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Cleaner wrasse prefer client mucus: support for partner control mechanisms in cleaning interactions

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Recent studies on cleaning behaviour suggest that there are conflicts between cleaners and their clients over what cleaners eat. The diet of cleaners usually contains ectoparasites and some client tissue. It is unclear, however, whether cleaners prefer client tissue over ectoparasites or whether they include client tissue in their diet only when searching for parasites alone is not profitable. To distinguish between these two hypotheses, we trained cleaner fish *Labroides dimidiatus* to feed from plates and offered them client mucus from the parrotfish *Chlorurus sordidus*, parasitic monogenean flatworms, parasitic gnathiid isopods and boiled flour glue as a control. We found that cleaners ate more mucus and monogeneans than gnathiids, with gnathiids eaten slightly more often than the control substance. Because gnathiids are the most abundant ectoparasites, our results suggest a potential for conflict between cleaners and clients over what the cleaner should eat, and support studies emphasizing the importance of partner control in keeping cleaning interactions mutualistic.

Keywords: cleaning behaviour; cooperation; mutualism; partner control; *Labroides dimidiatus*; diet preference

1. INTRODUCTION

During cleaning behaviour, so-called cleaners remove and eat ectoparasites from other animals called clients (Côté 2000). However, gut analyses of cleaner fish have also found client tissue such as mucus and scales (Gorlick 1980; Grutter 1997; Arnal & Côté 2000; Arnal & Morand 2001), and oxpecker birds feed on blood from wounds on hippopotami and cattle (Weeks 2000), shedding doubt on the mutualistic nature of the association.

In the cleaning symbiosis between *Labroides dimidiatus* and client fishes, there is accumulating evidence that the overall outcome is mutualistic. Cleaners eat more than 1200 parasites (mostly gnathiid isopods) per day (Grutter 1996), reduce parasite densities on clients (Grutter 1999) and have a positive impact on local reef fish diversity (Bshary 2003; Grutter *et al.* 2003). The outcome, however, does not reveal the degree of conflict between cleaners and clients over what the cleaner should eat. As long as cleaners prefer to eat ectoparasites, conflicts will be small, particularly if there are enough parasites on the client to make searching for them efficient (basic prey model; Hughes 1997). By contrast, if cleaners are attracted to client material that is energetically costly for the client to produce, a major conflict would need to be overcome to yield a mutualistic outcome: clients would need strategies to make cleaners feed against their foraging preferences. This latter scenario now seems a plausible possibility because recent evidence shows that clients indeed control cleaner fish behaviour in various ways, i.e. through partner switching (Bshary & Schäffer 2002) or punishment (Bshary & Grutter 2002). In the latter, punishment served to control cleaners that readily fed on client mucus in the absence of client control.

To evaluate the degree of conflict between cleaners and clients, we offered cleaner fish equal amounts of a variety of food items: mucus of the parrotfish *Chlorurus sordidus*, parasitic monogenean flatworms, parasitic juvenile gnathiid isopods and boiled flour glue as a control.

2. MATERIAL AND METHODS

The study was conducted in March and April 2002 at Lizard Island Research Station, Great Barrier Reef, Australia. We obtained gnathiids by capturing fishes, mostly labrid species, following Grutter (1994). Fishes were placed in plastic bags underwater then held in 10 litre aerated buckets overnight. As these isopods drop off from the fishes within an hour (Grutter 2003), by filtering all liquids at 57 μm we could recover all gnathiids. Gnathiid total lengths ranged from 1.1 mm to 2.1 mm.

To obtain relatively large numbers of monogeneans, we collected 30 surgeonfish *Ctenochaetus striatus* and kept them in 1.3 m diameter tanks for four to five weeks. We used several 'egg-nets' to capture and retain monogenean eggs in tanks (see Ernst & Whittington 1996; Bshary & Grutter 2002). After four to five weeks, we gave each fish a 2 min freshwater bath, brushing the fish with a soft plastic brush during and after the bath to remove the old and new generation of monogeneans from the skin. Monogenean total lengths ranged from 1.4 mm to 2.2 mm.

Mucus was obtained from five parrotfish, *C. sordidus*, which were killed with a sharp blow to the head. Fishes were refrigerated in plastic bags for several hours and the mucus gently scraped off their bodies with a scalpel blade. All parasites and mucus were frozen immediately after collection. Mucus did not appear to change in appearance after its extraction and freezing.

We caught 14 cleaners with total lengths of 6.3 cm to 8.2 cm which were held in pairs or alone in aquaria for a minimum of four weeks prior to the experiment. They were fed mashed prawns and fish flakes spread onto grey Plexiglas plates (8 cm × 15 cm). The fish flakes made the texture and colour different from all test food items. One week before the experiment, we painted a 4 cm × 3 cm grid system on the plates. We placed food within the grid only, so that cleaners learned to search the grid for food. At least 2 days before a given cleaner was tested, it was placed alone in one of six experimental aquaria (45 cm × 90 cm × 45 cm) to acclimatize. Finally, on the day before each cleaner was tested, we offered cleaners each food source (gnathiids, monogeneans and mucus on the grid, see below) in succession. All items, including mucus, were glued to the plates using similar amounts of glue made from boiled flour and water. We also offered cleaners the glue alone as a control. No parasites were present in the mucus, which was examined for parasites when it was placed onto the grid. Observations confirmed that cleaners had eaten mucus, monogeneans, gnathiids and the control substance prior to the preference test.

Each cleaner was tested three times. In each trial, we placed three food items each of mucus, monogeneans, gnathiids and boiled flour (control) on the plate such that each square on the grid contained one food item, with a total of 12 items on the grid. The position of each food item was determined with a random table, and each plate ($n = 42$ for 14 cleaners) had a different distribution of food items. Each food item was placed in a different grid cell. Using a stereomicroscope, we placed a drop of mucus or a monogenean or a gnathiid on top of a drop of boiled flour. Because of the small sizes of all items involved, it was impossible to weigh each item. Using an eye piece micrometer, we ensured that each food item covered the same area as the others. The amounts of mucus and glue were adjusted to monogenean sizes. Gnathiids were often smaller than

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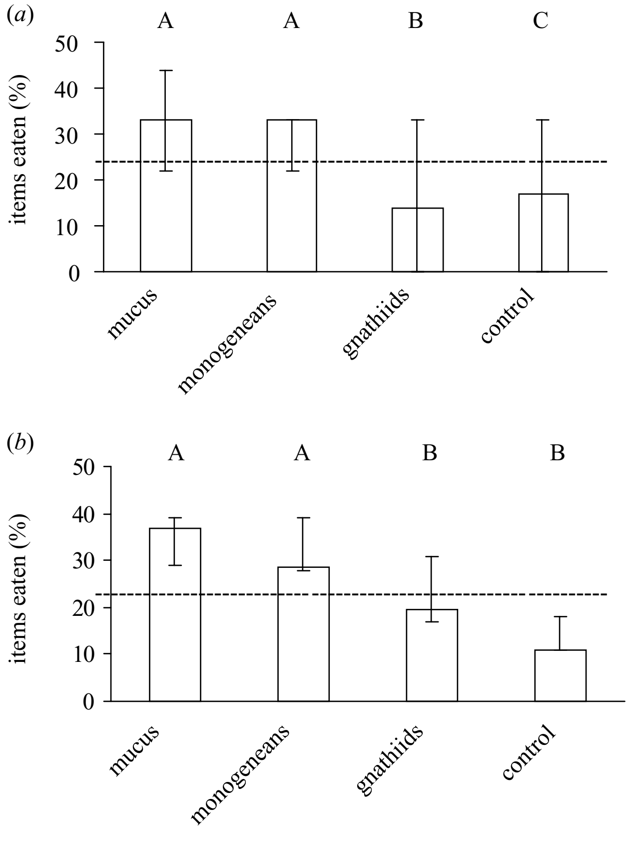

Figure 1. Median per cent of four food items eaten by *Labroides dimidiatus* when (*a*) the first three items eaten are considered and (*b*) the first six items eaten are considered. Error bars are interquartiles. Similar letters above bars represent non-significantly different means from *post hoc* multiple comparisons.

monogeneans, and in such cases two gnathiids were selected to cover the same area as the monogenean.

Plates were placed inside a cleaner's aquarium against one of the aquarium walls. The sequence of the first six food items that cleaners ate in each trial was usually determined by direct observation. We also recorded the trials with a Panasonic HI8 camera to re-examine cases in which cleaners ate very quickly. The three trials for each cleaner were conducted on the same day, between 08.00 and 12.00, with 1 h intervals between trials. All fishes were finally released to the original collection site.

To analyse the data, we added the first three items eaten out of the 12 in each trial and determined how often each cleaner had eaten each of the four food types. The data for each cleaner were ranked for a Friedman test and *post hoc* multiple comparisons (Conover 1999) between food items. Using the first three items only allowed for the possibility of a 100% preference for one food item over the others. In a second analysis, we counted the first six items eaten out of the 12 in each trial to obtain a higher resolution for potential cleaner preferences between less-preferred food items.

3. RESULTS

When the first three food items eaten per round were considered, we found that cleaners ate food items with different probabilities (Friedman test: $n = 14$, $\chi^2 = 7.9$, d.f. = 3, $p = 0.049$). Multiple comparisons revealed that cleaners ate more mucus and monogeneans than gnathiids or the control substance (mucus = monogeneans > gnathiids = control, all $p < 0.05$; figure 1*a*). When the first six food items eaten per round were considered, we found again that cleaners ate food items with different probabilities (Friedman test: $n = 14$, $\chi^2 = 17.6$, d.f. = 3, $p = 0.0005$). Multiple comparisons again revealed that cleaners ate mostly mucus and monogeneans (mucus = monogeneans > gnathiids > control; figure 1*b*) and both significantly more often than gnathiids (both $p < 0.01$). Gnathiids, by contrast, were eaten more often than the control substance ($p < 0.05$).

4. DISCUSSION

We found that the preferences of cleaner fish *L. dimidiatus* for mucus and monogeneans did not differ, with both of these preferred over gnathiids and a glue control. This suggests that cleaners apparently feed against their preferences under natural conditions. Although gnathiids are the most abundant ectoparasites in *L. dimidiatus* gut contents (Grutter 1997), mucus loads of most fishes are higher than gnathiid loads. For example, fishes have more than 1 ml of mucus (Gorlick 1980) whereas a common gnathiid load (10 per fish; Grutter & Poulin 1998) consists of not much more than 30 mm^3 (Grutter 2003). This indicates a huge potential for conflict between cleaners and clients over what the cleaners should eat. The conflict is likely to be more pronounced under natural conditions, as in our experiment we offered them equal amounts of food whereas in nature mucus is abundant all over the clients' surface. Mucus is also easily detectable, while parasites are localized and need to be searched for. When given access to unparasitized fishes in captivity, the Hawaiian cleaner *L. phthirophagus* ingests 2 ml h^{-1} of client mucus (Gorlick 1980), which is probably a significant proportion of the client's total mucus load (A. S. Grutter, personal observation). Such a loss should be costly to the client, as mucus is known to be a rich source of nitrogen (Gorlick 1980).

Mucus quality, however, varies with fish species (Gorlick 1980; Arnal & Morand 2001) and could affect a cleaner's preference. For example, the parrotfish mucus used in this study was ranked 10 out of 15 common reef species based on its mucus load (mean ± s.e. 0.64 ± 0.08 mg dry weight cm^{-2}) and ranked 10.5 based on its quality, which was calculated by using a mean of the ranks obtained from mucus protein richness (mean dry weight (DW): 69.5% ± 2.22% protein DW) and mucus calorific value (4.77 ± 0.26 cal mg^{-1} DW) (Arnal 2000). Whether cleaners would still prefer mucus over gnathiids if they were offered a lower-quality mucus, however, needs to be determined.

Other calculated caloric values of client fish mucus are 2.6–4.7 cal mg^{-1} DW (Gorlick 1980) and 2.25–6.14 cal mg^{-1} DW (Arnal 2000), while carbon : nitrogen ratios are 3.8 : 1 to 4.3 : 1, which are somewhat lower than that of zooplankton (5.1 : 1 to 6.0 : 1) (Parsons *et al.* 1961), the latter a rough estimate for crustacean parasites. Protein levels of client mucus are lower (1.2 mg ml^{-1}) than that of fish flesh (Geiger & Borgstrom 1962). Mucus from freshwater fishes, however, is high in phospholipids (Lewis 1970). Fish mucus may therefore provide cleaners with a potentially rich energy source, with ectoparasites providing more of the protein (Gorlick 1980).

Our results emphasize the importance of partner control in keeping cleaning interactions mutualistic. Client control mechanisms of cleaner fish behaviour, like punishment (Bshary & Grutter 2002) and partner switching (Bshary & Schäffer 2002), appear to be very efficient: stomach analyses show that the cleaners *L. dimidiatus* in the wild feed mainly on gnathiids (Grutter 1997), a food source the experimental cleaners ate significantly less frequently than mucus when in a free-choice situation. Local parasite densities (Grutter 1994; Grutter & Poulin 1998) and cleaner to client ratios may further influence the outcome of interactions. For example, in areas where there are few parasites and thus more incentive for cleaners to eat mucus, the benefits for the clients should be reduced (Johnstone & Bshary 2002). A comparsion between Heron and Lizard Islands fits this prediction. Parasite densities on clients from Heron Island are low compared with sites around Lizard Island (Grutter & Poulin 1998). Accordingly, on Heron Island cleaners eat more mucus (Grutter 1997) and clients respond to a cleaner's bite with a jolt more frequently than on Lizard Island (Bansemer *et al.* 2002). Jolts are a correlate of cleaner fish cheating (Bshary & Grutter 2002).

That gnathiids were less preferred than monogeneans is surprising since monogeneans have rarely been found in cleaner fish dietary studies (Grutter 2002), although *L. dimidiatus* can affect their abundance on captive fishes (Grutter *et al.* 2002). Possibly, monogeneans are difficult to detect in diet analyses (Grutter 1997) and/or are camouflaged on the host (Whittington 1998). They are, however, common on reef fishes (Grutter 1994; Whittington 1998). These results suggest that their role in cleaning interactions needs to be re-evaluated.

In conclusion, we show that there are strong conflicts of interest in cleaning symbiosis. This warrants developing theoretical models involving asymmetric cooperation games, as only predatory clients may reciprocate cheating by cleaners by eating them, while non-predatory clients lack any options to exploit cleaners. Thus, theories based on the iterated Prisoner's Dilemma game (Axelrod & Hamilton 1981) cannot be applied to cleaning symbioses. The preference for mucus also has implications for theories regarding the evolution of marine cleaning symbioses. Although it is generally assumed that cleaning in labrid fishes evolved as an extension of their feeding behaviour on small invertebrates (Poulin & Grutter 1996), our results suggest a different route, that it may have originated in opportunistic mucus feeding off clients, a feeding mode not unknown in other fishes (Gerking 1994).

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