RESEARCH PAPERS

# The Meaning of Jolts by Fish Clients of Cleaning Gobies

Marta C. Soares*, Redouan Bshary†, Sónia C. Cardoso* & Isabelle M. Côté‡

* School of Biological Sciences, University of East Anglia, Norwich, UK
† Institut de Zoologie, Eco-Ethologie, Université de Neuchâtel, Neuchâtel, Switzerland
‡ Department of Biological Sciences, Simon Fraser University, Burnaby, BC, Canada

**Correspondence**

Isabelle M. Côté, Department of Biological Sciences, Simon Fraser University, Burnaby, BC V5A 156, Canada. E-mail: imcote@sfu.ca

**Abstract**

Cooperative interactions offer the inherent possibility of cheating by each of the interacting partners. A key challenge to behavioural observers is to recognize these conflicts, and find means to measure reliably cheating in natural interactions. Cleanerfish *Labroides dimidiatus* cheat by taking scales and mucus from their fish clients and such dishonest cleaning has been previously recognized in the form of whole-body jolts by clients in response to cleaner mouth contact. In this study, we test whether jolts may be a general client response to cheating by cleaners. We experimentally varied the ectoparasite loads of yellowtail damselfish (*Microspathodon chrysurus*), a common client of the cleaning goby *Elacantinus evelynae*, and compared the rates of jolts on parasitized and deparasitized clients. As predicted if jolts represent cleaner cheating, deparasitized clients jolted more often than parasitized clients, and overall jolt rates increased over time as client parasite load was presumably reduced by cleaning activity. Yellowtail damselfish in the wild jolted significantly less frequently than those in captivity, which is consistent with a loss of ectoparasites during capture. Our results suggest that jolts by clients of cleaning gobies are not related to the removal of ectoparasites. Client jolts may therefore be a generally accurate measure of cheating by cleanerfish.

## Introduction

The evolution of cooperation has long appeared paradoxical because of the so-called 'cheating problem' (Noë 2006). In any cooperative interaction, there is strong selection for partners derive benefits from the interaction without incurring costs. 'Cheating' therefore occurs when either of the cooperative partners deceives the other by providing a dishonest service, which can range from a subtle reduction in service value to not delivering the expected commodity at all (Dugatkin 1997, 2002; Noë 2001, 2006; Bshary & Noë 2003; Sachs et al. 2004).

One biological system in which conflicts over cheating have been demonstrated is the interactions between cleanerfish and their fish clients. Clients visit cleaning stations, i.e. small territories held by cleanerfish, to have their parasites and dead or infected tissues removed (reviewed by Losey et al. 1999; Côté 2000) but cleaners potentially have access to other commodities, such as mucus and scales, on their clients' bodies. Conflicts between cleaners and clients over what cleaners should feed on are evident (Bshary & Würth 2001). In an experiment in which anaesthetized parasite-free surgeonfish clients were presented to bluestreak cleaner wrasses *Labroides dimidiatus*, the commonest Indo-Pacific cleanerfish, most cleaners scraped the body surface of their clients rather than feed on prawns which were provided as an alternative food source (Bshary & Grutter 2002). In addition, in food preference trials in the laboratory, *L. dimidiatus* fed on mucus rather than on gnathiid ectoparasites (Grutter & Bshary 2003), and preferred energy-rich mucus

(e.g. of parrotfish) over energy-poor mucus (e.g. snapper) (Grutter & Bshary 2004). Cleaners therefore appear to prefer mucus over ectoparasites, whereas clients would prefer them to forage only on the latter.

A key challenge for empirical biologists is to find means to reliably measure cheating in natural interactions. For marine cleaning mutualism involving the cleaner wrasse *L. dimidiatus*, client jolts – whole-body shudders that appear to be painful – in response to cleaner fish mouth contact appear to be a good correlate of cheating by cleaners, i.e. the eating of mucus or scales (Bshary 2001). The fact that experimentally deparasitized clients of *L. dimidiatus* jolt more often than highly parasitized clients supports this interpretation (Bshary & Grutter 2002).

Stomach content analyses of cleanerfish species other than *L. dimidiatus* have shown that all cleaner species feed not only on ectoparasites but also mucus and scales to varying extents (Randall 1958; Gorlick 1984; Cheney & Côté 2005; M. C. Soares, pers. obs.). It therefore becomes of interest to determine whether incidences of cheating by cleanerfish other than *L. dimidiatus* can also be detected through client behaviour.

Cleaning gobies (*Elacatinus* spp.) are the most ubiquitous cleaners in the Caribbean region. The clients of cleaning gobies also jolt during cleaning interactions (e.g. Côté & Molloy 2003; Soares et al. 2007), but the significance of this behaviour has not yet been determined. In a field study, Soares et al. (2007) failed to find a relationship between client jolting rate and client ectoparasite loads. Moreover, predatory clients, which should be able to enforce honesty in cleaners through risk of predation, did not jolt less frequently during cleaning interactions than harmless clients. Both results suggest that jolts by clients of cleaning gobies could be unrelated to the delivery of cheating bites.

To investigate the meaning of jolts by clients of cleaning gobies, we examined the interactions between cleaning gobies and both parasitized and experimentally deparasitized yellowtail damselfish (*Microspathodon chrysurus*) under captive conditions. We tested three main predictions. First, if client jolts are indeed linked to cleaner cheating, then deparasitized clients should jolt more than parasitized clients. Second, differences in jolt rates between parasitized and unparasitized clients should diminish as cleaners clean, thus reducing the ectoparasite load differences between both groups. Third, client jolt rate should increase over time as client parasite load is reduced by cleaning activity. Finally, to verify the extent to which behaviours observed in captivity reflect occurrences in the wild, we compared our experimentally controlled data to field observations of yellowtail damselfish behaviour during interactions with cleaning gobies.

## Methods

### Study Species and Fish Collection

The study was carried out between Apr. and Nov. 2005 at the Bellairs Research Institute, in Barbados, West Indies. We focussed on the sharknose goby (*Elacatinus evelynae*), one of two species of cleaning gobies present on Barbadian fringing reefs. These cleaning gobies are small (1.2–3.5 cm total length) and found solely on coral (usually *Siderastrea* spp. and *Montastrea* spp.), where individuals or pairs of fish establish small territories that act as cleaning stations. The bulk of the sharknose goby diet consists of ectoparasites gleaned from visiting client species (Whiteman & Côté 2002). As a focal client, we selected yellowtail damselfish because they are among the most frequent visitors to cleaning stations and they usually have a relatively higher ectoparasite loads than other client species at this location (Sikkel et al. 2000, 2005; Soares et al. 2007).

### Behavioural Observations in the Wild

To provide a comparison for experimental trials with captive fish, in situ observations of interactions between yellowtail damselfish and sharknose cleaning gobies were carried out while diving or snorkelling on eight fringing reefs near the Bellairs Research Institute. Forty-three cleaning stations were selected haphazardly across the reefs, with four to eight stations per reef. Each cleaning station was observed once for 30 min, between 10.00 and 17.00 hours. In fact, most of our field observations were carried out in the early afternoon, similarly to our laboratory experimentation trials, to make comparisons more meaningful. Observations were made from a distance of 2–3 m and began after a 2- to 5-min delay to allow the fish to become accustomed to the presence of the observer. During each observation period, we recorded on plastic slates client total length (estimated visually to the nearest cm), the duration (in s) of inspection and the number of jolts, i.e. apparently painful reactions to a cleanerfish bite, for each visiting yellowtail damselfish. Because yellowtail damselfish were unmarked, we assumed that all visits during an observation period were made by

different individuals. Focal observations carried out on 23 individual yellowtail damselfish on three of the study reefs revealed that fewer than half (10 of 23 individuals) repeatedly visited the same cleaner in a 30-min period and successive visits to cleaners were made to different cleaning stations (M. C. Soares, pers. obs.), suggesting a limited extent of double counting in our main observations.

### Experimental Design

We caught cleaning gobies and yellowtail damselfish from three of the reefs on which the in situ behavioural observations were carried out. Twelve cleaning gobies were collected 3–4 wk prior to the beginning of experiments to acclimatize to laboratory conditions. A mixture of clove oil (a natural anaesthetic), ethanol and water was sprayed over each individual cleaning goby in order to induce a temporary reduction in activity. Gobies were then easily caught with hand nets and placed individually in sealed plastic bags filled with seawater. Once in the laboratory, gobies were measured (total length to the nearest mm) and placed in individual glass aquaria (61 cm long × 38 cm wide × 46 cm high) with running seawater. Each aquarium had 1–2 cm of sand and gravel at the bottom. Several pieces of dead coral (10–40 cm diameter) were placed in a mound at one end of each aquarium to provide shelter and a vantage point. Aquaria were separated by opaque partitions. During the acclimation period, cleaning gobies, which ranged in total length from 2.2 to 3.3 cm, were fed daily with brine shrimp *Artemia* spp.

We collected 24 yellowtail damselfish from three of the study reefs between 07.00 and 12.00 hours. Individuals were targeted haphazardly and herded into a barrier net placed near the edge of their territories. The fish were then caught with a hand net, rapidly placed individually into hermetically sealed plastic bags filled with seawater, and immediately brought to the laboratory. Damselfish were randomly assigned to one of two groups: ectoparasite removal or control procedure. The ectoparasite removal method was similar to that of Sikkel et al. (2004), and has been shown to remove 98–100% of all ectoparasites present on coral reef fish (Grutter 1995a,b). Damselfish were placed into individual containers with variable amounts of seawater and two to three drops of clove oil. The damselfish were then transferred to a freshwater bath for 10 min, during which their body surface was gently brushed with a soft-bristle paintbrush. Finally, fish were

placed in seawater-filled containers to recover for a minimum of 10 min. Full recovery was deemed to have occurred when the fish were swimming actively. The control procedure was identical, except that damselfish were placed in a seawater bath rather than a freshwater bath, which is less effective at removing ectoparasites (see Results). Capture, parasite removal (or control procedure) and behavioural testing occurred on the same day.

After each behavioural trial, damselfish were measured to the nearest mm (total length) and released at their capture location. All fluids from the freshwater and saltwater baths were filtered separately for each fish. These samples were later examined under a binocular microscope. Ectoparasites were counted and identified to family, focussing on the families Bomolochidae, Caligidae, Ergasilidae, Gnathiidae and Hatschekiidae.

### Experimental Behavioural Observations

The yellowtail damselfish used for experimentation ranged in standard length from 9.2 to 12.3 cm. Of these, 12 had been deparasitized (i.e. subjected to a freshwater bath) and 12 were parasitized (i.e. subjected to a saltwater bath). There was no size difference between the two groups (independent t-test: $t_{20} = -0.69$, $p = 0.50$). Each damselfish was used in a single trial. Each of the 12 cleaning gobies was used twice: once with a parasitized client and once with a deparasitized client, with testing occurring on different days. All trials were carried out between 12.00 and 15.00 hours.

Each damselfish client was placed in a test aquarium with one cleaning goby for 30 min. Cleaning interactions were videotaped with a Sony Handycam digital videocamera (model DCR-TRV10E), which was placed 60 cm from the front wall of the aquarium, allowing all interactions to be clearly observed. The videotapes were played back on a 35-cm television. We recorded the duration of each inspection bout and the number of jolts by yellowtail damselfish.

### Statistical Analysis

Because the ectoparasite loads of experimental yellowtail damselfish were low (see Results), we surmised that any difference in parasite numbers between parasitized and deparasitized clients would be rapidly reduced by cleaning activity. We therefore initially considered only the first interaction between cleaners and damselfish under experimental

conditions. Client jolt rates (always expressed as the number of jolts per 100 s of inspection time) were compared between parasitized and deparasitized clients using paired tests centred on individual cleaning gobies. We also examined how jolt rates changed over time by considering the first three interactions between cleaners and both parasitized and deparasitized clients. These were analysed using two-way repeated measures analyses of variance (ANOVA) with time as a within-subject factor and parasite load as a between-subjects factor. Finally, to compare the jolt rates of captive and wild fish, we obtained jolt rates for individuals across all observed cleaner–client interactions and carried out pairwise comparisons between wild fish and each of the two categories of captive fish (parasitized and unparasitized) using unpaired t-tests.

## Results

### Effect of Ectoparasite Removal and Control Procedures

Significantly more ectoparasites were removed from yellowtail damselfish in the deparasitized group (i.e. subjected to a freshwater bath) than from those in the parasitized group (i.e. subjected to a seawater bath) (independent t-test: $t_{22} = 2.61$, p = 0.02; Fig. 1). Assuming that fish in both groups had similar numbers of ectoparasites upon capture, damselfish in the parasitized group entered the behavioural trials with more ectoparasites than those in the deparasitized group.
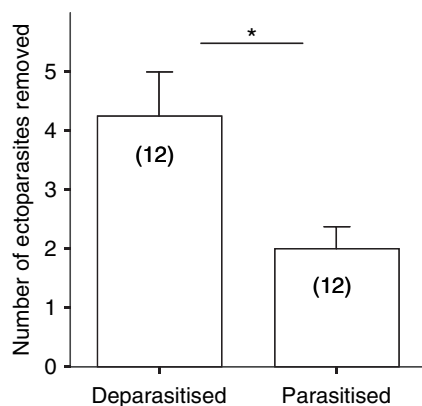


**Fig. 1:** Number of ectoparasites removed from yellowtail damselfish subjected to a freshwater bath (= deparasitized) or to a saltwater control bath (= parasitized) before entering behavioural trials. Means are shown ± 1 SE. *p < 0.05 with paired t-test. Sample sizes (= number of individuals) are given in parentheses.

### Effect of Client Ectoparasite Load on Client Jolt Rate

Parasitized damselfish jolted significantly less often during the first cleaning interaction than deparasitized damselfish (paired t-test: $t_{11} = -2.37$, p = 0.04; Fig. 2). When considering the first three interactions, jolt rate increased significantly over time (two-way RM-ANOVA: $F_{2,44} = 3.98$, p = 0.03), but did not vary between deparasitized and parasitized clients (two-way RM-ANOVA: $F_{1,22} = 1.24$, p = 0.28). There was no interaction between time and client parasite load (two-way RM-ANOVA: $F_{2,44} = 0.42$, p = 0.66).

Yellowtail damselfish in the wild jolted significantly less frequently than captive damselfish. Jolt rates in the wild were 79% lower than in captive, deparasitized damselfish (independent-samples t-test: $t_{44.53} = 4.34$, p < 0.0001), and 66% lower than in captive, parasitized fish (independent-samples t-test: $t_{45.36} = 2.36$, p = 0.02; Fig. 2).

## Discussion

Our results provide the first experimental evidence that jolts by clients of cleaning gobies are unlikely to be related to ectoparasite removal. If jolts by clients were responses to the physical removal of ectoparasites by cleaning gobies, then clients with higher parasite loads should have exhibited more frequent jolt reactions during cleaning inspection. Instead, we found that deparasitized clients jolted significantly more than their parasitized counterparts. This result supports our first prediction and corroborates previous work carried out with the cleaner wrasse *L. dimidiatus* (Bshary & Grutter 2002). Neither we nor previous authors have unambiguously shown that jolt-inducing bites are dishonest, i.e. that they result in the removal of items such as scales and mucus, which may be costly for clients to replace and/or make clients more vulnerable to disease. However, given the frequency with which such items are found in the gut contents of cleanerfish (Randall 1958; Gorlick 1984; Grutter 1997; Cheney & Côté 2005, M. C. Soares, pers. obs.), it seems parsimonious to conclude that cheating by cleanerfish is prevalent and that client jolts are a reflection of this dishonest behaviour.

We had also predicted that as cleaning gobies clean, and thereby reduce the ectoparasite load differences between parasitized and unparasitized clients, differences in jolt rates between both groups should diminish, and overall jolt rate should increase. Only the latter prediction was supported. The significant ANOVA interaction between time and

**Fig. 2:** Number of jolts per 100 s of inspection by captive deparasitized (white bars) and parasitized (black bars) yellowtail damselfish, in their first three interactions with cleaning gobies, and by wild yellowtail damselfish (grey bar). Means are shown ± 1 SE. Sample sizes (= number of individuals) are given in parentheses.
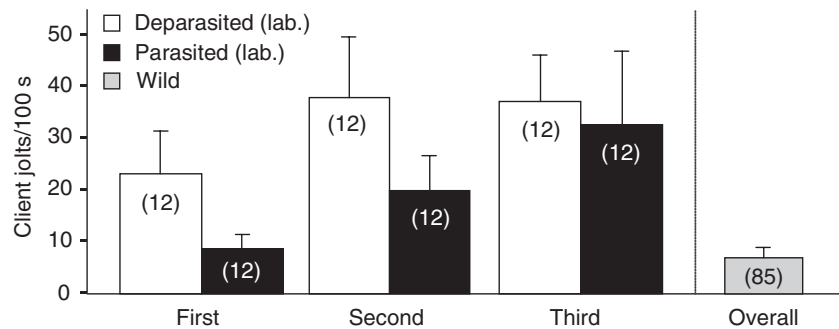
parasite load that would have supported the former was not observed. Experiments similar to ours, but carried out using clients that were heavily parasitized (x ± 1 SD: 42 ± 33 parasites) on one side of their body and deparasitized on the other, showed that client jolting rate was significantly higher after 25 h than in the first 15 min of exposure to *L. dimidiatus* cleaners (Bshary & Grutter 2002). Moreover, clients jolted at similar rates regardless of which side of the body the cleaner wrasses were foraging, suggesting that over the course of the day, the cleaners had reduced parasite numbers to equal levels on both sides of their clients (Bshary & Grutter 2002). Given that our parasitized clients entered the behavioural trials with relatively few parasites, as expected with the low ectoparasite intensities found in the Caribbean (Arnal et al. 2001; Cheney & Côté 2005; Soares et al. 2007), they probably became deparasitized quickly. This rapid levelling of parasites loads across fish groups may explain the absence of statistical difference in jolt rates between parasitized and deparasitized clients over time.

Despite the fact that jolt behaviour has often been used to infer cheating by cleanerfish under experimental conditions and in the wild (Bshary & Würth 2001; Bshary & Grutter 2002; Bshary & Schäffer 2002; Soares et al. 2007), there have been to date no direct comparisons of jolt rates by captive and wild clients of the same species in the same location. Wild yellowtail damselfish jolted significantly less frequently than both captive deparasitized and parasitized fish, suggesting that wild fish may have more parasites than those held in captivity. This is possible as gnathiids, in particular, are highly mobile ectoparasites (Davies & Johnston 1976; Grutter 1995a), which will readily leave disturbed hosts (Grutter 1994, 1995a). The capture procedure may therefore have reduced ectoparasite loads on the experimental damselfish, prior to further reductions during deparasitization.

Given the link established here between clients jolting and cleaning goby cheating in captivity, and given that jolting is frequently observed in the wild, one may predict that the clients of cleaning gobies will have strategies to control cleaner cheating. Such strategies have been documented in *L. dimidiatus*. For example, clients of *L. dimidiatus* that have the possibility to choose among several cleaners, end cleaning interactions after a jolt-inducing bite and withhold revisiting the cheating cleaner in favour of alternative cleaners (Bshary 2001; Bshary & Schäffer 2002). By contrast, clients that have access to a single cleaning station, owing to a small territory size, respond to jolt-inducing bites by chasing aggressively the cheating cleaners, which appears to reduce the likelihood of cleaner dishonesty in subsequent encounters (Bshary & Grutter 2002; Bshary & Noë 2003). Partner control, through punishment strategies that enforce honesty, is an essential feature in the maintenance of interspecific mutualistic interactions (Bshary & Grutter 2005). Further studies are needed to determine if and how the clients of cleaning gobies control cheating by their cleaners.

## Acknowledgements

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