Verge 12	Erin Collins and Kat Quintanilla
Characterization of the Interactions within Cleaning Statio	ns of the Mesoamerican Barrier Reef
and their Vertebrate Clie	ents

ABSTRACT

Cleaning stations are a beneficial service to the many occupants of the coral reef. Like the reef itself, the interactions between species at such cleaning stations can be quite diverse. Several dives were completed off the coast of Roatán, Honduras in order to observe coral reef cleaning stations and characterize the species-specific interactions between cleaner and client fish. Our study had three objectives: (1) to determine the species that frequented stations the most, (2) to characterize the orientation at which specific species posed when cleaned, and (3) to quantify the frequency of flinching behavior and whether it correlates with how many other clients are nearby. Herbivorous species like parrotfish and surgeonfish appeared to frequent the stations the most, but fish surveys would be needed to relate this frequency to overall abundance. Parrotfish species and Creole Wrasse posed with relative consistency at +45° and -45°, respectively, while the orientation of surgeonfish varied greatly. Less than four percent of the interactions observed resulted in flinching movements by the client. Seventy-five percent of these events occurred in the presence of other clients who responded by relocating from the station. Previous studies have related low instances of cleaners biting their clients with higher parasitic infection in clients. Further studies may inspect the rates of infection within fish of Roatán's reef system to affirm this correlation.

INTRODUCTION

Coral reefs are massive underwater ecosystems that host an incredible amount of biodiversity in marine life. Barrier reefs in particular are usually located off eastern shores of landmasses, such as the Mesoamerican Barrier Reef found off the coast of Central America (Harborne *et al.*, 2001). These tropical regions stabilize the environment of the coral reefs so

their conditions stay relatively unchanged throughout the year, with the exception of the effects of rainy season weather and hurricanes. These events can cause heavy sedimentation and physical damage to the reef in addition to stress-induced coral bleaching events - the most recent of which took place in 1998 (Harborne *et al.*, 2001). The Bay Islands of Honduras constitute a part of the southern region of the Mesoamerican Barrier Reef System. Most of the Bay Islands have mostly fringing reef around their shores, but the largest island, Roatán, is an ideal location for study due to its presence of barrier reef as well as fringing reef (Harborne *et al.*, 2001), allowing for study of associations in both locales. Roatán allows access to complete interdependent marine ecosystems that consist of mangrove forests, seagrass lagoons, and coral reef flats and slopes (Harborne *et al.*, 2001). This complete reef system makes it convenient to study fish interactions, including the cleaner-client relationship within cleaning stations.

Cleaning stations are a unique area of symbiotic behavior found mostly in coral reef systems. Certain types of cleaner fish will "clean" other fish species by picking off dead cells, debris, and parasites from their bodies (Sikkel *et al.*, 2004). This kind of symbiotic behavior is considered to increase overall reef health by reducing the amount of diseased fish (Waldie *et al.*, 2011). Both predatory and non-predatory species of fish are included in the clientele of the cleaner fish, which shows immense trust and cooperative behavior. The cleaner trusts predatory clients not to harm them and the clients cooperate by remaining still while the cleaners work (Soares *et al.*, 2007). Most client fish will adopt a specific pose in order to signal cleaners that they would like to be cleaned. This pose is usually very species-specific (Côté *et al.*, 1998). Client fish appear able to distinguish cleaner fish from distances, usually following fish that have blue and yellow striped colorations (Cheney *et al.*, 2009). These colorations are found in the most common types of cleaner fish: bluehead wrasse, yellowhead wrasse, neon gobies, and

cleaner gobies (Stummer *et al.*, 2004). An exception to this is the juvenile spanish hogfish, which is also known to clean clients (Arnal and Côté, 1998).

While cleaning stations are generally considered to be symbiotic, some interactions are not always beneficial for the clients. Cleaner fish may take advantage of the client fish by taking bites of live tissue, scales, or mucus. This often causes the client fish distress and is evident from a flinching reaction made by the client (Bansemer *et al.*, 2002). As a result, client fish sometimes become wary of cleaners and will avoid them (Bansemer *et al.*, 2002). More parasite-ridden client fish seem less likely to be exploited by the cleaner fish because they are host to an abundant food source (Bansemer *et al.*, 2002). Adversely, predatory client fish can exploit the cleaners by eating them, which can cause other cleaner fish in the area to avoid certain clients (Bansemer *et al.*, 2002).

Our study will observe client-cleaner interactions at cleaning stations within Roatán's coral reef system. Cleaning stations will be observed in order to determine how often a client species is cleaned in addition to illustrate species-specific tendencies to pose in a given orientation for cleaning. Special note will be made regarding whether cleaners are more likely to "cheat" their clients, as indicated by flinching behavior, with or without witnesses nearby. The responses of fish witnesses to a cleaner that has exploited its client will be noted to affirm a client's recognition and objection to the abuse. These results will help determine whether there are defined and predictable behaviors regarding client-cleaner signaling and client exploitation.

METHODS

The Bay Islands of Honduras are located northward of the mainland in the Caribbean Sea. These islands constitute a part of the southern region of the Mesoamerican Barrier Reef System, which supports a high diversity of marine animal and plant life. Roatán is the largest of the Bay Islands, bordered by both barrier and fringing reefs. Mangrove nurseries and lagoons along the coasts of the island provide habitat for juvenile fishes and invertebrates, where they grow before migrating to the open reef areas. The intimate associations within these active reef systems are easily accessible from the island.

Our study focused specifically on the operations of cleaning stations, which are a frequent occurrence especially in more populated areas of the reef. Data was collected from barrier and fringing reefs on both the north and south side of Roatán. Both the fore reef slope and walls were searched for cleaning stations. The species and quantity of cleaner and client fish were noted. The orientation of each fish serviced was noted, along with any possible flinching movement. Orientations were described as an angle to the horizontal in either the downwards (negative) or upwards (positive) direction. If flinching was observed, the reactions of fish within approximately three feet of the event were monitored. Time spent observing each cleaning station depended on the activity level of the station. If a station did not appear to be particularly active, another was located. A maximum of approximately 18 hours over two weeks in January 2015 was spent observing cleaning stations between 10-60 feet via snorkeling and SCUBA.

RESULTS

Cleaning stations of Roatán barrier and fringing reefs were observed to quantify and characterize behavioral tendencies between client and cleaner fish interactions. Eighteen

different species were observed posing at cleaning stations over the course of the study, amounting to 111 individuals serviced (Table 1). Cleaning stations were managed primarily by Bluehead Wrasse juveniles (Table 1). Rare cleaning activity was exhibited by cleaner gobies and spanish hogfish (Table 1). Parrotfish species, surgeonfish species and creole wrasse were the most frequently serviced species, accounting for 29.2%, 28.3%, and 20.8% of total cleaner-client interactions, respectively (Fig. 1). There was a greater diversity of parrotfish clients than any other family, yielding six to seven species, including: stoplight, redband and princess/striped parrotfish (Table 1). Interactions with other species all fell below 6% (Fig. 1). These included black durgon, angelfish species, as well as damselfish species like the blue and brown chromis and yellowtails (Fig. 1). Sergeant majors, blue and brown chromis, and bicolor damselfish were often seen in high abundance near stations, but rarely serviced.

The orientation of each fish served was noted in order to characterize species-specific posing behavior. Ninety percent of all parrotfish posed at +45° to the horizontal while the remaining fraction posed at a complete +90° (Fig. 2). Juvenile parrotfish posed at the same angles, but the fractions were more equivalent (Fig. 2). Surgeonfish species presented the greatest variety in orientation with individuals posing anywhere between -45° and +45° (Fig. 2). All creole wrasse posed in a downwards configuration of -45° (Fig. 2). Blue and brown chromis also posed downwards at -45° and -75° angles, respectively (Fig. 2). All other species observed exhibited +45° orientation; however, population sizes were very low for these species (Fig. 2; Table 1).

While being cleaned, clients were monitored for flinching movements. Of 111 cleaning interactions, only four potential flinching events were observed, amounting to less than four percent of total interactions (Fig. 3). These events are described as "potential" because client

reactions were relatively mild. Of these events, three were in proximity of other fish. In two of these instances, the client and witness fish left the cleaning station (one witness however did return within a few seconds after). In the event that there were no witnesses, the affected client did not relocate immediately. The final potential event resulted in no client or witness fish relocating.

Table 1. Distribution of client and cleaner fish participation detailed by species. Cleaning stations were observed along reefs of Roatán, Honduras for approximately 18 hours over the course of two weeks.

Clients	Quantity	Cleaners	Quantity
Princess/Striped Parrotfish*	9	Bluehead Wrasse juvenile	110
Princess/Striped Parrotfish juvenile*	8	Cleaner Goby	12
Stoplight Parrotfish	14	Spanish Hogfish juvenile	1
Redband Parrotfish	3		
Redtail Parrotfish	4		
Yellowtail Parrotfish	2		
Queen Parrotfish	1		
Creole Wrasse	22		
Ast. Surgeonfish	10		
Blue Tang	20		
Blue Chromis	6		
Brown Chromis	4		
Black Durgon	3		
Yellowtail Damselfish juvenile	2		
Chub	1		
Ast. Angelfish	1		
Total	111		123

^{*}No distinction was made between Princess and Striped Parrotfish due to an inability to

make confident identification.

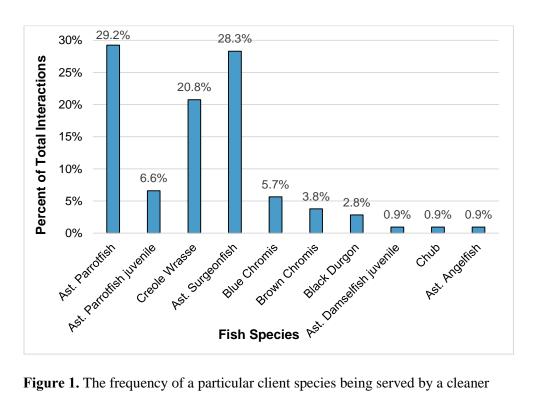


Figure 1. The frequency of a particular client species being served by a cleaner fish. Percentages were based off 111 cleaning interactions. Assorted (Ast.) groups combined values for closely related species. For exact values associated with each group, see Table 1.

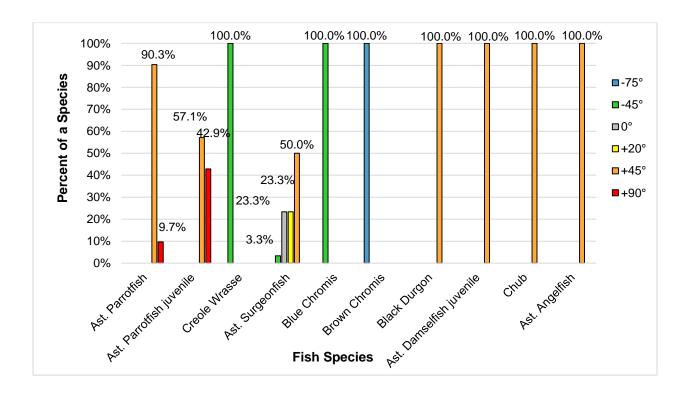


Figure 2. Distribution of posing orientation organized by client species. Orientation was characterized as a change in body angle from the horizontal in either the downwards (negative) or upwards (positive) direction. Percentages were based off 106 cleaning interactions. Assorted (Ast.) groups combined values for related species. For exact values associated with each group, see Table 1. Five individuals were

excluded from percentages due to a lack of data: one yellowtail damselfish juvenile, one surgeonfish, two adult parrotfish and one parrotfish juvenile.

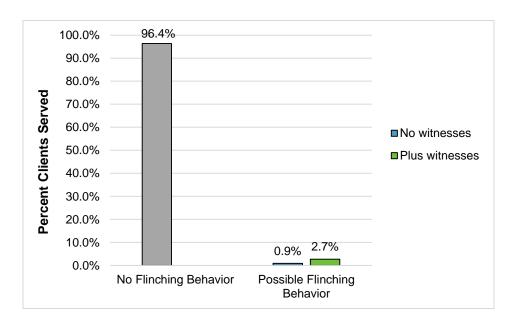


Figure 3. Frequency of potential flinching behavior observed in client fish when serviced by a cleaner species. Flinching behavior was defined as any sudden jerking movement by a client while being serviced. Potential reactions by clients while being cleaned were detailed by the presence or lack of witnesses.

Witnesses were defined as any fish within approximately 3ft of a flinching event. Percentages were based off 111 cleaning interactions.

DISCUSSION

Cleaning stations within the coral reefs of Roatán, Honduras were observed in order to characterize species-specific behaviors between cleaners and their clients. Parrotfish, surgeonfish

and Creole Wrasse were the most frequently sighted posing at cleaning stations. A 2007 study by Soares *et al.* predicted that cleaning gobies would prefer to serve less "risky" clients such as herbivores over potential predators. Our data supports this theory; however, Soares' results found that cleaners interacted with a higher proportion of predators that visited than non-predators. Because the vast majority of our clients were not piscivores, and therefore not considered a threat to the cleaners, we cannot adequately support that the increased frequency of interactions within these species is due to a specific relationship with cleaner fish or simply a greater abundance of herbivorous clients on the reef. Drift surveys of the area may be adequate to measure species abundance so that client interactions may be assessed relative to their abundance.

Conversely, damselfish, including the bicolor, chromis and sergeant majors, were noted to be frequently abundant (although not quantified explicitly) around cleaning stations, especially at depths around 20ft. The numbers of chromis that were serviced did not appear proportional to their abundance. Likewise, despite their abundance, bicolor damselfish and sergeant majors were never observed being cleaned. These species would be additional candidates for special observation in a drift survey to produce more relative cleaner-client interaction frequencies.

Although known to be territorial, bicolor damselfish appeared to tolerate the presence of cleaner fish (Robertson, 1984). This is likely due to not being recognized as a threat because of the beneficial services the cleaners offer to territorial damselfish. Although we observed no cleaning behavior between these damselfish and the bluehead wrasse, a 1998 study of the interactions between cleaning gobies and the dusky damselfish indicated that the damselfish claiming the territory was the primary client to the gobies, partly because the damselfish would chase away other potential clients (Arnal and Côté, 1998). If these damselfish were frequently serviced, their rates of infection by ectoparasites may be reduced, and thus have a lesser need to

be serviced in the future. Closer observations would need to be made on these species in order to avoid speculation regarding their interactions.

Previous studies have affirmed a variety of species-specific posing behaviors as well as varying tendencies for species to pose (Côté, 1998). Our results indicate that parrotfish, creole wrasse, and chromis species all have a strong tendency to pose with one orientation. Parrotfish nearly always posed at a +45° angle to the horizontal while creole wrasse tended to pose at a -45° angle. Juvenile parrotfish, however, appeared to exaggerate their orientation to nearly +90°. This may be due to inexperience as older individuals tended to pose at a lower, less strained angle. A study focusing explicitly on orientations exhibited by parrotfish of different lifestages may help to further explain this behavior. Meanwhile creole wrasse generally are always found in large schools, so their posing behavior is potentially learned much more readily.

Surgeonfish populations included a number of different species, like parrotfish populations, but a greater variety was seen in their posing behavior. Four different angles were commonly exhibited by surgeonfish, most of which were neutral (where the fish abruptly stopped and spread its fins) or at some positive angle upward. Future studies may look further into this family to potentially identify specific species with more consistent posing tendencies. No conclusions can be made on the tendencies of remaining clients identified given their low occurrence rate. These fish are not as commonly seen on the reef and so more time would need to be dedicated to obtaining stronger evidence to characterize their posing tendencies.

Other studies have suggested deviant behavior by cleaner fish where, instead of consuming parasites off the client, they "cheat" by feeding off the client's mucus or tissues (Bansemer *et al.*, 2002). Of the 111 cleaner-client interactions observed, only four clients were potentially exploited by their cleaners. Clients witness to this event reacted by swimming away

when they were present in three of the four cases, which is consistent with other studies (Bshary, 2002). It was expected that if more client witnesses were near the station, cleaners would be less inclined to take advantage of their clients. However, the presence of witnesses did not appear to inhibit exploitative behavior by the cleaners. The fraction of total interactions in which flinching possibly occurred is not significant enough to draw strong conclusions regarding when cleaners are inclined to exploit their clients.

Alternatively, Bansemer's (2002) study suggested that a greater frequency of ectoparasites in fish reduces the likelihood of a cleaner exploiting its client. Given our results, this theory would indicate that the fish within Roatán's reef system are providing their cleaners an adequate source of ectoparasites. The rate of infection within susceptible species in Roatán would be worth exploring given that illness is often linked with water quality and stress. Any differences in the frequency of illness between protected and unprotected areas of the reef could be compared.

Cleaning stations are a useful service to a wide variety of species on the reef. Herbivores such as parrotfish and surgeonfish were the most frequent visitors of the stations in Roatán's reefs. Future studies should quantify a species' overall abundance on the reef in order to more relatively access client participation. Creole wrasse posed with perfect consistency at -45° to the horizontal while surgeonfish species showed large variability in posing tendencies. The posing tendencies of juvenile parrotfish were notably different from their adult counterparts. Further studies may dissect the tendencies of specific surgeonfish species or alternatively explore why juvenile parrotfish behavior differs from the adults. Finally, of the flinching events that occurred all fish that witnessed the event left the station. However, because such few replicates of the behavior were obtained, strong supporting evidence is lacking. Studies showing that less

flinching behavior correlates with higher rates of parasitic infection would compel further investigation into the health of Roatán's reef fish.

REFERENCES

- Arnal, C., and I. Côté. (1998). Interactions between cleaning gobies and territorial damselfish on coral reefs. *Animal Behaviour*, 55,1429–42.
- Bansemer, C., Grutter, A. S., and R. Poulin. (2002). Geographic variation in the behaviour of the cleaner fish *Labroides dimidiatus* (Labridae). *Ethology*. 108, 353–366.
- Cheney, K. L., Grutter, A. S., Blomberg, S. P., and N. J. Marshall. (2009). Blue and yellow signal cleaning behavior in coral reef fishes. *Current Biology*. 19(15),1283–1287.
- Côté, I. M., Arnal, C., and J. D. Reynolds. (1998). Variation in posing behaviour among fish species visiting cleaning stations. *Journal of Fish Biology*. 53, 256–266.
- Harborne, A. R., Afzal, D. C., and M. J. Andrews. (2001). Honduras: caribbean coast. *Marine Pollution Bulletin*. 42(12),1221–1235.
- Robertson, D. R. (1984). Cohabitation of competing territorial damselfishes on a caribbean coral reef. *Ecology*. 65,1124–1135.
- Sikkel, P. C., Cheney, K. L., and I. M. Côté. (2004). In situ evidence for ectoparasites as a proximate cause of cleaning interactions in reef fish. *Animal Behaviour*. 68,241–247.
- Soares, M., Cardoso, S., and I. Côté. (2007). Client preferences by caribbean cleaning gobies: food, safety or something else? *Behavioral Ecology and Sociobiology*. 61(7),1015–1022.
- Stummer, L. E., Weller, J. A., Johnson, M. L., and I. M. Côté. (2004). Size and stripes: how fish clients recognize cleaners. *Animal Behaviour*. 68,145–150.
- Waldie, P. A., Blomberg, S. P., Cheney, K. L., Goldizen, A. W., and A. S. Grutter. (2011). Long-term effects of the cleaner fish *Labroides dimidiatus* on coral reef fish communities. *PLOS ONE*. 6(6), e21201.