LETTERS

Pairs of cooperating cleaner fish provide better service quality than singletons

Redouan Bshary[1], Alexandra S. Grutter[2], Astrid S. T. Willener[1] & Olof Leimar[3]

Service providers may vary service quality depending on whether they work alone or provide the service simultaneously with a partner. The latter case resembles a prisoner's dilemma[1–4], in which one provider may try to reap the benefits of the interaction without providing the service. Here we present a game-theory model based on the marginal value theorem[5], which predicts that as long as the client determines the duration, and the providers cooperate towards mutual gain, service quality will increase in the pair situation. This prediction is consistent with field observations and with an experiment on cleaning mutualism, in which stable male–female pairs of the cleaner wrasse *Labroides dimidiatus* repeatedly inspect client fish jointly. Cleaners cooperate by eating ectoparasites[6] off clients but actually prefer to cheat and eat client mucus[7]. Because clients often leave in response to such cheating, the benefits of cheating can be gained by only one cleaner during a pair inspection. In both data sets, the increased service quality during pair inspection was mainly due to the smaller females behaving significantly more cooperatively than their larger male partners. In contrast, during solitary inspections, cleaning behaviour was very similar between the sexes. Our study highlights the importance of incorporating interactions between service providers to make more quantitative predictions about cooperation between species.

Many cooperative interactions can be seen as an exchange of goods, services or commodities between two classes of traders[8–10]. Here we investigated traders that provide a service to a second class of traders, such as an ant partner species—for example, lycaenid butterfly larvae—providing a sugary solution to ants[11], rhizobial bacteria fixing nitrogen for leguminous plants[12] or cleaner fish removing ectoparasites from client reef fish[13]. We have used the last example as our model system. Cleaners prefer the mucus of some client species more than gnathiid isopods[7], the most commonly found ectoparasites of reef fishes[14]. Clients use various actions to make cleaners forage against their preference[15,16], the simplest form of control being to terminate the interaction by swimming off in response to a cheating bite[17]. Adult cleaners often live in pairs of a male and the largest female in his harem[18] and they commonly inspect larger clients simultaneously. Pair inspections result in cleaners facing a problem: a visiting client may leave after a cheat, even though only one cleaner was responsible for the cheating whereas the second cleaner cooperated. Hence, the cooperative cleaner loses a foraging opportunity owing to its partner's action, whereas the cheating cleaner gains a bite of mucus. We explored both mathematically and empirically how these pay-off asymmetries influence the service quality provided in paired compared with solitary inspections.

We explored a game in which one class of individuals provides a service (cleaners remove ectoparasites) to a second class of individuals (the clients). The service entails benefits to both the providers and the clients. However, the providers may also try to perform acts of exploitation (taking a bite of mucus) that yield a high immediate gain for them but are detrimental to the clients. Providers can decide on the level of service quality they provide, expressed as the frequency of exploits (the ratio of ectoparasites eaten per bite of mucus). Clients can decide on the duration of the interaction. In the cleaning mutualism, clients terminate interactions in response to cheating with about a 50% probability[19]. In the model, we assumed that clients have a 50% probability of terminating the interaction in response to an exploit. Under these conditions, we could find the optimal exploitation rate for the service provider. We assumed that the gains for providers from giving the service show diminishing returns with increasing duration (removal of ectoparasites leads to patch depletion). We used the expected value of all ectoparasites on a client as unit of benefit. We solve the problem for the cases in which: (1) one provider interacts with a client; (2) two providers cooperate with each other while interacting with the same client; and (3) two providers each try to maximise immediate individual gains while interacting with the same client.

For a singleton cleaner with a mucus bite rate (probability per unit time of taking a bite) of λ, the expected cleaning bout duration is $t = 2/\lambda$. The cleaner gain from ectoparasite removal is assumed to be $g_s(t)$, to which the benefit $2b$ of two bites per client on average is added. For large t, $g_s(t)$ approaches 1. For a cooperative pair of cleaners, each having the bite rate of $\lambda/2$, the expected cleaning duration is again t and the per capita gain is assumed to be $g_p(t)$, to which the per capita benefit b is added, because each cleaner has an equal chance of biting. We assume that $g_p(t) = g_s((1 + a)t)/2$, where a measures the added search efficiency when two cleaners service the client. Search efficiency doubles for $a = 1$, but interference between cleaners could lead to smaller values of a. As a fitness measure, we used the long-term gain rate. For an expected inter-client interval of t_0, the optimal bite rate for singletons and cooperative pairs (that is, the cleaning duration giving the maximum gain rate), satisfy the equations:

$$g_s'(t) = \frac{g_s(t) + 2b}{t + t_0} \tag{1}$$

$$g_p'(t) = \frac{g_p(t) + b}{t + t_0} \tag{2}$$

which are instances of the so-called marginal value theorem[5] (Fig. 1a). A cooperative pair maximises the total gain rate. For a non-cooperative pair, in which each cleaner attempts to maximise its own gain rate, an evolutionarily stable gain rate satisfies the equation

$$g_s'(t) = \frac{b}{t} + \frac{g_p(t) + b}{t + t_0} \tag{3}$$

provided that $t > 0$ holds. Immediate biting will be an evolutionarily

[1]University of Neuchâtel, Department of Zoology, Rue Emile-Argand 11 Case postale 158, 2009 Neuchâtel, Switzerland. [2]University of Queensland, School of Integrative Biology, St Lucia, Queensland 4072, Australia. [3]Stockholm University, Department of Zoology, SE-10691 Stockholm, Sweden.

stable strategy (ESS) for any $b > 0$, but there can also be an alternative ESS for which $t > 0$ (Fig. 1b; see Supplementary Information for derivation and analysis of equations (1), (2) and (3)). For small b there is an alternative non-cooperative ESS, for which the cleaning duration is smaller than the cooperative optimum (it approaches the cooperative optimum as b approaches zero). The client–cleaner mutualism involving pairs of cleaners is thus akin to a prisoner's dilemma, in which mutual cooperation yields higher pay-offs than mutual defection but defecting is dominant when cooperating in single rounds, at least for larger values of b. A general conclusion from our analyses is that cooperative pairs provide higher service quality than singletons (Fig. 1b; service quality is expressed as the proportion of removed ectoparasites per cheating bite) as long as there is added search efficiency for a pair ($a > 0$), whereas service quality would decrease (for $a < 1$) or stay the same (for $a = 1$) if each cleaner in the pair uses its singleton strategy (Fig. 1b).

We observed male–female pairs of cleaner fish in the field using standard interaction protocols (see Methods). We distinguished three situations—females inspecting alone, males inspecting alone, and pair inspection—and we compared client jolt rates (a response to cheating cleaners[19]) between the three situations. In the pair situation, we also distinguished between jolts caused by females and by males. In a laboratory experiment, we tested whether established cleaner fish pairs altered their willingness to feed against their preference (which translates into cooperative behaviour under natural conditions[7]) depending on whether they fed alone or with the partner. We considered three different possible outcomes: cleaners cheat more when cleaning in pairs (non-cooperative solution); cleaners behave in pairs how they behave when inspecting alone (solitary solution); or cleaners become more cooperative (cooperative solution). In the last case, client jolt rates during pair inspections should be lower than the sum of solitary male and female inspections, and the ratio of preferred to non-preferred items eaten in the experiment (prawn items eaten per fish flake item—called 'flake' hereafter[15]) should decrease in the pair situation. A tit-for-tat-like solution to the iterated prisoner's dilemma[1–4] would further suggest that males and females should equally adjust their behaviour during joint inspections.

Clients jolted significantly less frequently when interacting with a pair of cleaners compared to the sum of them interacting with females and males alone (Wilcoxon test, $n = 12$, $T = 13$, $P = 0.04$; Fig. 2). There were no significant differences in client jolt rates when interacting with either male or female alone (Wilcoxon test, $n = 12$, $T = 36$, $P > 0.05$), but females caused significantly less client jolts than males in the pair situation (Wilcoxon test, $n = 12$, $T = 6$, $P = 0.01$). Females also caused significantly less jolts when inspecting in a pair than when inspecting alone, whereas there was only a trend in the same direction for males (Wilcoxon test, females: $n = 12$, $T = 11$, $P = 0.023$; males: $n = 12$, $T = 16$, $P = 0.07$).

In the experiment, the ratio of (preferred) prawn items eaten per flake items eaten almost halved in pair trials compared with singleton trials (Fig. 3). There was no significant difference between male and female foraging behaviour when feeding on their own (Wilcoxon test, $n = 10$, 1 tie, resulting $n = 9$, $T = 19$, $P < 0.05$); both sexes ate significantly less prawn items per flake items in the pair situation compared with the singleton situation (Wilcoxon tests, males: $n = 10$, $T = 4$, $P = 0.017$; females: $n = 10$, $T = 0$, $P = 0.002$); and females ate significantly more against their preference than males in the pair situation (Wilcoxon test, $n = 10$, $T = 4$, $P = 0.017$). In the pair situation, males ate the prawn items significantly more often (on average 70%) than females (Wilcoxon rest, $n = 10$, 1 tie, resulting $n = 9$, $T = 2.5$, $P = 0.015$).

Males regularly chased females immediately after the removal of the plate on which food items were offered. Males were significantly more likely to chase females in trials in which the female had eaten the prawn item (median 100%) than in trials in which the male had eaten the prawn (median 50%; Wilcoxon test, $n = 10$, 2 ties, resulting $n = 8$, $T = 0$, $P = 0.008$).

Our model showed that two providers interacting simultaneously with the same client can face a situation similar to a prisoner's dilemma. The marginal value theorem allowed us to explore theoretically how diminishing returns influence the frequency of cheating. Exploring the time course of interactions may thus make models of cooperation more applicable to real life interactions, such as cooperative territorial defence in lionesses[20] or predator inspection in
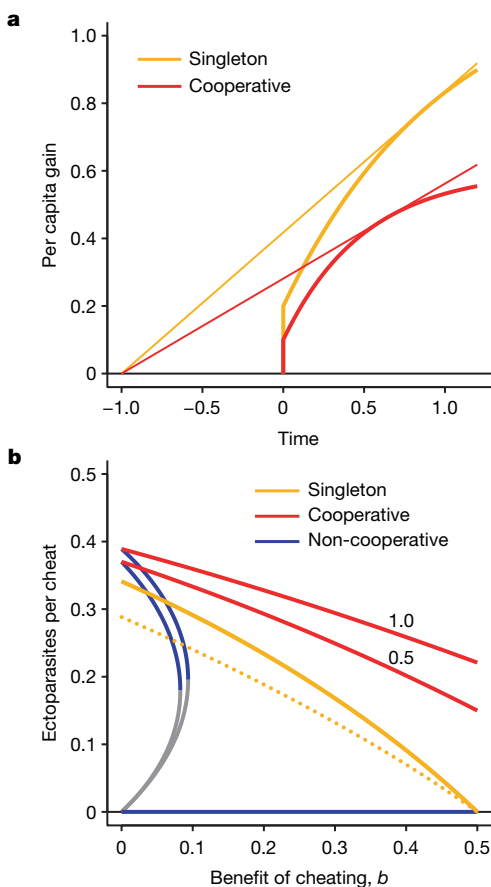
Figure 1 | The marginal value theorem for service providers. a, For singletons and cooperative pairs, the optimal cleaning duration occurs where a line from $(-t_0, 0)$ is tangent to the gain curve (inter-client interval $t_0 = 1$; $b = 0.1$). **b**, Service quality (measured as the proportion of removed ectoparasites per cheating bite) is higher for cooperative pairs than for singletons if pairs have higher search efficiency (cases with $a = 1$ and $a = 0.5$ are shown). Service quality is lower if each cleaner uses the singleton strategy (dotted curve, $a = 0.5$). Zero service quality (immediate biting) is a non-cooperative ESS for $b > 0$. For small b there is another ESS (the grey curve separates the basins of attraction of these ESSs).
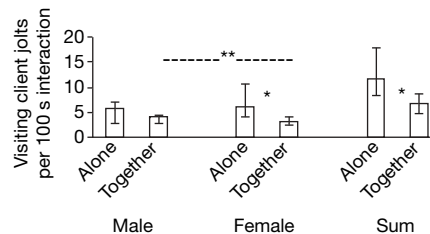
Figure 2 | Client jolt rates in response to singleton and pair inspections. Median and interquartiles of client jolt rates for 12 cleaner-fish pairs, with separate values for males when inspecting alone or with the female partner, for females when inspecting alone or with the male partner, for summed values of males and females inspecting alone, and total values for males and females inspecting together. Asterisk, $P < 0.05$; double asterisk, $P < 0.01$.

965
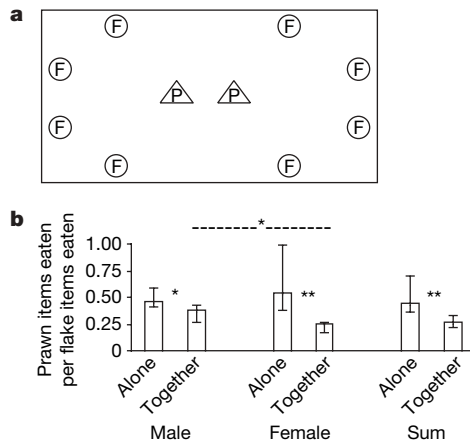
Figure 3 | Cleaner fish feed more against their preference when in pairs than when alone. a, The distribution of prawn items (P, in triangles) and of flake items (F, in circles) on the experimental Plexiglas plate is shown. **b**, Median and interquartile values of the ratio of prawn items eaten per flake items eaten for ten cleaner-fish pairs, with separate values for males when inspecting alone or with the female partner, for females when inspecting alone or with the male partner, for total values of males and females inspecting alone, and total values for males and females inspecting together. Asterisk, $P < 0.05$; double asterisk, $P < 0.01$.

fishes[21,22]. Our field observations and our aquarium experiment on cleaner fish pairs yielded consistent results that support the cooperative solution predicted by the model. Our results help to explain the observation that clients with choice options seem to preferentially visit stations with pairs of cleaner fish[23].

Although standard game theoretical analyses of iterated prisoner's dilemmas propose tit-for-tat-like solutions[1–4], these kinds of strategies are rarely found outside of humans[24]. Our two data sets superficially fit a tit-for-two-tats model in the sense that males cheated about twice as often as females during pair inspections. Alternatively, the unilateral aggression of the larger males towards females for cheating may function as punishment[25,26]. Although the importance of punishment as a control mechanism ensuring cooperation in symmetrical interactions has been disputed[27], we suggest that both empirical and theoretical future research should explore how dominance relationships affect solutions to the iterated prisoner's dilemma when punishment is a strategic option for the dominant individual.

METHODS SUMMARY

The model is described in Supplementary Information.

During field observations at Ras Mohammed National Park, Egypt, we noted cleaner identity (male, female or both), client species, duration of interaction and whether clients jolted in response to cleaner fish mouth contact (using methods as described previously[28]). Jolts provide a strong correlate of cleaners' cheating[19]. We calculated client jolt rates when interacting with cleaner pairs and when interacting with the male or the female only. In the pair situation, we also calculated the relative contribution of males and females to the total amount of client jolts.

In the laboratory experiment, cleaners were offered plates with two different food types, namely prawn and fish flakes mixed with prawn. The cleaners prefer prawn to flakes[16]. However, they could continue to eat as long as they ate only flakes, whereas eating a prawn item led to the immediate removal of the plate. Immediate reaction to prawn feeding was possible because the plate was attached to a lever held by the observer[16]. Cleaners thus had to eat against their preference to increase their foraging success. In the test trials, a brown Plexiglas plate (18 × 12 cm) with flake items placed within 8 black circles (each 1 cm diameter) drawn near the edges of the plate and prawn items placed within two black triangles in the centre (Fig. 3) was offered to cleaners when alone, and when paired in male–female pairs, and the sequence was balanced across individuals. For each cleaner and situation, the average ratio of prawn to flake items eaten in each round was calculated as a measure of how much cleaners were willing to feed against their preference. For the pair situation, we calculated the total number of prawn items eaten by males and by females, and we noted any aggression between partners.

1. Axelrod, R. & Hamilton, W. D. On the evolution of co-operation. *Science* **211**, 1390–1396 (1981).
2. Nowak, M. A. & Sigmund, K. Tit for tat in heterogeneous populations. *Nature* **355**, 250–253 (1992).
3. Nowak, M. & Sigmund, K. A strategy of win-stay, lose-shift that outperforms tit-for-tat in the prisoner's dilemma game. *Nature* **364**, 56–58 (1993).
4. Dugatkin, L. A. *Cooperation Among Animals: An Evolutionary Perspective* (Oxford Univ. Press, 1997).
5. Charnov, E. L. Optimal foraging, the marginal value theorem. *Theor. Popul. Biol.* **9**, 129–136 (1976).
6. Grutter, A. S. Parasite removal rates by the cleaner wrasse *Labroides dimidiatus. Mar. Ecol. Prog. Ser.* **130**, 61–70 (1996).
7. Grutter, A. S. & Bshary, R. Cleaner wrasse prefer client mucus: support for partner control mechanisms in cleaning interactions. *Proc. R. Soc. Lond. B* **270** (Suppl.), S242–S244 (2003).
8. Noë, R., Van Schaik, C. P. & Van Hooff, J. A. R. A. M. The market effect: an explanation for pay-off asymmetries among collaborating animals. *Ethology* **87**, 97–118 (1991).
9. Noë, R. & Hammerstein, P. Biological markets. *Trends Ecol. Evol.* **10**, 336–339 (1995).
10. Bowles, S. & Hammerstein, P. in *Genetic and Cultural Evolution of Cooperation* (ed. Hammerstein, P.) 153–165 (MIT Press, 2003).
11. Pierce, N. E. *et al.* The ecology and evolution of ant associations in the Lycaenidae (Lepidoptera). *Annu. Rev. Entomol.* **47**, 733–771 (2002).
12. Kiers, E. T., Rousseau, R. A., West, S. A. & Denison, R. F. Host sanctions and the legume–rhizobium mutualism. *Nature* **425**, 78–81 (2003).
13. Côté, I. M. Evolution and ecology of cleaning symbioses in the sea. *Oceanogr. Mar. Biol. Ann. Rev.* **38**, 311–355 (2000).
14. Grutter, A. S. Relationship between cleaning rates and ectoparasite loads in coral reef fishes. *Mar. Ecol. Prog. Ser.* **118**, 51–58 (1995).
15. Bshary, R. & Grutter, A. S. Punishment and partner switching cause cooperative behaviour in a cleaning mutualism. *Biol. Lett.* **1**, 396–399 (2005).
16. Bshary, R. & Grutter, A. S. Image scoring and cooperation in a cleaner fish mutualism. *Nature* **441**, 975–978 (2006).
17. Johnstone, R. A. & Bshary, R. From parasitism to mutualism: partner control in asymmetric interactions. *Ecol. Lett.* **5**, 634–639 (2002).
18. Robertson, D. R. Social control of sex reversal in a coral-reef fish. *Science* **177**, 1007–1009 (1972).
19. Bshary, R. & Grutter, A. S. Asymmetric cheating opportunities and partner control in a cleaner fish mutualism. *Anim. Behav.* **63**, 547–555 (2002).
20. Heinsohn, R. & Packer, C. Complex cooperative strategies in group-territorial African lions. *Science* **269**, 1260–1262 (1995).
21. Milinski, M. TIT FOR TAT in sticklebacks and the evolution of cooperation. *Nature* **325**, 433–435 (1987).
22. Dugatkin, L. A. Do guppies play TIT FOR TAT during predator inspection visits? *Behav. Ecol. Sociobiol.* **23**, 395–399 (1988).
23. Bshary, R. & Schäffer, D. Choosy reef fish select cleaner fish that provide high-quality service. *Anim. Behav.* **63**, 557–564 (2002).
24. Hammerstein, P. *Why is Reciprocity so Rare in Social Animals? A Protestant Appeal* in *Genetic and Cultural Evolution of Cooperation* (ed. Hammerstein, P.) 83–93 (MIT Press, 2003).
25. Clutton-Brock, T. H. & Parker, G. A. Punishment in animal societies. *Nature* **373**, 209–215 (1995).
26. Clutton-Brock, T. H. & Parker, G. A. Sexual coercion in animal societies. *Anim. Behav.* **49**, 1345–1365 (1995).
27. Dreber, A., Rand, D. G., Fudenberg, D. & Nowak, M. A. Winners don't punish. *Nature* **452**, 348–351 (2008).
28. Bshary, R. & Würth, M. Cleaner fish *Labroides dimidiatus* manipulate client reef fish by providing tactile stimulation. *Proc. R. Soc. Lond. B* **268**, 1495–1501 (2001).

METHODS

Field observations. Observations were made on 12 cleaner-fish pairs at Ras Mohammed National Park, Egypt from September 2006 to November 2006. Females were identified as the smaller individual in each pair. Each pair was observed for 400 min, with 200 min being focused on each individual. In client interactions with the pair that resulted in a jolt we noted which cleaner had caused it. In 32% of 1,296 cases, the identity could not be determined. These cases were attributed evenly to males and females for the statistical analyses. The statistical analyses required three steps of data processing. First, for each cleaning station, we identified the client species that had interacted with the pair and with the male and female only. We then determined for each species the jolt frequency when interacting with the male only or the female only, and when interacting with the cleaner pair the jolt frequency caused by the male and by the female. These values were used to calculate means of client jolt rates caused by males and by females when inspecting either alone or in pairs. In addition, we calculated the sums of client jolts when the male and female inspected alone and when the male and female inspected in pairs. The final analyses were conducted using Wilcoxon tests in which n was the number of cleaning stations.

Aquarium experiments. Experiments were conducted at the Lizard Island Research Station, Great Barrier Reef, Australia. Ten pairs of cleaners (total length of females 6.2–7.1 cm; males 7.0–8.9 cm; size difference within pairs 0.9–2.6 cm) were kept in aquaria of varying sizes (minimal size $50 \times 30 \times 25$ cm) for a minimum of 30 days before the experiments. All aquaria had running sea water and fish were provided with a PVC tube (1 cm diameter $\times$ 8 cm) for shelter. All cleaners were released after the experiment at the site of capture. Cleaners were trained to feed off Plexiglas plates. With the help of a temporary partition, they were individually familiarised with the main aspects of the experimental protocol—that is, that eating preferred prawn led to the removal of the plate. Within six rounds designed as learning trials, all cleaners ate flake items before eating a prawn item; the experiment was conducted afterwards.

In a sequence of 16 test trials distributed over 2 days, cleaners were confronted with the plate either alone or with their partner. The order of treatments was balanced: half of the individuals started with four pair trials, followed by eight singleton trials and another four pair trials; conversely, the other half started with four singleton trials, followed by eight pair trials and four singleton trials. During the day, a time interval of 40 min was maintained between trials. We also quantified how often male and female partners ate the prawn item in the pair situation and noted any aggressive behaviour of the partners (identity of aggressor and of victim) immediately after the removal of the plate.