





Client reef fish tolerate closer human approaches while being cleaned

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Introduction

Many reef fishes visit cleaning stations where a cleaner (fish or shrimp) removes and consumes ectoparasites from the body of clients, thus obtaining food, while clients are thought to benefit from ectoparasite removal (Côté, 2000). Cleaning interactions increase fish diversity on coral reefs because the cleaner presence (Grutter, Murphy & Choat, 2003) benefits fish health by removing parasites from clients while providing food for cleaners (Côté, 2000). Yet, tactile stimulation from cleaners may reduce cortisol levels (Soares *et al.*, 2011), and this may decrease clients' ability to respond to disturbances like predators (Schirmer, Jesuthasan & Mathuru, 2013).

Flight initiation distance – FID – is a common way to quantify behavioral responses to disturbance stressors. FID is defined as the distance from an approaching predator at which prey first initiates an escape (Cooper, 2008). The theory of animal escape behavior predicts that individuals assess risk associated with a predatory encounter and trade off the costs versus the benefits of fleeing (Cooper, 2008). There is growing literature on how humans modulate risk perception in fishes and thus have impacts on FID as well (Samia *et al.*, 2019). Such

Abstract

On tropical reefs, cleaning interactions, in which a fish or shrimp cleaner removes ectoparasites from a client fish, are common. Such cleaning interactions have been shown to reduce physiological stress in the clients. We asked whether the process of cleaning by a cleaner wrasse *Thalassoma noronhanun* modified a fish client's risk assessment, hypothesizing that the benefits of being cleaned may modify the cost avoiding a potential disturbance or predator. We experimentally approached fish of two species (a parrotfish *Sparisoma amplum* and a squirrelfish *Holocentrus adscensionis*) when they were being cleaned and when they were not being cleaned, and measured the client's flight initiation distance (FID) – a metric of risk assessment. Both client fish species tolerated ~30% closer approach when being cleaned. The body size of the client did not affect FID in either species, and the number of cleaners present did not influence FID of squirrelfish, but parrotfish who received cleaning from two cleaners had longer FID. These findings imply that fish being cleaned modify their risk assessment, and these results add to a list of potential costs fish clients face in this fascinating mutualism.

escape behavior data have helped us understand the fundamental trade-offs animals make as well as the impacts of human disturbance on fish assemblages.

Despite the increasing body of knowledge on cleaning interactions, little is known about risk perception and escape behavior of cleaners and clients from disturbances during cleaning interactions. Aggressive behavior from territorial fish may change the duration of cleaning interactions and decrease feeding rate on client fish, having impacts on the dynamic of cleaning interactions (Arnal & Côté, 1998). Human drivers like diver disturbance may suppress cleaning interactions by more than 50% and thus have long-term impacts on reef dynamics (Titus *et al.*, 2015). If cleaning increases client vulnerability to predators and/or human disturbances, this mutualistic relationship may have a heretofore unidentified cost.

We studied how being cleaned influenced two fish client species' perceptions of risk from approaching humans by quantifying their FID. More specifically, we asked if: (1) cleaning interactions by a facultative cleaner modify risk assessment from human interference by reducing FID; and (2) the body size of the client and number of cleaners are associated with FID. Because cleaning provides benefits to clients and cleaning

is a limited resource (Côté, 2000), we expect that cleaning interactions modify risk assessment. Furthermore, more cleaners may increase tactile stimulation, thus reducing cortisol levels and thereby reducing fear in clients (Schirmer *et al.*, 2013). We hypothesize a negative relationship among the clients' body size and FID because larger fish might be relatively safer from predation. We also expect a negative relationship between the number of cleaners and FID because more cleaners would translate to greater benefits to the cleaner, influencing the trade-off between being cleaned and avoiding predators.

Materials and methods

Study site

Fieldwork was conducted in May and June 2018 at Rocas atoll (03°50'S, 33°49'W), a 5.5 km², well-enforced marine reserve located 230 km off the north-eastern coast of Brazil. Rocas atoll is formed mainly by red coralline algae and vermetid mollusks (Gherardi & Bosence, 2001) and exhibits one of the largest reef fish biomass in the Brazilian biogeographic province (Morais, Ferreira & Floeter, 2017).

Experiments were conducted between 08:00 and 15:00 h during low tide, which is the peak time for cleaning interactions between the studied species (Campos *et al.*, 2006). Two closed pools with perimeters of 0.3 and 0.5 km and depth ranging 0.3–3 m were sampled. Closed pools remain isolated from the exterior area during low tide, being less exposed to tidal variations. Visibility and temperature during the data collection were recorded but varied little (8 to 10 m visibility; 29 to 31°C).

Data collection and analysis

We estimated the FID as a response to human presence in two client fish species. In each species, we tested FID in individuals who were either receiving cleaning (cleaning treatment) or not receiving cleaning (no cleaning treatment). Fish clients were the squirrelfish *Holocentrus adscensionis* and the reef parrotfish *Sparisoma amplum*. Criteria for selecting the client species were that these fish were common in cleaning stations, they differ in time of day at which they are active and in diet type, and they were widely distributed in the Brazilian province (Pinheiro *et al.*, 2018). The squirrelfish is nocturnal and feeds mainly on crabs and other small crustaceans, being found during the day inside or near shelter (Carvalho-Filho, 1999), making occasional movements to receive cleaning. The parrotfish is diurnal and herbivorous/detritivorous occurring off oceanic islands, dwelling mainly in shallow and turbulent areas on coral, algal and rocky reefs (Moura, Figueiredo & Sazima, 2001).

We surveyed cleaning interactions of those aforementioned fish species with a Brazilian endemic fish species, the Noronha wrasse, *Thalassoma noronhanum*. This is a common wrasse off Brazilian oceanic islands (Pereira-Filho *et al.*, 2011; Quimbayo *et al.*, 2017), very abundant species in closed pools of the Rocas atoll (Longo *et al.*, 2015), and is considered a facultative cleaner, in that only juveniles of the species are known

to clean (Francini-Filho, Moura & Sazima, 2000; Sazima *et al.*, 2005; Francini-Filho & Sazima, 2008).

For both treatments, a trained snorkeler swam at a constant speed (~0.7 m/s⁻¹) directly toward a focal fish to measure FID of the sampled individual (Fig. 1). The snorkeler remained at the same depth as observed fish. We began all experimental approaches 5 m from the focal subject. When fish fled, the snorkeler measured FID (with a tape measure) as distance (cm) between snorkeler and the place where the fish was before fleeing. Flight was defined when the fish increased its swim speed to greater than the approach speed of the data collector (Nunes *et al.*, 2019). For each client subject, we visually estimated the body size (total length in cm) at the closest approach before the fish moved away. The snorkeler was previously trained to conduct fish body size estimations with limited error. For subjects actively being cleaned, we also recorded the number of cleaners interacting with the subject. We focused sampling in different cleaning stations to avoid pseudoreplication and standardized observations in parts of the reef with similar reef complexities for both treatments to ensure that fish had reasonably similar access to refuge. We avoided sampling individuals in locations that overlapped with territorial species (e.g., damselfishes) because they can impact cleaner behavior (Arnal & Côté, 1998). We surveyed 87 squirrelfish (46 with cleaning treatment; 41 with no cleaning treatment) and 106 parrotfish (52 with cleaning treatment; 54 with no cleaning treatment). During four observations, the cleaner fled before the client. We removed these data from analysis because it was uncommon and we were interested in investigating client behavior.

We used a t-test to examine body size differences between treatments for each species and for differences in FID. A Kruskal–Wallis test was fitted to explain variation in FID as a function of the number of cleaners observed in the interaction. Dunn's test was conducted to verify differences between groups. Linear regression was fitted to ask whether FID was associated with body size in the no cleaning treatment. FID was $\ln(x + 1)$ transformed to ensure that residual variation was normally distributed. Analyses were conducted in the R software version 3.5.1 (R Core Team, 2018).

Results

Squirrelfish at cleaning stations ranged from 14 to 26 cm (mean 18.7 ± 2.8 (\pm SD)), and those not at cleaning stations ranged from 15 to 25 cm (mean 19.4 ± 2.9). For parrotfish, the body size of individuals at cleaning stations ranged from 12 to 34 cm (mean 24.4 ± 5.4), and those not at cleaning stations ranged from 12 to 32 cm (mean 22.9 ± 6.6). There were no significant differences in body size for between treatments for both squirrelfish ($t = 1.2$, d.f. = 82, $P = 0.23$) and parrotfish ($t = -1.3$, d.f. = 103, $P = 0.18$).

For both species, FID was significantly shorter for those fish receiving cleaning (squirrelfish $t = 5.7$, d.f. = 78, $P < 0.001$, Fig. 2a; parrotfish: $t = 8.5$, d.f. = 105, $P < 0.001$, Fig. 2b). Squirrelfish reduced FID from 76 ± 22 cm to 50 ± 19 cm when being cleaned, and parrotfish reduced FID from 103 ± 20 cm to 72 ± 17 cm. FID of squirrelfish did not

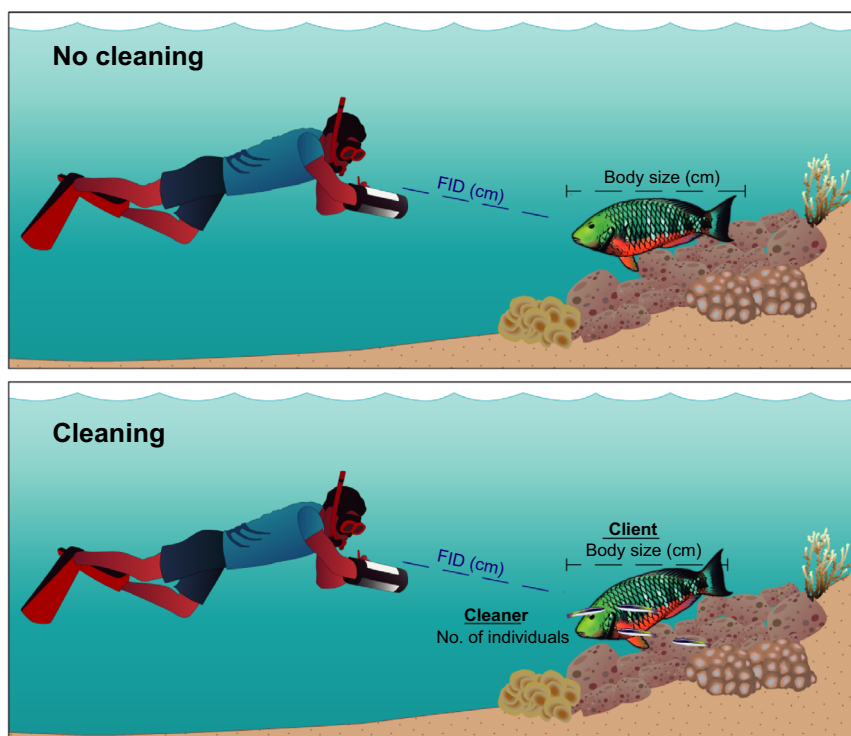


Figure 1 Experimental design for both no cleaning and cleaning treatments. FID was measured as the distance between the data collector and the place where the fish was before fleeing. Symbols courtesy of the Integration and Application Network (<http://ian.umces.edu/symbols/>).

differ as a function of the number of cleaners ($H = 1.3$, $P = 0.51$; Fig. 3b), but FID varied significantly for parrotfish ($H = 8.9$, $P = 0.03$) where interactions with two cleaners had longer FIDs (79 ± 8.8 cm; Fig. 3a). Body size did not explain variation in FID in either species (squirrelfish $R^2 = 0.001$, $P = 0.94$; parrotfish $R^2 = 0.003$, $P = 0.89$ Fig. 4a,b).

Discussion

Our results showed that fish individuals being actively cleaned tolerated closer human approaches. It could be that being cleaned modifies the decisions of client fish to flee, since they must trade off the benefits of remaining and being cleaned versus escaping from a potential predator. In our study, in place of a natural predator, we used humans, which may sometimes be perceived by animals as predators (Frid & Dill, 2002). The presence of cleaners has been suggested to influence long-term patterns in growth and recruitment of reef fishes (Waldie *et al.*, 2011). Being cleaned may increase the opportunity cost of leaving (cleaners are a limited resource); hence, individuals tolerate a greater risk to gain benefits of cleaning before fleeing, resulting in the reduced FID.

Alternatively, allowing humans to approach more closely while being cleaned could be related to a reduction in basal cortisol levels as a consequence of tactile stimulation (Bshary *et al.*, 2007; Soares *et al.*, 2011; Schirmer *et al.*, 2013). If so, this result provides support for the role of glucocorticoids modifying risk assessment in animals (Geffroy *et al.*, 2015) by

decreasing FID of reef fishes. Clients advertise their cleaning service to clients through conspicuous dances or tactile stimulation, but the communication strategy is poorly known for many species (Francini-Filho *et al.*, 2000; Stummer *et al.*, 2004). Tactile stimulation from cleaner wrasses may modify risk perception and hence permit a closer approach. However, our findings need to be interpreted with caution because it is unknown whether *Noronha wrasse* communicates with clients via tactile stimulation during cleaning (Francini-Filho *et al.*, 2000). Regardless of the mechanism, this antipredator response may affect individual fitness and potentially population dynamics due to energetic and lost opportunity costs of disturbance avoidance (Frid & Dill, 2002).

One further possibility is that the increased approach tolerance while being cleaned could be explained by the 'safe havens' hypothesis, whereby cleaning stations are actually relatively safe places to be due to reduced aggressive behavior and predatory interactions (Cheney *et al.*, 2008). It is currently unknown whether predation is lower at the cleaning stations that we investigated in comparison with predation at other points on the reef. However, if predation is indeed reduced at cleaning stations, then increased tolerance of potential predators could be an adaptive response to reduced risk and may not represent a cost of being cleaned. Further research is needed to distinguish between the mechanisms that may explain increased approach tolerance at cleaning stations in the species we investigated, for example, investigating whether cleaning leads to reduced physiological stress levels,

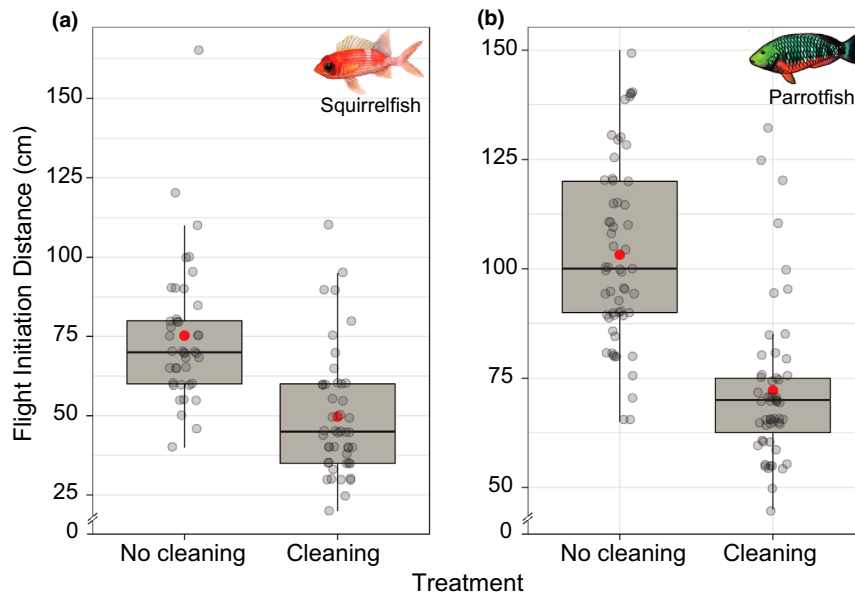


Figure 2 Flight initiation distance of (a) squirrelfish *Holocentrus adscensionis* and (b) parrotfish *Sparisoma amplum* during the two treatments: no cleaning and cleaning. Gray points are the raw data, the red dot is the mean, and black points outliers. Note that scales differ on the Y axes.

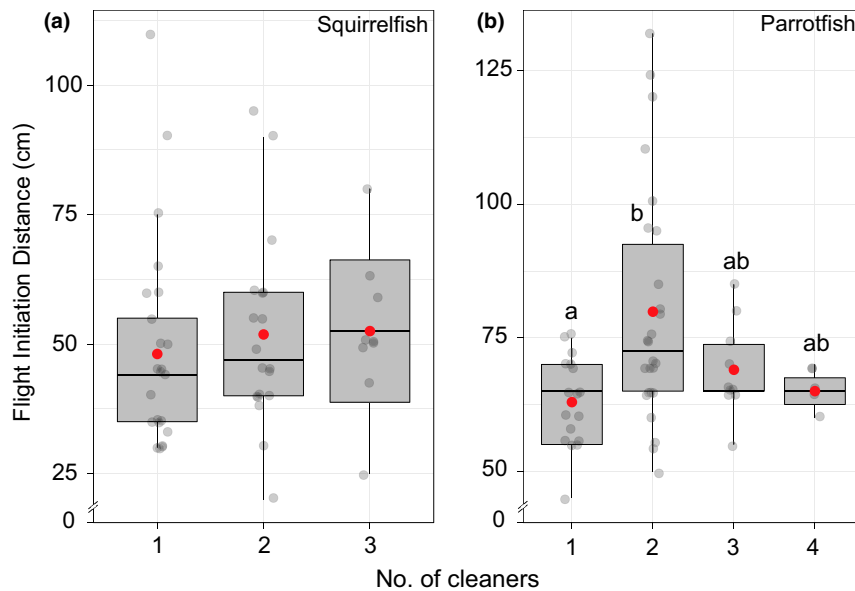


Figure 3 Flight initiation distance of client fishes as a function of the number of cleaners observed for (a) squirrelfish *Holocentrus adscensionis* and (b) parrotfish *Sparisoma amplum*. Gray points are the raw data, and the red dot is the mean. Different letters above boxplots indicate significant differences (Dunn’s test, $P < 0.05$). Note that scales differ on the Y axes.

changed escape behavior and/or differences in predation levels.

Contrasting with our predictions, we found no negative relationship between the number of cleaners and FID. For instance, significantly higher FID for parrotfish was verified for two cleaners instead of four, the highest cleaner abundance.

Instead, being cleaned was associated with increased tolerance to human approach, regardless of the number of fish cleaners. Cleaning by one individual has physiological and behavioral effects on clients (Côté, Arnal & Reynolds, 1998; Soares *et al.*, 2011); thus, the increase in the number of cleaners may not reflect in increased effects. Similarly, we found no positive

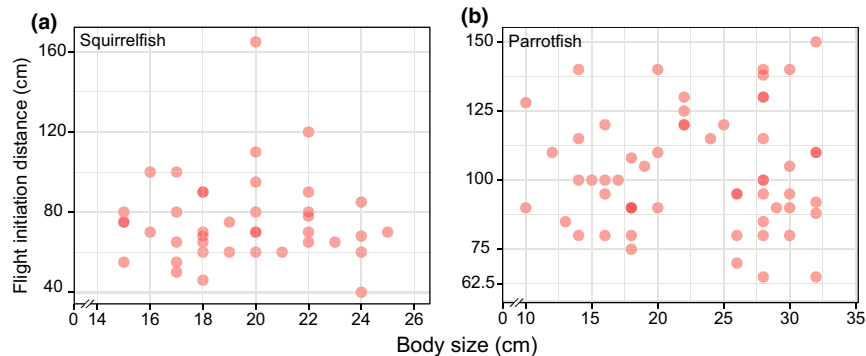


Figure 4 Relationship (nonsignificant) between body size (cm TL) and flight initiation distance (cm) for (a) squirrelfish *Holocentrus adscensionis* and (b) parrotfish *Sparisoma amplum* between treatments. Note that scales differ on both X and Y axes.

relationship between fish body size and FID for either fish species. This is in accordance with Nunes *et al.* (2019) hypothesis that fish risk assessment on remote oceanic islands differs from that on coastal human-impacted reefs. It is expected that as the fish grow, they will accumulate predator recognition experiences, which would likely increase their FID. An explanation for the lack of a relationship is that in isolated oceanic islands, humans may not be directly recognized as predators by the fish. On the other hand, we could also expect that larger prey would have tolerated closer approach from predators than small prey, at both intra- and interspecific levels (Samia *et al.*, 2019). Our results provide additional evidence that human threats modulate the relationship between body size and FID (Januchowski-Hartley *et al.*, 2011).

Clients receive benefits (parasite removal; Côté, 2000) but also may experience costs associated with cleaning. Previous work has focused on the costs to clients associated with cheating cleaners (Grutter & Bshary, 2003), having to enter in foreign territories and/or leaving a territory empty which would permit invasion by competitors (Cheney & Côté, 2001), and lost time and effort spent during cleaning interactions (Bshary *et al.*, 2007). Our study has identified a potential novel cost and decreased flight initiation distance from a possible threat while being cleaned.

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