situ, in that reef fishes oriented towards and attacked non-cleaner shrimp *A. armatus* significantly more than cleaner shrimp *A. pedersoni*. These results indicate that visual characteristics of the cleaner shrimp act to deter predation by reef fishes. Our methods allowed us to rule out chemical or tactile (ie: potentially detected toxicity when fish attempt to contact shrimps) cues in deterring fish predation, because all shrimp were enclosed in plastic bags, preventing the transmission of either chemical or tactile cues.

Additionally, fishes oriented towards and attacked the potential cleaner mimic *P. yucatanicus* significantly less than non-cleaner shrimp *A. armatus*, indicating that visual traits of the mimic result in lower predation pressure than on other non-cleaner anemoneshrimp. The non-significant differences in fish orient and attack rates toward cleaner and mimic shrimp confirm that both these species exhibit visual signals which deter fish predation on them at reef cleaning stations. Given the high level of similarity in the spectral and color shape of both their uropod and abdomen spots, which are the largest color spots on their bodies, these 2 types of color signals may be the main ones that deter predatory attacks by fishes on both shrimps. In addition, both species of anemoneshrimps have long white (or red-and-white banded) antennae, which

they vibrate as distinctive signals in response to the approach of client fishes (see Chapter 3), and which may contribute to fish recognition of both species as potential cleaners. However, alpheid shrimp also possess long red and white banded antennae, which did not appear to deter attacks by fishes in our study. Further, antenna vibration behavior was not exhibited by any shrimps in our experimental setup, so behavioral differences in antennae use among these species could not have caused the differences in fish attack rates.

Additional work is needed to confirm whether mimic shrimp *P. yucatanicus* receive protection from predation based on their visual mimicry of actual cleaners *A. pedersoni*, or instead because they function as infrequent (and as yet undetected except for a single field observation, Spotte 1999) facultative cleaners of reef fishes. However, both laboratory experimental evidence in different settings (Bunkley-Williams and Williams 1998; McCammon et al. 2010), and field evidence (Huebner and Chadwick 2012; Titus et al. 2015) in which substantial cleaning was observed by *A. pedersoni* and not by any *P. yucatanicus* even though both were present, do not support the idea that the latter function as cleaners on Caribbean reefs. The most parsimonious explanation to date thus appears to be that *P. yucatanicus* function as visual mimics of cleanershrimp *A. pedersoni*, which allows them to appear to be potential cleaners to some visiting reef fishes, even though they do not offer actual cleaning services. Their color patterns that are less bright than those of cleaners, and are more similar to their background anemone hosts, may also allow them to employ a certain level of camouflage on their hosts. Their coloration pattern thus appears to exhibit a potential balance between color signals that allow them to both avoid detection and to appear as a beneficial species.

Our quantification of the color reflectance patterns of the host anemone and guest shrimp species in this symbiotic network, coupled with our field experimental evidence on fish predation

patterns on the shrimp, lead us to conclude that a cleaner mimicry system occurs in this complex. These data also are supported by similarities in the antennae-vibration behavioral signals employed by both shrimp species in response to the visual stimulus of simulated fish visits (Chapter 3). As such, this system appears to parallel the behavioral and color-pattern cleaner mimicry systems that have been described for some cleaner fishes on Indo-Pacific coral reefs.

References

Aksnes, D. L. (2007). Evidence for visual constraints in large marine fish stocks. *Limnology and Oceanography, 52*(1), 198-203. doi: 10.4319/lo.2007.52.1.0198

Aksnes, D. L., & Utne. A. C. W. (1997). A revised model of visual range in fish. *Sarsia*, *82*(2), 137-147. http://dx.doi.org/10.1080/00364827.1997.10413647

Arvedlund, M., & Nielsen, L. E. (1996). Do the anemonefish *Amphiprion ocellaris* (Pisces: Pomacentridae) imprint themselves to their host sea anemone *Heteractis magnifica* (Anthozoa: Actinidae)? *Ethology*, *102*(2), 197−211. doi: 10.1111/j.1439-0310.1996.tb01118.x

Barry, K. L. (1996). *Visual communication between the Hawaiian cleaner wrasse (Labroides phthirophagus) and host fish in Kaneohe Bay, Hawaii* (master's thesis). University of Victoria, Victoria.

Barry, K. L., & Hawryshyn, C. W. (1999). Spectral sensitivity of the Hawaiian saddle wrasse, *Thalassoma duperrey*, and implications for visually mediated behaviour on coral reefs. *Environmental Biology of Fishes*, *56*(4), 429-442. doi: 10.1023/A:1007556112449

Bauer, R. T. (2004). Remarkable shrimps: Adaptations and natural history of the carideans. Norman: University of Oklahoma Press.

Becker, J. H., & Grutter, A. S. (2004). Cleaner shrimp do clean. *Coral Reefs*, *23*(4), 515-520. doi: 10.1007/s00338-004-0429-3

Bradshaw, C. J. A., & White, W. W. (2006). Rapid development of cleaning behaviour by Torresian crows *Corvus orru* on non-native banteng *Bos javanicus* in northern Australia. *Journal of Avian Biology*, *37*(4), 409-411. http://www.jstor.org/stable/30243298

Briones-Fourzan, P., Perez-Ortiz, M., Negrete-Soto, F., Barradas-Ortiz, C., & Lozano-Alvarez, E. (2012). Ecological traits of Caribbean sea anemones and symbiotic crustaceans. *Marine Ecology Progress Series*, *470*, 55-68. doi: 10.3354/meps10030

Bshary, R. (2003). The cleaner wrasse, *Labroides dimidiatus*, is a key organism for reef fish diversity at Ras Mohammed National Park, Egypt. *Journal of Animal Ecology*, *72*(1), 169-176. doi: 10.1046/j.1365-2656.2003.00683.x

Bshary, R., & Bronstein, J. L. (2004). Game structures in mutualisms: What can the evidence tell us about the kind of models we need? *Advances in the Study of Behavior*, *34*, 59-101. doi: 10.1016/S0065-3454(04)34002-7

Bshary, R., & Grutter, A., S. (2002). Asymmetric cheating opportunities and partner control in a cleaner fish mutualism. *Animal Behaviour*, *63*(3), 547-555. doi: 10.1006/anbe.2001.1937

Bshary, R., & Grutter, A. S. (2005). Punishment and partner switching cause cooperative behaviour in a cleaning mutualism. *Biology Letters*, *1*(4), 396–399. doi: 10.1098/rsbl.2005.0344

Bshary, R., & Schäffer, D. (2002). Choosy reef fish select cleaner fish that provide high-quality service. *Animal Behaviour, 63*(3), 557–564. http://dx.doi.org/10.1006/anbe.2001.1923

Bunkley-Williams, L., & Williams, E. H. (1998). Ability of Pederson cleaner shrimp to remove juveniles of the parasitic cymothoid isopod, *Anilocra haemuli*, from the host. *Crustaceana*, *71*(8), 862-869. http://www.jstor.org/stable/20106067

Cantrell, C. E., Henry, R. P., & Chadwick, N. E. (2015). Nitrogen transfer in a Caribbean mutualistic network. *Marine Biology*, *162*(12), 2327-2338. doi: 10.1007/s00227-015-2764-z

Chadwick, N. E., Duris, Z., & Horka, I. (2008). Biodiversity and behavior of shrimps and fishes symbiotic with sea anemones in the Gulf of Aqaba, northern Red Sea. In F. D. Por (Ed.), *The improbable gulf: History, biodiversity, and protection of the Gulf of Aqaba (Eilat)* (pp. 209-223). Jerusalem: Magnes Press, Hebrew University.

Chapuis, L., & Bshary, R. (2009). Strategic adjustment of service quality to client identity in the cleaner shrimp, *Periclimenes longicarpus*. *Animal Behavior*, *78*(2), 455-459. http://dx.doi.org/10.1016/j.anbehav.2009.06.001

Cheney, K. L., & Côté, I. M. (2007). Aggressive mimics profit from a model-sign receiver mutualism. *Proceedings of the Royal Society B*, *274*(1622), 2087-2091. doi: 10.1098/rspb.2007.0543

Cheney, K. L., Grutter, A. S., Blomberg, S. P., & Marshall, N. J. (2009). Blue and yellow signal cleaning behavior in coral reef fishes. *Current Biology*, *19*(15), 1283-1287. doi: 10.1016/j.cub.2009.06.028

Cheney, K. L., & Marshall, N. J. (2009). Mimicry in coral reef fish: How accurate is this deception in terms of color and luminance? *Behavioral Ecology*, *20*(3), 459-468. doi: 10.1093/beheco/arp017

Chockley, B. R., & St. Mary, C. M. (2003). Effects of body size on growth, survivorship, and reproduction in the banded coral shrimp *Stenopus hispidus*. *Journal of Crustacean Biology*, *23*(4), 836-848. http://www.jstor.org/stable/1549665

Clark, D. L., Roberts, J. A., Rector, M. & Uetz, G. W. (2011). Spectral relflectance and communication in the wolf spider, *Schizocosa ocreata* (Hentz): Simultaneous crypsis and background contrast in visual signals. *Behavioral Ecology and Sociobiology. 65*(6), 1237-1247. doi: 10.1007/s00265-011-1137-y

Côté, I. M. (2000). Evolution and ecology of cleaning symbioses in the sea. *Oceanography and Marine Biology: An Annual Review*, *38*, 311-355. doi: 10.1201/b12589

Cournoyer, B. L., & Cohen, J. H. (2011). Cryptic coloration as a predator avoidance strategy in seagrass arrow shrimp colormorphs. *Journal of Experimental Marine Biology and Ecology*, *402*(1), 27-34. doi: 10.1016/j.jembe.2011.03.011

Cuthill, I. C., Bennet, T. D., Partridge, J. C., & Maier, E. J. (1999). Plumage reflectance and the objective assessment of avian sexual dichromatism. *The American Naturalist*, *153*(2), 183-200. http://www.jstor.org/stable/10.1086/303160

Eggers, D. M. (1977). The nature of prey selection by planktivorous fish. *Ecology*, *58*(1), 46-59. doi: 10.2307/1935107

Endler, J. A. (1993). The color of light in forests and its implications. *Ecological Monographs*, *63*(1), 1-27. http://www.jstor.org/stable/2937121

Endler, J. A., Westcott, D. A., Madden, J. R., & Robson, T. (2005). Animal visual systems and the evolution of color patterns: Sensory processing illuminates signal evolution. *Evolution*, *59*(8), 1795-1818. http://www.jstor.org/stable/3449083

Francihi-Filho, R. B., Moura, R. L., & Sazima, I. (2000). Cleaning by the wrasse *Thalassoma noronhanum*, with two records of predation by its grouper client *Cephalopholis fulva*. *Journal of Fish Biology, 56*(4), 802-809. doi: 10.1111/j.1095-8649.2000.tb00873.x

Grill, C. P., & Rush, V. N. (2000). Analyzing spectral data: Comparison and application of two techniques. *Biological Journal of the Linnean Society, 69*(2), 121-138. doi: 10.1111/j.1095-8312.2000.tb01194.x

Grutter, A. S. (1997). Spatio-temporal variation and feeding selectivity in the diet of the cleaner fish *Labroides dimidiatus*. *Copeia*, *2*, 346-355. http://www.jstor.org/stable/1447754

Grutter, A. S., Deveney, M. R., Whittington, I. D., & Lester, R. J. G. (2002). The effect of the cleaner fish *Labroides dimidiatus* on the capsalid monogenean *Benedenia lolo* parasite on the labrid fish *Hemigymnus melapterus*. *Journal of Fish Biology*, *61*(5), 1098-1108. doi: 10.1111/j.1095-8649.2002.tb02458.x

Grutter, A. S., Murphy, J. M., & Choat, J. H. (2003). Cleaner fish drives local fish diversity on coral reefs. *Current Biology*, *13*(1), 64-67. doi: 10.1016/S0960-9822(02)01393-3

Grutter, A. S., & Poulin, R. (1998). Cleaning of coral reef fishes by the wrasse *Labroides dimidiatus*: Influence of client body size and phylogeny. *Copeia*, *1*, 120–127. http://www.jstor.org/stable/1447707

Horth, L., Campbell, L., & Bray, R. (2014). Wild bees preferentially visit *Rudbeckia* flower heads with exaggerated ultraviolet absorbing floral guides. *Biology Open*, *3*(3), 221-230. doi: 10.1242/bio.20146445

Huebner, L. K., & Chadwick, N. E. (2012a). Reef fishes use anemones as visual cues for cleaning interactions with shrimp. *Journal of Experimental Marine Biology and Ecology*, *416-417*, 237-242. http://dx.doi.org/10.1016/j.jembe.2012.01.004

Huebner, L. K., & Chadwick, N. E. (2012b). Patterns of cleaning behavior on coral reef fish by the anemoneshrimp *Ancylomenes pedersoni. Journal of the Marine Biological Association of the United Kingdom, 92(*7) 1557-1562. http://dx.doi.org/10.1017/S0025315411001822

Humann, P., & DeLoach, N. (2002). *Reef fish identification: Florida, Caribbean, Bahamas* (2nd ed.). Jacksonville: New World Publications.

Lettieri, L., Cheney, K. L., Mazel, C. H., Boothe, D., Marshall, N. J., & Streelman, J. T. (2009). Cleaner gobies evolve advertising stripes of higher contrast. *Journal of Experimental Biology*, *212*, 2194-2203. doi: 10.1242/jeb.025478

Losey, G. S., McFarland, W. N., Loew, E. R., Zamzow, J. P., Nelson, P. A., & Marshall, N. J. (2003). Visual biology of Hawaiian coral reef fishes. I. ocular transmission and visual pigments. *Copeia*, *3*, 433-454. http://dx.doi.org/10.1643/01-053

Macedonia, J. M. (2001). Habitat light, colour variation and ultraviolet reflectance in the Grand Cayman anole, *Anolis conspersus*. *Biological Journal of the Linnean Society*, *73*(3), 299-320. doi:10.1006/bijl.2001.0545

Mahnken, C. (1972). Observations on cleaner shrimps of the genus *Periclimenes*. *Bulletin of the Natural History Museum of Los Angeles County, 14*, 71-83.

Marshall, J. N. (2000). Communication and camouflage with the same 'bright' colours in reef fishes. *Philosophical Transactions of the Royal Society B*, *355*(1401), 1243-1248. Doi: 10.1098/rstb.2000.0676

Mascaro, M., Rodriguez-Pestana, L., Chiappa-Carrara, X., & Simoes, N. (2012). Host selection by the cleaner shrimp *Ancylomenes pedersoni:* Do anemone host species, prior experience or presence of conspecific shrimp matter? *Journal of Experimental Marine Biology and Ecology*, *413*, 87-93. doi: 10.1016/j.jembe.2011.11.026

McCammon, A., Sikkel, P. C., & Nemeth, D. (2010). Effects of three Caribbean cleaner shrimps on ectoparasitic monogeneans in a semi-natural environment. *Coral Reefs*, *29*(2), 419-426. doi: 10.1007/s00338-009-0583-8

McFarland, W. N., & Munz, F. W. (1975). Part III: The evolution of photopic visual pigments in fishes. *Vision Research*, *15*(10), 1071-1080. doi: 10.1016/0042-6989(75)90003-6

Oates, J., Manica, A., Bshary, R., & Grutter, A. S. (2012). Relationship between roving behaviour and the diet and client composition of the cleaner fish *Labroides bicolor*. *Journal of Fish Biology*, *81*(1), 210-219. doi: 10.1111/j.1095-8649.2012.03330.x

Ostlund-Nilsson, S., Becker, H. A., & Nilsson, G. E. (2005). Shrimps remove ectoparasites from fishes in temperate waters. *Biology Letters*, *1*(4), 454-456. doi: 10.1098/rsbl.2005.0363

Partridge, J. C., & Cummings, M. E. (1999). Adaption of visual pigments to the aquatic environment. In S. Archer, M. B. Djamgoz, E. Loew, J. C. Partridge, & S. Vallerga (Eds.), *Adaptive Mechanisms in the Ecology of Vision* (pp. 251-283). Great Britain: Kluwer Academic Publishers.

Pike, T. W., Bjerkeng, B., Blount, J. D., Lindstrom, J., & Metcalfe, N. B. (2011). How integument colour reflects its carotenoid content: A stickleback's perspective. *Functional Ecology*, *25*(1), 297-304. doi: 10.1111/j.1365-2435.2010.01781.x

Potts, G, W. (1973). The ethology of *Labroides dimidiatus* (Cuv. And Val.) (Labridae, Pisces) on Aldabra. *Animal Behavior, 21*(2), 250-291. doi: 10.1016/S0003-3472(73)80068-5

Roopin, M., & Chadwick, N. E. (2009). Benefits to host sea anemones from ammonia contribution of resident anemonefish. *Journal of Experimental Marine Biology and Ecology*, *370*(1), 27-34. doi: 10.1016/j.jembe.2008.11.006

Siebeck, U. E., & Marshall, N. J. (2001). Ocular media transmission of coral reef fish – Can coral reef fish see ultraviolet light? *Vision Research*, *41*(2), 133-149. doi: 10.1016/S0042-6989(00)00240-6

Siebeck, U. E., & Marshall, N. J. (2007). Potential ultraviolet vision in pre-settlement larvae and settled reef fish – A comparison across 23 families. *Vision Research*, *47*(17), 2337-2352. doi: 10.1016/j.visres.2007.05.014

Sikkel, P. C., Shaumburg, C. S., & Mathenia, J. K. (2006). Diel infestation dynamics of gnathiid isopod larvae parasitic on Caribbean reef fish. *Coral Reefs*, *25*(4), 683-689. doi: 10.1007/s00338-006-0154-1

Sluka, R., Chiappone, M., Sullivan, K. M., & De Garine-Wichatitsky, M. (1999). Benthic habitat characterization and space utilization by juvenile Epinepheline groupers in the Exuma Cays Land and Sea Park, Central Bahamas. *Proceedings of the Forty-Fifth Gulf and Caribbean Fisheries Institute, 45,* 23-36. Retrieved from http://www.gcfi.org/proceedings/

Soares, M. C., Bshary, R., Cardoso, S. C., Côté, I. M., & Oliveira, R. F. (2012). Face your fears: Cleaning gobies inspect predators despite being stressed by them. *PLOS ONE*, *7*(6). doi:10.1371/journal.pone.0039781

Soares, M. C., Côté, I. M., Cardoso, S. C., & Bshary, R. (2008). The cleaning goby mutualism: A system without punishment, partner switching, or tactile simulation. *Journal of Zoology, 276*(3)*,* 306-312. doi: 10.1111/j.1469-7998.2008.00489.x

Soares, M. C., Côté, I. M., Cardoso, S. C., Oliveira, R. F., & Bshary, R. (2010). Caribbean cleaning gobies prefer client ectoparasites over mucus. *Ethology*, *116*(12), 1244-1248. doi: 10.1111/j.1439-0310.2010.01838.x

Sornes, T. A., & Aksnes, D. L. (2004). Predation efficiency in visual and tactile predators. *Limnology and Oceanography, 49*(1), 69-75. doi: 10.4319/lo.2004.49.1.0069

Spotte, S. (1997). Sexual and regional variation in the morphology of the spotted anemone shrimp *Periclimenes yucatanicus* (Decapoda: Caridea: Palaemonidae). *Journal of Crustacean Biology*, *17*(3), 389-397. http://www.jstor.org/stable/1549433

Stambler, N., & Shashar, N. (2007). Variation in spectral reflectance of the hermatypic corals, *Stylophora pistillata* and *Pocillopora damicornis*, from the Red Sea. *Journal of Experimental Marine Biology and Ecology*, *351*, 143-149. http://dx.doi.org/10.1016/j.jembe.2007.06.014

Stummer, L. E., Weller, J. A., Johnson, M. L., & Côté, I. M. (2004). Size and stripes: How fish clients recognize cleaners. *Animal Behaviour*, *68*(1), 145-150. doi: 10.1016/j.anbehav.2003.10.018

Sun, D., Blomberg, S. P., Cribb, T. H., McCormick, M. I., & Grutter, A. S. (2012). The effects of parasites on the early life stages of a damselfish. *Coral Reefs*, *31*(4), 1065-1075. doi: 10.1007/s00338-012-0929-5

Szczebak, J. T., Henry, R. P., Al-Horani, F. A., & Chadwick, N. E. (2013). Anemonefish oxygenate their anemone hosts at night. *Journal of Experimental Biology*, *216*, 970-976. doi: 10.1242/jeb.075648

Titus, B. M., Marymegan, D., & Exton, D. A. (2015). Temporal patterns of Pederson shrimp (*Ancylomenes pedersoni* Chace 1958) cleaning interactions on Caribbean coral reefs. *Marine Biology, 162*(8), 1651-1654. doi 10.1007/s00227-015-2703-z

Utne, A. C. W. (1997). The effect of turbidity and illumination on the reaction distance and search time of a marine planktivore *Gobiusculus flavescens*. *Journal of Fish Biology*, *50*(5), 926-938. doi: 10.1111/j.1095-8649.1997.tb01619.x

Vorobyev, M., Brandt, R., Peitsch, D., Laughlin, S. B., & Menzel, R. (2001). Colour thresholds and receptor noise: Behaviour and physiology compared. *Vision Research*, *41(5)*, 639-653. doi: 10.1016/S0042-6989(00)00288-1

Vorobyev, M., & Osorio, D. (1998). Receptor noise as a determinant of colour thresholds. *Proceedings of the Royal Society B*, *265*(1394), 351-358. doi: 10.1098/rspb.1998.0302

Waldie, P. A., Blomberg, S. P., Cheney, K. L., Goldizen, A. W., & Grutter, A. S. (2011). Long-term effects of the cleaner fish *Labroides dimidiatus* on coral reef fish communities. *PLOS ONE*, *6*(6): e21201. doi: http://dx.doi.org/10.1371/journal.pone.0021201

Wicksten, M. K. (1998). Behaviour of cleaners and their client fishes at Bonaire, Netherlands Antilles. *Journal of Natural History*, *32*(1), 13-30. http://dx.doi.org/10.1080/00222939800770021

Wirtz, P. (1997). Crustacean symbionts of sea anemone *Telmatactis crioides* at Maderia and the Canary Islands. *Journal of Zoology*, *242*(4), 799-811. doi: 10.1111/j.1469-7998.1997.tb05827.x

Table. 1. Pairwise comparisons of PC1 scores from the PCA analysis of the normalized reflectance spectra of the shrimps *A. pedersoni*, and *P. yucatanicus* uropods and abdomen, and the anemones *B. annulata* and *C. gigantea* oral discs and tentacles. a.-g. in bold indicate the reference for comparisons in each table subset.

PC1				
(a.) A. pedersoni uropod	***Std. Error***	**DF**	**t-value**	**p-value**
A. pedersoni abdomen	1.501	62	2.538	0.014
P. yucatanicus uropod	1.327	62	4.414	<0.001
P. yucatanicus abdomen	1.352	62	3.191	0.002
B. annulata Oral Disc	1.644	62	10.778	<0.001
B. annulata Tentacles	1.382	62	8.406	<0.001
C. gigantea Oral Disc	1.417	62	12.109	<0.001
C. gigantea Tentacles	1.399	62	6.284	<0.001
(b.) *A. pedersoni* abdomen	***Std. Error***	**DF**	**t-value**	**p-value**
P. yucatanicus uropod	1.568	62	1.306	0.196
P. yucatanicus abdomen	1.589	62	0.318	0.751
B. annulata oral disc	1.844	62	7.543	<0.001
B. annulata tentacles	1.615	62	4.836	<0.001
C. gigantea oral disc	1.645	62	8.115	<0.001
C. gigantea Tentacles	1.630	62	3.058	0.003
(c.) *P. yucatanicus* uropod	***Std. Error***	**DF**	**t-value**	**p-value**
P. yucatanicus abdomen	1.201	62	-1.285	0.204
B. annulata oral disc	1.611	62	7.364	<0.001
B. annulata tentacles	1.343	62	4.292	<0.001
C. gigantea oral disc	1.378	62	8.199	<0.001
C. gigantea Tentacles	1.360	62	2.158	0.035
(d.) *P. yucatanicus* abdomen	***Std. Error***	**DF**	**t-value**	**p-value**
B. annulata oral disc	1.631	62	8.216	<0.001
B. annulata tentacles	1.367	62	5.342	<0.001
C. gigantea oral disc	1.402	62	9.158	<0.001
C. gigantea Tentacles	1.384	62	3.234	0.002
(e.) *B. annulata* oral disc	***Std. Error***	**DF**	**t-value**	**p-value**
B. annulata tentacles	1.554	62	-3.923	<0.001
C. gigantea Oral Disc	1.685	62	-0.333	0.7405
C. gigantea Tentacles	1.671	62	-5.342	<0.001
(f.) *B. annulata* tentacles	***Std. Error***	**DF**	**t-value**	**p-value**
C. gigantea oral disc	1.432	62	3.867	<0.001
C. gigantea tentacles	1.414	62	-2.000	0.0499
(g.) *C. gigantea* oral disc	***Std. Error***	**DF**	**t-value**	**p-value**
C. gigantea tentacles	1.323	62	-6.320	<0.001

Table. 2. Pairwise comparisons of PC2 scores from the PCA analysis of the normalized reflectance spectra of the shrimps *A. pedersoni*, and *P. yucatanicus* uropods and abdomen, and the anemones *B. annulata* and *C. gigantea* oral discs and tentacles. a.-g. in bold indicate the reference for comparisons in each table subset.

PC2				
(a.) *A. pedersoni* uropod	***Std. Error***	**DF**	**t-value**	**p-value**
A. pedersoni abdomen	1.032	62	1.833	0.072
P. yucatanicus uropod	1.212	62	2.979	0.004
P. yucatanicus abdomen	1.228	62	4.618	<0.001
B. annulata oral disc	1.432	62	1.040	3.021
B. annulata tentacles	1.263	62	1.850	0.069
C. gigantea oral disc	1.290	62	3.056	0.003
C. gigantea tentacles	1.278	62	2.952	0.005
(b.) *A. pedersoni* abdomen	***Std. Error***	**DF**	**t-value**	**p-value**
P. yucatanicus uropod	1.286	62	1.258	0.213
P. yucatanicus abdomen	1.382	62	2.735	0.008
B. annulata oral disc	1.087	62	-0.256	0.798
B. annulata tentacles	1.566	62	0.315	0.754
C. gigantea oral disc	1.437	62	1.426	0.159
C. gigantea tentacles	1.427	62	1.315	0.192
(c.) *P. yucatanicus* uropod	***Std. Error***	**DF**	**t-value**	**p-value**
P. yucatanicus abdomen	0.791	62	2.605	0.012
B. annulata oral disc	1.400	62	-1.516	0.135
B. annulata tentacles	1.227	62	-1.040	0.303
C. gigantea oral disc	1.254	62	0.262	0.795
C. gigantea tentacles	1.243	62	0.130	0.897
(d.) *P. yucatanicus* abdomen	***Std. Error***	**DF**	**t-value**	**p-value**
B. annulata oral disc	1.414	62	-2.958	0.004
B. annulata tentacles	1.243	62	-2.685	0.009
C. gigantea oral disc	1.269	62	-1.364	0.177
C. gigantea tentacles	1.258	62	-1.509	0.136
(e.) *B. annulata* oral disc	***Std. Error***	**DF**	**t-value**	**p-value**
B. annulata tentacles	1.072	62	0.790	0.433
C. gigantea oral disc	1.468	62	1.670	0.100
C. gigantea tentacles	1.458	62	1.567	0.122
(f.) *B. annulata* tentacles	***Std. Error***	**DF**	**t-value**	**p-value**
C. gigantea oral disc	1.303	62	1.231	0.223
C. gigantea tentacles	1.292	62	1.112	0.270
(g.) *C. gigantea* oral disc	***Std. Error***	**DF**	**t-value**	**p-value**
C. gigantea Tentacles	0.869	62	-0.192	0.849

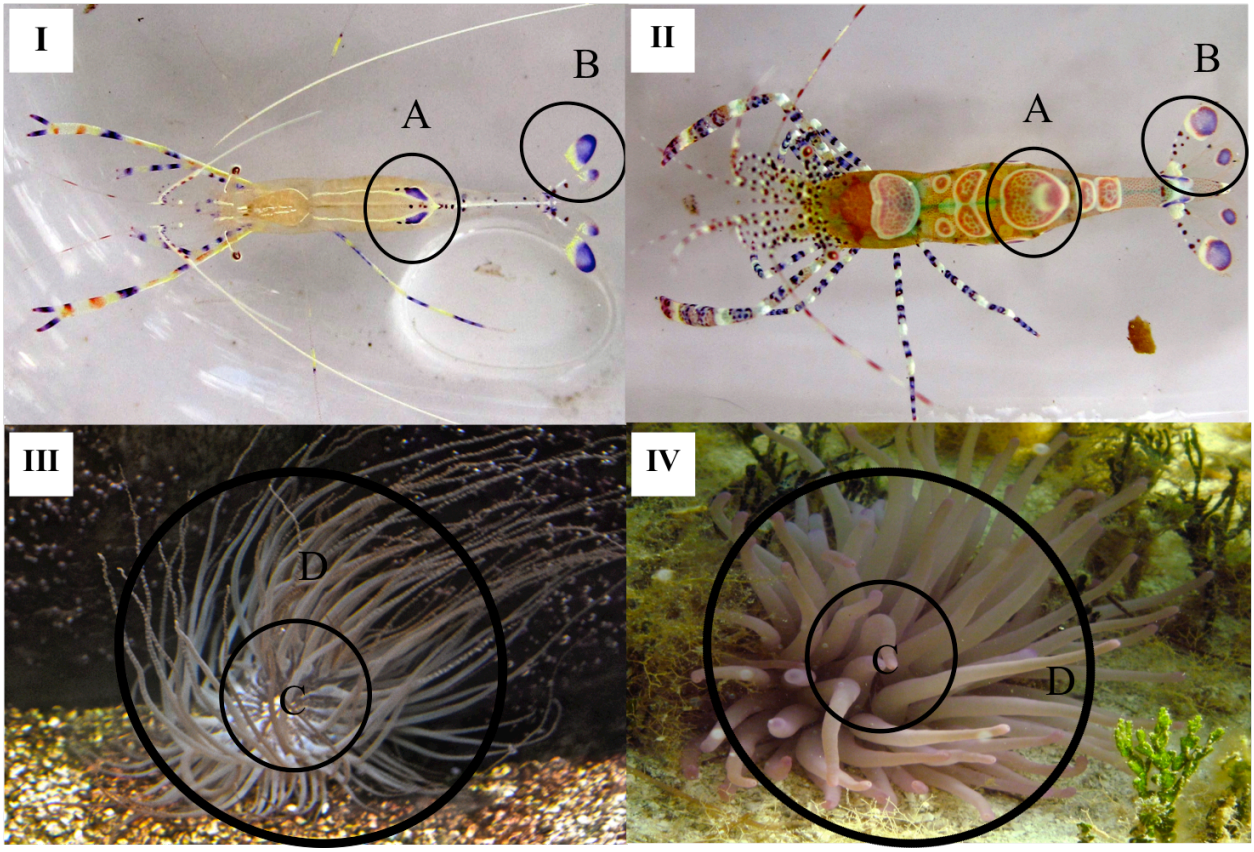

Fig. 1. Locations of reflectance measurements on the anemone shrimps *A. pedersoni* (I), and *P. yucatanicus* (II) as well as the host anemones *B. annulata* (III) and *C. gigantea* (IV). The circles indicate reflectance locations (a) shrimp abdomen, (b) shrimp uropod, (c) anemone oral disc, and (d) anemone tentacles.

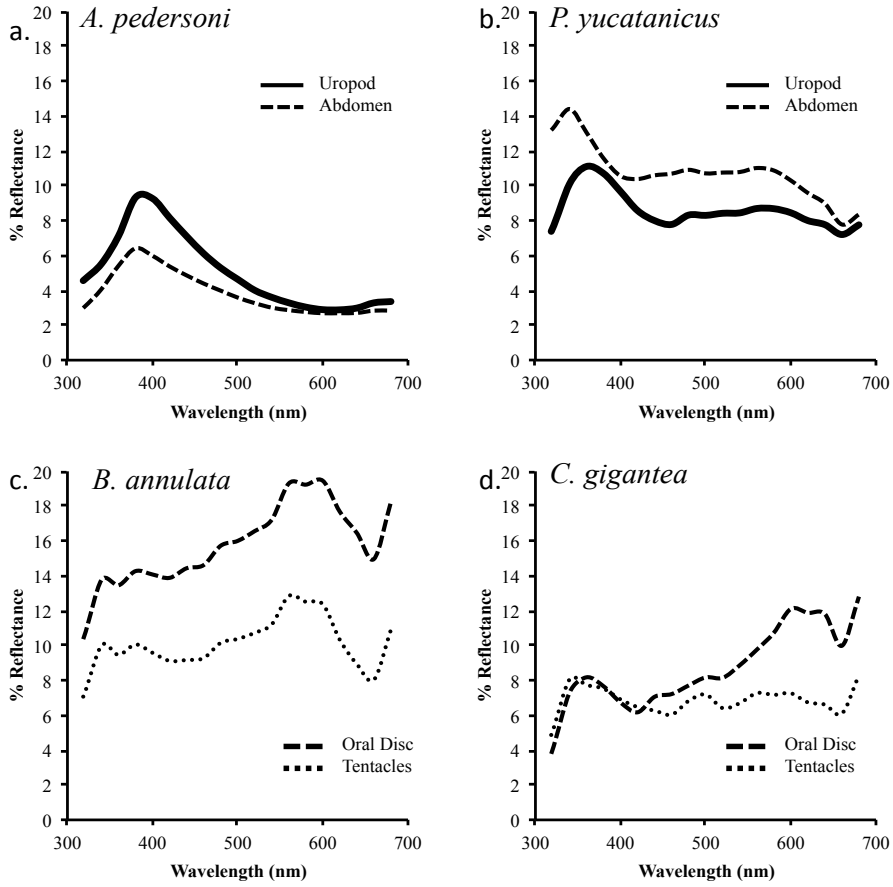

Fig. 2. Mean % reflectance of the anemone shrimps *A. pedersoni* (a.), and *P. yucatanicus* (b.) uropods (solid line) and abdomens (dashed line) as well as the anemones *B. annulata* (c.) and *C. gigantea* (d.) oral discs (dashed line) and tentacles (dotted line) in UV and visible light spectrum (300-700nm).

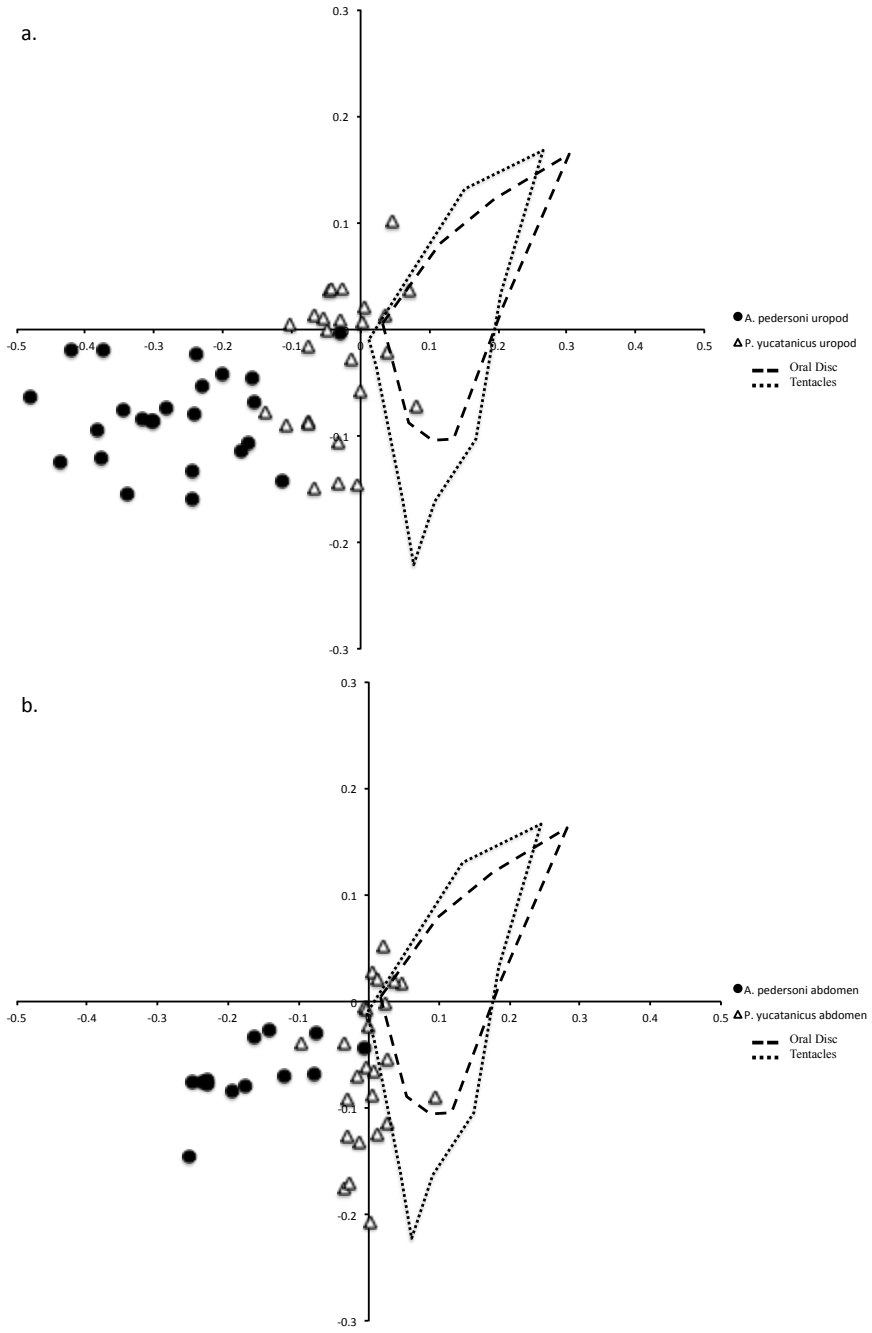

Fig. 3. Color space scores comparing the anemone *B. annulata* oral disc (dashed line) and tentacles (dotted line) to the anemone shrimps *A. pedersoni* and *P. yucatanicus* uropods (a) and abdomens (b).

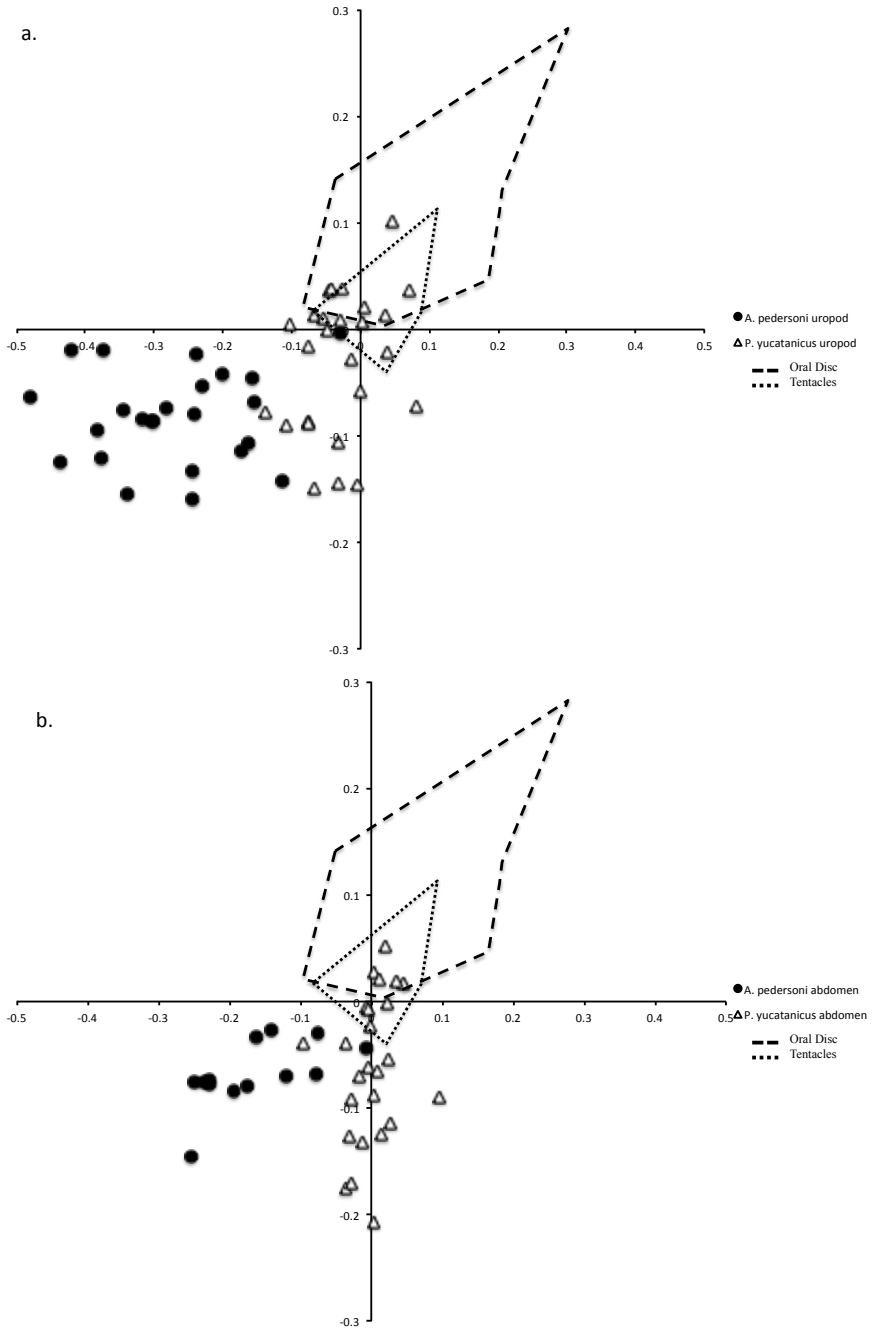

Fig. 4. Color space scores comparing the anemone *C. gigantea* oral disc (dashed line) and tentacles (dotted line) to the anemone shrimps *A. pedersoni* and *P. yucatanicus* uropods (a) and abdomens (b).

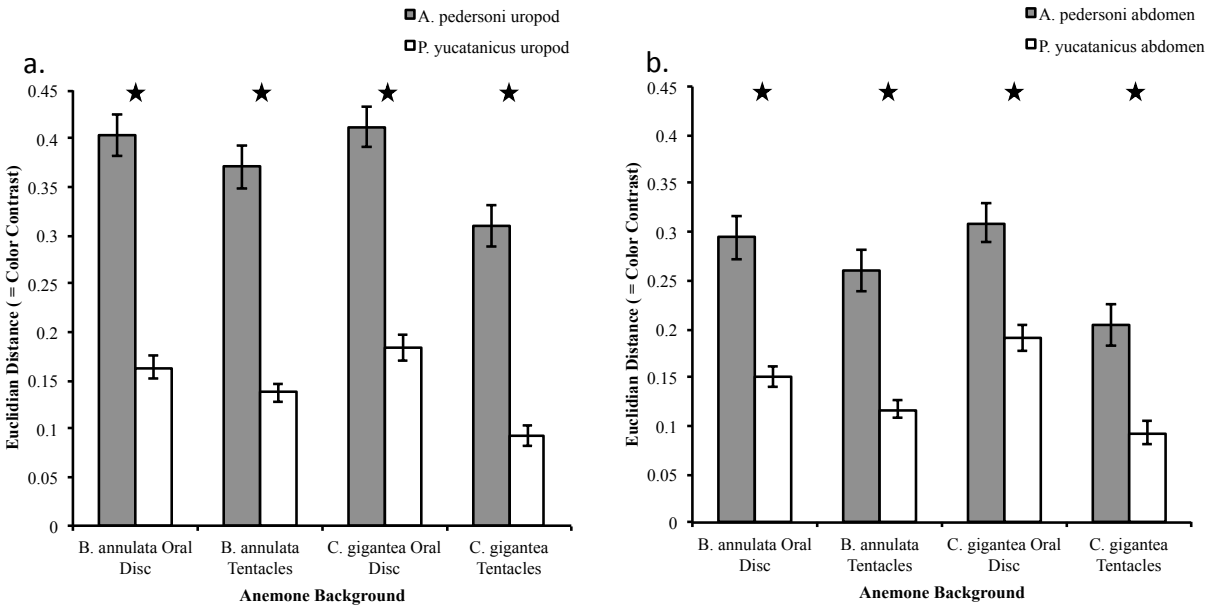

Fig. 5. Mean Euclidean distance (color) contrast (± s.e.) comparing anemone shrimps *A. pedersoni* and *P. yucatanicus* uropods (a) and abdomens (b) against the host anemones *B. annulata*, and *C. gigantea*, oral discs, and tentacles. Statistical results are from the general linear model with ⋆ indicating significant differences ($p < 0.05$) between *A. pedersoni* and *P. yucatanicus* against that anemone body part.

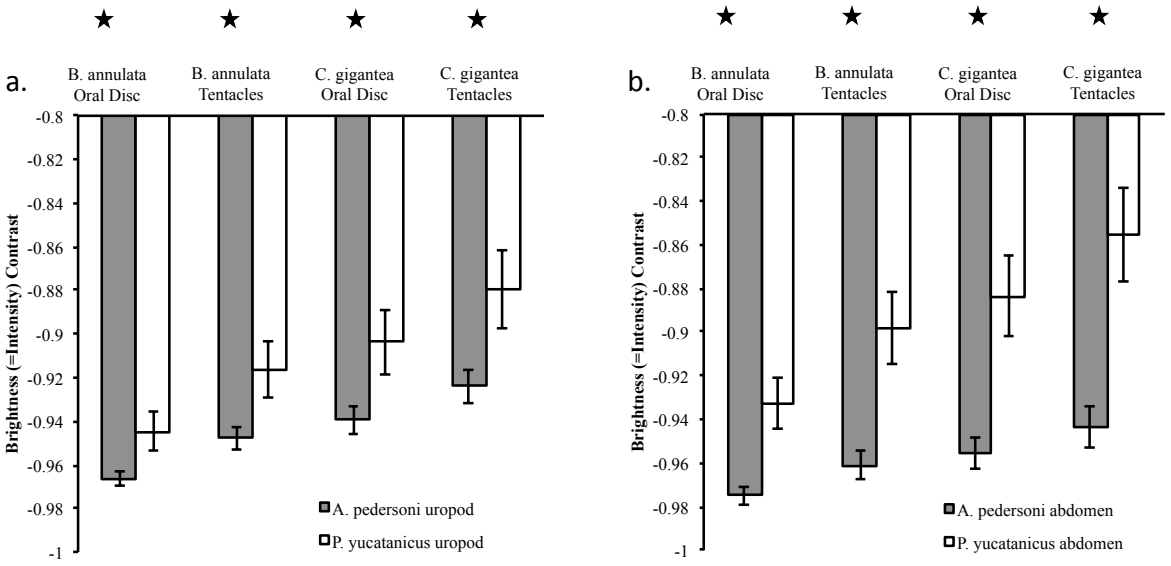

Fig. 6. Mean brightness (intensity) contrast (± s.e.) comparing anemone shrimps *A. pedersoni* and *P. yucatanicus* uropods (a) and abdomens (b) against the host anemones *B. annulata*, and *C. gigantea*, oral discs, and tentacles. Statistical results are from the general linear model with ⋆ indicating significant differences ($p < 0.05$) between *A. pedersoni* and *P. yucatanicus* against that anemone body part.

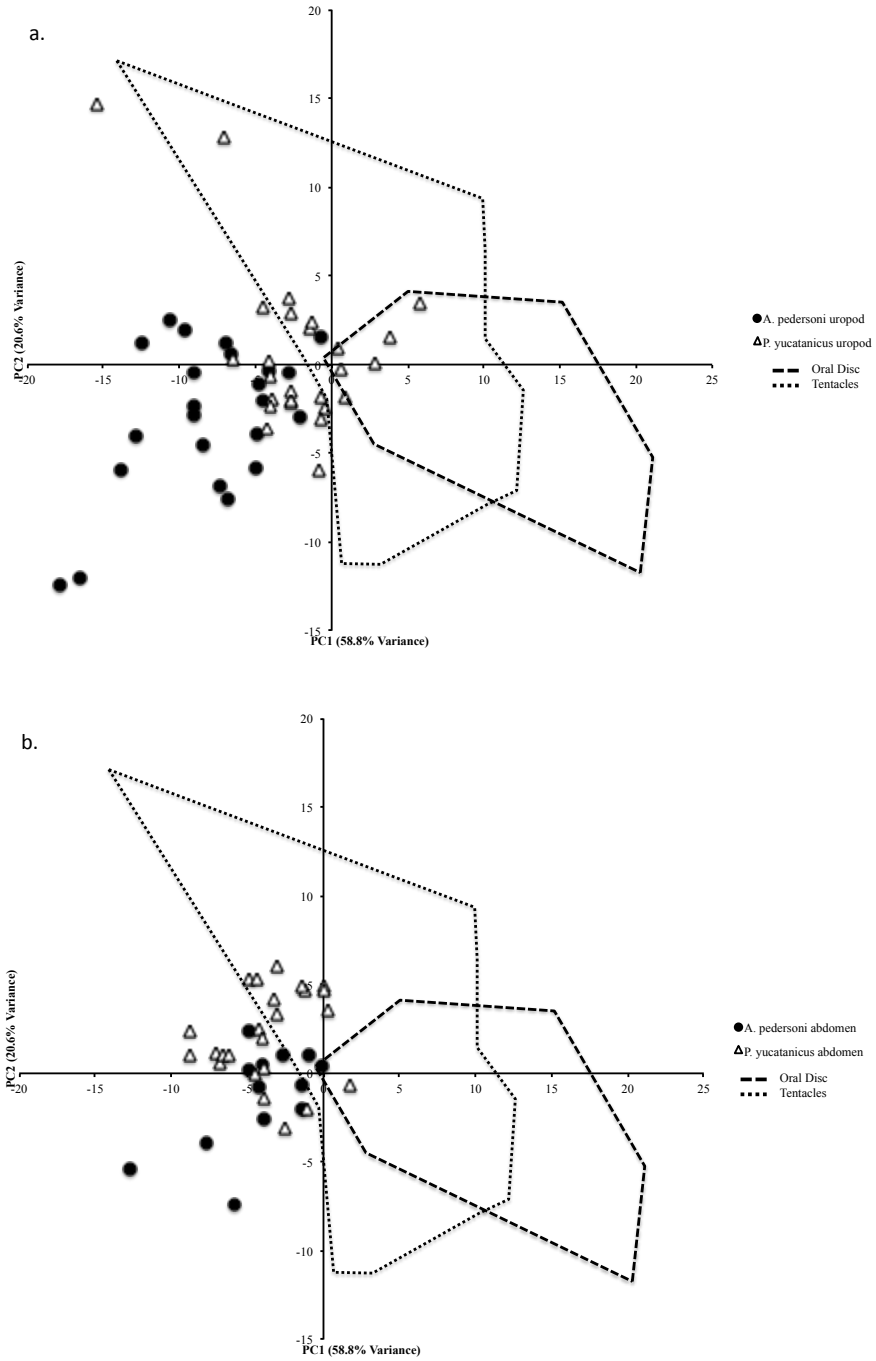

Fig. 7. PC1 and PC2 of normalized reflectance values comparing the outline of the anemone *B. annulata* oral disc (dashed line) and tentacle (dotted line) values to the anemone shrimps *A. pedersoni* and *P. yucatanicus* uropods (a) and abdomens (b).

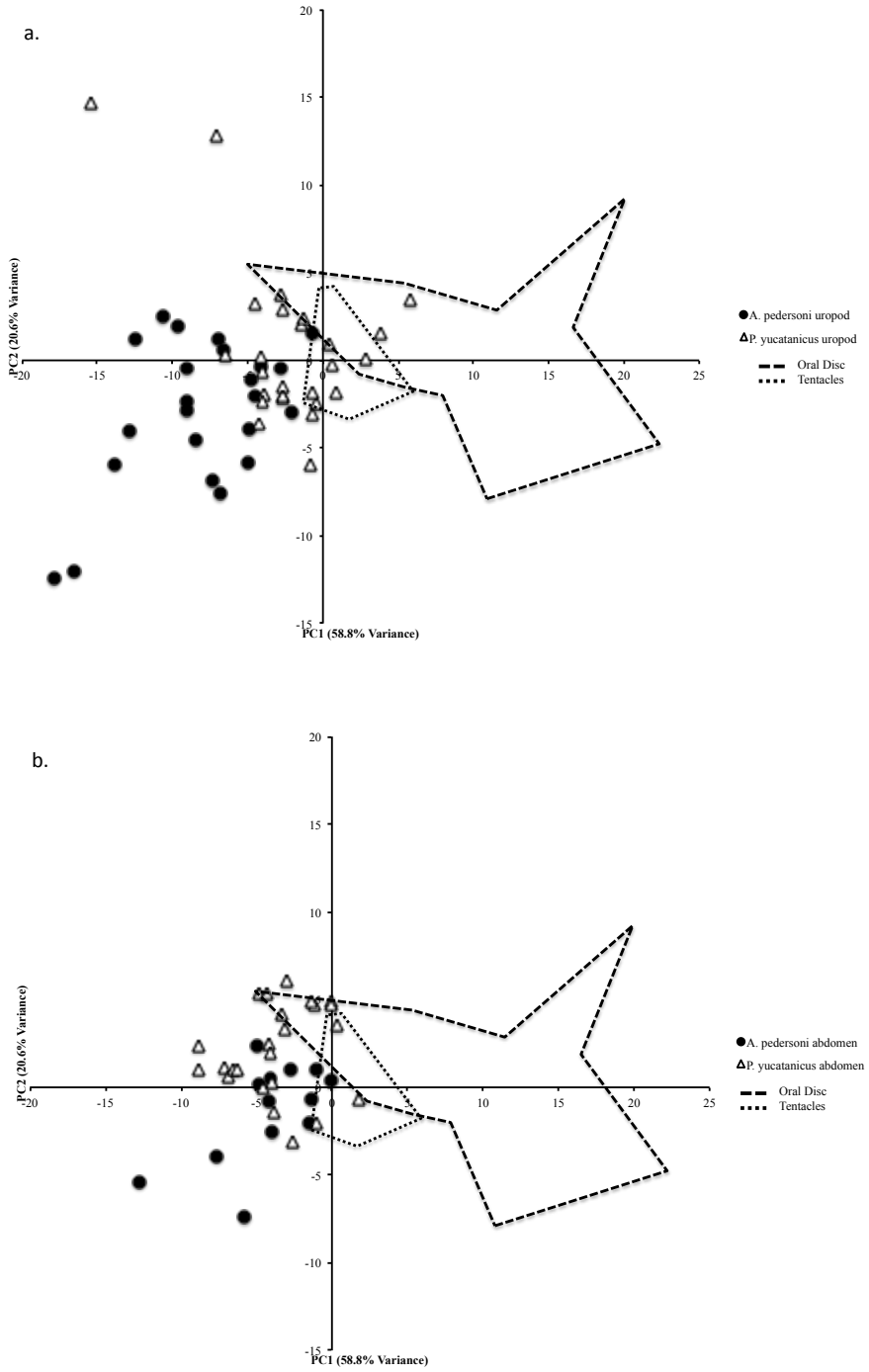

Fig. 8. PC1 and PC2 of normalized reflectance values comparing the outline of the anemone *C. gigantea* oral disc (dashed line) and tentacle (dotted line) values to the anemone shrimps *A. pedersoni* and *P. yucatanicus* uropods (a) and abdomens (b).

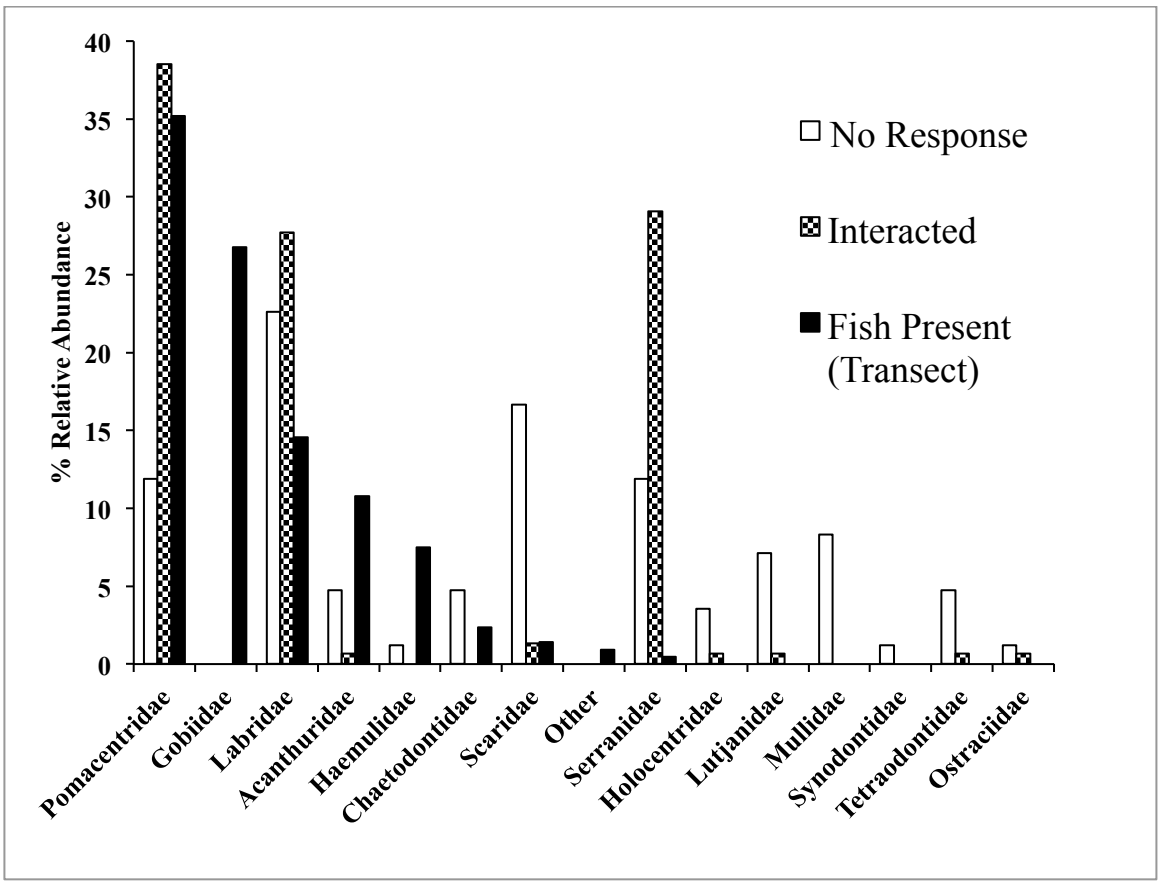

Fig. 9. Relative abundance (%) of fish families that were present (black, N = 5, 50m^2 transects), and fish families that interacted with the shrimp treatments (gray, N = 148 total interactions), or did not respond to the treatments (white, N = 84) in Brewer's Bay, St. Thomas, U. S. Virgin Islands, during July 2015.

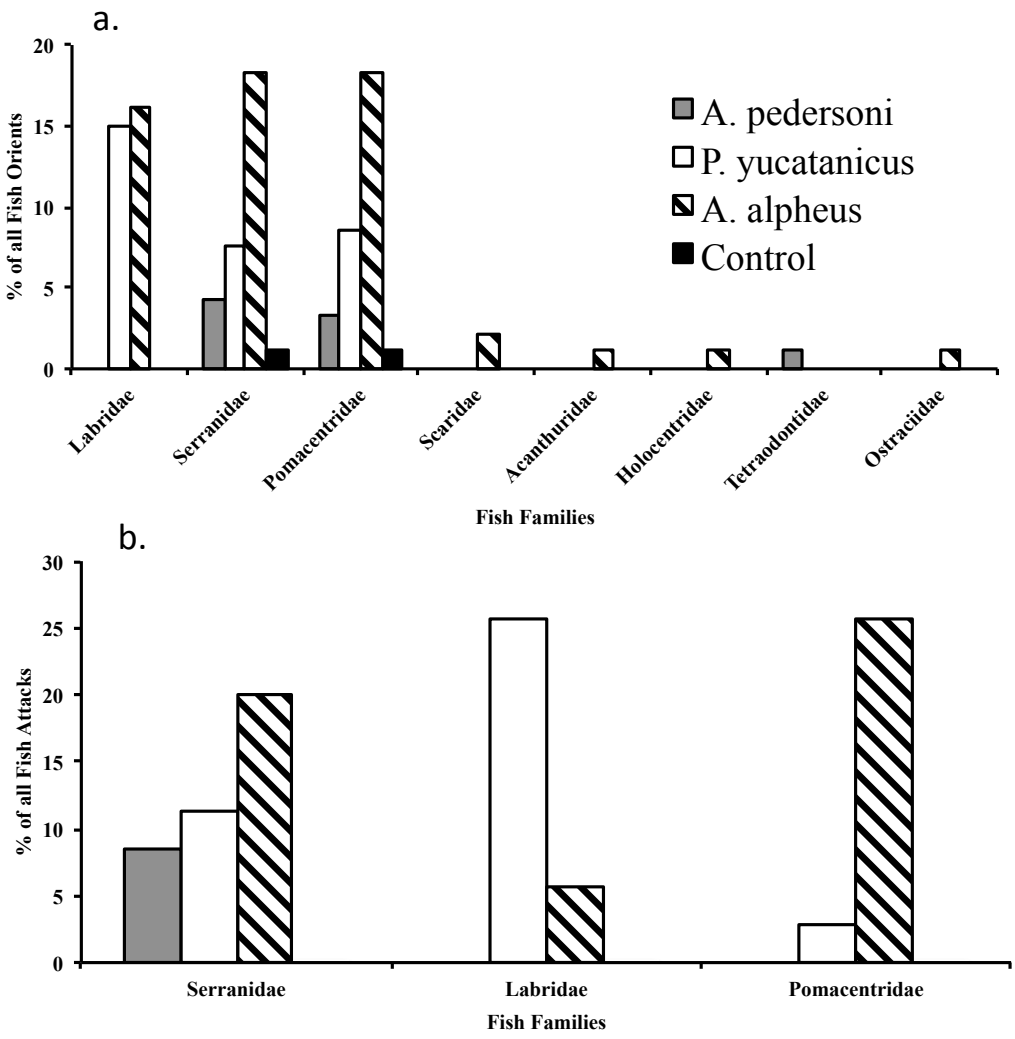

Fig. 10 Relative abundance (%) of fish families that oriented (a, n = 93), and attacked (b, n = 35) the shrimp the shrimp treatments *A. pedersoni* (gray), *P. yucatanicus* (white), *A. armatus* (striped), and *the control* (black) in Brewer's Bay, St. Thomas, U. S. Virgin Islands, during July 2015.

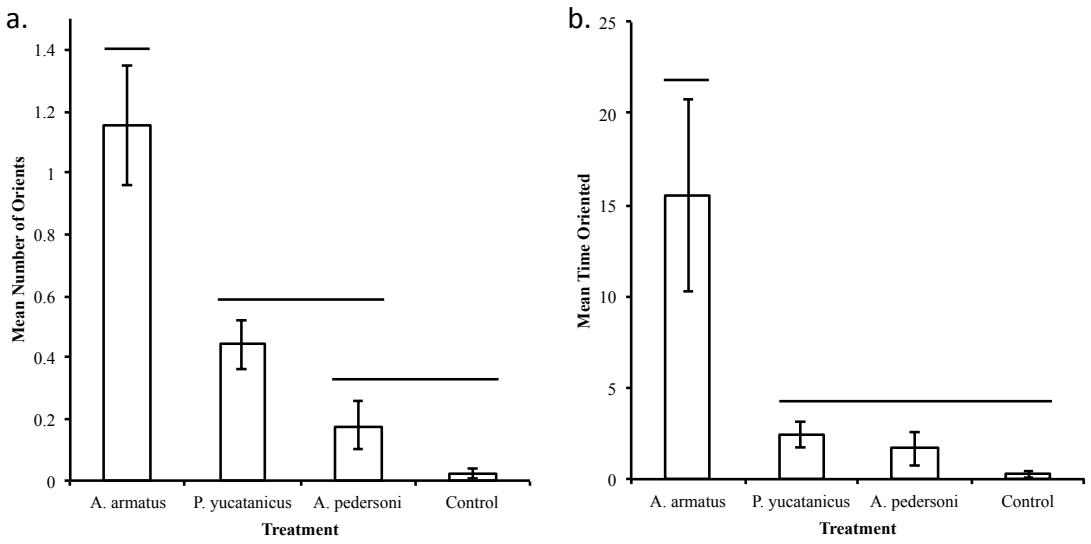

Fig. 11. Mean total number of orients (a) and mean duration of orients (b) towards shrimp treatments of (N = 36) trials in Brewer's Bay, St. Thomas, U. S. Virgin Islands, during July 2015. Statistical results are from a general linear model analysis, with separation of bars indicating a significant difference ($p \leq 0.05$) in responses to the treatments.

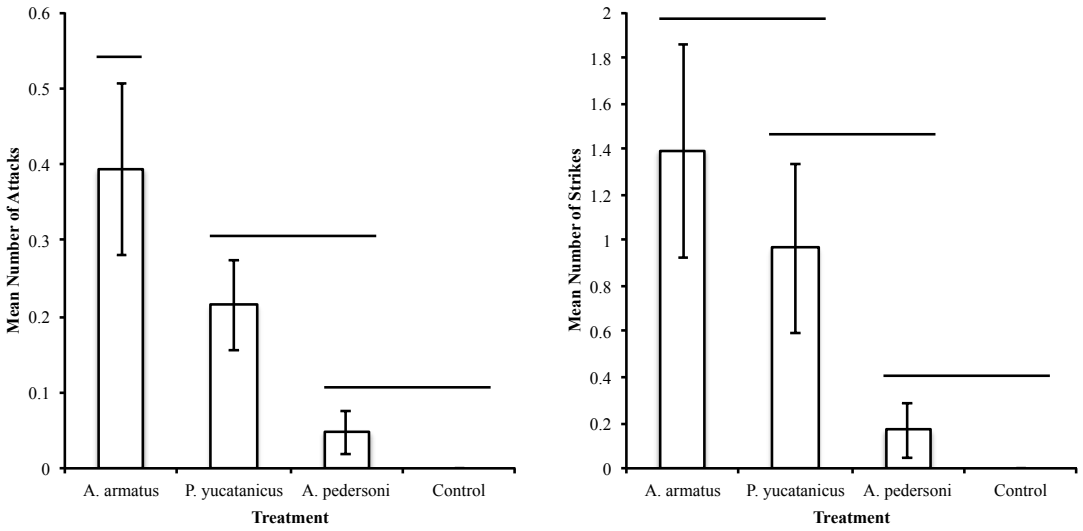

Fig. 12. Mean total number of attacks (a) and mean number of strikes/attack (b) towards shrimp treatments of (N = 36) trials in Brewer's Bay, St. Thomas, U. S. Virgin Islands, during July 2015. Statistical results are from a general linear model analysis, with separation of bars indicating a significant difference ($p \leq 0.05$) in responses to the treatments.

Chapter 3

Microhabitat Utilization and Behavioral Signaling Patterns of Two Anemoneshrimps: How do Mimics Compare with True Cleaners?

Introduction

Symbiotic relationships between organisms can be characterized as commensal, parasitic, or mutualistic, and all three types of symbiosis are prevalent in benthic marine environments. Especially common are relationships between small crustaceans and larger, often sessile host organisms, due to the high degree of competition for nutrients and space among crustaceans, as well as intense predation on these small invertebrates in marine environments (Roughgarden 1975; Trench 1979; Ambrosio and Brooks 2011; Cantrell et al. 2015). Crustaceans form symbiotic relationships with a wide variety of host invertebrates such as sea anemones (Mahnken 1972; Bauer 2004; Chadwick et al. 2008; Huebner and Chadwick 2012a;), corals (Blackall et al. 2015), scyphozoans (Goncalves et al. 2016), bivalves (Douglas et al. 2013), echinoideans (Baeza 2007), holothuroideans (Vandenspiegel et al. 1992), corallimorpharians (Williams and Williams 1982), and ascidians (Ambrosio and Brooks 2011). Benefits that the partners provide or receive in relationships that are mutualistic may vary depending on environmental conditions, and some relationships that have been assumed to be mutualistic may in some situations be better classified towards the parasitic side of the symbiotic spectrum (Guo et al. 1996). Potential benefits to partners in crustacean-host symbioses may include shelter from predators (Godwin and Fautin 1992; Huebner and Chadwick et al. 2012a), nutrient transfer (Spotte 1996; Roopin and Chadwick 2009; Cleveland et al. 2011; Cantrell et al. 2015; Verde et al. 2015), ventilation (Szczebvck et al. 2013), transport (Cutress et al. 1970 in Roughgarden 1975), and cleaning services (Becker and

Grutter 2004; Ostlund-Nilsson et al. 2005; Chapuis and Bshary 2009; McCammon et al. 2010; Huebner and Chadwick 2012a).

Most studies of cleaning symbioses on coral reefs have focused on small fishes that clean larger client fishes (reviewed in McCammon et al. 2010). This cleaner/client relationship is a form of mutualism, in which both partners receive a fitness benefit (Dickman 1992; Becker and Grutter 2005; Huebner and Chadwick 2012a). In marine cleaning symbioses, the cleaner receives food (ectoparasites), such as the gnathid isopods and monogeneans commonly found on reef fishes (Grutter and Poulin 1998; Grutter et al. 2002), and the client receives a reduced parasite load (Cheney et al. 2009; Huebner and Chadwick 2012b). Parasite removal improves the overall health of client fishes, and enhances fish diversity in coral reef environments (Bshary 2003; Grutter et al. 2003; Waldie et al. 2011; Sun et al. 2012). Marine cleaners are not always fishes; crustaceans such as shrimps also can be effective cleaners (Becker and Grutter 2004; Ostlund-Nilsson et al. 2005; Chapuis and Bshary 2009; McCammon et al. 2010). However, the abilities of reported cleaner shrimps to actually clean have been debated. In a recent study, the Caribbean cleaner shrimps *Ancylomenes pedersoni*, *Periclimenes yucatanicus*, and *Stenopus hispidus* were tested for their abilities to remove the monogenean ectoparasite *Neobenednia melleni* from host reef fish *Acanthurus coeruleus* (Blue Tang). Of the three shrimp species examined, only *A. pedersoni* significantly reduced the size and abundance of parasites on host fish (McCammon et al. 2010). Additionally in a similar study, only individuals of *A. pedersoni* were able to remove fish-parasitic cymothoid isopods *Anilocra haemuli* from French grunts *Haemulon flavolineatum* in laboratory trials when compared to several other Caribbean cleaners (Bunkley-Williams and Williams, 1998). These studies indicate that some shrimps reported as cleaners may not actually be effective cleaners of fishes at an ecological scale on reefs.

To attract the attention of potential client fishes to reduce predatory attacks, cleaner shrimps typically perform stereotypical dances that signal their status as cleaners, and which also may indicate shrimp willingness to clean (Becker et al. 2005; Chapuis and Bshary 2010). In response to the approach of a large object such as a client fish, or even of a scuba diver due to the shrimps' poor visual shape resolution (Caves et al. 2016), individuals of *A. pedersoni* wave their antennae and laterally rock their bodies (Wicksten 1998). In the Indo-Pacific cleaner shrimp *P. longicarpus*, a stereotypical clapping of claws apparently is not used to attract potential clients from a distance, but to signal cleaner identity (as distinct from prey) after clients have arrived at the station; individuals of *P. longicarpus* use their claw-clapping behavior to signal more frequently to predatory clients than to non-predatory clients, presumably to communicate their identity more clearly to predators and thus avoid being attacked (Chapuis and Bshary 2010). Also, some cleaner shrimp signal their motivation or willingness to clean (satiation level) by increasing their frequencies of dancing behaviors (body rocking and antennae waving) when hungry (Becker et al. 2005). Shrimp behaviors may communicate reinforcing signals to fish clients that are similar to signals conveyed by their body coloration patterns, or alternately may convey different or contrasting types of signals. When advertising their services, cleaner organisms also usually perch near or on large symbiotic hosts such as sea anemones, sponges, or massive corals, or on prominent knolls or in large crevices on coral reefs (Soares et al. 2008a; Huebner and Chadwick 2012b; Mascaro et al. 2012). Association with a large reef organism or prominent physical feature may make small cleaners more visible to passing clients (Huebner and Chadwick 2012a; Chapter 2). This is especially apparent for the anemoneshrimps *A. pedersoni* and *P. yucatanicus* on their two host anemones *Bartholomea annulata* and *Condylactis gigantea*. Examining how these two anemone shrimps signal behaviorally to client

fishes, and utilize their anemone microhabitat space, could lead to insights about the extent to which they use anemones as cues for cleaning interactions versus for protection from predators.

Some cleaning symbiotic systems include cleaner mimics such as the Indo-Pacific fangblennies *Plagiotremus rhinorhynchos* which are color mimics of cleaner fish *Labroides dimidiatus*, Cheney and Cote 2007; Cheney and Marshall 2009). I propose that a similar but more passive type of mimicry, based on predator avoidance rather than aggressive attack of predators, occurs between two Caribbean anemoneshrimps: *A. pedersoni* (cleaners) and *P. yucatanicus* (mimics). These shrimps exhibit similar but not identical patterns in both their body coloration (spots on the abdomen and uropods, Chapter 2), and behavioral signaling (stereotypical dances of body rocking and antennae waving, Limbaugh et al. 1961; Mahnken 1972) to passing fishes. In addition, they also occupy overlapping but not identical types of microhabitat zones on host sea anemones *B. annulata* and *C. gigantea*, Fig. 1). As such, they are very similar in both their color and behavioral signals to fishes, as well as their habitat use patterns related to using anemones as shelter habitats, suggesting potentially similar ecological roles as well as possible interspecific mimicry.

Only one reported cleaning observation exists of *P. yucatanicus* removing parasites from a single fish individual (Spotte et al. 1991); subsequent replicated trials indicated no substantial cleaning by this shrimp, in contrast to *A. pedersoni* which consumes large amounts of fish parasites (McCammon et al. 2010). Thus, individuals of *P. yucatanicus* may receive benefits by visually and behaviorally mimicking *A. pedersoni* thus avoiding predation by fishes (Chapters 1 and 2) without providing any regular services to client fishes that approach sea anemone cleaning stations on Caribbean coral reefs.

I address here 2 hypotheses concerning patterns of microhabitat use and behavioral signaling by cleaner shrimp *A. pedersoni* in comparison with mimic shrimp *P. yucatanicus* on Caribbean coral reefs:

1. Cleaner shrimp *A. pedersoni* occupy microhabitats further from the center of host anemones than do mimic shrimp *P. yucatanicus*, because of their relatively low risk of predation and high requirement for access to client fishes.
2. Antennae waving and body rocking patterns of mimic shrimp *P. yucatanicus* are similar to those of cleaner shrimp *A. pedersoni*, but are displayed less frequently and do not indicate their willingness or ability to clean fishes.

Methods

To determine the species of sea anemone hosts selected by shrimps, and their patterns of microhabitat use on each species of host anemone, laboratory experiments were conducted at Auburn University during February 2015 to July 2016. Anemoneshrimps *A. pedersoni* (N = 24) and *P. yucatanicus* (N = 23), as well as host sea anemones *Bartholomea annulata* (N = 26) and *Condylactis gigantea* (N = 18) were obtained from KP Aquatics Inc, (Key Largo, Florida, USA), and Dynasty Marine (Marathon Key, Florida, USA), at least 1 week prior to their use in experiments. All shrimps then were cultured separately from sea anemones for > 15 days, because this is the known duration for these shrimps to lose protection from the nematocysts of their former host sea anemones, thus preventing any pre-collecting bias in host choice trials (Rodriguez-Pestana 2007, Mascaro et al. 2012). During culture, each shrimp was housed in a separate cage that floated in a tank containing other shrimps in cages; each cage was a 0.5 L plastic container with holes cut in it to allow water flow. Shrimps were fed individually inside their cages, allowing identification and isolation of individual shrimps during culture. Shrimp

and anemones were cultured in closed-system aquaria; shrimp were fed every other day with Formula One Marine Pellets (Ocean Nutrition, San Diego CA, USA), and anemones were fed once a week with pieces of raw cocktail shrimp (after Szczebak et al. 2013; culture system details in Roopin and Chadwick 2009; Cantrell et al. 2015). The shrimps and anemones grew, some of them sexually reproduced (female shrimp produced egg masses on their abdomens), and almost all of them appeared to remain healthy during culture in this system.

At the start of each behavioral experiment, each shrimp was transferred into one half of a treatment tank that was divided into 2 sections with a plastic mesh barrier (due to limited tank space; see details above about tank size and setup), and randomly assigned to a treatment. Each treatment consisted of exposure to a group of 2-6 sea anemones, consisting of individuals of: (1) *B. annulata* (N = 17 *A. pedersoni*, N = 19 *P. yucatanicus*), (2) *C. gigantea* (N = 13 *A. pedersoni*, N = 14 *P. yucatanicus*), or (3) both *B. annulata* and *C. gigantea* (one individual of each species) (N = 11 *A. pedersoni*, N = 9 *P. yucatanicus*). Due to shrimp mortality (see above), some shrimps were exposed to only some treatments (79.2% of *A. pedersoni* individuals, and 69.6% of *P. yucatanicus* of individuals), but 5 shrimp replicates of *A. pedersoni*, and 7 of *P. yucatanicus* were observed in each of the 3 above treatments. Groups of anemones rather than only one individual per treatment were used, because the anemones were being used in a concurrent growth study, and tank space was limited in the lab. The individual anemones were cultured separately from the shrimps between treatments (see above), and were assigned randomly to each treatment. Each treatment of one shrimp housed with a group of anemones lasted for 14 days, after which each shrimp was cultured separately again for a minimum of 15 days in its cage, before beginning the next treatment.

In a randomized subset of the above 3 treatments, each shrimp (*A. pedersoni* N = 12, *P. yucatanicus* N = 13) also was exposed to a common Caribbean reef fish cultured in the same tank with the sea anemones. This treatment was performed to determine how fish presence impacted microhabitat choice and distance occupied from host anemones by the shrimps. Yellowtail damselfish (*Microspathodon chrysurus*) or longfin damselffish (*Stegastes diencaeus*) were used because they are common on Caribbean reefs and easy to culture (Cantrell et al. 2015 and references therein). Fish were obtained from the same suppliers (see above), and cultured under the same conditions. Shrimps were exposed to fish for 7 days, with randomized order in terms of whether the fish exposure was for the first or second 7 days in the above 3 treatments. Overall, treatment order for each shrimp was randomized, based on the availability of treatment tanks in the laboratory (N = 9 tanks total).

Microhabitat use was quantified as shrimp location each day in one of 5 location categories on the host anemones (Fig. 13): (1) along the column underneath tentacle crown, (2) oral disc or inner half of tentacle crown, (3) outer half of the tentacle crown, (4) not in contact with the anemone on soft substrate, (5) not in contact with anemone on hard substrate. In the case of categories 4 and 5, the distance from the shrimp to the nearest sea anemone tentacle was measured in centimeters. If the shrimp was in categories 1-3, or off the anemone (categories 4-5) but still touching the tentacles, the distance from the anemone was recorded as 0. Each day, the anemone which each shrimp was closest to was also recorded. Although microhabitat use was distinguished between hard and soft substrate (categories 4 and 5) during field studies on these shrimps (Huebner and N. E. Chadwick unpublished data,), during the present laboratory studies these 2 categories were grouped because both categories offered the same amount of protection by the host anemone (distance from tentacles), and there was limited hard substrate in the

experimental tanks. Each of the 3 types of responses (number of days associated with each anemone species and in each type of microhabitat, as well as distance from the closest anemone each day) were examined for variation with shrimp species (N=2) and anemone species (N=2).

Visual Signaling Frequency and Patterns

A field experiment was conducted during August 2 – 9, 2016, on shallow coral reefs (2 to 10 m depth) in Brewer's Bay, St. Thomas, U. S. Virgin Islands (see Huebner and Chadwick 2012a,b for detailed study site description). The experiment was conducted using SCUBA, with the nearby MacLean Marine Science Center (MMSC) of the University of the Virgin Islands as a base for operations, in accordance with AAUS (American Academy of Underwater Sciences) scientific diving standards.

The experiment focused on determining the frequencies and patterns of behavioral signaling by the anemone shrimps *A. pedersoni* and *P. yucatanicus* (both body rocking and antenna waving) towards client fishes who approached their host anemones for cleaning. Individuals of anemones *B. annulata* (n = 76) and *C. gigantea* (N = 5) that contained *A. pedersoni* or *P. yucatanicus* shrimps were selected haphazardly at the study site, and marked with a numbered small orange flag. A total of 157 shrimps of *A. pedersoni* (N = 131) and *P. yucatanicus* (N = 26) were observed during the trials. Fewer of the latter species were observed, due to their relatively lower abundance at the study site.

These anemone shrimps have poor eyesight and are able to visually resolve only large looming objects (Caves et al. 2015), thus they readily signal to passing fishes and to human SCUBA divers alike (Limbaugh et al. 1961; Mahnken 1972; Humann and Deloach 2006; Stuart

personal observations). Thus, at the start of each trial, the shrimps were presented with a diver's hand as the stimulus for the cleaning interaction (after Chapuis and Bshary 2010), and the shrimps reactions were recorded. Shrimp reactions were classified as retreat (backing away from the hand), approach (moving towards the hand), signaling (rocking and or antennae waving), cleaning (physically picking at the divers hand with their chelepeds in an attempt to clean), and no response (not moving or reacting to the hand stimulus). Trials were concluded when the shrimps stopped reacting toward the hand (retreating, approaching, signaling, or cleaning), which was usually between 40-180 seconds after presentation of the stimulus. For shrimps that exhibited no reaction, the hand was removed after 90 seconds. A GoPro Hero4 Black edition set to 1080p wide view at 30 fsp was mounted on a weighted tripod and placed about 30cm distant from the shrimps for the duration of each trial. The recorded videos were later reviewed in slow motion (frame by frame) using Quick-Time Player 10.3 computer software, in order to accurately quantify aspects of the shrimp signaling patterns.

Statistical Analysis: Microhabitat Experiment

Separate statistical analyses were conducted for each of the three laboratory treatments on microhabitat use by the shrimps, due to some individual shrimps participating in combinations of multiple anemone treatments (*B. annulata*, *C. gigantea*, or *B. annulata* and C. *gigantea*) while others did not. Additionally a subset of shrimps (N = 8 *A. pedersoni*, N = 10 *P. yucatanicus*) that participated in both *B. annulata* and *C. gigantea* treatments were also analyzed separately for a direct comparison for each shrimp species with the two anemone treatments. For the individual treatment analysis, a general linear model was used to test for variation in shrimp distance (cm) from the anemone tentacles with shrimp species and length of trial (days), with a random effect of individual to account for repeated measures. To test the distribution of shrimp occupation of

anemone microhabitats (*B. annulata* and *C. gigantea* treatments, Fig.13), a Chi-Square analysis was conducted comparing effect of shrimp species (*A. pedersoni* and *P. yucatanicus)* on microhabitat occupied for the majority of days during each 14-day trial. Additionally the percent of microhabitats occupied by each shrimp species on each the anemone species was calculated using the most frequent habitat selected over 14 days of individual shrimps.

For the anemone choice experiment between *B. annulata* and *C. gigantea*, a binomial was conducted for each of the shrimp species using the mode anemone species choice of the 14 trial days for each shrimp individual. For the subset of shrimps that participated in the *B. annulata* and *C. gigantea* treatments, a general linear model was used to test the average distance from the anemone of shrimp species as explained by the anemone species, and duration of the trial with a random effect for individual shrimp. Lastly in a randomized subset of the 3 (whole) anemone treatments (*A. pedersoni* N = 12, *P. yucatanicus* N = 13) to test the effect of fish presence on mean distance from anemone tentacles, a general linear model was conducted testing the effect of shrimp species, anemone species, fish presence, and duration of trial on the distance from anemone tentacles with a random effect of individual to account for the repeated measures.

Statistical Analysis: Shrimp Signaling Patterns

For the shrimp signaling patterns in response to a stimulus, a Chi-Square analysis was conducted comparing the shrimp species *A. pedersoni* and *P. yucatanicus* of the response categories approach, retreat, wave antennae, clean, wave antennae and clean, and no response. Due to low frequencies in some categories the data were consolidated into three categories, (1) signaled (approach, rock/wave, clean, rock/wave and clean), (2) retreat, and (3) no response

All statistical analyses were conducted using R vs.3.1.1, R Core Team (2013; R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/).

Results

In laboratory experiments, the 2 anemoneshrimps did not differ significantly in the overall distance of microhabitat that they occupied away from the tentacles of host anemones *B. annulata*, but did vary in the timing duration of their distances from this host (d.f. = 455, F = 37.77, p <0.0001) (Fig. 14a). In contrast, the mimic shrimp occurred significantly closer to host anemones *C. gigantea* than did the cleaner shrimp (d.f. = 25, F = 11.96, p = 0.002), and this distance differed significantly over time for both shrimp species (d.f. = 341, F = 7.46, p = 0.007) (Fig. 14b). Similarly, the mode habitat distribution did not differ significantly between the 2 shrimps *A. pedersoni* and *P. yucatanicus* in relation to *B. annulata* (Table. 3 a, Fig. 15), but did when they occurred with *C. gigantea* hosts (d.f. = 3, $X^2 = 16.93$, p = 0.0007, Table 3b, Fig. 15). In the subset of shrimps that participated in both the *B. annulata* and *C. gigatantea* treatments, there was a not a significant effect of anemone species for the average distance from the anemone for *A. pedersoni*, but there was for the duration trial (d.f. = 208, F = 7.95, p = 0.0053). For *P. yucatanicus* there was a significant effect of anemone species (d.f. = 251, F = 30.11, p <0.001) and duration of the trial (d.f. = 251, F = 19.37, p < 0.001). Additionally there were no significant difference in the Chi-square analysis of the distribution of the shrimps occupying the anemone microhabitats for the subset of *A. pedersoni* and *P. yucatanicus* shrimps that participated in both the *B. annulata* and *C. gigantea* treatments.

In terms of laboratory choice of host species, the shrimp species did not significantly differ in their mode preferred choice (binomial) of the host anemones *B. annulata* or *C. gigantea*, but *A. pedersoni* favored *B. annulata* 64% of the time, and conversely *P. yucatanicus* favored *C. gigantea* 78% of the time. Only once, a single shrimp (*A. pedersoni*) switched its preferred anemone species (*B. annulata* to *C. gigantea*) during the choice trial period (14 days), with all other shrimps residing on or near the initial anemone species choice for the duration of the trial.

During the field behavioral trials, the probability of the shrimps signaling, retreating, or no responding to the hand stimulus varied significantly with the species of shrimp (Chi-square test, d.f. = 2, $X^2 = 67.74$, $p < 0.0001$, Table 5). Individuals of *A. pedersoni* signaled more frequently than did those of *P. yucatanicus*, with *A. pedersoni* signaling for 24.4% of its total number of interactions (N = 131) to *P. yucatanicus*' 11.5% of its total number of interactions (N = 26). *A. pedersoni* did not respond to the stimulus for 66.4% of total interactions, and retreated from the stimulus for 9.2% of the total interactions. *P. yucatanicus* did not respond to the stimulus for 7.7% of its total interactions, and retreated for 80.8% of its total interactions.

Discussion

We demonstrate here that cleaner shrimp *A. pedersoni* and potential mimic shrimp *P. yucatanicus* occupy similar microhabitats in relation to their host sea anemones, but that overall *A. pedersoni* occur more distant from the centers of host anemone tentacle crowns. In addition, both species exhibit highly similar behavioral patterns of antennae vibration in response to simulated fish approach, with some important differences. These patterns, together with their similar body coloration patterns and visual deterrence of fish predation (Chapter 2), provide strong evidence that non-cleaners *P. yucatanicus* mimic the behavior and color patterns of

cleaner shrimp *A. pedersoni* without actually providing any cleaning services, thereby gaining the immunity from fish predation that is afforded to cleaners on coral reefs.

Results from our laboratory experiments generally supported the hypothesis that cleaner shrimp *A. pedersoni* occupy microhabitats further from the center of host anemones than do mimic shrimp *P. yucatanicus*. Interestingly, cleaner shrimp *A. pedersoni* remained significantly farther away from the protection of anemone tentacles than did mimic shrimp *P. yucatanicus* when they associated with anemone host *C. gigantea*, but not with *B. annulata*, where both shrimp remained off the anemone. This pattern indicated that cleaner shrimp did not vary in their distance from hosts depending on the anemone species, but that mimics *P. yucatanicus* remain significantly closer to *C. gigantea* than to *B. annulata*. These differences may be explained in part by the strong preference of *P. yucatanicus* shrimp for *C. gigantea* as a host (78% of *P. yucatanicus* shrimps in our study), whereas *A. pedersoni* do not exhibit a strong preference for either host anemone species, and occupy similar microhabitats relative to both. These patterns are consistent with the results of other host selection experiments conducted on these species (Silbiger and Childress 2008; Mascaro et al. 2012). They may be driven by characteristic of *C. gigantea* that offer a greater degree of physical protection, camouflage, or other benefits to the mimic shrimp than do corkscrew anemones *B. annulata*. They also are supported by the lower visual contrast exhibited by mimic shrimp against *C. gigantea* anemones than *B. annulata* anemones, in that *C. gigantea* may serve as a better camouflage host for *P.yucatanicus*. The pink/purple coloration of spots on *P. yucatanicus* (Chapter 2) may relate to this closer visual matching to and behavioral preference for *C. gigantea* over *B. annulata*.

The lack of impact from the presence of damselfish in the experimental tanks on shrimp distance from the anemones may have been due to laboratory artifact. In contrast, under natural

field conditions, fish presence likely exerts more impact on shrimp microhabitat use, due to frequent visits to shrimp-occupied anemones by a wide diversity of reef fishes (Huebner and Chadwick 2012; Titus et al. 2015), some of which may attack the shrimps (Chapter 2). In our laboratory experiments, the damselfish were not ever observed to attack acclimated shrimps (a few shrimp deaths occurred when shrimp were newly introduced to a tank), and rarely oriented towards them, possibly because they were well-fed in the laboratory with dried and frozen foods. Field observations indicate that mimic shrimp *P. yucatanicus* usually occupy microhabitat regions near to the center of anemones (regions 1, 2, and 3, see Fig. 13) than they did in the laboratory, regardless of whether the anemone host species is *B. annulata* or *C. gigantea* (L. Huebner and N. E. Chadwick unpublished data, Stuart personal observation).

Microhabitat use by cleaner shrimp on the outer tentacles or off the anemone may occur due to their low risk of predation and their high requirement for access to client fishes, relative to mimic shrimp. In contrast, because the mimic shrimp rarely if ever actually approach or clean fishes, they do not need to perch on the outer anemone tentacles for quick access to passing fishes. As well, they likely benefit more from remaining in the tentacle crown where they are both more camouflaged (thus avoiding detection) and can more easily prevent attack if detected (due to the stinging properties of the anemone tentacles). Thus, the benefits appear to outweigh the costs for cleanershrimp to occupy outer microhabitats on anemone hosts, and likewise for mimic shrimp to remain on the inner habitats, given their different ecological functions relative to passing reef fishes.

Our results also supported the hypothesis that the antennae waving and body rocking patterns of mimic shrimp *P. yucatanicus* are similar to those of cleaner shrimp *A. pedersoni*. Both shrimps were observed to display both types of behavioral signals under field conditions in

response to a simulated fish stimulus, and also have been observed to display them frequently under laboratory conditions as well, even when no stimulus is present (Stuart and Chadwick personal observations). We also demonstrate that mimics display these signals less frequently than do models (cleaner shrimp), and that the display of behavioral signals by the mimic does not indicate a willingness or ability to clean fishes, as only two of the mimic shrimp were observed to attempting to briefly clean the simulated client fish that was presented to them in the field, and did not leave the vicinity of the anemone tentacles in the attempt to do so. The significantly different distributions among the 6 possible responses to the stimulus (approach, rock/wave, clean, rock/wave and clean, retreat, or no-response) that were exhibited by the two shrimp species indicated that even though their rocking and waving behaviors may be similar, their frequencies of displaying them vary dramatically. Cleaner shrimp *A. pedersoni* behaviorally interacted with the presented stimulus in all 4 ways examined (approach, rock/wave, clean, or rock/wave and clean) more than twice as frequently as did mimic shrimp (overall 24.4% of interactions versus only 11.5%), indicating a greater willingness to engage in signaling behavior and engagement with a visual stimulus. It is not surprising that most of the time cleaners *A. pedersoni* did not respond to the stimulus (66.4% of interactions), because they may have been satiated from recent consumption of fish parasites at the field site, and cleanershrimp willingness to signal is known to decreases with satiation (Becker et al. 2005; Chapuis and Bshary 2010). Their very low frequency of retreat from the stimulus (only 9.2% of interactions) contrasts dramatically to mimic shrimp *P. yucatanicus* retreating during most interactions (80.8% of interactions), and reflects a major difference in the behavioral responses of these two shrimp species to visual stimuli. The frequent retreat by *P. yucatanicus shrimp* from visual stimulus suggests that despite their ability to mimic cleaner behavioral signals, they prefer most of the

time to retreat into the interior of the anemone tentacle crown, rather than to signal when approached. This behavioral pattern supports the idea that they may rely on camouflage and avoidance of detection by visiting fishes, as an alternate and more frequently-employed strategy to deter predation, in addition to their infrequently employing behavioral and color mimicry of cleaners. It is possible that they use the latter strategy mainly when they perceive that they already have been detected by fishes (ie: when they are perched farther out on the edges of tentacles), so that camouflage is no longer an effective option during that particular interaction. Further experimental testing could explore this possibility.

Our observations that *A. pedersoni* are more likely to signal potential client fishes than are *P. yucatanicus*, and are less wary when large organisms approach their anemones, are in keeping with their functional role as major fish cleaners on Caribbean reefs. This observation is supported by their occupation of microhabitats further from the protection of anemone tentacles than by *P. yucatanicus*, most likely related to their need to gain quick access to client fishes visiting their cleaning stations. Together, both types of behavioral evidence (patterns of microhabitat occupation and behavioral signaling to visual stimuli) support the idea that *P. yucatanicus* functions as a mimic of cleaner shrimp *A. pedersoni* on Caribbean reefs, but that it also employs behavioral camouflage strategies of background matching when approached by reef fishes. Together with evidence concerning the coloration patterns of both shrimps and their likelihoods of visually eliciting fish attacks (Chapter 2), this study reveals an interesting and complex mimicry system based on cleaner shrimp in the Caribbean Sea.

References

Ambrosio, L. J., & Brooks, W. (2011). Recognition and use of ascidian hosts, and mate acquisition by the symbiotic pea crab *Tunicotheres moseri* (Rathbun, 1918): The role of chemical, visual and tactile cues. *Symbiosis, 53*(2)*,* 53-61. doi: 10.1007/s13199-011-0112-8

Baeza, J. A. (2007). The origins of symbiosis as a lifestyle in marine crabs (genus Petrolisthes) from the eastern Pacific: Does interspecific competition play a role? *Revista de Biologia Marina y Oceanografia, 42*(1), 7-21. Retrieved from http://www.revbiolmar.cl/index.php?lang=en

Bauer, R. T. (2004). Remarkable shrimps: Adaptations and natural history of the carideans. Norman: University of Oklahoma Press.

Becker, J. H., & Grutter, A. S. (2004). Cleaner shrimp do clean. *Coral Reefs*, *23*(4), 515-520. doi: 10.1007/s00338-004-0429-3

Becker, J. H. A., & Grutter, A. S. (2005). Client fish ectoparasite loads and cleaner shrimp *Urocaridella* sp. c hunger levels affect cleaning behaviour. *Animal Behaviour*, *70*(5), 991-996. doi: 10.1016/j.anbehav.2005.01.004

Blackall, L. L., Wilson, B., & Van Oppen, M. J. H. (2015). Coral – The world's most diverse symbiotic ecosystem. *Molecular Ecology, 24*(21), 5330-5347. doi: 10.1111/mec.13400

Braithwaite, V. A., & de Perera, T. B. (2006). Short-range orientation in fish: How fish map space. *Marine and Freshwater Behavioral Physiology*, *39*(1), 37-47. doi: 10.1080/10236240600562844

Bruce, A. J., & Svoboda, A. (1983). Observations upon some pontoniine shrimps from Aqaba, Jordan. *Zoologische Verhandelingen, 205*, 3-44.

Bunkley-Williams, L., & Williams, E. H. (1998). Ability of Pederson cleaner shrimp to remove juveniles of the parasitic cymothoid isopod, *Anilocra haemuli*, from the host. *Crustaceana*, *71*(8), 862-869. http://www.jstor.org/stable/20106067

Bshary, R., & Bronstein, J. L. (2004). Game structures in mutualisms: What can the evidence tell us about the kind of models we need? *Advances in the Study of Behavior*, *34*, 59-101. doi: 10.1016/S0065-3454(04)34002-7

Calado, R., Dionísio, G., & Dinis, M. T. (2007). Decapod crustaceans associated with the snakelock anemone *Anemonia sulcata*. Living there or just passing by? *Scientia Marina, 71*(2), 287-292. Retrieved from http://scimar.icm.csic.es/scimar/index.php

Cantrell, C. E., Henry, R. P., & Chadwick, N. E. (2015). Nitrogen transfer in a Caribbean mutualistic network. *Marine Biology*, *162*(12), 2327-2338. doi: 10.1007/s00227-015-2764-z

Caves, E. M., Frank, T. M., & Johnsen, S. (2016). Spectral sensitivity, spatial resolution, and temporal resolution and their implications for conspecific signaling in cleaner shrimp. *Journal of Experimental Biology, 219*, 597-608. doi: 10.1242/jeb.122275

Chadwick, N. E., Duris, Z., & Horka, I. (2008). Biodiversity and behavior of shrimps and fishes symbiotic with sea anemones in the Gulf of Aqaba, northern Red Sea. In F. D. Por (Ed.), *The improbable gulf: History, biodiversity, and protection of the Gulf of Aqaba (Eilat)* (pp. 209-223). Jerusalem: Magnes Press, Hebrew University.

Chapuis, L., & Bshary, R. (2009). Strategic adjustment of service quality to client identity in the cleaner shrimp, *Periclimenes longicarpus*. *Animal Behavior*, *78*(2), 455-459. http://dx.doi.org/10.1016/j.anbehav.2009.06.001

Chapuis, L., & Bshary, R. (2010). Signalling by the cleaner shrimp *Periclimenes longicarpus*. *Animal Behaviour*, *79*(3), 645-647. doi: 10.1016/j.anbehav.2009.12.012

Cheney, K. L., & Côté, I. M. (2007). Aggressive mimics profit from a model-sign receiver mutualism. *Proceedings of the Royal Society B*, *274*(1622), 2087-2091. doi: 10.1098/rspb.2007.0543

Cheney, K. L., Grutter, A. S., Blomberg, S. P., & Marshall, N. J. (2009). Blue and yellow signal cleaning behavior in coral reef fishes. *Current Biology*, *19*(15), 1283-1287. doi: 10.1016/j.cub.2009.06.028

Cheney, K. L., & Marshall, N. J. (2009). Mimicry in coral reef fish: How accurate is this deception in terms of color and luminance? *Behavioral Ecology*, *20*(3), 459-468. doi: 10.1093/beheco/arp017

Cleveland, A., Verde, E. A., & Lee, R. W. (2011). Nutritional exchange in a tropical tripartite symbiosis: Direct evidence for the transfer of nutrients from anemonefish to host anemone and zooxanthallae. *Marine Biology, 158*(3), 589-602. doi: 10.1007/s00227-010-1583-5

Corredor, L. (1978). Notes on the behavior and ecology of the new fish cleaner shrimp *Brachycarpus biunguiculatus* (Lucas) (Decapoda Natania, Palaemonidae). *Crustaceana*, *35*(1)*,* 35-40. doi: 10.1163/156854078X00187

Côté, I. M. (2000). Evolution and ecology of cleaning symbioses in the sea. *Oceanography and Marine Biology: An Annual Review*, *38*, 311-355. doi: 10.1201/b12589

Cutress, C., Ross, M., & Sutton, L. (1970). The association of Calliactis tricolor with its pagurid, calappid, and Majiid partners in the Caribbean. *Canadian Journal of Zoology*, *48*(2), 371-376. doi: 10.1139/z70-059

Debelius, H. (1999). *Crustacea guide of the world*. Frankfurt: Ikan.

Dickman, C. R. (1992). Commensal and mutualistic interactions among terrestrial vertebrates. *Trends in Ecology & Evolution, 7*(6), 194-197. http://dx.doi.org/10.1016/0169-5347(92)90072-J

Douglas, P., Baeza, A. J., & Montelatto, F. L. (2013). Host-use pattern and sexual dimorphism reveals the mating system of the symbiotic pea crab *Austinixa aidae* (Crustacea: Brachyura: Pinnotheridae). *Journal of the Marine Biological Association of the United Kingdom*, *93*(3), 715-723. http://dx.doi.org/10.1017/S0025315412000720

Godwin, J., & Fautin, D. G. (1992). Defense of host actinians by anemone-fishes. *Copeia*, 3, 902-908. http://www.jstor.org/stable/1446171

Goncalves, G. R. L., Wolf, M. R., da Costa, R. C., & Castilho, A. L. (2016). Decapod crustacean associations with scyphozoan jellyfish (Rhizostomeae: Pelagiidae) in the Southeastern Brazilian coast. *Symbiosis*, *69*(3), 193-198. doi: 10.1007/s13199-016-0395-x

Grutter, A. S., & Bshary, R. (2003). Cleaner wrasse prefer client mucus: Support for partner control mechanisms in cleaning interactions. *Proceedings of the Royal Society B, 270*(S2)**,** S242 – S244. doi: 10.1098/rsbl.2003.0077

Grutter, A. S., Deveney, M. R., Whittington, I. D., & Lester, R. J. G. (2002). The effect of the cleaner fish *Labroides dimidiatus* on the capsalid monogenean *Benedenia lolo* parasite on the labrid fish *Hemigymnus melapterus*. *Journal of Fish Biology*, *61*(5), 1098-1108. doi: 10.1111/j.1095-8649.2002.tb02458.x

Grutter, A. S., & Poulin, R. (1998). Cleaning of coral reef fishes by the wrasse *Labroides dimidiatus*: Influence of client body size and phylogeny. *Copeia*, *1*, 120–127. http://www.jstor.org/stable/1447707

Guo, C. C., Hwang, J. S., & Fautin, D. G. (1996). Host selection by shrimps symbiotic with sea anemones: A field survey and experimental laboratory analysis. *Journal of Experimental Marine Biology and Ecology*, *202*(2), 165-176. doi: 10.1016/0022-0981(96)00020-2

Gwaltney, C. I., & Brooks, W. R. (1994). Host specificity of the anemoneshrimp *Periclimenes pedersoni* and *P. yucatanicus* in the Florida Keys. *Symbiosis, 16*, 83-93.

Huebner, L. K., & Chadwick, N. E. (2012a). Reef fishes use anemones as visual cues for cleaning interactions with shrimp. *Journal of Experimental Marine Biology and Ecology*, *416-417*, 237-242. http://dx.doi.org/10.1016/j.jembe.2012.01.004

Huebner, L. K., & Chadwick, N. E. (2012b). Patterns of cleaning behavior on coral reef fish by the anemoneshrimp *Ancylomenes pedersoni. Journal of the Marine Biological Association of the United Kingdom, 92(*7) 1557-1562. http://dx.doi.org/10.1017/S0025315411001822

Humann, P., & DeLoach, N. (2006). *Reef creature identification: Florida, Caribbean,*

Bahamas (2nd ed.). Jacksonville: New World Publications.

Jonsson L. G., Lundälv T., & Johannesson K. (2001). Symbiotic associations between anthozoans and crustaceans in a temperate coastal area. *Marine Ecology Progress Series, 209*, 189-195. doi: 10.3354/meps209189

Khan, R. N., Becker, J. H. A., Crowther, A. L., & Lawn, I. D. (2004). Spatial distribution of symbiotic shrimps (*Periclimenes holthuisi, P. brevicarpalis, Thor amboinensis*) on the sea anemone *Stichodactyla haddoni*. *Journal of the Marine Biological Association of the United Kingdom, 84*(1)*,* 201-203. http://dx.doi.org/10.1017/S0025315404009063h

Kulbicki, M., & Arnal, C., (1999). Cleaning of fish cctoparasites by a Palaemonidae shrimp on soft bottoms in New Caledonia. *Cybium*, *23*(1), 101-104. Retrieved from http://sfi.mnhn.fr/cybium/homecybiumgb.html

Lettieri, L., Cheney, K. L., Mazel, C. H., Boothe, D., Marshall, N. J., & Streelman, J. T. (2009). Cleaner gobies evolve advertising stripes of higher contrast. *Journal of Experimental Biology*, *212*, 2194-2203. doi: 10.1242/jeb.025478

Lieske, E., & Meyers, R. F. (2004). *Coral reef guide: Red Sea*. London: HarperCollins.

Limbaugh, C. (1961). Cleaning symbiosis. *Scientific American*, *205*, 42-49. Retrieved from https://www.scientificamerican.com/

Mahnken, C. (1972). Observations on cleaner shrimps of the genus *Periclimenes*. *Bulletin of the Natural History Museum of Los Angeles County, 14*, 71-83.

Mascaro, M., Rodriguez-Pestana, L., Chiappa-Carrara, X., & Simoes, N. (2012). Host selection by the cleaner shrimp *Ancylomenes pedersoni:* Do anemone host species, prior experience or presence of conspecific shrimp matter? *Journal of Experimental Marine Biology and Ecology*, *413*, 87-93. doi: 10.1016/j.jembe.2011.11.026

McCammon, A., Sikkel, P. C., & Nemeth, D. (2010). Effects of three Caribbean cleaner shrimps on ectoparasitic monogeneans in a semi-natural environment. *Coral Reefs*, *29*(2), 419-426. doi: 10.1007/s00338-009-0583-8

Mihalik, M. B., & Brooks, W. R. (1995). Protection of the symbiotic shrimps *Periclimenes pedersoni*, *P. yucatanicus*, and *Thor* spec. from fish predators by their host sea anemones. In J. C. den Hartog, L. P. van Ofwegen, & S. van der Spoel (Eds.), *Proceedings of the 6th International Conference on Coelenterate Biology* (pp. 337-343). Leiden: Nationaal Naturhistorisch Museum.

Oates, J., Manica, A., Bshary, R., & Grutter, A. S. (2012). Relationship between roving behaviour and the diet and client composition of the cleaner fish *Labroides bicolor*. *Journal of Fish Biology*, *81*(1), 210-219. doi: 10.1111/j.1095-8649.2012.03330.x

Ostlund-Nilsson, S., Becker, H. A., & Nilsson, G. E. (2005). Shrimps remove ectoparasites from fishes in temperate waters. *Biology Letters*, *1*(4), 454-456. doi: 10.1098/rsbl.2005.0363

Roopin, M., & Chadwick, N. E. (2009). Benefits to host sea anemones from ammonia contribution of resident anemonefish. *Journal of Experimental Marine Biology and Ecology*, *370*(1), 27-34. doi: 10.1016/j.jembe.2008.11.006

Roughgarden, J. (1975). Evolution of marine symbiosis – A simple cost-benefit model. *Ecology*, *56*(5), 1201-1208. http://www.jstor.org/stable/1936160

Sargent, R. C., & Wagengach, G. E. (1975). Cleaning behavior of the shrimp, *Periclimenes anthopilus* Holthuis and Eibl-Eibesfeldt (Crustacea: Decapoda: Natania). *Bulletin of Marine Science*, *25*(4), 466-472. Retrieved from http://www.rsmas.miami.edu/bms/index.html

Soares, M. C., Bshary, R., Cardoso, S. C., & Côté, I. M. (2008a). The meaning of jolts by fish clients of cleaning gobies. *Ethology*, *114*(3), 209-214. doi: 10.1111/j.1439-0310.2007.01471.x

Soares, M. C., Côté, I. M., Cardoso, S. C., & Bshary, R. (2008b). The cleaning goby mutualism: A system without punishment, partner switching, or tactile simulation. *Journal of Zoology, 276*(3)*,* 306-312. doi: 10.1111/j.1469-7998.2008.00489.x

Soares, M. C., Côté, I. M., Cardoso, S. C., Oliveira, R. F., & Bshary, R. (2010). Caribbean cleaning gobies prefer client ectoparasites over mucus. *Ethology*, *116*(12), 1244-1248. doi: 10.1111/j.1439-0310.2010.01838.x

Spotte, S. (1996). Supply of regenerated nitrogen to sea anemones by shrimp. *Journal of Experimental Marine Biology and Ecology*, *198*(1)*,* 27-36. doi: 10.1016/0022-0981(95)00169-7

Spotte, S. (1998). "Cleaner" shrimps? *Helgolander Meerresuntersuchungen*, *52*(1)*,* 59-64. doi: 10.1007/BF02908736

Stanton, G. (1977). Habitat partitioning among associated decapods with *Lebrunia danae* at Grand Bahama. *Proceedings of the Third International Coral Reef Symposium, 1*, 169-175. Retrieved from http://www.reefbase.org/main.aspx

Stanton, M. L. (2003). Interacting guilds: Moving beyond the pairwise perspective on mutualisms. *The American Naturalist, 162*(S4), S10-S23. http://www.jstor.org/stable/10.1086/378646

Stevens, B. G., & Anderson, P. J. (2000). An association between the anemone, *Cribrinopsis fernaldi*, and shrimps of the families Hippolytidae and Pandalidae. *Journal of Northwest Atlantic Fishery Science, 27*, 77-82. Retrieved from http://journal.nafo.int/dnn/

Stummer, L. E., Weller, J. A., Johnson, M. L., & Côté, I. M. (2004). Size and stripes: How fish clients recognize cleaners. *Animal Behaviour*, *68*(1), 145-150. doi: 10.1016/j.anbehav.2003.10.018

Sun, D., Blomberg, S. P., Cribb, T. H., McCormick, M. I., & Grutter, A. S. (2012). The effects of parasites on the early life stages of a damselfish. *Coral Reefs*, *31*(4), 1065-1075. doi: 10.1007/s00338-012-0929-5

Szczebak, J. T., Henry, R. P., Al-Horani, F. A., & Chadwick, N. E. (2013). Anemonefish oxygenate their anemone hosts at night. *Journal of Experimental Biology*, *216*, 970-976. doi: 10.1242/jeb.075648

Titus, B. M., Marymegan, D., & Exton, D. A. (2015). Temporal patterns of Pederson shrimp (*Ancylomenes pedersoni* Chace 1958) cleaning interactions on Caribbean coral reefs. *Marine Biology, 162*(8), 1651-1654. doi 10.1007/s00227-015-2703-z

Trench, R. K. (1979). The cell biology of plant-animal symbiosis. *Annual Review of Plant Physiology, 30,* 485-531. doi: 10.1146/annurev.pp.30.060179.002413

Vandenspiegel, D., Ovaere, A., & Massin, D. L. (1992). On the association between the crab *Hapalonotus reticulatus* (Crustacea, Brachyura, Eumedonidae) and the sea cucumber *Holothuria* (*Metriatyla*) *scabra* (Ecinodermata, Holothuridae). *Biologie, 62*, 167-177.

Verde, A. E., Cleveland, A., & Lee, R. W. (2015). Nutritional exchange in a tropical tripartite symbiosis II: Direct evidence for the transfer of nutrients from host anemone and zooxanthellae to anemonefish. *Marine Biology, 158*(3), 589-602. doi: 10.1007/s00227-010-1583-5

Waldie, P. A., Blomberg, S. P., Cheney, K. L., Goldizen, A. W., & Grutter, A. S. (2011). Long-term effects of the cleaner fish *Labroides dimidiatus* on coral reef fish communities. *PLOS ONE*, *6*(6): e21201. doi: http://dx.doi.org/10.1371/journal.pone.0021201

Wicksten, M. K. (1995). Associations of fishes and their cleaners on coral reefs of Bonaire, Netherlands Antilles. *Copeia*, *2*, 477-481. http://www.jstor.org/stable/1446914

Wicksten, M. K. (1998). Behaviour of cleaners and their client fishes at Bonaire, Netherlands Antilles. *Journal of Natural History*, *32*(1), 13-30. http://dx.doi.org/10.1080/00222939800770021

Wicksten, M. K. (2009). Interactions of fishes of five species of Lysmata (Decapoda, Caridea, Lysmatidae). *Crustaceana, 82*(9), 1213-1223. http://www.jstor.org/stable/27743377

Williams, E. H., & Williams, L. B. (1982). First report of *Periclimenes yucatanicus* (Ives) (Decapoda, Palaemonidae) in association with a corallimorpharian anemone. *Crustaceana 42*(3), 318-319. http://www.jstor.org/stable/20103733

Wirtz, P. (1997). Crustacean symbionts of sea anemone *Telmatactis crioides* at Maderia and the Canary Islands. *Journal of Zoology*, *242*(4), 799-811. doi: 10.1111/j.1469-7998.1997.tb05827.x

Table 3. The distribution of mode microhabitat choice (Fig. 13) of anemone shrimps *A. pedersoni* and *P. yucatanicus* on the anemones *B. annulata* (a.) and *C. gigantea* (b.) during experimental trials at Auburn University.

a.	B. annulata microhabitat zones				
Shrimp Species	1	2	3	4+5	Total
A. pedersoni	1	0	0	16	17
P. yucatanicus	1	0	0	19	20
Total	2	0	0	35	37

b.	***C. gigantea microhabitat zones***				
Shrimp Species	1	2	3	4+5	Total
A. pedersoni	0	1	0	12	13
P. yucatanicus	7	4	1	2	14
Total	9	5	1	14	29

Table. 4. Distribution of anemone shrimps *A. pedersoni* and *P. yucatanicus* responses to a pseudo client fish stimulus (a hand) at Brewer's Bay, St. Thomas, U. S. Virgin Islands. For statistical analysis the responses, approach, wave/rock, clean, and wave/rock and clean were combined as a signal response due to low frequencies.

Shrimp Species	Approach	Wave/Rock	Clean	Wave/Rock and Clean	Retreat	No Response	Total
A. pedersoni	1	11	13	7	12	87	131
P. yucatanicus	0	1	0	2	21	2	26
Total	1	12	13	9	33	89	157

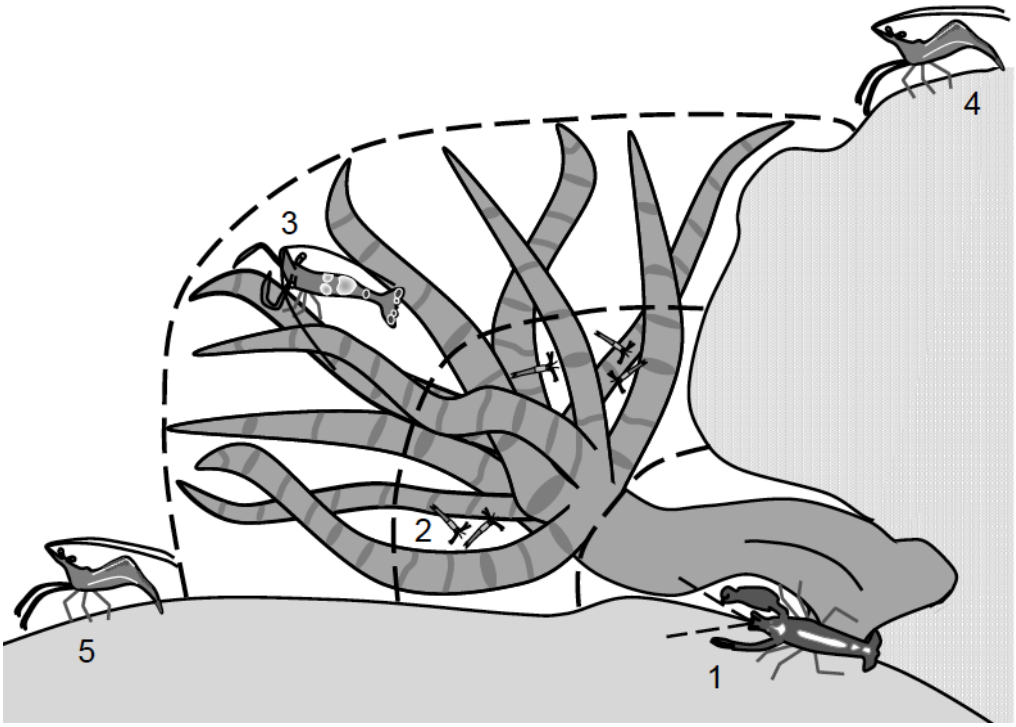

Figure 13. Anemone microhabitat locations of the anemone shrimps *A. pedersoni* and *P. yucatanicus* on the anemones *B. annulata* and *C. gigantea*. Microhabitat locations were defined as (1) along the column underneath tentacle crown, (2) oral disc or inner half of tentacle crown, (3) outer half of the tentacle crown, (4) not in contact with the anemone on soft substrate, (5) not in contact with anemone on hard substrate. Diagram created by Lindsay Huebner.

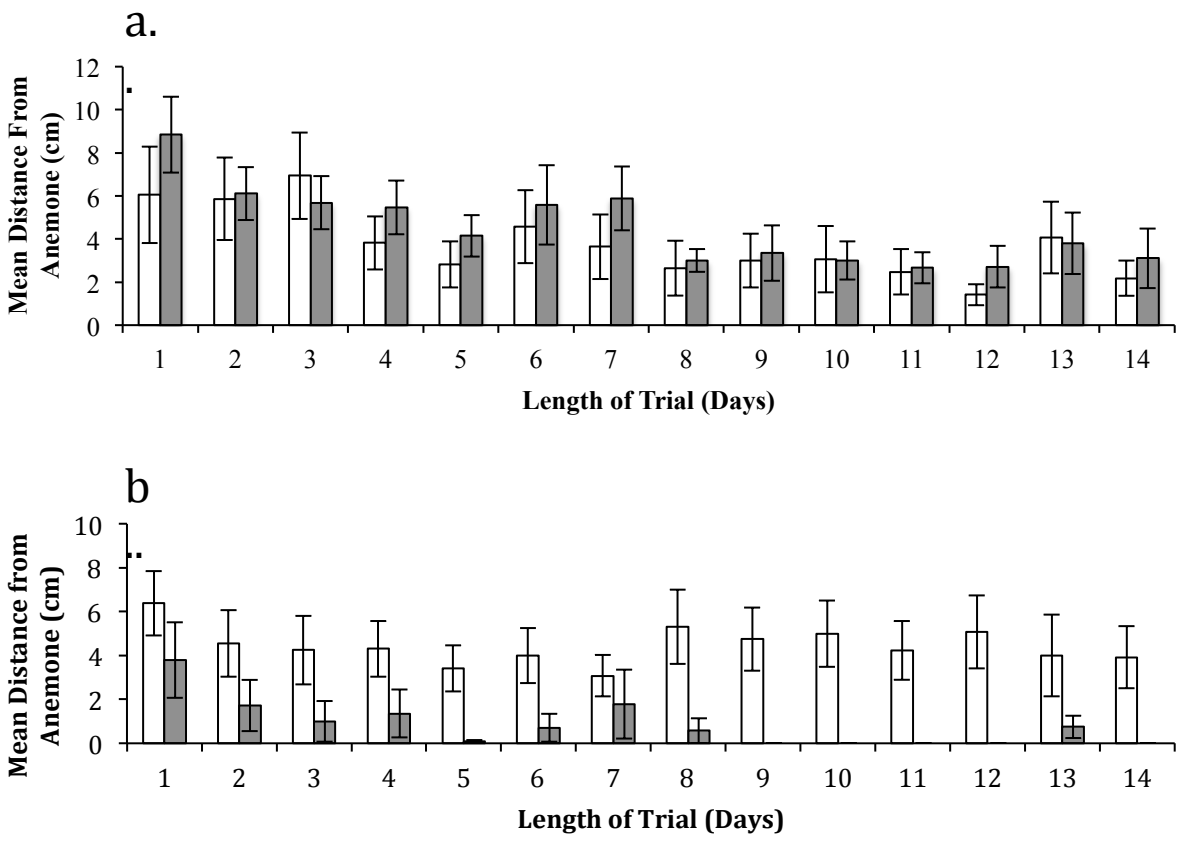

Figure 14. Mean distance (cm) from anemone tentacles (± s.e.) for each day of the experimental trials of the anemone shrimp *A. pedersoni* (white, N = 12 a, N = 13 b) and *P. yucatanicus* (gray, N = 19 a, N = 14 b) on the anemones *B. annulata* (a.) and *C. gigantea* (b.) at Auburn University, Auburn AL, USA.

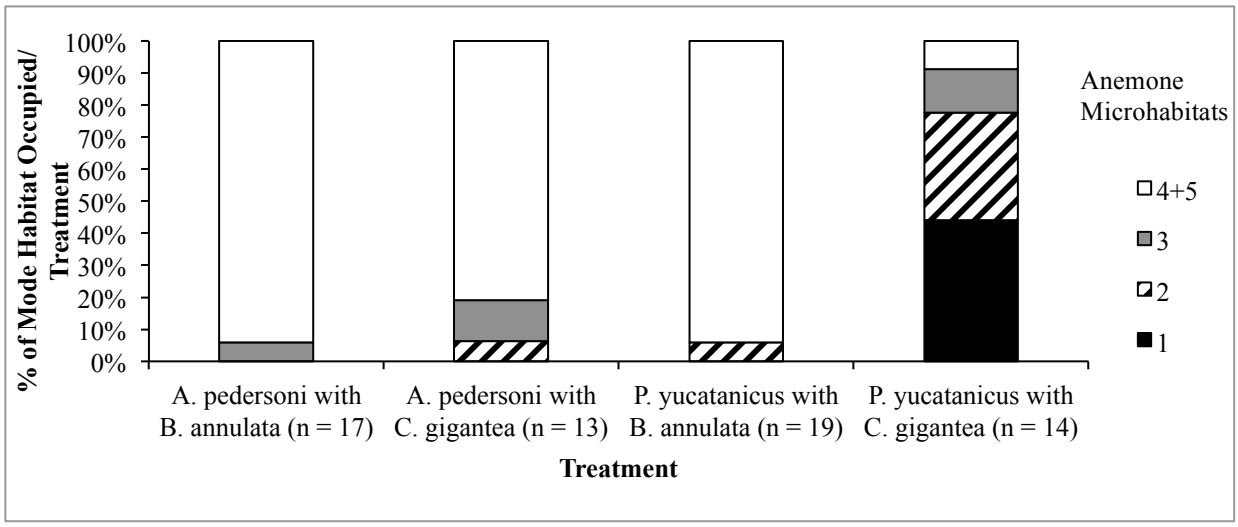

Figure 15. % Mode anemone microhabitat (fig. 13) occupied by anemone shrimps *A. pedersoni* and *P. yucatanicus* with the anemones *B. annulata* and *C. gigantea* during experimental trials at Auburn University, Auburn AL, USA.

.