Cleaner-fish use tactile dancing behavior as pre-conflict management strategy

Alexandra S. Grutter

School of Life Sciences

University of Queensland

Brisbane, Queensland, 4072, Australia

Telephone: 61 7 3365 7386

Fax: 61 7 3365 1655

Email: a.grutter@uq.edu.au

Key words: cleaning behavior, cooperation, conflict management, mutualism, predators, cleaner-fish

Summary

The most commonly asked question about cooperative interactions is how they are maintained when cheating is theoretically more profitable [1]. In cleaning interactions, where cleaners remove parasites from apparently cooperating clients, the classical question asked is why cleaner-fish can clean piscivorous client-fish without being eaten, a problem Trivers [2] used to explain reciprocal altruism. Trivers [2] suggested predators refrain from eating cleaners only when the repeated removal of parasites by a particular cleaner results in a greater benefit than eating the cleaner. Although several theoretical models have examined cheating behaviour in clients [3,4], no empirical tests have been done (but see Darcy [5]). That cleaners are susceptible to predation is not unheard of [6,7]. Thus cleaners should have evolved strategies to avoid conflict or being eaten. In primates, conflicts are often resolved using conflict or pre-conflict management behaviour [8]. Here I show that cleaner-fish tactically stimulate clients while swimming in an oscillating 'dancing' manner (tactile dancing) more when exposed to hungry piscivorous clients than satiated ones, regardless of the client's parasite load. Tactile dancing thus may function as a pre-conflict management strategy that enables cleaner-fish to avoid conflict with potentially 'dangerous' clients.

Results and Discussion

The iterated prisoner's dilemma has long been used to explain the evolution of cooperation between unrelated individuals, although, some of its limitations have been illustrated with the cleaner-fish mutualism [9]. Recently, biological market theory, where traders exchange goods and or services, was proposed as an alternative for understanding cooperation in many systems, including cleaning symbioses [10]. Client ectoparasites and cleaning services are the main goods traded in the cleaner-fish market [10]. Thus, the probability of aggression directed at a cleaner by a client is likely to increase as a client’s ectoparasite load decreases and its need for the cleaner's services subsequently declines. Also, as client hunger levels increase, the benefits of eating a cleaner for a client should also increase. Thus, I hypothesized that cleaners should respond differently to hungry piscivorous clients compared with satiated ones and that this should vary according to client parasite load. Field observations suggest that cleaner-fish use pre-conflict management behaviour which involves the use of tactile stimulation, where they rub clients with their body or fins, as a strategy to reduce conflict that might arise from a client's decision [11]. Because of the severe consequences of conflicts with predators (death) it was also proposed cleaner-fish should provide tactile stimulation unconditionally to predators [11]. This has never been tested experimentally. Most other anti-predator behaviors, such as predator inspection, harassment, and mobbing [12], however, are costly. So identifying potentially dangerous individuals would be advantageous to cleaners. Another controversial form of interspecific communication in cleaning interactions involves cleaner-fish 'dancing' behaviour, where cleaner-fish swim in an oscillating fashion [13]. Although assumed to play a role in advertising cleaning services [6,14,15], others propose ‘dancing’ may reduce aggression [14, 16, 13, 15], yet this has not been tested. One of the most striking behaviors of clients during cleaning interactions is the highly stereotyped posturing of clients [17]. Posing appears to signal a client’s desire to be cleaned as it increases the client’s likelihood of being cleaned [17]. However, it has been proposed that posing might also serve as an appeasement behavior by potentially dangerous clients towards cleaners [17]. Ectoparasite load is known to affect a client's desire to seek a cleaner [18] but whether it influences it's posing behavior is unknown. To determine whether cleaner and client behaviors differed according to client hunger (satiated or hungry) and ectoparasite loads (few or many parasites) I tested the effect of these factors, and the interaction between the two, on the behaviors between the cleaner-fish *Labroides dimidiatus* and the large piscivorous client [19], the coral trout *Plectropomus leopardus*. A non-cleaner-fish, *Halichoeres melanurus*, was included to determine whether another fish perceived the client as a potential threat. Tactile stimulation by cleaners, with and without cleaner dancing, and client posing rates were measured.

I found that the frequency of tactile dancing was higher in cleaner-fish exposed to hungry piscivorous client-fish than cleaner-fish exposed to satiated clients, regardless of client parasite level, and this was consistent in both experiments (Figure 1). As conflicts with hungry clients are likely to be more risky, tactile dancing may function as a pre-conflict management strategy [8, 11], conditional on client characteristics. The results also support the hypothesis that cleaners exploit the sensory system of clients with tactile stimulation [20]. Parasite load, however, did not affect the rate of tactile dancing towards hungry clients as was predicted. This suggests that for hungry client-fish, the potential benefits of eating a cleaner-fish relative to the benefits of parasite removal are higher. Tactile dancing appears reminiscent of predator inspection behavior where the most vulnerable prey approach predators more closely [12].

How *Labroides dimidiatus* identified the hungry fish is unclear, as no effect of hunger on client behavior was detected. It has been proposed that cleaner-fish may recognise predators that aren't intent on feeding or predators may posture and advertise this state [21], however, the latter was not apparent.

The use of tactile stimulation while dancing as a pre-conflict management strategy differed in appearance, but apparently not functionally, from that previously proposed where tactile stimulation was directed at the dorsal fin area only (not observed in this study) and dancing was not considered [11]. Possibly, cleaner-fish need to use different conflict management behaviors with different client-fish.

The frequency of tactile stimulation without dancing was higher on fish with many parasites than those with few, regardless of hunger level, and this was also consistent in both experiments (Figure 2). That foraging tactile stimulation was higher on fish with many parasites suggests it is related to feeding.

Client posing frequency, a measure of a client’s desire to be cleaned [17], was higher for fish with many parasites, regardless of hunger level, and this also was consistent in both experiments (Figure 3). This is the first study to show that client posing rates are related to client ectoparasite load. Whether posing was initiated by the cleaner-fish or the client-fish [17], however, could not be determined. Thus, whether posing was a possibly a response to ectoparasite irritation or to the tactile stimulation without dancing, which was higher on fish with many parasites, is unclear. That client posing did not vary with hunger level, however, suggests that it does not serve as a form of appeasement behavior by potentially dangerous fish towards cleaner-fish [17].

Tactile dancing as interspecific signalling to manage conflicts, in addition to manipulating, reconciling [11], using altruism [22], and recognizing clients [23], adds to the increasing list of the cleaner's abilities to deal with complex social environments, abilities usually the focus of cognitive studies of primates [24].

Experimental Procedures

Fish Collection

Clients (n=56) were collected using hook and line at Lizard Island, Great Barrier Reef, 6 to 26 days before trials. Fish were initially held in 4 pools (3 x 1 m) and fed pilchards every other day. Cleaner-fish (n = 48), a mean (s.e.) 7.4(0.1) cm in total length (TL), and non-cleaner-fish (n=48), 5.85(0.1) cm TL, were collected [25] at the same time and held in aquaria.

Fish Handling and Experimental Treatments

Up to 10 days before trials, only "satiated" fish were fed, daily, while ‘hungry’ ones were not. Two to 8 days before trials, fish randomly allocated to the treatment ‘few parasites’ were treated for parasites using two baths (0.5 g Praziquantal in 3 ml ethanol/50 L seawater for 2.5 h, ICN Biomedicals Inc., Aurora, USA, then 3 min in freshwater 24 h later) and those allocated to ‘many parasites’ given a two control seawater baths. Six clients, plus one for ectoparasite estimates (not tested), were transferred to each of 8 pools with 2 pools per treatment combination. The 8 untested fish and the last 8 fish tested were examined for parasites after experiment 1 and 2, respectively. Parasites were removed with a 5 min freshwater bath and filtered at 62 μm (the former were first killed with an overdose of clove oil and their gills and fins examined separately with a microscope).

Behavioral observations

Two identical, 6 day long experiments, were conducted 5 days apart (n = 12 trials per treatment combination for a total of 96 trials and 144 h). One fish from each of the pools was tested each day. Clients were placed in test aquaria (37 high x 90 long x 36 cm wide) the evening before trials. Two upright covered clear Plexiglas tubes (10 x 50 cm) were each placed in 2 aquaria on the night before the trials, then 90 min prior to trials for the remaining trials, to allow clients to acclimate to them. Test aquaria had shelters (2 x 15 cm opaque pipes) for the cleaner and non-cleaner-fish. Recording began when a cleaner and the non-cleaner-fish were placed in the tube to acclimate to the client and aquarium. After 5 min, fish were released from the tube. Two 90 min trials were recorded at 0700, 0900, 1100, and 1300 h with two video cameras (Sony Hi8 TRV89E and TRV87E). The time of day was random but balanced across treatments with each treatment by time of day combination tested 3 times. Thus, all treatments were tested each day and at all 4 times of the day. At 1700 h, tested clients were offered a chopped pilchard fish to estimate hunger levels. The proportion of hungry clients that fed on pilchards was higher for hungry (88, 55%) than for satiated fish (50, 11%,) in experiments 1 and 2, respectively. Tested clients were returned to the same pool. To identify them, they were anaesthetized with 2 g /25 L of MS222 (Sigma) in seawater for 7 min, measured, and individual markings recorded. Fish were also tagged by threading a piece of flexible plastic (1.5 x 1.5 cm) with surgical thread into the dorsal fin, but some of the tags were lost during the course of the study. Separate anaesthetic baths were used for fish with few and many parasites. Mean (s.e.) client total length was 44.0(0.8) cm and did not vary among treatments (ANOVA P>0.05). All test aquaria were emptied after trials and soaked in freshwater for 30 min to kill any detached parasites. The experiment was repeated using randomly selected fish. Behaviors were analysed using Observer Video Pro® 4 (Noldus).

Behaviors recorded

Behaviors were defined as follows: Cleaner tactile dancing involved dorsal-ventral oscillation of the posterior body [6, 14] within 15 cm of client and mainly in one place, while often contacting the client with its body. Tactile stimulation without dancing: cleaner contacted client's body with pelvic fins, often also nibbling on client’s body. Posing involved the client opening the gills and/or mouth [17]. Non-cleaner-fish hiding involved sheltering in a pipe or in a corner.

Statistical analyses

Data were analysed with a repeated measures ANOVA with hunger level and parasite load as the between-subjects factors, experiment as the within subjects/repeated factor, and client pools as the factor nested within hunger level and parasite load. As the effects of pools were all highly not significant ($P > 0.25$) they was omitted from the final analyses to increase power following Underwood’s [26] rules. One trial where the client appeared agitated was omitted. Three clients (2 hungry client with many parasites, and one satiated with few parasites) attacked a cleaner and were omitted from the analyses. Three non-cleaner-fish spent no time hiding in a shelter and were omitted from the analysis to satisfy the assumption of homogeneity of variance of the analysis of variance. Non-cleaner-fish spent 98% of their time in a shelter; this was not affected by experiment, hunger, or parasites (all $P > 0.230$). Client-fish had 15 different types of ectoparasites, mostly monogeneans and copepods (A.S. Grutter and C. Fury, unpublished data). The mean (s.e.) parasite load for the sub-sample of fish in experiment 1 and 2 was 2041(715) and 1456(785) for fish with many parasites and 169(934) and 26(8) for fish with few parasites, respectively.

Acknowledgements

I thank C. Fury, F. Grutter, M. Johnson, E. Hunley, and the Lizard Island Research Station staff for field and laboratory assistance, N. Khan for statistical advice, J. Becker, M. Blows, R. Bshary, C. Jones, H. McCallum, and K. Warburton for comments on previous versions of the manuscript, and the Australian Research Council for funding.

1. Dugatkin, L.A. (1997). Cooperation Among Animals: An Evolutionary Perspective (New York: Oxford University Press).

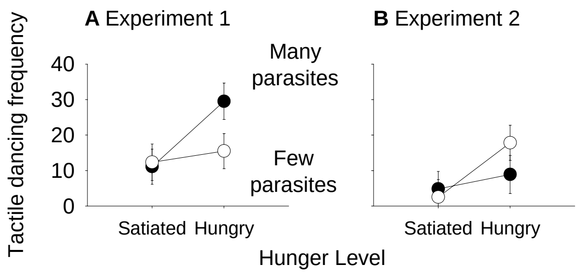
2. Trivers, R.L. (1971). The evolution of reciprocal altruism. The Quarterly Review of Biology *46*, 35-57.
3. Poulin, R., and Vickery, W.L. (1995). Cleaning symbiosis as an evolutionary game: to cheat or not to cheat? Journal of Theoretical Biology *175*, 63-70.
4. Freckleton, R.P., and Côté, I.M. (2003). Honesty and cheating in cleaning symbioses: evolutionarily stable stragies defined by variable pay-offs. Proceedings of the Royal Society of London Series B *270*, 299-305.
5. Darcy, G.H., Maisel, E., and Ogden, J.C. (1974). Cleaning preferences of the gobies *Gobiosoma evelynae* and *G. prochilos* and the juvenile wrasse *Thalassoma bifasciatum*. Copeia *2*, 375-379.
6. Wickler, W. (1968). The origin of the cleaner mimic. In Mimicry in plants and animals, W. Wickler, ed. (London: Weidenfeld and Nicolson), pp. 157 - 176.
7. Lobel, P.S. (1976). Predation on a cleanerfish (*Labroides*) by a hawkfish (*Cirrhites*). Copeia *1976*, 384-385.
8. Aureli, F., and Waal, F.B.M. (2000). Natural Conflict Resolution (Berkely: University of California Press).
9. Hammerstein, P., and Hoekstra, R.F. (1995). Mutualism on the move. Nature *376*, 121-122.
10. Bshary, R. (2001). The cleaner fish market. In Economics in Nature: Social Dilemmas, Mate Choice and Biological Markets, R. Noe, J.A.R.A.M. van Hooff and P. Hammerstein, eds. (Cambridge: Cambridge University Press), pp. 146-172.
11. Bshary, R., and Wurth, M. (2001). Cleaner fish *Labroides dimidiatus* manipulate client reef fish by providing tactile stimulation. Proceedings of the Royal Society of London. Series B *268*, 1495-1501.
12. Dugatkin, L.A., and Godin, J.-G. (1992). Prey approaching predators: a cost-benefit perspective. Annales Zoologici Fennici *29*, 233-252.
13. Potts, G.W. (1973). The ethology of *Labroides dimidiatus* (Cuv. & Val.) (Labridae, Pisces) on aldabra. Animal Behaviour *21*, 250-291.
14. Youngbluth, M.J. (1968). Aspects of the ecology and ethology of the cleaning fish, *Labroides phthirophagus* Randall. Zeitschrift Für Tierpsychologie *25*, 915-932.
15. Sazima, I., Moura, R.L., and Gasparini, J.L. (1998). The wrasse *Halichoeres cyanocephalus* (Labridae) as a specialized cleaner fish. Bulletin of Marine Science *63*, 605-610.
16. Losey, G.S. (1971). Communication between fishes in cleaning symbiosis (Baltimore: University Park Press).
17. Côté, I.M., Arnal, C., and Reynolds, J.D. (1998). Variation in posing behaviour among fish species visiting cleaning stations. Journal of Fish Biology *53*, 256 - 266.

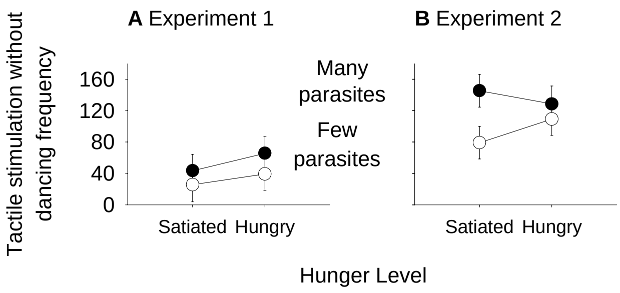
18. Grutter, A.S. (2001). Parasite infection rather than tactile stimulation is the proximate cause of cleaning behaviour in reef fish. Proceedings of the Royal Society of London. Series B *268*, 1361-1365.
19. Randall, J.E., Allen, G.R., and Steene, R.C. (1997). Fishes of the Great Barrier Reef and Coral Sea, second Edition (Bathurst: Crawford House Publishing).
20. Losey, G.S. (1987). Cleaning Symbiosis. Symbiosis *4*, 229-258.
21. Hobson, E.S. (1971). Cleaning symbiosis among California inshore fishes. Fishery Bulletin *69*, 491-523.
22. Bshary, R. (2002). Biting cleaner fish use altruism to deceive image-scoring client reef fish. Proceedings of the Royal Society Biological Sciences Series B *269*.
23. Tebbich, S., Bshary, R., and Grutter, A.S. (2002). Cleaner fish Labroides dimidiatus recognise familiar clients. Animal Cognition *5*, 139-145.
24. Bshary, R., Wickler, W., and Fricke, H. (2002). Fish cognition: a primate's eye view. Animal Cognition *5*, 1-13.
25. Grutter, A.S. (1997). Spatio-temporal variation and feeding selectivity in the diet of the cleaner fish *Labroides dimidiatus*. Copeia *1997*, 346-355.
26. Underwood, A.J. (1997). Experiments in ecology : their logical design and interpretation using analysis of variance (New York: Cambridge University Press).

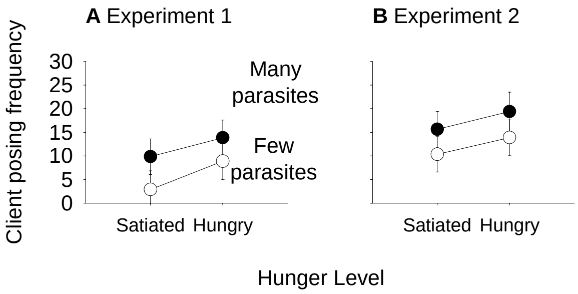
Figure 1. The frequency of tactile dancing of cleaner-fish exposed to piscivorous client-fish. (A) Experiment 1, (B) Experiment 2. Clients were satiated or hungry and had many or few parasites. There was a significant difference between satiated and hungry fish ($F_{1,91}$=8.26, P=0.005) and between the two experiments ($F_{1,91}$=5.77, P=0.019); all other factors and interactions were not significant (P>0.05). Data are least square means and standard errors (bars) per 90 min observation.

Figure 2. Frequency of foraging tactile stimulation of piscivorous client-fish by cleaner-fish. (A) Experiment 1, (B) Experiment 2. Clients were satiated or hungry and had many or few parasites. There was a significant difference between fish with many and few parasites ($F_{1,91}$=4.64, P=0.034) and between the two experiments ($F_{1,91}$=22.98, P=0.0001); all other factors and interactions were not significant (P>0.05). Data are least square means and standard errors (bars) per 90 min observation.

Figure 3. Frequency of client posing. (A) Experiment 1, (B) Experiment 2. Clients were satiated or hungry and had many or few parasites. There was a significant difference between fish with many and few parasites ($F_{1,91}$=4.33, P=0.040) and between the two experiments ($F_{1,91=4.76}$, P=0.032); all other factors and interactions were not significant (P>0.05). Data are least square means and standard errors (bars) per 90 min observation.


A Experiment 1
B Experiment 2
Tactile dancing frequency
40
30
20
10
0
Many parasites
Few parasites
Satiated
Hungry
Satiated
Hungry
Hunger Level


A Experiment 1
B Experiment 2
Tactile stimulation without dancing frequency
160
120
80
40
0
Many parasites
Few parasites
Satiated
Hungry
Satiated
Hungry
Hunger Level


A Experiment 1
B Experiment 2
Client posing frequency
30
25
20
15
10
5
0
Many parasites
Few parasites
Satiated
Hungry
Satiated
Hungry
Hunger Level