Short-Term Variation in the Level of Cooperation in the Cleaner Wrasse *Labroides dimidiatus*: Implications for the Role of Potential Stressors

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**Abstract**

There is a wealth of game theoretical approaches to the evolution and maintenance of cooperation between unrelated individuals and accumulating empirical tests of these models. This contrasts strongly with our lack of knowledge on proximate causes of cooperative behaviour. Marine cleaning mutualism has been used as a model system to address functional aspects of conflict resolution: client reef fish benefit from cleaning interactions through parasite removal, but cleaner fish *Labroides dimidiatus* prefer client mucus. Hence, feeding against their preference represents cooperative behaviour in cleaners. Cleaners regularly cheat non-predatory clients while they rarely cheat predatory clients. Here, we asked how precisely cleaners can adjust service quality from one interaction to the next. We found that non-predatory clients receive a better service if the previous client was a predator than if the previous client was a non-predator. In a related laboratory experiment, a hand-net used as a stressor resulted in cleaners feeding more against their preference in subsequent interactions. The combination of the cleaners’ behaviour in the two studies shows that the cleaners’ service quality for a given client species is not fixed, but it can be manipulated. The results suggest that short-term stress is one factor that causes cleaners to increase their levels of cooperation, a hypothesis that is amenable to further experiments manipulating the endocrine system.

**Introduction**

In recent years, our understanding of the evolution of cooperation among unrelated individuals has increased rapidly. New concepts like punishment (Clutton-Brock & Parker 1995), partner switching (Bshary & Schäffer 2002; Ferriere et al. 2002, McNamara et al. 2004), sanctions (Herre et al. 1999) and indirect reciprocity (Nowak & Sigmund 1998) have been added to the well-known tit-for-tat-like solutions to the iterated prisoner’s dilemma as potential explanations for why individuals cooperate in situations where cheating would yield a higher (short-term) benefit. These new theoretical concepts are also supported by experimental evidence (Kiers et al. 2003; Bshary & Grutter 2005, 2006).

These advances in our understanding of the ultimate questions regarding the evolution of cooperation have not been accompanied by similar research efforts regarding the proximate mechanisms underlying cooperative behaviour. This is unfortunate as proximate causes for inter- and intra-individual variation in the level of cooperation are both interesting in their own right, and they have important
implications for modelling. For example, if the level of cooperation is influenced by ontogenetic effects or by variation in internal states, there should be so-called phenotypic defectors present in populations at any point in time (Sherratt & Roberts 2001). The presence of such phenotypic defectors should select against unconditional cooperators and hence stabilise the evolutionary persistence of conditional cooperative strategies against genetic drift and the eventual switch to a population of defectors (Sherratt & Roberts 2001). Finally, with respect to animal cognition, it is important to know how decisions to cooperate or to defect are made: for example, what is the role of learning, physiology or genetics in such interactions?

Some studies on humans have specifically addressed the proximate causes of cooperative behaviour. First, de Quervain et al. (2004) found that an individual’s will to punish persons who have cheated in potentially cooperative interactions correlates with how much such an action stimulates the punisher’s reward system in the neocortex. The authors conclude that the more self-rewarding the action of punishment is the more people are willing to act that way. Second, Kosfeld et al. (2005) and Baumbgartner et al. (2008) found that levels of oxytocin in the blood influence how much trust subjects give to other people in situations where the risk of being cheated is imminent. Finally, Eisenegger et al. (2010) found that women proposed higher offers in a bargaining game when treated with testosterone, interpreted as testosterone positively affecting status-seeking behaviour. In other animals, the contribution of helpers in cooperatively breeding species has attracted some attention. In a multivariate analysis of hormone levels in meerkat helpers, Carlson et al. (2010) found that base-line cortisol levels correlated positively with helping. In contrast to earlier studies (Schöech et al. 1996), prolactin and testosterone had no significant influence when confounding variables are controlled. Similar studies, and in particular experimental manipulations, are needed to better understand the proximate causes of decision-making in potentially cooperative interactions in animals. Given the little we currently know about underlying physiological mechanisms (Soares et al. 2010) even studies that evaluate conditions that cause changes in the level of cooperation without determining the physiological changes will be useful.

The cleaner fish _L. dimidiatus_ has the potential for being a good animal model to study decision-making processes and the physiology of cooperative behaviour. Cleaners actually prefer client mucus (‘cheating’) over ectoparasites (‘cooperating’) (Grutter & Bshary 2003). The cost of exploiting clients is likely to vary among different clients, for example predators can retaliate by eating the cleaner (Trivers 1971), which has been termed the ‘threat of reciprocity’ (Bshary & Bronstein 2004). Therefore, the level of exploitation should also vary according to the client’s identity. A correlate of exploitation is the frequency of jolts, a short twitching of the body, that clients perform during inspection in response to cleaner fish mouth contact (Bshary & Grutter 2002). Indeed, field observations on jolt rates indicate that predatory clients, compared with non-predatory ones, are rarely cheated (Bshary 2001). Non-predatory clients jolt on average about three to five times per 100-s interaction (Bshary 2001), while predators rarely jolt under natural conditions (the median jolt frequency per predatory species, across 15 species, was 0–100-s interaction, Bshary 2001). As cleaners have more than 2000 interactions with clients per day (Grutter 1995), selection may favour cleaners that are able to adjust their level of exploitation within seconds to maximise the trade-off between caloric intake and risk of predation. Here, we investigate this idea by asking how well they are able to adjust the appropriate exploitation level from one interaction to the next. To do so, we observed sequences of cleaner–client interactions in the wild and asked whether or not the current service quality provided by a cleaner depended on the identity of the previous client.

We distinguished between predatory and non-predatory clients and asked whether or not the service quality a non-predatory client receives depended on whether the cleaner’s previous client was a predator. The reverse question how the service quality a predatory client receives is affected by the previous client’s identity was not addressed, as the predators almost always receive high quality service (Bshary 2001). We distinguished between two possible outcomes. First, if we assume that a cleaner’s decision-making processes function with high precision, then we should not find any effects of the previous client’s identity (predatory or non-predatory). Alternatively, predatory clients may affect a cleaner’s behaviour in the short-term differently than non-predatory clients.

There are likely many potential physiological explanations for carry-over effects of the identity of the current client on the cleaners’ level of cooperation during the next interaction. Interactions with a predator could, for example, cause different levels of arousal, satiation or stress. A stress response is likely to occur as this is the usual effect of predators on
prey (Remage-Healey et al. 2006), but this does not automatically imply that stress affects the level of cooperation in cleaners. To identify candidate physiological processes that affect levels of cooperation in cleaners, amenable to future testing through specific manipulation of the physiological system, we conducted a laboratory experiment in which we confronted cleaners with a stressor and then measured how this affected their willingness to feed against their preference, as they have to feed against their preference under natural conditions if they are to cooperate (Grutter & Bshary 2003). In our experiment, eating a preferred item led to the immediate removal of the food source. In half of the trials, a hand-net was presented as a stressor (Brown & Wurburry 1999; Brown et al. 2007). If short-term stress induces more cooperative behaviour (either directly through the stress response or indirectly via a general arousal that affects foraging behaviour), we predicted that cleaners should feed more against their preference when exposed to a hand-net than when not exposed to one.

Methods

Field observations

Observations were made at Ras Mohammed National Park, Egypt from May to July 1998 and 1999. Twelve cleaners were each observed for four hours and an additional four cleaners each for 3 h. During observations, we noted client species, duration of interaction and the number of client jolts. Jolts are small abrupt body movements of clients in response to cleaner fish mouth contact, experimentally shown to correlate with cheating by cleaners both in our study species (Bshary & Grutter 2002) and in Caribbean cleaner gobies (Soares et al. 2008) and so are an easy measure of a correlate of cheating behaviour by cleaners. Full methodological details are described in Bshary & Würth (2001).

Data were entered in the sequence in which interactions took place; therefore, we could determine for each interaction between a cleaner and a non-predatory client whether the previous client was a predator or a non-predator. Predators are defined as species that according to Randall (1983) feed on fish whereas non-predators feed on invertebrates, plankton, corals or algae. A list of client species in the study area and their classification as predators or non-predators are published in Bshary (2001). Overall, we had 269 interactions between cleaners and predators and 3431 interactions between cleaners and non-predatory clients in the data file. Information on the exact time intervals between subsequent interactions was not available. Intervals may vary between 1 and 120 s (R. Bshary, pers. comm.). For the analyses, we first identified for each cleaner the non-predatory client species for which we had observations both after a cleaner’s interaction with a predator and with a non-predatory client. Controlling for client species identity is appropriate, as it is known that client species show strong variation with respect to jolt rates (Bshary 2001), and the data paired for each species take care of this variation. We then analysed our data in two ways and asked whether we would get consistent results.

In the first analysis, we determined for each species and each cleaner the jolt frequency after a predatory client and after a non-predatory client. If the jolt rate of the current client was lower when the cleaner’s previous client had been a predator, we gave the species a ‘+’ for that particular cleaner; if it was higher in interactions following an interaction with a predator, we gave the species a ‘−’ for that cleaner. We then counted the numbers of ‘+’ and ‘−’ for each cleaner, with the final sign for the cleaner depending on which sign was more frequent. The final analysis was conducted using a Sign test where \( N \) was the number of cleaners. The problem with this approach is that as long as the duration of interactions per species per situation is low, one must expect many extreme jolt rate values, including many 0 values. The average jolt rate is about 4 jolts/100 s. Hence, a 10-s interaction can only produce a clearly lower value (0) or much higher values (10, 20 jolts/100 s, etc.). To control for the effects of short interaction durations, we conducted a second analysis where we summed up for each cleaner the jolts and total duration for all client species that had interacted both after a predator and after a non-predatory client. These values were then used to calculate mean values per cleaner for the two situations. We only used data from cleaners that had spent in total more than 50 s with clients in each of the two situations to avoid the high variance to be expected when sample size is small. The criterion reduced the data set to 10 individuals. As the resulting data are interval-like in nature, we conducted a Wilcoxon matched-pairs signed-ranks test.

Laboratory experiments

Experiments were conducted from May to July 2004 at the Lizard Island Research Station, Great Barrier Reef, Australia. Fifteen cleaners were caught in the
adjacent lagoon with hand and barrier nets and transported back to the station. Fish were kept singly or in pairs in aquaria of varying sizes (minimal size 50 × 30 × 25 cm). All aquaria had running seawater and fish were provided with a polyvinyl chloride (PVC) tube (1 cm diameter × 8 cm) for shelter. All cleaners were released after the experiment at the site of capture. Cleaners were trained to feed off Plexiglas plates of various colours, and they familiarised with the experimental protocol. In the experiment, we offered cleaners a Plexiglas plate (12 × 7 cm) with six black circles drawn on the plate (each 1 cm diameter), each of which contained a food item. Three food items were prawn, and the other three items were tropical fish flakes mixed with prawn (called ‘flake’ hereafter). The plate remained in the tank as long as a cleaner ate flake items, but was removed immediately as soon as a cleaner ate one prawn item. Immediate reaction to prawn feeding was possible because the plate was attached to a lever held by the observer (Bshary & Grutter 2005). Previously, experiments have shown that cleaners have an almost 100% preference for prawn over flake (Bshary & Grutter 2005). Previously, experiments have shown that cleaners have an almost 100% preference for prawn over flake (Bshary & Grutter 2005). Therefore, cleaners had to feed against their preference if they wanted to increase their food intake.

In a sequence of 20 trials distributed over 2 d, cleaners were alternately confronted with a hand-net immediately prior to the feeding session or left undisturbed. The order of treatments was balanced: half of the individuals began with the hand-net situation, and the other half began with the undisturbed situation. A time interval of 40 min was left between trials. The hand-net evoked flight responses and the ‘dancing’ behaviour of cleaners which consisted of a whipping of the body while remaining stationary (Eibl-Eibesfeldt 1955). Our criterion for offering the Plexiglas plate was that cleaners were dancing at the moment we inserted the plate. As individual cleaners responded quite differently to the hand-net, we had to vary duration, movement and location of the hand-net for each cleaner and trial to produce the dancing behaviour. With the boldest individuals, the hand-net had to remain in the aquarium after a quick 1-s chase while the plate was offered, while the net could only be shown few seconds outside the aquarium with the shyest individuals or else they would not forage. For each cleaner and situation, the average number of flake items eaten in each round before eating a prawn item caused the termination of the interaction was calculated as a measure of how much cleaners were able to inhibit their preference for prawn. Note that the number of prawn items eaten in each trial always equalled one. Therefore, any significant variation between the two conditions in the number of flake items eaten per trial translated into significant differences in the ratio of flake items eaten per prawn items eaten, or total amount of food items eaten.

We also calculated the expected number of flake item eaten on average per trial under the assumption that cleaners forage in an indiscriminative way. As the plate was removed as soon as a cleaner ate one prawn item, indiscriminative foraging would lead to a 50% probability that a cleaner ate 0 flake items in any given round. The probability of a cleaner eating one flake item was $\frac{3}{6} \times \frac{3}{5}$ (three flakes and two prawn items left) = 30%. The probability of a cleaner eating two flake items was $\frac{3}{6} \times \frac{2}{5} \times \frac{3}{4}$ = 15%. Finally, the probability of a cleaner eating all three flake items was $\frac{3}{6} \times \frac{2}{5} \times \frac{1}{4}$ = 5%. Combining these probabilities, the ‘indiscriminate foraging hypothesis’ predicts that cleaners eat on average $0 \times 0.5 + 1 \times 0.3 + 2 \times 0.15 + 3 \times 0.05 = 0.75$ flake items per trial. For both experimental situations (with and without hand-net), we tested the actual number of flake items eaten per cleaner per round against 0.75 to determine whether cleaners ate according to their preference (value for flakes eaten significantly lower than expected), against their preference (value for flakes significantly higher than expected) or in an indiscriminate way (value for flakes not significantly different from expectation).

**Results**

**Field observations**

We found that in most cleaner individuals, the majority of non-predatory client species jolted less frequently when the cleaner had previously interacted with a predatory client species compared with a non-predatory client (Sign test: $n = 16$ cleaners, two ties, remaining $N = 14$, $x = 2$, $p = 0.012$). Similarly, when we used the ten individual cleaners for which we had sufficient observations as units for analyses, we found that they caused less jolts per time unit if the previous client had been a predator (Wilcoxon test: $n = 10$ cleaners, $z = -1.99$, $p = 0.047$, Fig. 1).

**Laboratory experiment**

Cleaners ate significantly more against their preference during the trials where they were exposed to a hand-net just prior to testing than in trials where
they were undisturbed (Wilcoxon test, n = 15, two
ties, remaining N = 13, \( T = 10.5, p = 0.013 \), Fig. 2). In both situations, cleaners are more flake items
than would have been predicted by indiscriminate
feeding (Wilcoxon tests, with hand-net: N = 15, one
tie, remaining N = 14, \( T = 2.5, p < 0.001 \); without
hand-net: N = 15, one tie, remaining N = 14, \( T = 17, p = 0.03 \), Fig. 2).

Discussion

We had asked whether the service quality a client
receives merely depends on its species identity or
whether service quality is modified by events that
may be stressful for cleaners. Our field results show
that the jolt rate of non-predatory clients does not
simply vary around a mean but changes predictably
according to a client’s position in the chain of clea-
er–client interactions. There are several potential
explanations as to why non-predatory clients may
have jolted less frequently when the previous client
was a predator than when it was a non-predatory
client. A null hypothesis, with respect to overall ser-
vice quality, is that the ratio of parasite to mucus
feeding does not change in interactions following a
cleaner–predator interaction, but that the cleaners
are just less efficient because of the arousal of their
internal state. Alternatively, interactions with preda-
tors may affect the cleaners’ hunger level differently
than interactions with non-predatory clients, which
in turn may influence their level of cooperation.
Indeed, current evidence suggests that cleaners gain
less preferred food from interactions with predators
than non-predators; although the duration of inter-
actions is very similar (Bshary 2001), cleaners cheat
predators less frequently and hence should eat less
mucus than in interactions with non-predatory cli-
ents (Bshary 2001). Finally, another hypothesis is
that interactions with predators cause a physiologi-
cal reaction in cleaners that specifically reduces
their cheating behaviour, either as a mechanism
that discourages cleaners from cheating predators or
as a side product of the interaction. In this scenario,
the physiological product would persist for some
time thereby leading to carry-over effects that influ-
ence the cleaners’ level of cooperation in the near
future.

While our field data do not allow us to distinguish
between the three aforementioned hypotheses, our
laboratory experiment suggests that a cleaner’s level
of cooperation can indeed be affected by evoking a
physiological response. It is important to note that
cleaners are selectively against their preference in
our experiment. Therefore, we can exclude the pos-
sibility that the hand-net as a stressor interfered with
the cleaners’ capacity to feed selectively (Olla &
Davis 1989, Brown 2001), and that our results are
not because of cleaners shifting from eating preferred
items in the control situation to indiscriminate feed-
ing in the test situation. The nature of the physiolog-
ic reaction, however, has to be examined in a
future study that manipulates physiology directly
rather than merely observing the behaviour of
cleaners. The hand-net could have led, for example,
to a general arousal that altered foraging behaviour.
We consider it more likely, however, that the hand-
net evoked a stress response (Brown & Warburton
1999; Brown et al. 2007). We chose a hand-net for
our experiments to mimic the likely physiological
response that predators could cause in cleaners, as
predators typically cause a stress response in prey
(Remage-Healey et al. 2006).

Fig. 1: Frequency (n/100-s interaction) of non-predatory client jolts
when the previous client had been a non-predator or a predator.
Mean and SD of the values for 10 cleaners.

Fig. 2: Number of flake items eaten per round by 12 cleaners with a
hand-net either present or absent. Median and interquartiles of the 12
individual cleaner values are shown. The dashed line indicates the
number of flake items eaten per round that would be predicted if
cleaners ate items indiscriminately.
Our experimental results suggest that stress may indeed cause increased levels of cooperation in cleaners, as cleaners indeed altered their foraging behaviour in the presence of a hand-net in the predicted way; i.e. they fed more against their preference. Under natural conditions, such a change in behaviour would translate into a better service quality for the client fish, as cleaners would feed more on parasites rather than on mucus (Grutter & Bshary 2003). We note, however, that the effect of the hand-net on the foraging behaviour of cleaners was relatively small and certainly did not lead to cleaners refraining from eating preferred prawn. Therefore, our data suggest that it is not immediate stress that causes virtually unconditional cooperative behaviour of cleaners towards predators in the wild, unless it could be shown in a future study that predators cause higher stress levels than a hand-net. In any case, it would be nice to investigate whether variation in stress levels correlates well with variation in service quality. If that was the case one would expect that the effect of interactions with a predator diminishes with the time elapsed until the next interaction, a parameter that was not measured in the current study.

The results add a new dimension to a previously documented case study on one particular female cleaner that produced average client jolt rates when cleaning at her cleaning station but cheated non-predatory clients much more frequently when visiting the male at his station (Bshary & D’Souza 2005). This idiosyncratic study demonstrates that individual L. dimidiatus may have the potential to alter service quality for non-predatory clients quickly in an apparently functional way. A similar conclusion has been proposed for variation in service quality in the closely related cleaner wrasse L. bicolor. The latter species roves over much larger areas (Oates et al. 2010a), and it readily adjusts service quality to location: in its core area where interactions with individual clients occur more frequently, levels of cooperation are higher than in the periphery where future interactions with the same client will be delayed (Oates et al. 2010b). The current study cautions that not all changes in service quality may be adaptive and emphasises the need to better understand the mechanisms underlying such changes.

The next important step will be to manipulate the endocrine system to test the hypothesis that stress affects levels of cooperation. Putative physiological mechanisms underlying this process must act on a very short-time scale which makes central neurotransmitter and neuromodulator systems the most likely candidates, because the behavioural effects of stress hormones (i.e. glucocorticoids) typically only occur within minutes (Wendelaar Bonga 1997). Telencephal fish brain monoamines, in particular serotonin, have been shown to increase in response to social stressors (Winberg & Lepage 1988; Winberg et al. 1992, 1997), including the exposure to a predator (Winberg et al. 1993), and to mediate the expression of aggressive behaviour (Winberg et al. 2001; Lepage et al. 2005). Thus, they meet the conditions needed to fine tune the behaviour of cleaners. Other neurochemical systems likely to influence the cleaner’s level of cooperation are the neuropeptides arginine, vasotocin and isotocin, which are the teleost homologues to the mammalian arginine, vasopressin and oxytocin. Neuropeptides play key roles in the control of aggressive and pro-social behaviours (Thompson & Walton 2004; Santangelo & Bass 2006).

In conclusion, our study identified predictable short-term variation in cleaner fish foraging behaviour, where the nature of the stimuli — predators and a hand-net — suggests that short-term stress may be a factor promoting cooperative behaviour. The validity of this hypothesis remains to be tested. If it were confirmed, one would have to start thinking about the potential adaptive value of such a mechanism as opposed to alternative solutions. There is clearly a need for more experimental studies that manipulate the endocrine system both through changing environmental settings (this study) and through the application of hormones, neurotransmitters and neuromodulators (Soares et al. 2010 in press). The study of the causal mechanisms underlying the social modulation of the cleaner’s behaviour is a promising research model for the experimental study of the physiological basis of cooperative behaviour in vertebrates. Only a thorough understanding of how physiological processes contribute to decision-making will allow us a proper appreciation of the putative cognitive mechanisms underlying cooperative behaviour (Brosnan et al. 2010).

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**Literature Cited**


