

Potential effects of fishing on cleaning interactions in a tropical reef

R. A. M. Silvano · I. R. Tibbetts · A. S. Grutter

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Abstract We investigated the effect of fishing on the behavior and abundance of the cleaner fish *Labroides dimidiatus* on the Great Barrier Reef, Australia, by comparing cleaner fish density (underwater visual census) and behavior (focal animal observations) at a fished and a no-take site. Cleaner fish density, as well as the number of individuals and species cleaned, did not differ significantly between sites; however, *L. dimidiatus* spent more time interacting (physical contact involving feeding bites) with individual clients at the no-take site, indicating that fishing can indirectly affect mutualistic interactions on tropical reefs.

Keywords Fish behavior · Fish ecology · Conservation · Mutualism · Marine protected areas · *Labroides dimidiatus*

Introduction

Cleaning interactions are among the most readily observed and better-known marine interspecific mutualisms, especially on tropical reefs (Poulin and Grutter 1996; Bshary 2003). These interactions involve a cleaner (fish or shrimp) that removes parasites and other host material from clients (usually other fishes). Cleaners get food and clients benefit

from the removal of parasites (Grutter 1999, 2002). Although cleaning interactions can range from mutualistic to parasitic (Poulin and Grutter 1996), at least some clients have their parasite loads reduced by the activity of the blue-streak cleaner wrasse, *Labroides dimidiatus* (Grutter 1996, 1999). Indeed, the presence of *L. dimidiatus* influences the diversity and distribution of client reef fishes (Waldie et al. 2011), including those that are targeted for human consumption.

Fishing pressure may have adverse ecological effects on tropical reefs, including changes in the size and structure of target fish populations, depletion of top predators and a concomitant increase in the abundance of their fish or invertebrate prey (cascade effects) (Roberts 1995; Bellwood et al. 2003; Williamson et al. 2004). Factors that affect client fishes, such as fishing, may indirectly affect cleaning interactions through changes in the size range or the species composition of client fishes available for the attention of cleaners, which obtain nearly all of their food from clients (Grutter 1997). Despite this, the effects of fishing on cleaning interactions have yet to be addressed. Here, we investigated the potential effects of fishing on the behavior and abundance of the cleaner fish *L. dimidiatus* on the Great Barrier Reef, Australia, by testing the hypotheses that cleaner density, cleaning rate, number of clients cleaned, client size and diversity of clients would be higher and composition of client species would differ in a no-take site compared with a fished one. We also tested whether an effect of fishing on cleaning interactions (amount of time that cleaners spent interacting with clients) would be due to: (1) differences in the size range or species composition between a fished and a no-take site or (2) differences in the cleaning interaction (cleaner behavior) between the two sites when controlling for any differences in clients' size and community structure.

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R. A. M. Silvano (✉)
Department of Ecology, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS 91501-970, Brazil
e-mail: renato.silvano@ufrgs.br

I. R. Tibbetts · A. S. Grutter
School of Biological Sciences, The University of Queensland,
Brisbane, QLD 4072, Australia

Materials and methods

The Great Barrier Reef Marine Park (GBRMP), located along the eastern Australian coast, is the largest reef conservation zone in the world and has contiguous fished and no-take zones. These no-take zones have been effective in increasing the population of at least some of the targeted reef fish species in the GBRMP (Evans and Russ 2004; Williamson et al. 2004). Considering the close proximity of no-take and fished habitats, and that cleaning by the blue-stripe cleaner wrasse, *L. dimidiatus* has been extensively studied there (Grutter 1996, 1999, 2002), GBRMP reefs provide an ideal setting to analyze influences of fishing on cleaning interactions.

We conducted our study in adjacent fringing reef sites corresponding to a no-take zone (closed to all forms of fishing) and a fished zone (line fishing allowed) on the western and sheltered side of Orpheus Island, in the central section of GBRMP (18°34'S, 146°29'E) in March 2008. We chose this island because previous studies found that fishing had resulted in a lower abundance and size of the target reef fishes *Plectropomus* spp. (coral trout) and *Lutjanus carponotatus* at fished compared with no-take sites (Evans and Russ 2004; Williamson et al. 2004).

We estimated the density of cleaners (adults and juveniles) using underwater visual censuses on 16 band transects of 50 × 4 m (200 m² each transect, 3.2 km² of total area surveyed), at the same fished and no-take sites where we also recorded cleaning behavior (see below). We recorded the composition of the substrate in general categories (live coral, dead coral, other sessile invertebrates, algae or sand) at each 2 m of the transect, considering the percent coverage of live coral per transect as an indicator of habitat complexity. Transects were conducted during daylight between 0953 and 1717 hours. The time of each transect was converted into a continuous variable measured as hours past 0600 hours, based on the time each transect commenced. We compared mean depth of transects between fished and no-take sites using a nonparametric Wilcoxon test (*Z*), and we used a nonparametric Spearman (ρ) correlation to examine the relationship between depth and number of cleaners. We then used a general linear model (GLM) to examine the relationship between the dependent variable (number of cleaners recorded in each transect) and the independent variables: period of observation, percent of live coral cover and zoning system: fished or no-take.

The same researcher made focal observations of cleaner fish in the no-take ($n = 18$) and a fished ($n = 9$) area, through snorkeling ($n = 25$) and SCUBA ($n = 2$, to gather additional observations at depths up to 5 m). We considered the whole reef areas of the studied bays as study sites, but we scattered the observations to decrease the likelihood

of observing the same individual cleaner fish more than once. Larger host fishes are more mobile, and some are likely to have been observed more than once. We haphazardly selected the sites (bays) according to diving conditions (visibility, tides and currents) and observed cleaners from 1000 to 1700 hours. We converted the time of observation in a continuous variable in the same way we did for transects, calculated as hours past 0600 hours.

We recorded cleaning behavior (species and size of clients, time and frequency of interactions) during 15-min observations, through focal animal observations, following an adult cleaner and recording its interactions, after allowing a 2-min habituation period (Grutter 1995, 1996). While client inspection is considered part of the cleaning interaction (Grutter 1995, 1996), on this survey we recorded only those interactions where the cleaner had taken what appeared to be at least one feeding bite on the surface of the client. The total duration of each interaction was measured from the cleaner's first bite to the time that either the cleaner or the client retreated. We also identified and estimated the size (10-cm size classes) of all fish clients.

Time of observation (hours past 0600 hours) was not normally distributed even after transformation. Therefore, we compared this variable between the fished and the no-take site using a nonparametric Wilcoxon test (*Z*). We also examined nonparametric Spearman (ρ) correlation coefficients between time of observation and four variables: number of attended clients, the total time spent interacting (s) per observation, the duration of each interaction (s) and average client size. For analysis of cleaning behavior, we considered individual cleaners as replicates and calculated the average client size for each cleaner: we considered the midpoint range of each client size class ($>09 = 5$ cm, 10–20 = 15 cm and so on) and calculated the average size of clients that interacted with each cleaner, making client size into a continuous variable. We then ran analysis of covariance (ANCOVA) using independent variables as the zoning system (fished and no-take, factor with two levels) and the covariate average client size (cm) (continuous variable). The dependent variables analyzed (one analysis for each) were the number of attended clients, the total time spent interacting (s) and the duration of each interaction (s).

Data were \log_{10} transformed to achieve normality and homogeneous variance, when needed. Statistical analyses were conducted using JMP software.

The species richness of clients interacting with cleaners was compared through rarefaction curves based on the number of cleaners that interacted with each client species (to avoid double counting of the same client individual attended by the same cleaner), using the software Ecological Methodology (Kenney and Krebs 2000).

Finally, we made a nonmetric multidimensional scaling (NMDS) ordination (1,000 permutations, Bray–Curtis

similarity index), grouping the individual cleaners (sampling units) observed in the fished and in the no-take site according to the total time (s) that the cleaners interacted with client species. This analysis makes an ordination of cleaners (sampling units) according to the composition of clients (rows of the matrix) in a two-dimensional space, in the same way sampling units (for example sites) are arranged according to their similarity in species composition. We used percent similarity analyses (SIMPER) to check for the dissimilarities between the fished and the no-take site and the relative contribution of client species. Ordination analyses were made using PRIMER 5 (Clarke and Gorley 2006).

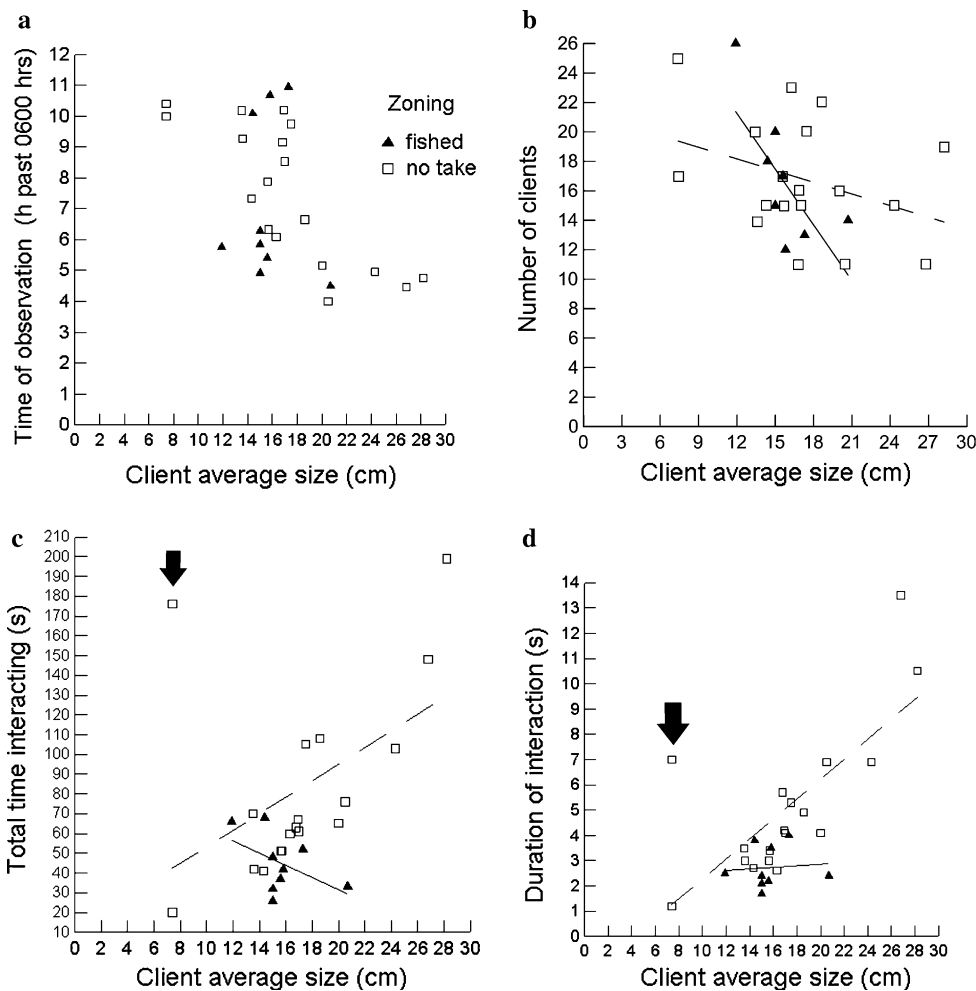
Results and discussion

We recorded 46 adult cleaners (*L. dimidiatus*) in the transects. Mean depth of transects did not differ significantly between fished and no-take sites ($n = 16$, $Z = 0.3$,

$p = 0.8$), and cleaner density was not related to depth ($n = 16$, $\rho = 0.45$, $p = 0.08$). The density of cleaners did not differ between the no-take ($n = 9$ transects, mean \pm SD = 3 ± 0.8 cleaners per 200 m²) and fished sites ($n = 7$, 2.7 ± 0.6 cleaners per 200 m²), and cleaner density was neither related to coral cover ($p = 0.83$) nor to period of observation ($p = 0.4$) (GLM $r^2 = 0.07$, $F_{3,12} = 0.31$, $p = 0.82$, zoning effect $p = 0.76$).

We observed and analyzed 452 interactions (302 in the no-take site and 150 in the fished site) involving 68 client species (24 interactions were not analyzed because we could not identify the client species). Average client size did not differ between fished (15.7 ± 2.4 cm) and no-take (17.3 ± 5.5 cm) sites ($t_{8,17} = 1.1$, $p = 0.85$); frequency distributions of client average sizes also did not differ between fished and no-take sites (Kolmogorov–Smirnov test for two samples, $p = 0.25$, Fig. 1a). The time of observation did not differ between fished and no-take sites ($n = 27$, $Z = -0.18$, $p = 0.86$, Fig. 1a), but time of observation was inversely related to average client’s size:

Fig. 1 Effects of zoning (fished and no-take sites) and average client size (cm) on the cleaning behavior of observed individuals ($n = 27$) of the cleaner fish *L. dimidiatus* in Orpheus Island, Great Barrier Reef: **a** time of observation (hours past 0600 hours); **b** number of clients cleaned; **c** total time interacting with clients (s) (\log_{10} transformed data); **d** duration of each interaction (s) (\log_{10} transformed data). Outliers are shown by an arrow



Cleaners observed earlier in the morning interacted with larger clients ($n = 27$, $\rho = -0.48$, $p < 0.05$, Fig. 1a). Time of observation of individual cleaners was not related to number of clients attended ($n = 27$, $\rho = 0.13$, $p = 0.5$), to total time spent interacting with clients ($n = 27$, $\rho = -0.08$, $p = 0.69$), nor to duration of each interaction ($n = 27$, $\rho = -0.03$, $p = 0.87$). We thus considered that time of observation did not influence these three behavioral variables that we analyzed.

The number of clients attended by the observed cleaners did not differ between fished and no-take sites (Fig. 1b, $F_{1,23} = 0.94$, $p = 0.34$), but the number of clients attended was negatively related to average client's size ($F_{1,23} = 7$, $p < 0.05$): More clients were attended by those cleaners interacting with smaller clients (Fig. 1b). The interaction between zoning and client size was nonsignificant ($F_{1,23} = 3$, $p = 0.1$) (Fig. 1b).

Total time that cleaners spent interacting with all clients per observation was greater at the no-take site (Fig. 1c, $F_{1,23} = 8.6$, $p < 0.01$) and was not related to client size ($F_{1,23} = 0.001$, $p = 0.97$), and there was no interaction effect ($F_{1,23} = 3.1$, $p = 0.09$) (Fig. 1c). The duration of each interaction with clients was also longer at the no-take site (Fig. 1d, $F_{1,23} = 8.7$, $p < 0.01$) and was not related to client size ($F_{1,23} = 2$, $p = 0.17$), and there was no interaction effect ($F_{1,23} = 1.4$, $p = 0.26$) (Fig. 1d). However, if we excluded from the above analyses one single cleaner (outlier) that interacted longer with clients of low average size, client size was significantly and positively related to duration of each interaction ($F_{1,22} = 9.3$, $p < 0.01$). Also, when excluding this outlier, we observed a significant

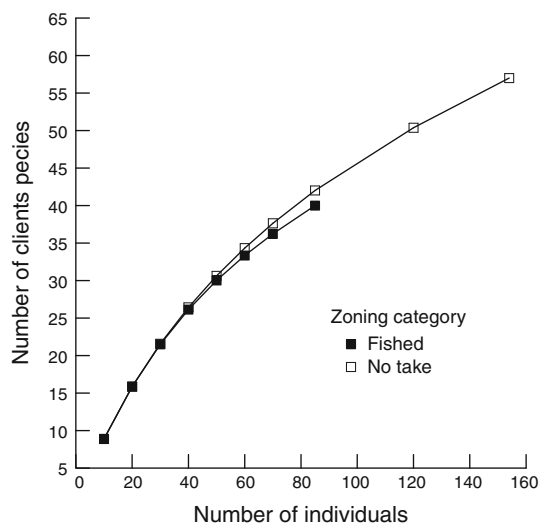


Fig. 2 Expected number of client species (rarefaction curves) based on the number of cleaning interactions with individual clients in a fished (85 interactions) and a no-take (154 interactions) site in Orpheus Island, Great Barrier Reef

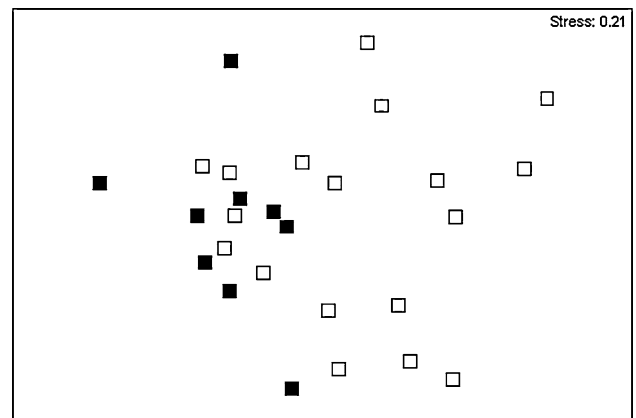


Fig. 3 Nonmetric multidimensional scaling (NMDS) ordination plot of cleaners (*L. dimidiatus*) sampled in a fished (solid squares $n = 9$) and a no-take (open squares $n = 18$) site, based on total time interacting (s) with client species (stress value = 0.21, Table 1) in Orpheus Island, Greater Barrier Reef. The plot indicates the extent to which cleaners are similar based on the joint (averaged) contributions of variables (total time interacting with clients) in two dimensions

interaction effect between zoning and client size for total time spent cleaning ($F_{1,22} = 14.3$, $p < 0.01$) and duration of each interaction ($F_{1,22} = 6.3$, $p < 0.05$). Other effects remained the same. The excluded cleaner (outlier) interacted with 23 small clients (<10 cm) for short periods (1.6 ± 0.7 s) and with only two larger clients (31–40 cm) for longer periods (20 and 120 s), which may have biased the interactions of this cleaner and hence the size effect.

The number of species of clients did not differ between no-take and fished sites: In both sites about 40 client species were expected to be cleaned in 85 interactions with individual clients (Fig. 2).

The SIMPER analysis indicated a high average dissimilarity (82.8 %) between the fished and the no-take site, possibly because at least some cleaners in both sites interacted with different clients (Fig. 3). Among the client species that contributed to this dissimilarity between the fished and the no-take site were the larger, but seldom fished *Scarus* spp., *Diagramma picta* and *Pomacanthus sexstriatus*, as well as two species that are commonly the target of line fishing: *L. carponotatus* and *Plectropomus* spp. (Table 1). Cleaners interacted more frequently with larger fishes, *D. pictum*, *P. sexstriatus* and *Plectropomus* spp., in the no-take site (Table 1). Conversely, five of the six species of the smaller fishes not susceptible to line fishing (e.g., Pomacentridae) were more frequently the subject of cleaner fish attention at the fished than at the no-take site (Table 1).

Overall, cleaner fish spent more time interacting with larger clients, including commercial fishes, at the no-take site, while at the fished site cleaners spent less time interacting with clients, and many interactions were directed

Table 1 Relative contribution of the client fish species that contributed most (up to 80 %) to the average dissimilarity between cleaners (*L. dimidiatus*) sampled in fished and no-take sites in Orpheus Island, Great Barrier Reef, according to the SIMPER analysis

Client species	Average abundance (s)		Average dissimilarity (\pm SD)	% Contribution
	No-take	Fished		
Scaridae				
Scarus spp.	14.3	12.4	12.3 (\pm 1.3)	14.8
Scarus rivulatus	4.9	0.8	4.3 (\pm 0.6)	5.2
Scarus flavipectoralis	4.4	0	3 (\pm 0.7)	3.7
Pomacentridae				
<i>Neopomacentrus</i> spp.	4	0.6	2.9 (\pm 0.7)	3.5
<i>Stegastes</i> spp.	1.1	2.1	2.6 (\pm 0.8)	3.1
<i>Stegastes apicalis</i>	0.1	3.1	2.4 (\pm 0.5)	2.9
<i>A. polyacanthus</i>	0.5	2.1	2.5 (\pm 0.9)	3.1
<i>A. curacao</i>	0.3	2	1.7 (\pm 0.7)	2.1
<i>Abudefduf whitleyi</i>	0.1	0.9	1.4 (\pm 0.7)	1.6
<i>Abudefduf bengalensis</i>	0.2	1.7	1.3 (\pm 0.6)	1.6
Haemulidae				
<i>D. picta</i>	12	0	4 (\pm 0.4)	4.83
Lutjanidae				
<i>Symphorus nematophorus</i>	0.2	0.8	1.2 (\pm 0.5)	1.5
<i>L. carponotatus</i>	3.3	4.7	5 (\pm 0.8)	6
Pomacanthidae				
<i>P. sexstriatus</i>	8.8	0	4.2 (\pm 0.6)	5
Serranidae				
<i>Plectropomus</i> spp.	4.5	0.6	2.9 (\pm 0.6)	3.5
<i>Cephalopholis boenak</i>	1.7	0.2	1.5 (\pm 0.4)	1.8
Labridae				
<i>Choerodon schoenleinii</i>	1.1	1.3	2.3 (\pm 0.7)	2.7
<i>Thalassoma lunare</i>	0.9	1.2	2.1 (\pm 0.7)	2.6
<i>Hemigymmus melapterus</i>	0.7	0.8	1.8 (\pm 0.4)	2.2
Caesionidae				
<i>Caesio teres</i>	2.7	0	1.8 (\pm 0.4)	2.2
Nemipteridae				
<i>Scolopsis bilineatus</i>	2.2	0	1.7 (\pm 0.6)	2.1
Centropomidae				
<i>Psammoperca waiigiensis</i>	0.8	1.3	2.2 (\pm 0.5)	2.7

The species that showed the highest contribution (more than 4 %) are in bold

toward small clients, such as damselfishes. Cleaning interactions usually increase in duration with client size, possibly because smaller fish have fewer parasites and less surface area to clean (Grutter 1995). The reduced abundance, and thus availability for cleaners, of larger fishes in fished sites may be reflected by observed shifts in the cleaning behavior of *L. dimidiatus* (longer interaction time with clients in the no-take site and a reduced duration of interactions in the fished site). The cleaners may also have responded to such effects by interacting with the more abundant species at the fished site, including the smaller

damselfishes *Acanthochromis polyacanthus* and *Amblyglyphidodon curacao* (Table 1), which are not fished (Holmes 2007). These two species also have higher abundances on fished reefs in Orpheus Island due to a reduction in the abundance of their predator (*Plectropomus* spp.) (Graham et al. 2003).

Although cleaners maintained the same number of clients, the quality (duration) of cleaning interactions may have decreased as a result of fishing at the fished site. This may represent a decrease in the clients' parasite removal rates (Grutter 1996), and thus, this important ecological

service provided by cleaners (Grutter 1999) may have been affected by fishing. However, the generalization of this conclusion is limited by lack of spatial replicates: We addressed only one no-take and one fished site, which are close to each other. Further studies would be desirable to assess the extent to which fishing effects may influence cleaning interactions over a larger spatial scale.

Our study addressing the consequences of fishing to cleaning interactions provides future avenues for research into other important mutualistic and antagonistic behavioral interactions among reef fishes, such as following behavior (Silvano 2001; Sazima et al. 2007) and protective or aggressive mimicry (Sazima 2002). Because the presence of cleaners can affect the behavior and density of client fishes (Waldie et al. 2011), it would be interesting to determine how alterations in cleaning interactions would affect the spillover of adult fish from no-take to fished areas in coral reefs (reviewed in Gell and Roberts 2003).

According to our findings, in sites subjected to high fishing pressure, cleaners would spend less overall time cleaning their clients, but this was at least partially influenced by the interaction with some larger clients in the no-take site (Fig. 1c, d). This indicated possible fishing effects on cleaning behavior, as changes in the species composition, size and size range of fish may also be a fishing effect (Graham et al. 2003; Evans and Russ 2004). Therefore, cleaners in the fished site may have habituated to interact with smaller clients, which usually require shorter interactions. When cleaners found larger clients in the fished site, they tended to interact with these for shorter periods of time, possibly because cleaners have less experience with cleaning large fish there. Cleaner fish spend more time near familiar than unfamiliar clients, possibly to improve the benefits of the interaction to both partners (Tebbich et al. 2002). To our knowledge, this is the first study to show fishing effects on fish interspecific mutualisms in tropical reefs.

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