Adjustments of levels of cooperation in cleaner wrasse *Labroides dimidiatus*: the effects of an audience and satiation

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Résumé général

La coopération, soit l’entraide entre individus sans lien de parenté, est une énigme évolutive. Cela est dû au fait que l’aide est souvent un investissement qui doit générer des avantages futurs pour pouvoir faire l’objet d’une sélection positive. Généralement, la sélection naturelle favorise les individus qui adoptent un comportement égoïste. La tricherie devient donc une question conceptuelle majeure. Cependant, les exemples de coopération sont nombreux dans la nature. De ce fait, la recherche d'explications réconciliant la coopération avec la théorie de l'évolution a longtemps été d’une importance majeure en biologie, mais également dans les sciences sociales, l’objectif étant d'expliquer la complexité sociale chez l'homme. Divers mécanismes dits de contrôle du partenaire - des réponses comportementales qui entraînent une réduction des gains d'un partenaire tricheur de sorte qu'un partenaire coopérant gagne davantage - se sont avérés efficaces pour stabiliser la coopération. Mes recherches sur ce sujet ce sont axées en particulier sur le rôle du prestige social dans un réseau de communication. En effet, de nombreuses interactions animales sont observées par des tiers («spectateurs»), qui peuvent obtenir des informations extrêmement utiles sur les interactants. Dans le contexte de la coopération, les spectateurs doivent ainsi essayer d'identifier des individus singulièrement coopératifs comme futurs partenaires, ce qui permet de sélectionner les individus particulièrement coopératifs s'ils sont observés.

Mon système modèle a consisté d’un mutualisme de nettoyage marin impliquant des labres nettoyeurs (*Labroides dimidiatus*) et ses poissons de récif dit «clients» qui leur rendent visite pour se faire enlever les ectoparasites. Cependant, des conflits surgissent car les nettoyeurs préfèrent la couche de mucus protectrice des clients aux ectoparasites, où se nourrir de mucus est préjudiciable au client et est donc fonctionnellement considéré comme de la tricherie. Par conséquent, les clients doivent faire en sorte que les nettoyeurs mangent contre leur préférence afin de pouvoir bénéficier d'un bon service de nettoyage. Des observations sur le terrain et une expérience de laboratoire utilisant des plaques en plexiglas en remplacement de clients avaient déjà suggéré que les clients spectateurs étaient attentifs à la manière dont un nettoyeur traite son client actuel et que les nettoyeurs sont plus coopératifs s’ils sont observés. J'ai pu démontrer ce concept de prestige social pour la première fois dans le cadre d'une expérience de laboratoire contrôlée utilisant de véritables interactions client-nettoyeur. Dans deux autres expériences utilisant soit des plaques en plexiglas soit de vrais clients, j'ai montré que les nettoyeurs peuvent ajuster la qualité de leurs services à l'importance relative du client actuel par rapport au client spectateur: plus le spectateur a de la valeur, plus le service envers le client actuel est peaufiné. Dans une troisième expérience, j’ai manipulé le niveau de satiété des nettoyeurs afin de tester la prédiction de la théorie du marché biologique selon laquelle un besoin d’interactions temporairement faible entraîne une baisse de la qualité du service. De manière quelque peu surprenante, cette prédiction n’a pas été confirmée, les nettoyeurs rassassiés ayant augmenté leur niveau de coopération envers leurs clients, c’est-à-dire qu’ils ont moins triché lors des interactions avec leurs clients et se sont nourris davantage contre leur préférence sur les plaques en plexiglas. Ainsi, les nettoyeurs rassassiés investissent fonctionnellement dans leur relation avec les clients pour des avantages futurs.

En conclusion, mon travail de recherche démontre que le labre nettoyeur *L. dimidiatus*, est capable de prendre des décisions sophistiquées, adaptées aux spécificités de la situation. Les résultats soulèvent des questions concernant les processus cognitifs sous-jacents, car ils remettent en cause la notion selon laquelle des cerveaux plus larges sont nécessaires pour une coopération sophistiquée. Au lieu de cela, il semble qu'une approche écologique par rapport à la cognition soit plus appropriée pour expliquer mes résultats. Mon étude s'inscrit dans une longue tradition selon laquelle les poissons constituent des systèmes modèles idéaux pour tester la théorie de la coopération, dont les résultats prometteurs devraient inspirer de nouvelles analyses théoriques.

mots clés: coopération, mutualisme, *Labroides dimidiatus*, reputation.

General Abstract

Cooperation, the mutual helping between unrelated individuals, is an evolutionary puzzle. This is because helping is often an investment that must yield future benefits in order to be under positive selection. Generally, natural selection favours individuals that perform self-serving behaviour, and hence cheating is a major conceptual issue. However, examples of cooperation are abundant in nature. As such, finding explanations that reconcile cooperation with evolutionary theory has long been a major focus in biology but also in the social sciences with their aim to explain the social complexity in humans. A variety of so-called partner control mechanisms – behavioural responses that cause a reduction in the payoffs of a cheating partner such that a cooperating partner gains more – have been shown to stabilise cooperation. My research has focused in particular on the role of social prestige in a communication network. Many animal interactions are observed by third parties ("bystanders"), who may gain valuable information about the interactants. In the context of cooperation, bystanders should try to identify particularly cooperative individuals as future partners, which selects for individuals being particularly cooperative if they are observed.

My model system has been marine cleaning mutualism involving bluestreak cleaner wrasses (*Labroides dimidiatus*) and their so-called "client" reef fishes that visit to have ectoparasites removed. However, conflict arises as cleaners prefer the protective mucus layer of clients over ectoparasites, where mucus feeding is detrimental to the client and hence functionally constitutes cheating. Thus, clients have to make cleaners feed against their preference in order to receive a good cleaning service. Field observations and a laboratory experiment using Plexiglas plates as client surrogates had already suggested that bystander clients pay attention to how a cleaner treats its current client, and that cleaners are more cooperative if observed. I could demonstrate this social prestige concept for the first time in a controlled laboratory experiment in real cleaner-client interactions. In two further experiments using either plates or real clients I showed that cleaners can fine-tune their service quality to the relative importance of current client versus bystander: the more valuable the bystander the better the current service. Finally, I manipulated the cleaners' level of satiation in order to test the prediction from biological market theory that a temporarily low need for interactions causes a decrease in service quality. Somewhat surprisingly, this prediction was not met as satiated cleaners increased their cooperation levels towards their clients, i.e. they caused less jolts during interactions with their clients and fed more against their preference on plates. Thus, satiated cleaners functionally invest into their relationship with clients for future benefits.

In conclusion, my work shows that cleaner wrasse *L. dimidiatus* show sophisticated decision rules that are fine-tuned to the specifics of the situation. The results raise questions concerning the underlying cognitive processes as they challenge the notion that large brains are necessary for sophisticated cooperation. Instead, it appears that an ecological approach to cognition is better suited to explain my results. My study fits into a long tradition that fish yield ideal model systems to test cooperation theory, where results hopefully inspire further theoretical analyses.

keywords: cooperation, mutualism, *Labroides dimidiatus*, reputation.

General Introduction

Cooperation as a conundrum in the evolutionary theory

Helping between unrelated individuals is a conundrum in biology and therefore has attracted a lot of research interest and continued debate ever since Darwin pointed out that helping other individuals is difficult to reconcile with his theory of evolution through natural selection (Darwin 1859). Why is that individuals that should be competing for resources instead help other individuals under certain conditions, and consequently increase the recipients' fitness? Hamilton (1964) showed that biological altruism can be under positive selection as long as the recipients are genetically related to the helper. However, there are many cases in which interacting individuals are unrelated and even belong to different species (Boucher et al. 1982; Bergstrom et al. 2003; Bronstein 2003). In these cases, helping must lead to direct fitness benefits to the helper, i.e. to cooperation (for terminology see Lehmann & Keller 2006). As there are many examples of cooperative behaviours in a wide range of taxa, including bacteria, plants and invertebrates (Pierce et al. 2002; Kiers et al. 2003) it is clear that the initial emphasis on high cognitive abilities as found in humans or primates (Byrne & Whiten 1989) is misleading. Instead, cooperation can be achieved by very simple means (West et al. 2007; Clutton-Brock 2009; Brosnan et al. 2010). The diversity of examples for cooperation is reflected in a great diversity of theoretical concepts that have been put forward to explain the evolution and persistence of cooperation.

Concepts to explain cooperation

The problem of cooperation is best illustrated with the prisoner's dilemma game. The game consists of two players playing against each other and they have two available options: they can either cooperate or defect. The payoffs are such that each player receives a higher payoff if both cooperate than if both defect but defecting yields a higher payoff than cooperating irrespective of the partner's action. Thus, defection is dominant over cooperation in each single round. In other words, to cooperate is an investment that needs to yield future benefits to be under positive selection. Therefore, defection is the only evolutionary stable strategy if partners only interact once. Following Trivers' ideas about reciprocal investments in iterated interactions (Trivers

1971), Axelrod & Hamilton (1981) analysed an iterated version of the prisoner's dilemma game and concluded that repeated interactions allow cooperative strategies to evolve and persist. This seminal paper led to a major effort to find evolutionarily stable cooperative strategies for this game; with generous tit-for-tat and pavlov being the most famous solutions (Nowak & Sigmund 1992, 1993). Generous tit-for-tat is a probabilistic strategy where the notion of "benefice of doubt" is involved, that is, individuals cooperate with a certain probability even when the opponent defects (Nowak & Sigmund 1992). Pavlov is also probabilistic and cooperates on the first move. If a reward or temptation payoff is received in the last round then players repeat their last choice, otherwise they switch to the alternative behavioural option. Therefore, pavlov players can temporarily exploit unconditionally cooperative partners (Nowak & Sigmund 1993).

The cooperative strategies that can solve an iterated prisoner's dilemma are invariably conditional on the partner's behaviour, i.e. cooperating as long as your partner cooperates and defecting in the next round if your partner defected in the previous round. Despite these theoretical discoveries, empirical examples of tit-for-tat-like strategies are relatively scarce. The most detailed evidence for reciprocal strategies has been provided by studies on predator inspection by small prey fish (Turner & Pitcher 1986; Milinski 1987; Dugatkin 1988) where groups of fish approach predators closer than singletons (Pitcher et al. 1986). Some colleagues propose that predator inspection in sticklebacks fit the positive pseudoreciprocity concept where the best strategy would be to approach the predator a bit closer than the partner which empowers him to approach even further and so on (Hammerstein 2003; Clutton-Brock 2009). However, there is evidence that and it has also been showed that it is beneficial to remain behind a cooperative partner (Huntingford et al. 1994; Milinski et al. 1997; Krause et al. 1998). Also, the tendency of a fish to approach a predator is dependent on its partner's behaviour (Dugatkin 1991; Milinski et al. 1990a, 1990b; Külling & Milinski 1992; Milinski et al. 1997; Walling et al. 2004), favouring the reciprocity concept. Recently, some further examples have been published though often without ecological validity (reviewed by Raihani & Bshary 2011). Nevertheless, various authors have pointed out that many examples of cooperation do not fit the assumptions of an iterated prisoner's dilemma game (Bergstrom et al. 2003; Hammerstein 2003; Leimar & Hammerstein 2010; Bshary & Bronstein 2011). For example, to date, there is no empirical evidence from interspecific cooperative interactions that fulfils the assumptions of the IPD (Bergstrom et al. 2003).

Instead, various other concepts may explain the maintenance and evolution of cooperation between unrelated, including by-product mutualism (Brown 1983), pseudo-reciprocity (Connor 1986), punishment (Clutton-Brock & Parker 1995; Raihani et al. 2012; Raihani & Bshary in press), or partner switching (Ferriere et al. 2002; Johnstone & Bshary 2008). Indeed, many empirical examples have been matched to one of these concepts (Leimar & Hammerstein 2010; Bshary & Bronstein 2011). Furthermore, it has become evident that as interactions often take place within a communication network (McGregor 1993) individuals may gain reputation from their actions which will in turn affect the behaviour of current bystanders during future interactions. In the context of cooperation, reputation may lead to indirect reciprocity via image scoring (Alexander 1987; Nowak & Sigmund 1998) or to indirect pseudoreciprocity (Zahavi 1995; Roberts 1998; Lotem et al. 2003). As cooperation within communication networks is central to my PhD thesis, these concepts will be developed in more detail in the next section.

Indirect reciprocity and indirect pseudoreciprocity

Indirect reciprocity based on image scoring has been modelled as a game in which individuals repeatedly face the decision to help someone else in the presence of bystanders. Helping will raise their image score, while failure to help will decrease their image score (Nowak & Sigmund 1998). Under these conditions, a strategy that helps recipients who have helped others in the past may spread and hence lead to stable cooperation (Nowak & Sigmund 1998). The model has been criticised and an alternative reputation strategy proposed (Leimar & Hammerstein 2001): a “standing strategy” that proposes that failing to help an uncooperative individual should be good for the standing (while it is bad for the image score). Nevertheless, current evidence on humans suggests that the image scoring strategy fits the data better (Wedekind & Milinski 2000; Milinski et al. 2001). Various additional theoretical models show that reputational mechanisms can facilitate the evolution and maintenance of cooperation (Pollock & Dugatkin 1992; Nowak & Sigmund 2005; Ohtsuki & Iwasa 2006; Roberts 2008; Barclay 2011). In addition, many studies have confirmed that humans are more cooperative when others can observe or will be informed of their actions (Bixenstine et al. 1966; Kurzban 2001; Milinski et al. 2002a, 2002b; Andreoni & Petrie 2004; Barclay 2004; Rege & Telle 2004; Barclay & Willer 2007). Important empirical expansions of the initial theories include the demonstration that reputation building may explain contributions to public goods (Nowak & Sigmund 1998; Milinski et al. 2002b; Sylwester & Roberts 2013), and that reputation can be built not just through direct observations but also through gossip (McAndrew & Milenkovic 2002; Hess & Hagen 2006; McAndrew et al. 2007; Sommerfeld et al. 2007; Sommerfeld et al. 2008). Even subtle cues of observation have been shown to affect cooperation, given that human decision-making is influenced not only by conscious, reasoned evaluation of explicit knowledge (e.g. the rules of a game), but also by non-conscious, intuitive judgements based on implicit cues (Haidt 2001; DeBruine 2002; Hagen & Hammerstein 2006). Haley and Fessler (2005) investigated whether implicit cues of observation influence cooperative decision-making by exposing participants to eye-like images just before they completed a generosity task, finding that generosity was higher in the eyes condition relative to control conditions that they did not contain a visual cue to observation (henceforth the ‘eyes effect’).

In indirect reciprocity, each act of helping is an investment. Thus, this mechanism is apparently quite complicated, i.e. potentially cognitively demanding. In line with this interpretation, indirect reciprocity has so far only been found in humans (Wedekind & Milinski 2000; Milinski et al. 2002a, 2002b; Semmann et al. 2003). Indirect pseudoreciprocity (“social prestige”: Zahavi 1995; Roberts 1998; Lotem et al. 2003) is a second more simple mechanism. In this scenario the bystander will use the information obtained through observation to choose a cooperative partner that will provide the highest benefits. The image scoring of the bystander is thus self-serving, while the individual that helped benefits because it will be chosen as a cooperative partner (Zahavi 1995; Roberts 1998; Bshary & Bergmüller 2008). The social prestige concept has been demonstrated to work in humans (Sylwester & Roberts 2010, 2013). Outside humans, social prestige has been documented in the cleaning mutualism involving my study species *L. dimidiatus* (Bshary 2002; Bshary & Grutter 2006). In the first paper field observations revealed that there is strong variation between cleaners with respect to cheating rates and that bystanders avoid cleaners that they observed cheating their current client (Bshary 2002). As an apparent adjustment to image scoring bystanders, biting cleaners behaved more cooperatively towards small resident clients and had access to large visitor clients that they exploited. In the second paper Bshary and Grutter (2006) conducted laboratory experiments where they found that bystanders spent more time close to cleaners that seemed to be cooperative (they ate mashed prawn of a laminated client picture) in comparison to cleaners that presented an unknown cooperative level. In addition, cleaners learn to eat against their preference – flake items instead of prawn items – of a plate when in the presence of a second image scoring plate rather than a second non-image scoring Plexiglas plate. These two papers provided the starting point of my PhD thesis.

State dependent cooperation

The cleaners' incorporation of the presence of an audience represents a flexible adjustment of levels of cooperation to current circumstances. But also other variables may affect levels of cooperation. For example, levels of cooperation may be state dependent. For example, Lotem and colleagues (2003) linked social prestige to the handicap principle: only high quality individuals may be able to pay the cost of signalling their status through helping. Sherratt and Roberts (2001) developed the concept of phenotypic defectors: individuals that are for ontogenetic reasons in poor state are not able to help and hence defect. The presence of phenotypic defectors is an alternative to probabilistic strategies like generous tit-for-tat or pavlov to explain the persistence of conditionally cooperative strategies in an IPD. State dependent levels of cooperation in cleaner wrasse are interesting in the context of biological market theory and its emphasis on partner choice. The concept accounts for the presence of numerous potential cooperators belonging to different classes of traders. Potential cooperating partners offer and require differing resources. Most importantly, shifts in the supply and demand ratio cause changes in the exchange value of the commodities traded (Noë & Hammerstein 1994, 1995). Hence, in the course of my PhD I complemented research on the effects of an audience to effects of satiation on the cleaners' levels of cooperation.

Study species – the cleaner wrasse Labroides dimidiatus

The cleaner wrasse *Labroides dimidiatus* is a territorial, reef-associated marine fish belonging to the family *Labridae* (Randall et al. 1997). This species is widespread across the Indo-Pacific Ocean and can be found in various reef habitats, ranging from sheltered lagoons to exposed seaward reefs, between depths of 1 to 40 m (Myers 1991; Lieske & Myers 1994; Kuiter & Tonozuka 2001). Adult individuals can reach up to 11.5 cm in length (TL) (Randall et al. 1997), with a maximum reported age of four years (Robertson 1974). Cleaners can be solitary, yet are often found living in pairs or in small groups, consisting of a single male with a harem of several smaller females (Robertson 1972; Potts 1973; Kuiter & Tonozuka 2001). As in other labrid species, *Labroides dimidiatus*, is capable of changing sex (generally protogynous, but see Kuwamura et al. 2002), where the largest and most dominant female replaces a single dominant male, if/when he disappears (Robertson 1972; Ross 1990).

The mutualism involving the cleaner wrasse L. dimidiatus

In the mutualism involving the cleaner wrasse *Labroides dimidiatus*, clients regularly visit cleaners at their small territories (so called 'cleaning stations'). These territories often contain a distinctive feature, such as an adjacent cave or large coral head, which acts as a visual cue for returning visitor clients (Kuiter & Tonozuka 2001) and are generally confined to a few cubic meters of reef (Grutter 1995). Cleaners are diurnal feeders which remove ectoparasites, mucus and infected or dead tissue removed from the surface, the gills and sometimes the mouth of their clients (reviewed by Losey et al. 1999; Côté 2000). Clients, which largely consist of teleost or bony fish, generally visit these territories for inspection between 5 to 30 times a day (some over 100 times in a single day), and may spend up to 30 min per day being 'cleaned' (i.e. Siganus doliatus, Grutter 1995). Unlike other labrid genera, such as *Labropsis* and *Bodianus*, which only clean as juveniles (Randall et al. 1997), *L. dimidiatus* are considered obligate cleaners as they also clean as adults. They engage in between 800 to 2'000 interactions per day (Grutter 1995, 1997; Wismer et al. 2014) and consume more than 1'200 parasites per day (predominantly gnathiid isopod larvae; Grutter 1995, 1996).

Interactions between cleaners and clients

Various evidence supports the view that the overall relationship between *L. dimidiatus* and its client fish is mutualistic. By removing large amounts of parasites (Grutter 1996), cleaners benefit from a rich and untapped food source (Poulin & Grutter 1996). Consequently, client parasite loads are significantly reduced (Grutter 1999), that otherwise may potentially lead to a significant reduction in growth, survival and reproductive output (Cusack & Cone 1986; Lehmann 1993; Poulin & Grutter 1996; Waldie et al. 2010). Furthermore, it has been shown that cleaner wrasses drive local reef fish biodiversity (Bshary 2003; Grutter et al. 2003). As clients without access to cleaner have higher cortisol levels (Bshary et al. 2007) and lower body condition (Ros et al. 2011), it appears that physiological measures may yield good correlates of the mutualistic effects of cleaning interactions on client fitness. Nevertheless, while several studies suggest that clients indeed benefit from interactions with cleaners (see also Grutter & Lester 2002; Cheney & Côté 2003), it is also evident that there is a main conflict between cleaners and clients over what cleaners should eat. As demonstrated by Grutter and Bshary (2003), cleaners prefer nitrogen rich client mucus, the protective layer that covers the skin, over clients' parasites. A laboratory experiment revealed that feeding on client tissue is not a by-product of parasite removal, and client jolts in response to cleaner fish mouth contact correlates with mucus feeding by cleaners and hence indicate cheating (Bshary & Grutter 2002a; Bshary & Noë 2003). Thus, cleaner wrasse individuals have to feed against their preference in order to provide a good service to clients (Grutter & Bshary 2003) but ideally cheat non-vigilant clients. For example, anaesthetised clients are readily exploited by cleaners (Bshary & Grutter 2002a), and client jolts are readily observable in nature (Bshary 2001). Clients counteract such cheating to ensure overall cooperative cleaner behaviour by employing various partner control mechanisms (Bshary 2010).

The typical reaction of a client species to cheating by cleaners depends on the clients' strategic options. Interactions between *Labroides dimidiatus* and predatory clients tend to be highly cooperative (Bshary 2001). In such interactions, both partners have symmetrical strategic options, i.e. each can choose to either cooperate or defect (eating mucus versus eating the cleaner), yet the outcome of such cheating would be highly asymmetrical (i.e. the predator is relatively unaffected, yet the cleaner loses its life) (Bshary 2001; Bshary & Grutter 2002a). Such interactions can therefore not be controlled by positive reciprocity, as cleaners will not have the option of cheating in subsequent rounds if a predator was to cheat first (Bshary & Grutter 2002a). Instead, rather unconditional cooperation is maintained probably because as maximal service quality by cleaners means that it is in the self-interest of predators to cooperate as well (Bshary 2001; Bshary & Bronstein 2011). This is in marked contrast to non-predatory, harmless clients, which have been observed to “jolt” at much higher rates than predators do under natural conditions (Bshary 2001; Bshary & Grutter 2002a). Approximately 85% of client species that visit cleaner stations are harmless reef fish, such as planktivores and herbivores, which lack the ability to cheat, or consume the cleaner (Bshary & Grutter 2002a; Bshary & Noë 2003). Thus, the strategic options in this scenario are highly asymmetric (Bshary & Noë 2003). Control mechanisms, such as terminating interactions and aggressively chasing the cheating cleaner (as a form of punishment) have been shown to increase the frequency of cooperative behaviour during subsequent interactions between the same individuals (Bshary & Grutter 2002a). Visitor fish, also called to as ‘choosy’ fish, refer to clients with large home ranges that encompass several cleaner stations and include roving reef fish and non-reef associated pelagic fish (i.e. roving herbivores, chaetodontids, carangids) (Bshary 2001, 2003; Bshary & Grutter 2002b). Visitor fish typically swim away in response to cheating by a cleaner and will access other cleaning stations for future interactions (Bshary & Grutter 2002a; Bshary & Schäffer 2002). Such behaviours support market theory (Noë et al. 1991) and suggest partner switching is actively used as a control mechanism for cooperative behaviour in this cleaning mutualism (Bshary & Schäffer 2002): a cheating cleaner stands to lose a potential future food source if it chooses to defect (Bshary & Grutter 2002a). In contrast, resident fish, such as territorial, coral dwelling and site attached species (i.e. pomacentrids, apogonids, and holocentrids), refer to clients who have small home ranges and generally only have access to one cleaner station (Bshary 2001, 2003; Bshary & Grutter 2002b). Hence, resident fish will queue for service, where visitor fish will leave the territory if not inspected immediately (Bshary 2001). Since resident fish are spatially restricted, they generally chase a defecting cleaner, which imposes an immediate energetic cost on the cleaner. Chasing functions as punishment as cleaners will provide such individual clients a particularly good service during follow-up interaction (Bshary & Grutter 2002a, 2005).

In all cleaner-client interactions, both with predatory and harmless species, one component is always asymmetrical, either the consequences of being cheated (loss of mucus versus loss of life) or the strategic options (Bshary & Noë 2003): non-predatory clients lack any option to cheat a cleaner. Due to this inequality, cooperative interactions between cleaners and clients cannot be scrutinized in the context of the IPD, or derivatives thereof (Bshary & Grutter 2002a; Bshary & Noë 2003). Instead, asymmetrical games, based on empirical data, are the appropriate paradigms needed to adequately explain or predict the evolution or the strategies played in this particular cleaning mutualism (Bshary & Grutter 2002a; Bshary & Noë 2003).

The conflict of interest between cleaners and clients over what the cleaner should eat and how clients respond does not only affect cleaner foraging behaviour. As it stands, cleaners of the genus *Labroides* have evolved a peculiar behaviour named tactile stimulation. It involves the cleaner touching the client with its pectoral and specifically pelvic fins, typically while “hovering” above the client (“host stabilisation”: Potts 1973). Such behaviour is not associated with foraging, but rather a manipulative behaviour or an ‘extra service’ provided by a cleaner which clients seem to appreciate (Bshary & Würth 2001). Indeed, it has been shown that physical stimulation reduces client stress levels (Soares et al. 2011). *Labroides dimidiatus* may ensure conflict free interactions with predators by providing large amounts of tactile stimulation, and the cleaners use tactile stimulation to functionally reconcile with clients after a conflict caused by the cleaner cheating (Bshary & Würth 2001). Therefore, any study on cleaner – client interaction should take this component of cleaner strategy into account.

At the beginning of my PhD project, it was also already described that cooperative behaviour by cleaners may further be enhanced by indirect pseudo-reciprocity or social prestige (Zahavi 1995; Bshary 2002; Bshary & Grutter 2006; Bergmüller et al. 2007). Field observations suggested that clients arriving at a cleaning station pay attention to on-going interactions and are more likely to invite inspection if they observe an interaction without conflict than if they observe a conflict (Bshary 2002). In a laboratory experiment, Bshary & Grutter (2006) showed that clients indeed prefer a cleaner that interacted with a client model over a cleaner that ignored the client model. Image scoring by bystanders is self-serving because it helps them to seek cooperative cleaners and to avoid cheating ones. However, the story is more complicated as cleaners seem to respond to image scoring clients: field observations suggest that cleaners are more cooperative if observed by potential clients (Bshary 2002), and experiments in the lab established a causal link using a Plexiglas plate paradigm (Bshary & Grutter 2006). In this paradigm, clients, ectoparasites and mucus are replaced by Plexiglas plates with small items of fish-flakes and prawn. As cleaners prefer to eat prawn, this is the equivalent of mucus and hence constitutes cheating. Experimenters cause the appropriate response of the plate; in this case the plate is removed immediately if the cleaner eats a piece of prawn. Hence, cleaners need to feed against their preference to increase food intake. In the image scoring experiment, cleaners ate more against their preference in the presence of a second image scoring plate than in the presence of a non-image scoring plate or in the absence of a second plate (Bshary & Grutter 2006). Such behavioural adjustments in response to the presence of bystanders have been termed “audience effects” (Doutrelant et al. 2001; Johnstone 2001; McGregor 2005). However, a potential criticism is that possibly cleaners learned to solve a simple optimal foraging task that does not properly represent real interactions with clients. Moreover, in this study the clients models used for the experiments on bystander image scoring were not sufficient to define what cues bystanders use to choose a cooperative partner under natural conditions. This is because the models could not jolt or flee though such cues seemed to be important under natural conditions (Bshary 2002). Taken together, cleaners apparently benefit from the audience effects as they will be chosen as a future cooperative partner (Bshary 2002; Bshary & Grutter 2006). In extreme cases, temporarily biting cleaners provide tactile stimulation to small residents with the apparent function to attract large visitors that will then be exploited (Bshary 2002). This form of tactical deception can be induced by cortisol injections (Soares et al. 2014). Thus, hunger levels and body condition appear to affect service quality in interesting ways.

Aims of my PhD thesis

The aim of my PhD thesis was to study causes and consequences of variation in cleaner fish service quality. Initially, a major part was to study variation between individual cleaners and to correlate variation in cooperation with cognitive performance and body condition as a correlate of fitness. This line of research led to unexpected results that needed to be followed up beyond the scope of my thesis. Instead, Sharon

Wismer conducted first her Master thesis on the topic under my supervision in the field and now continues with her own PhD project. I am shared first author on her first chapter (Wismer et al. 2014), which is provided as supplement to my thesis (in agreement with my supervisor Professor Redouan Bshary and the dean of the faculty of sciences, Professor Bruno Colbois). My own thesis focusses on factors that cause cleaners to adjust service quality ad hoc. The first two chapters deal with image scoring by clients and how cleaners respond to image scoring. The third chapter investigates the effect of satiation on cleaners' levels of cooperation.

Chapter One: audience effects with real clients

When I started my PhD, all experimental evidence on image scoring was restricted to the demonstration that cleaner wrasse *L. dimidiatus* could learn to solve a foraging task (Bshary & Grutter 2006) by feeding against their preference on artificial Plexiglas plates in order to have access to further artificial clients. The logic of the task was built on the logic that cleaners have to feed against their preference under natural conditions in order to be cooperative as cleaners prefer client mucus over client ectoparasites (Grutter & Bshary 2003). Thus, the main conclusion was built on the inference that the cleaners' ability to feed against their preference in the presence of a bystander plate would translate into them feeding less on client mucus in the field when a bystander client is present. Therefore, an important extension would be to repeat the experiments with real clients in order to see whether the results from the "optimal foraging task" on the Plexiglas plates really translate into more cooperative behaviour towards clients. Ideally, cleaners should be able to show audience effects spontaneously rather than after several learning trials. Therefore, the first chapter of my thesis provides laboratory experiments on image scoring and audience effect where all interactions took place between real fish. For bystanders, I investigated what information they use to decide whether to seek or to avoid a cleaner. The lab experiments by Bshary & Grutter (2006) had focussed on interaction duration. However, in their case cheating by cleaners could not occur as the 'client' was a laminated fish picture. Therefore, I also quantified the absolute number of client jolts as correlate of cleaner cheating (Bshary & Grutter 2002a) and I calculated client jolt rate as a measure of average service quality. For the audience effects, I tested whether cleaners spontaneously improved service quality to current clients if we experimentally introduced a bystander. If this was the case, I predicted that clients would jolt less frequently in the presence of bystanders. Furthermore, I quantified the number of cleaner mouth contacts with the clients' surface in order to test whether a reduction in client jolt rates in the presence of an audience would be due to cleaners feeding less or really due to an increase in service quality. In the latter case, I would need to observe less jolts per mouth contacts.

Chapter Two: fine-tuned audience effects with real and artificial clients

For the second chapter of my thesis, I wanted to know whether the audience effects are fixed behavioural patterns or whether cleaners can fine-tune their behaviour as a function of current client and current bystander identity. If they do, I predicted that they should increase service quality in particular if the bystander is an attractive food source relative to the current client. The hypothesis was based on field observations which suggested that cleaners treat current clients particularly well if the bystander is a visitor species that could easily leave and interact with another cleaner instead (Bshary & D'Souza 2005). The chapter was an extension of my Master thesis project and integrates its key results. During my Master, I used Plexiglas plates as surrogates of real clients of varying size and containing different amounts of food. In order to gain access to a bystander plate cleaners had to eat against their preference on a first plate. I predicted that they would do so only if the bystander plate was more attractive (i.e. offered more food) compared to the first plate. During my PhD I extended the project to test if cleaners were able to flexibly adjust service quality to both current client and bystander identity in interactions with real clients. I used four different species of differing attractivity and strategic options. I used a small damselfish as a small resident client, a surgeonfish as a large resident client, a bream as a medium sized visitor client (similarly sized to the surgeonfish) and a wrasse as a large visitor client. I predicted that client species would affect service quality, which would be higher in the presence of an audience and that bystander species identity would matter as well.

Chapter Three: role of satiation as a state dependent variable

After completing experiments that focused on a more detailed understanding of image scoring in a communication network and resulting audience effects (McGregor 1993, 2005; Nowak & Sigmund 1998; Roberts 1998), I wanted to test another variable that would be state dependent and hence that could potentially affect levels of cooperation between the cleaners and its clients. Various models propose that levels of cooperation should be state dependent (Sherratt & Roberts 2001; Lotem et al. 2003). Also, field observations and a follow-up experiment show that cleaners cheat more when they are in high demand for energy (Bshary 2002; Bshary & D'Souza 2005; Soares et al. 2014). A negative energy balance should be more likely to occur when few clients visit. In contrast, when many clients visit cleaners should be able to obtain plenty of food. Thus, one might predict that cleaners with many clients are typically satiated and hence cheat less. However, this prediction is opposite to the prediction that would be made by biological market theory (Noë et al. 1991). Many studies have shown that cooperative exchanges in nature follow the logic of supply and demand, where high demand leads to an increase in prices (for reviews of the literature see Noë 2001; Van de Waal et al. 2013). Thus, cleaners should be less cooperative when many clients visit as this indicates that the demand for cleaning is high. Thus, I wanted to test what happened between a cleaner and its current client if cleaners had been given extra food, lowering their needs for interactions. The first part of the chapter integrates data collected by Redouan Bshary in 2005, based on the Plexiglas plate paradigm. I contributed the experiments on real clients. I predicted that if satiation leads to risk aversion, then satiated cleaner fish would be more cooperative to avoid punishment and visiting clients switching to other cleaners for future inspections. In other words, satiated cleaners would invest in their relationships with clients in order to obtain future benefits. In contrast, if current demand affects service quality, then satiation should lead to a decrease in service quality.

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Chapter One | Cleaner Wrasses *Labroides dimidiatus* Are More Cooperative in the Presence of an Audience

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Abstract

Humans may help others even in situations where the recipient will not reciprocate (Alexander 1987; Wedekind & Milinski 2000; Fehr & Gächter 2002; Milinski et al. 2002; Nowak & Sigmund 2005). In some cases, such behaviour can be explained by the helpers increasing their image score, which will increase the probability that bystanders will help them in the future (Nowak & Sigmund 1998, 2005; Leimar & Hammerstein 2001). For other animals, the notion that many interactions take place in an environment containing an audience of eavesdropping bystanders has also been proposed to have important consequences for social behaviour, including levels of cooperation (McGregor 2005). However, experimental evidence is currently restricted to the demonstration that cleaner fish *Labroides dimidiatus* can learn to solve a foraging task (Bshary & Grutter 2006). The cleaners learned to feed against their preference on artificial clients if that allowed them to access additional artificial clients, which would translate into cooperatively eating ectoparasites rather than cheating by eating client mucus under natural conditions (Haley & Fessler 2005). Here we show that cleaners immediately increase current levels of cooperation in the presence of bystander client reef fish. Furthermore, we find that bystanders respond to any occurrence of cleaners cheating their current client with avoidance. In conclusion, the results demonstrate, for the first time, that image scoring by an audience indeed leads to increased levels of cooperation in a non-human animal.

Introduction

A large body of recent literature demonstrates the great importance that humans give to their reputation or how their image is scored by others. For example, subtle cues indicating that a person is being watched, i.e., has an audience, lead to increases in levels of cooperation (Haley & Fessler 2005; Bateson et al. 2006). In economic experiments, humans benefit from helping others if this behaviour is known to future interaction partners (Wedekind & Milinski 2000), as a result of an increase in their image score that leads to indirect reciprocity (Nowak & Sigmund 1998). The concept of indirect reciprocity (“give and you shall be given to”) may even help to explain why people contribute to public goods: contributors receive more help in other contexts than non-contributors (Milinski et al. 2002; Semmann et al. 2004).

Evidence for indirect reciprocity based on image scoring is currently restricted to humans and, as far as we are aware, is not suspected in other species. A simpler scenario consists of self-serving image scoring, where bystanders directly benefit from choosing a cooperative partner for mutually beneficial interactions. The right choice can be based on information about how potential partners have behaved toward third parties. This form of cooperation is also known as “indirect pseudoreciprocity” (Bergmüller et al. 2007) and “social prestige” (Zahavi 1995; Roberts 1998; Lotem et al. 2003). As a consequence of the image scoring, individuals should increase levels of cooperation in the presence of potential partners in order to increase the probability of being chosen as a cooperative partner. Such adjustments of behaviour to the presence of bystanders are called audience effects (McGregor 2005).

Marine cleaning mutualism involving the cleaner fish *Labroides dimidiatus* appears to be a prime candidate for the occurrence of self-serving image scoring and audience effects. In this mutualism, cleaners remove ectoparasites from visiting reef fish called “clients”. Conflict arises because cleaners prefer the clients’ protective layer of mucus to ectoparasites, where eating the former constitutes “cheating” (Grutter & Bshary 2003). Therefore, clients have to make cleaners feed against their preference in order to receive a good service. Cleaners have more than 2’000 interactions per day (Grutter 1996). As a consequence, many interactions take place in the presence of bystanders who could potentially become the next client (Bshary & D’Souza 2005).

Field observations and laboratory experiments, involving in part artificial clients, support the idea that bystander clients prefer to invite inspections from cooperative cleaners and that cleaners are more cooperative in the presence of bystanders (Bshary 2002; Bshary & D’Souza 2005; Bshary & Grutter 2006). However, previous experimental evidence is rather indirect, because it is based on the cleaners’ ability to eat less-preferred food items off a plate in order to gain access to a second “image scoring” bystander plate, which would otherwise have been removed by the experimenter (Bshary & Grutter 2006). This approach leaves open the possibility that cleaners learned to solve an optimal foraging task that any species might be able to learn, contrary to the interpretation in Bshary and Grutter (2006) that cleaners learned to solve the task only because of its similarity to real-life interactions with client reef fish. Furthermore, although clients in the laboratory apparently used the duration of interactions with an artificial client as a criterion for the image score attributed to cleaners (Bshary & Grutter 2006), that does not necessarily mean that clients use this cue under natural conditions. In nature, clients often do not wait for inspection (Bshary & Schäffer 2002) and hence cannot gain much information on cleaning duration. Therefore, cheating behaviour seems to be a more appropriate candidate for image score determination. In the laboratory experiment (Bshary and Grutter 2006), this parameter could not be investigated, because cleaners invariably interacted “cooperatively” – their foraging behaviour could not cause any conflicts with the artificial client.

Therefore, in the current study, all interactions took place between real fish. We investigated the relative importance of cleaning duration and the occurrence of client “jolts” in response to cleaner wrasse mouth contact for a bystander’s decision to invite inspection. A jolt, i.e., an involuntary short twitch of the client’s body in response to some cleaner fish mouth contacts, is an established correlate of cheating by cleaners (Bshary & Grutter 2002; Soares et al. 2008). Furthermore, we tested whether cleaners spontaneously improved service quality to current clients if we experimentally introduced a bystander. If this was the case, we predicted that clients would jolt less frequently in the presence of bystanders.

Experimental Procedures

Experiments on image scoring were conducted in February and March 2008 and experiments on audience effects in July and August 2010 at the Lizard Island Research Station, Great Barrier Reef, Australia. All fish were wild caught from the surrounding reefs and released at their site of capture after the experiments. In total, we used 35 adult bluestreak cleaner wrasse (*Labroides dimidiatus*: total length 6.5 – 9.0 cm), 35 adult lined bristletooth (*Ctenochaetus striatus*: total length 12.0 – 22.0 cm) as clients, and 35 adult bridled monocle bream (*Scolopsis bilineatus*: total length 12.0 – 16.0 cm) as bystanders, caught with barrier nets and hand nets. Fish were fed commercial fish food (prawn and fish flakes) every day. All cleaners and bystanders were kept in aquaria of varying sizes (minimum size 50 x 30 x 25 cm) for a minimum of 7 days prior to experiments. The clients were kept for a minimum of 30 days in large tanks (1 m diameter) with mesh in order to keep monogenean flatworm eggs inside the system for reinfection of the fish.

Experiment for image scoring behavior of bystanders

Lined bristletooth were used as clients and bridled monocle bream as bystanders. Both are common clients of *Labroides dimidiatus* (Grutter 1995; Bshary 2001). Clients and bystanders were placed in their experimental aquaria 2 days before the experiment, with cleaners added to the client aquaria but separated from clients by a clear partition (Figure 1a). All aquaria had running seawater, and fish were provided with a PVC tube for shelter placed in the middle of each aquarium. Following established methods (Bshary & Grutter 2002), we created differences in cleaning duration and the cleaners' levels of cooperation by using parasitized and unparasitized clients. We removed the ectoparasites on half of the clients by placing them in a freshwater bath for 2 minutes and then brushing them with a paintbrush. The other half of the clients remained untreated and hence were parasitized. To further reduce the likelihood that cleaners would interact for significant amounts of time with the unparasitized clients during the experiment, we additionally allowed unparasitized clients (but not parasitized ones) to interact with cleaners for 6 hours by temporarily removing the clear partition.

A trial involved one bystander, one parasitized client and its cleaner, and one unparasitized client and its cleaner. We ran four parallel setups, with the position of the aquaria containing parasitized versus unparasitized clients counterbalanced. A trial started with the experimenter removing simultaneously the two cleaner partitions in the side aquaria that allowed cleaners and clients to interact with each other. The experimenter then left the room while three digital cameras recorded the behaviour of cleaners, clients, and bystanders for 10 minutes. We quantified the total time of the interactions in seconds and the number of jolts performed by clients. Interactions started when cleaners touched the client and ended when the cleaner or client swam away (i.e., movement not oriented toward the partner). As an indicator of the bystanders' choice, we measured the total amount of time in seconds that a bystander spent with its entire body outside its shelter near a cleaner-client pair. In nature, being close to a cleaner would typically lead to the cleaner starting to interact with the client. Hence, proximity appears to be a good proxy for the bystanders' choice.

Immediately after the trial, the bystander was replaced by another bystander, with the new individual being tested 2 days later with the same cleaner-client pairs. In between the two trials, cleaners and clients were separated by a clear partition. After the second trial, one round of the experiment on audience effects took place (see next section) before all fish were replaced. For the analysis, we calculated mean values for the two bystanders that shared the same cleaner-client pairs to avoid any pseudoreplication.

Experiment for Audience Effects of Cleaner Fish

A different set of fish was used in the audience experiment. Housing prior to the experiment was as described for the experiment on image scoring, and the same test aquaria were used (Figure 1b). Clients and bystanders were placed in their experimental aquaria 2 days before the experiment, with cleaners added to the client aquaria but separated from clients by a clear partition (Figure 1b). An opaque partition between the aquaria prevented visual contact between cleaner/client and bystander. All aquaria and tanks had running seawater, and fish were provided with a PVC tube for shelter placed in the middle of each aquarium. On the morning of an experiment, a second opaque partition was introduced in the middle of the bystander aquarium. The side facing the cleaner-client aquarium always contained a shelter but contained a bystander in only half of the experiments. For the experiment, both the cleaner partition that separated client and cleaner and the other opaque partition between the aquaria were removed. Thus, cleaner and client always saw half of the neighbouring aquarium and the shelter inside but only saw a bystander in half of the trials (Figure 1b). The experimenter then left the room while two digital cameras filmed the interactions. After 10 minutes, the experimenter reentered the room and put all partitions back into place. The bystander was then moved to the other compartment. After 60 minutes, the cleaner-client pair was then exposed to this new condition. The sequence of conditions was counterbalanced over all pairs. We quantified the total time that fish spent interacting and clients' jolts in both conditions in a matched-pair design. In addition, we quantified the visible number of times that a cleaner touched the client with its mouth (when the cleaner was in front or on the side of the client rather than behind from the camera's perspective) and determined the percentage of mouth contacts that caused jolts, again in a matched-pair design.

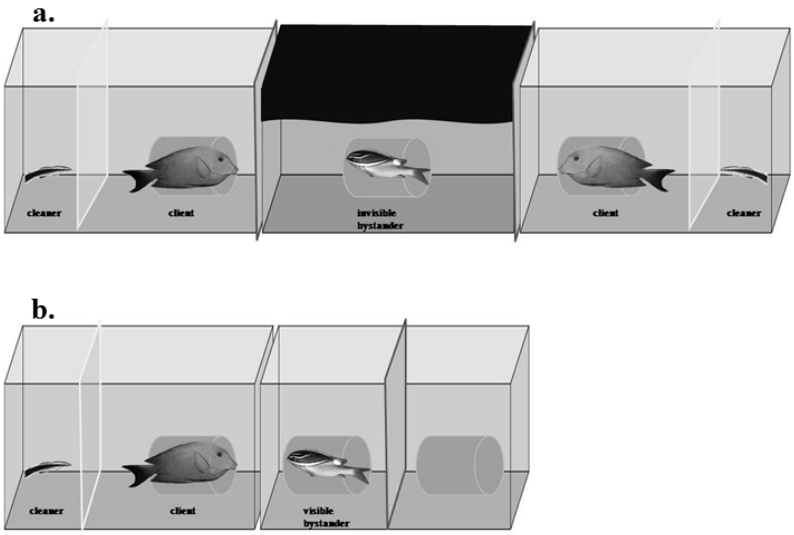

Figure 1 | Experimental Setup to Test for Image Scoring Behavior of Bystanders and Audience Effects of Cleaner Fish (a) A bystander in the central aquarium could observe and approach, behind one-way mirrors, a cleaner-parasitized client pair and a cleaner-unparasitized client pair in adjacent aquaria with the same dimensions (90 x 38 x 38 cm). PVC tubes were provided for fish to use as shelters. The clear partitions in the cleaner-client aquaria depicted in the figure were removed during trials. (b) For the audience effects, new individuals were used and cleaners interacted with their clients once with the bystander behind the opaque partition and once with the bystander visible. An opaque partition (not shown) was placed between the aquaria between trials to block visual contact.

Results

In the first experiment, we tested which cues client fish may use for the decision to approach or to avoid a cleaner. A bystander client in a central aquarium could observe through one-way mirrors in one adjacent aquarium a cleaner exposed to a parasitized client ("parasite treatment") and on the other side a cleaner exposed to an unparasitized client ("control treatment") (Figure 1a). The same ten cleaner and parasitized client pairs and ten cleaner and unparasitized client pairs were exposed to two different bystanders. To avoid pseudoreplication, we calculated mean values for the two tests that involved the same cleaner and client pairs.

We found that cleaners interacted about 17 times more with parasitized (median = 31 s) than with unparasitized clients (median = 0 s) (Mann-Whitney U test, $m = 10$, $n= 10$, $z = -3.1$, $p = 0.001$). However, there was strong variation among individual cleaners in both experimental groups (parasitized clients, 2–432 s; unparasitized clients, 0–41 s). Also, as a consequence of longer interactions, parasitized clients jolted about five times more often than unparasitized clients (101 compared to 21 jolts in total). Therefore, we adopted a correlative approach to evaluate separately for parasitized and unparasitized clients how the time interacting, jolt rate, and total number of jolts were related to the choice behaviour of bystanders. The time that bystanders spent near the cleaner-parasitized client pairs was negatively correlated with the time that cleaners inspected parasitized clients (Spearman rank correlation, $n = 10$, $r = -0.88$, $p = 0.001$; Figure 2). This result is the opposite of the previous study involving artificial clients (Bshary & Grutter 2006). The negative correlation was apparently due to bystanders avoiding cleaners that produced many jolts in their clients (Spearman rank correlation, $n = 10$, $r = -0.74$, $p = 0.014$; Figure 2b) because the number of jolts correlated positively with time spent interacting (Spearman rank correlation, $n = 10$, $r = 0.92$, $p < 0.001$; Figure 2d). In contrast, the bystanders' choice was not significantly correlated with jolt rate as a measure of the cleaners' actual level of cooperation (Spearman rank correlation, $n = 10$, $r = -0.17$, $p = 0.64$; Figure 2c). Jolts were rarely followed by the client fleeing or chasing the cleaner (3 occurrences out of a total of 101 jolts). Therefore, it appears that the mere occurrence of jolts is sufficient as a cue for the bystanders' decision making.

The course of interactions involving cleaners and unparasitized clients did not significantly affect bystander choices (Spearman rank correlations, interaction duration and bystander near control cleaner: n = 10, r = -0.54, p = 0.11; absolute number of jolts and bystander near control cleaner: n = 10, r = 0.03, p = 0.94; jolt rate and bystander near control cleaner: n = 10, r = -0.16, p = 0.65; number of client jolts and interaction duration: n = 7, r = -0.08, p = 0.87; Figure 3).

In the second experiment, new cleaner and client pairs were tested in the presence or absence of a bystander in an adjacent aquarium (Figure 1b) to test whether this manipulation affected the levels of cooperation of the cleaner fish. The duration of the cleaning interactions of the pairs did not differ according to whether they were in the presence or absence of bystanders (Wilcoxon test, n = 15, z = -0.17, p = 0.87; Figure 4a). In contrast, the client jolt frequency was lower when bystanders were present than when they were absent (Wilcoxon test, n = 14, z = -2.73, p = 0.006; Figure 4b). The lower jolt frequency was due to the cleaners feeding more cooperatively rather than feeding less frequently, because the number of jolts relative to all mouth contacts was significantly lower when bystanders were present than when they were absent (Wilcoxon test, n = 14, z = -2.12, p = 0.034; Figure 4c).

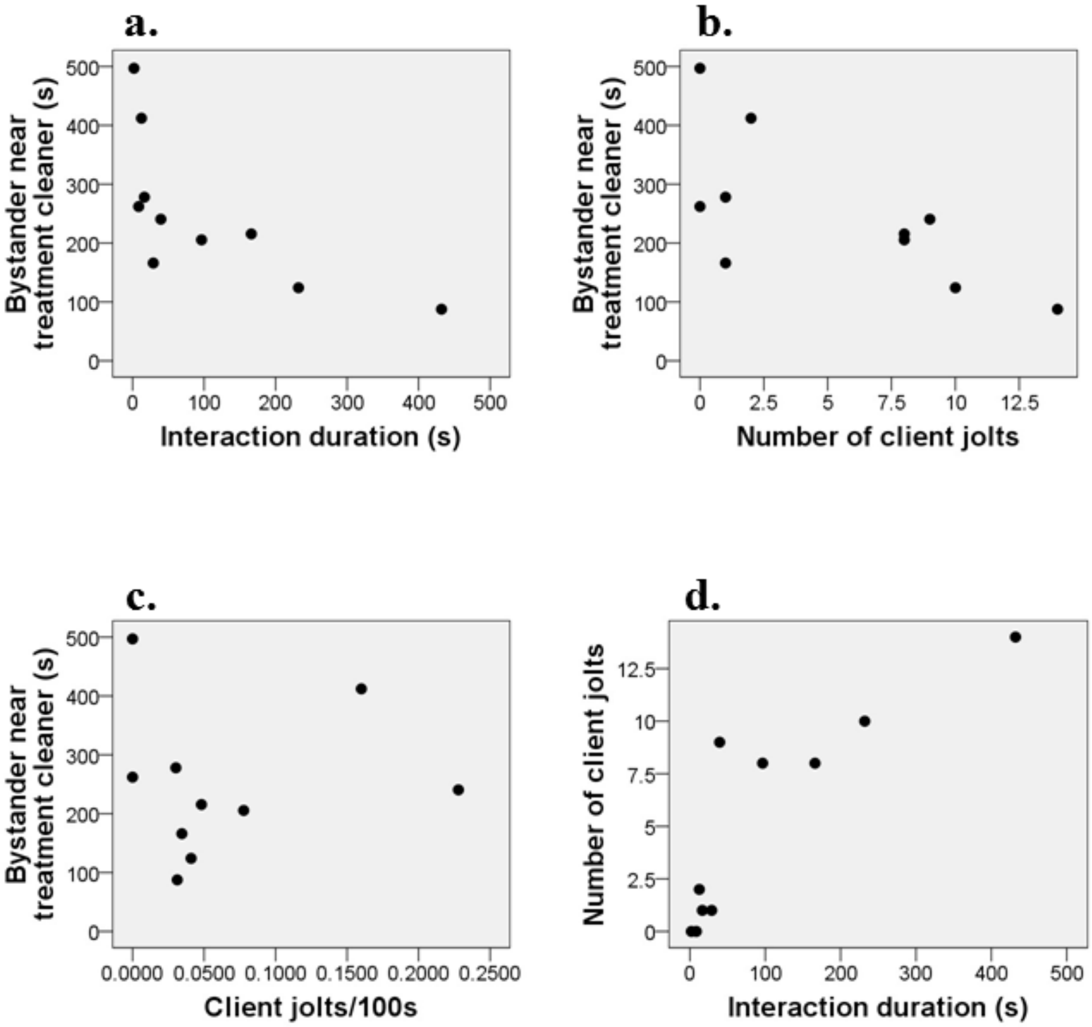

Figure 2 | Image Scoring Rules of Bystanders for Interactions with Parasitized Clients (a–c) Duration (in seconds) that the bystander spent near the parasitized client's aquarium relative to the duration that the cleaner spent interacting with the client (a), the absolute number of jolts by the client (b), and the jolt rate performed by the client (number of jolts per 100 s of interaction with client) (c). (d) Number of client jolts relative to the duration that the cleaner spent interacting with the client. Each point represents mean values of two bystanders being exposed to the same cleaner-client pairs.

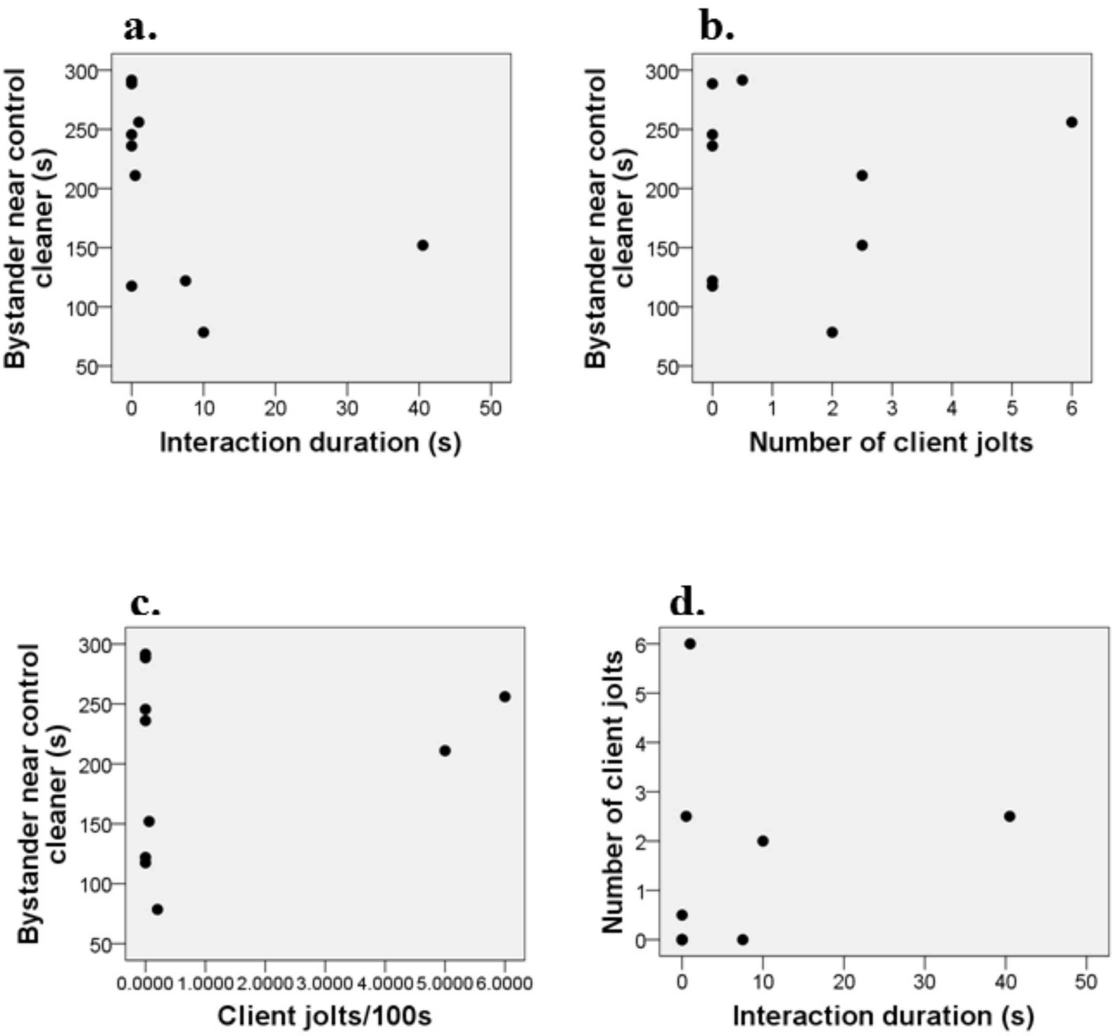

Figure 3 | Image Scoring Rules of Bystanders for Interactions with Unparasitized Clients (a–c) Duration (in seconds) that the bystander spent near the unparasitized client's aquarium relative to the duration that the cleaner spent interacting with the client (a), the absolute number of jolts by the client (b), and the jolt rate performed by the client (number of jolts per 100 s of interaction with client) (c). (d) Number of client jolts relative to duration that the cleaner spent interacting with the client. Three cleaner-client pairs never interacted, which explains why n = 7 in (d).

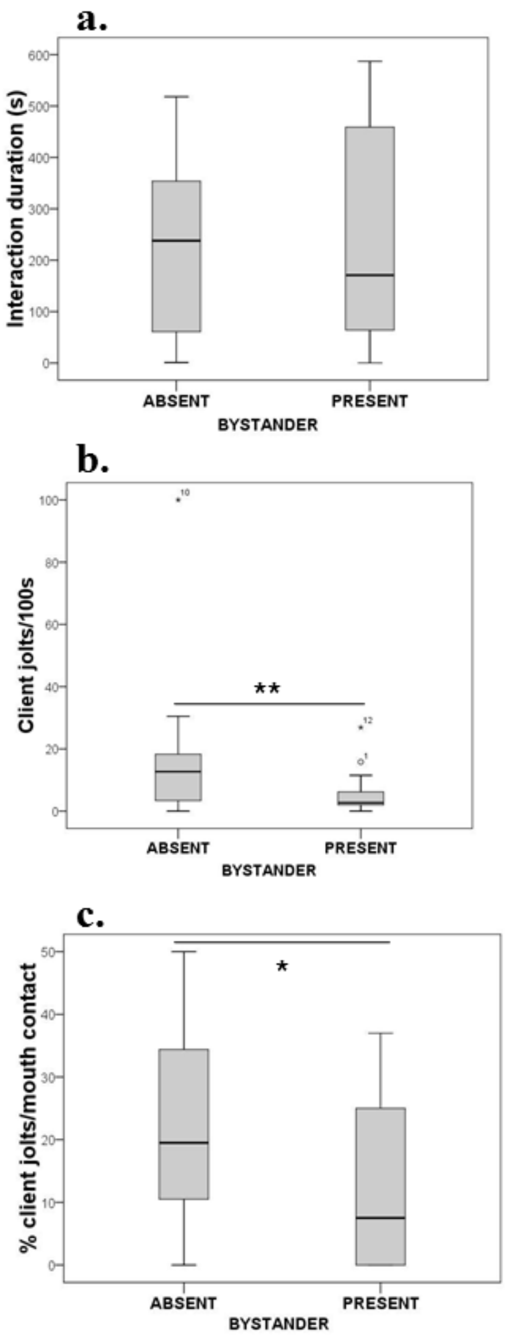

Figure 4 | Cleaner Wrasses Increase Cooperation with an Audience
Behaviours of cleaners in the absence (absent) or presence (present) of a bystander. Data are presented as box-and-whisker plots with boxes representing medians and interquartiles; error bars are 10^{th} and 90^{th} percentiles, and points are outliers. *$p < 0.05$; **$p<0.01$. (a) Duration (in seconds) that the cleaner spent interacting with the client. (b) Number of jolts (per 100 s) by client. (c) Percentage of jolts relative to all cleaner mouth contacts with the client.

Discussion

Although our results on image scoring generally confirm the previous conclusion that clients pay attention to cleaners' behaviour (Bshary & Grutter 2006), they also demonstrate that client models as used previously were not sufficient for determining what cues clients use under natural circumstances. Bystanders do not seem to prefer interacting over non-interacting cleaners per se but avoid interacting cleaners that exhibit cheating behaviour. Such a decision rule makes sense because under natural conditions, bystanders usually only see the end of an on-going interaction (Bshary & Schäffer 2002). Under these circumstances, bystanders cannot calculate cheating rates to assess a cleaner's level of cooperation, whereas it is easy to note whether a conflict occurs during the actual time that they observe cleaning.

Our experiment on audience effects provides, for the first time, conclusive evidence that an animal increases levels of cooperation as a result of the presence of bystanders. Cleaners showed such adjustment immediately, which precludes any learning during the experiments. Nevertheless, it could be that cleaners learned to behave this way in nature before they were caught. Cleaners may have more than 2'000 interactions per day (Grutter 1995, 1996), offering ample opportunities for operant associative learning (Thorndike 1917). Therefore, the importance of learning in order to produce adaptive behaviour remains a major open question in this system. Other important future questions are whether bystanders incorporate the current client's identity in their image scoring and how well cleaners may be able to fine tune current levels of cooperation depending on the bystanders' identity. For example, cleaners are very cooperative with predators (Bshary 2001), and hence little information can be gained from observations of interactions involving predators, whereas such information can be obtained if cleaners interact with non-predatory clients. From the cleaners' perspective, we note that they interact with a large number of species that differ with respect to size, parasite load, and mucus quality. Therefore, cleaners should pay selectively more attention to their image score if bystanders are an attractive food source, i.e., if they are large, highly parasitized, and covered with high-quality mucus. This is because the increased probability of access to an attractive food source would be more likely to offset the immediate reduction in payoffs due to increased levels of cooperation by the cleaner.

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Chapter Two | Cleaner wrasse *Labroides dimidiatus* adjust audience effect according to the stakes

Chapter to be published in *Current Biology*

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Abstract

Humans cooperate more in the presence of an audience to increase their image score, an adaptive strategy that has also been described in fish (Nowak & Sigmund 1998; Wedekind & Milinski 2000; Kurzban 2001; Milinski et al. 2002b; Andreoni & Petrie 2004; Semmann et al. 2004; Soetevent 2005; Barclay & Willer 2007; Bshary & Grutter 2006; Pinto et al. 2011; Desjardins et al. 2012). A hitherto untested aspect is that individuals should fine-tune levels of cooperation to the quality of both current partner and bystanders. Similar effects have been described in competitive situations in a variety of experiments on communication networks in birds and mammals (McGregor 2005), including sophisticated triadic awareness in chimpanzees (Slocombe & Zuberbühler 2007; Laporte & Zuberbühler 2010). Here we show such strategic adjustments in the context of cooperation in cleaner fish *Labroides dimidiatus*. Cleaner wrasses remove ectoparasites from visiting client reef fish but a conflict occurs as cleaners prefer to eat client mucus, which constitutes cheating. In the first experiment we used Plexiglas plates as artificial clients. Cleaners were challenged to eat against their preference on a first plate in order to gain access to a second bystander plate. They did so only if the bystander plate offered more food than the initial plate. Using four different real client species of differing attractivity and strategic options we confirmed that cleaners spontaneously adjust service quality to both current client and bystander identity. In conclusion, cleaners are able to fine-tune levels of cooperation to manage their social prestige in a way that has not even been demonstrated for humans so far.

Introduction

There is ample empirical evidence that humans seem to universally care about their image score or social prestige and therefore increase their levels of cooperation when in presence of an audience (Roberts 1998; Wedekind & Milinski 2000; Kurzban 2001; Milinski et al. 2002 a, b; Andreoni & Petrie 2004; Barclay 2004; Semmann et al. 2004; Soetevent 2005; Barclay & Willer 2007; Sylwester & Roberts 2010; 2013). The importance of social prestige is so strong that individuals even respond to subconscious cues like eye images or pictures of faces under various circumstances (Haley & Fessler 2005; Bateson et al. 2006; Rigdon et al. 2009; Ernest-Jones et al. 2011; Keller & Pfattheicher 2011; Oda et al. 2011; Francey & Bergmüller 2012; Powell et al. 2012; Nettle et al. 2013; Panagopoulos 2014a, 2014b; but see Fehr & Schneider 2010 and Raihani & Bshary 2012). In other animals, evidence for audience effects in the cooperative context is currently rather limited (Pinto et al. 2011; Desjardins et al. 2012). Nevertheless, such effects may be expected to be more widespread based on communication network experiments in competitive contexts, which rely on subjects' variable response to different observations to show the existence of a network in the first place. Thus, in wild chimpanzees was demonstrated that victims not only adjust the acoustic structure of their screams depending on the level of aggression experienced but also seem to exaggerate the real level of aggression if there is at least one bystander of equal or higher ranking in comparison to the aggressor (termed "triadic awareness": Slocombe & Zuberbühler 2007). In addition, they attenuate vocalizations depending on the audience and accordingly pant grunt in greeting only to individuals with a higher ranking (Laporte & Zuberbühler 2010).

Nevertheless, the many studies on reputation in humans are either set up in a way that all players are equal with respect to their strategic options or they investigate the effect of subconscious stimuli (Pollock & Dugatkin 1992; Nowak & Sigmund 1998; Haidt 2001; DeBruine 2002; Milinski et al. 2002a; Hagen & Hammerstein 2006). Also the models typically assume equality between players (Nowak & Sigmund 1998, 2005; Leimar & Hammerstein 2001; Ohtsuki & Iwasa 2006; Roberts 2008; Barclay 2011; Ghang & Nowak 2015). Alternatively, it is investigated how differences in state affect the signalling, i.e. the cooperative investment (Roberts 1998; Lotem et al. 2003; Sylwester & Roberts 2010, 2013). However, one would expect that in real life, individuals often differ in many ways, for example with respect to their quality as a partner. As soon as there are inequalities between individuals, a more sophisticated cooperative strategy should integrate the identity of the audience to choose the appropriate level of cooperation. A considerable amount of social research suggests an individual's initial perception and reaction to another individual depends on the other's attractiveness (Baker & Churchill 1977). In the presence of bystanders, individuals might be able to increase their payoff by exaggerating signals beyond their means (cheating) or investing to help others despite considerable costs. In doing so, individuals can gain immediate benefits by manipulating (or helping) individuals with whom they are currently interacting and delayed benefits by convincing bystanders that they are more cooperative than perhaps is warranted.

The mutualism involving the cleaner wrasse *Labroides dimidiatus* is an ideal model to test in the context of cooperation audience effects with differing bystanders' identity as cleaners have more than 2000 interactions per day and therefore interact up to 500 different species (Grutter 1995, 1997). Hence, they interact with a large number of species that differ with respect to size, parasite load and mucus quality.

Clients regularly visit cleaners at their small territories ("cleaning stations") in order to have their ectoparasites removed (Losey et al. 1999; Côté 2000). While several studies suggest that clients indeed benefit from interactions with cleaners (Grutter 1996, 1999; Grutter & Lester 2002; Cheney & Côté 2003; Bergmüller et al. 2007; Ros et al. 2011; Waldie et al. 2011), it is also evident that there is a main conflict between cleaners and clients over what cleaners should eat. *L. dimidiatus* feeds largely on parasitic gnathiid isopods (Grutter 1996) nevertheless cleaners prefer mucus of their clients (Grutter 1997; Grutter & Bshary 2003). Client jolts in response to cleaner fish mouth contact correlates with mucus feeding by cleaners and hence indicate cheating (Bshary & Grutter 2002). Clients have different mechanisms to ensure cooperative behaviour by cleaners during interactions (Bshary 2001; Bshary & Würth 2001; Bshary & Grutter 2002; Bshary & Schäffer 2002), one of which is image scoring (Bshary 2002; Bshary & Grutter 2006). Clients arriving at a cleaning station pay attention to ongoing interactions and are more likely to invite inspection if they observe an interaction without conflict than if they observe a conflict (Bshary 2002). As a consequence of this image scoring by clients, cleaners are more cooperative towards current clients in the presence of bystanders (Bshary 2002, Bshary & Grutter 2006; Pinto et al. 2011).

Therefore, in the current study, we investigated whether the audience effects are fixed behavioural patterns or whether cleaners can fine-tune their cooperative behaviour in response to experimentally introduced different bystanders either with artificial or with real clients. While there is no explicit model exploring these conditions, using the logic from optimal foraging theory we predict that they should increase current service quality in particular if the bystander is an attractive food source relative to the current client. The hypothesis was supported by early field observations which suggested that cleaners treat current clients particularly well if the bystander is a visitor species that could easily leave and interact with another cleaner instead (Bshary & D’Souza 2005). In the artificial experiment we varied plate size and accordingly the amount of food on them. We created a high value and a low value client, and varied who would be the current client and who the bystander. Furthermore, we tested cleaners in 6 situations based on three client species pairs. Large versus small resident clients as equivalent to the plate experiments, large resident with no choice option versus medium visitor species with choice option (emphasis on choice rather than quality as a food source) and finally a large and a medium visitor (differences in client value but not choice).

Based on previous research, we expected client species to affect service quality (Bshary 2001) and that service quality is higher in the presence of an audience (Pinto et al. 2011). Furthermore, we make the following predictions. a) If relative quality as a food patch plays a role we predict that cleaners will act more cooperatively towards their current clients if the bystander is more attractive than the current client. b) If absolute value plays a role we predict that the strength of the audience effect will correlate with the quality/size of the bystander species, thus increasing from damsels to breams to surgeons to thicklips as bystanders. Finally, c) if client/bystander choice options play a role we predict that cleaners will show higher cooperative levels to their current clients when in the presence of a visitor bystander, i.e. when in the presence of breams or thicklips.

Experimental Procedures

Experiments on fine-tuned audience effects using Plexiglas plates and real clients were conducted, respectively, from May to August 2006 at the lab of the University of Neuchâtel, Switzerland and from July to August 2010 at the Lizard Island Research Station, Great Barrier Reef, Australia. In Switzerland, cleaner fish were wild caught from the Island of Luzon (near Legaspi), Philippines and sent to the University of Neuchâtel in February 2006. In Australia, all fish were wild caught from the surrounding reefs with barrier and hand nets and released at their site of capture after the experiments. Fish were fed daily with mashed prawn and a mixture of mashed prawn and fish flakes (“flakes”). The later was prepared every day with two third volume of prawn and one third volume of flakes and was then spread on Plexiglas plates [Bshary & Grutter 2005]. Cleaners learned to feed from the plates within one to three days of exposure. Cleaners, bridled monocle breams and staghorn damsels clients, were kept in aquaria of varying sizes (minimum size: 50 x 30 x 25 cm) for a minimum of 7 days prior to experiments. Lined bristletooth surgeonfish and thicklip wrasse clients were kept for a minimum of 30 days in 1 m diameter plastic tanks.

Cleaners’ fine-tuned audience effects with Plexiglas plates

Each aquarium had a commercial aquarium heater (Eheim, Jäger 125 W for 200 l tanks) and an air supply. Across all aquaria (100 x 50 x 40 cm) the water condition was kept analogous and was pumped via flow through system from a larger cleaning tank (160 x 80 x 60 cm) holding pieces of hard coral *Scleractinia* serving as natural filter. Water salinity was kept at a definite gravity of 1.025 ± 0.005 at a temperature of 25 ± 1 °C, the nitrite concentration was always below 0.3 mg/l and the pH ranged between 8.1 and 8.4. Fractional water changes were accomplished monthly with a commercial salt water mixture *Aquamedic*. All cleaners were provided with a PVC tube (2.5 cm diameter by 10 cm long) for shelter placed in the middle of each aquarium.

For this experiment, we used 21 adult bluestreak cleaner wrasses. Cleaners had already completed the initial training sessions that involved the basic principles of the current study, i.e. that eating flakes was allowed but eating prawn would lead to the removal of the Plexiglas plate. More specifically, they had already been exposed to plates attached to levers so that they could be introduced and removed from the aquarium without major disturbance. The Plexiglas plates were all grey and had two different sizes: a small (5 x 10 cm) and a large (7 x 14 cm). On the smaller plate two items of food were offered (one prawn and one flake item) and the cleaner was allowed to eat just one of them. On the larger plate there were six items of food (three prawn and three flake items) and cleaners could continue to eat as long as they ate flakes, and up to a maximum of three items. Cleaners could choose until three possible items, depending on the first choices. Cleaners prefer client mucus over ectoparasites [Grutter & Bshary 2003] and prawn over flakes [Bshary & Grutter 2005]. Therefore, prawn items were used as the equivalent of mucus, and a cleaner that ate a prawn item was considered as having cheated, leading to the immediate removal of the plate by the experimenter. The cleaners were confronted with four differing situations as shown in Figure 1. In two situations only one plate was present. In the other two situations, one plate was immediately accessible while the "bystander plate" was waiting behind a transparent partition according to how the cleaner behave to the first plate. In these image scoring situations, cleaners only gain access to the second plate if they ate only flakes on the first plate (see Bshary & Grutter 2006). Otherwise the interacting plate was removed along with the "bystander" plate. To prevent cooperative cleaners to continue foraging on the interacting plate, it was turned against the wall of the aquarium after the removal of all flake items so that the cleaners could not eat remaining prawn items off the plate. We conducted 48 trials in each of the four experimental conditions, starting at 8 am and the order was counterbalanced between the 21 subjects. All tests were performed with SPSS version 14.0 and all results are two-tailed.

Cleaners' fine-tuned audience effects with real clients

We used 15 adult bluestreak cleaner wrasses (*Labroides dimidiatus*: 6.1-7.9 cm total length, further abbreviated TL). All the four species of fish used in this experiment are common clients of *L. dimidiatus* [Grutter 1995; Bshary 2001]. The staghorn damsel (*Amblyglyphidodon curacao*, further called "damsel") was chosen as a small resident (a species with access to only one cleaning station). Its body length (mean/SD = 5.2 cm/±1.33; min/max 3.2/7.4 cm TL) was significantly shorter than that of the lined bristletooth surgeonfish (*Ctenochaetus striatus*, further called "surgeon") with its mean 14.54 cm ±1.88SD, min/max 11.8/18.2 cm TL (Mann-Whitney-U Test, n=46, z=-5.45, p<0.0001). The surgeon was chosen as a large resident client based on information from the Red Sea [Bshary 2001. Furthermore, we had two visitor species. The bridle monocle bream (*Scolopsis bilineatus*, further called “bream”) is a relatively small visitor species where our caught individuals (mean 13.3 cm ±1.07SD, min/max 11.5/14.8 cm TL) were significantly smaller than the surgeons (Mann-Whitney-U Test, n=62, z=-2.54, p=0.011). Finally, the thicklip wrasse (*Hemigymnus melapterus*, further called “thicklip”; mean size = 18.4 ± 2.42SD, Min/max 14.2/21.3 cm TL) is of relative large size compared to both surgeon and bream (Mann-Whitney-U Tests, both n=46, z=-5.2 and -4.23, both p<0.0001). Each cleaner was exposed to the 15 individuals of the four client species in a counterbalanced way. Overall, we tested cleaners with six combinations of species (name of client species first, name of bystander species second): a) surgeon – bream, b) bream – surgeon, c) surgeon – damsel, d) damsel – surgeon, e) bream – thicklip, and f) thicklip – bream. The data on surgeon – bream are identical to the data published in Pinto et al. (2011) while all other combinations are new. Cleaners did not always interact with clients during our ten minute trials. Due to the described matched pair design of our study. This led to the removal of some data points, leaving us with 9 damsels with surgeons as bystanders, 15/14 surgeons with damsels/breams as bystanders, 15/12 breams with surgeons/thicklips as bystanders, and 13 thicklips with breams as bystanders.

Cleaners and clients were placed in their experimental aquaria 2 days before the experiment, with cleaners added to the client aquaria but physically separated from clients by a clear partition. An opaque partition between the aquaria prevented visual contact between cleaner/client and bystander. All aquaria (90 x 38 x 38 cm) had running seawater, and fish were provided with a PVC tube (16 cm diameter by 20 cm long for clients and bystanders, and 1 cm diameter by 10 cm long for cleaners) for shelter placed in the middle of each aquarium. On the morning of an experiment, a second opaque partition was introduced in the middle of the bystander aquarium. The side facing the cleaner-client aquarium always contained a shelter but contained a bystander in only half of experiments. For the experiment, both the clear partition that separated client and cleaner and the other opaque partition between the aquaria were removed. Thus, cleaner and client always saw half of the neighbouring aquarium and the shelter inside but only saw a bystander in half of the trials (Figure 2). The experimenter then left the room while two digital cameras filmed the interactions. After

10 min, the experimenter reentered the room and put all partitions back into place. The bystander was then moved to the other compartment. After 60 min, the cleaner-client pair was then exposed to this new condition. The sequence of conditions was counterbalanced over all pairs. We quantified the total time that fish spent interacting and clients' jolts in both conditions in a matched-pair design. In addition, we quantified the visible number of times that a cleaner touched the client with its mouth (when the cleaner was in front or on the side of the client rather than behind from the camera's perspective) and determined the percentage of mouth contacts that caused jolts, again in a matched-pair design. All tests were performed with R version 3.1.1. and all results are two-tailed.

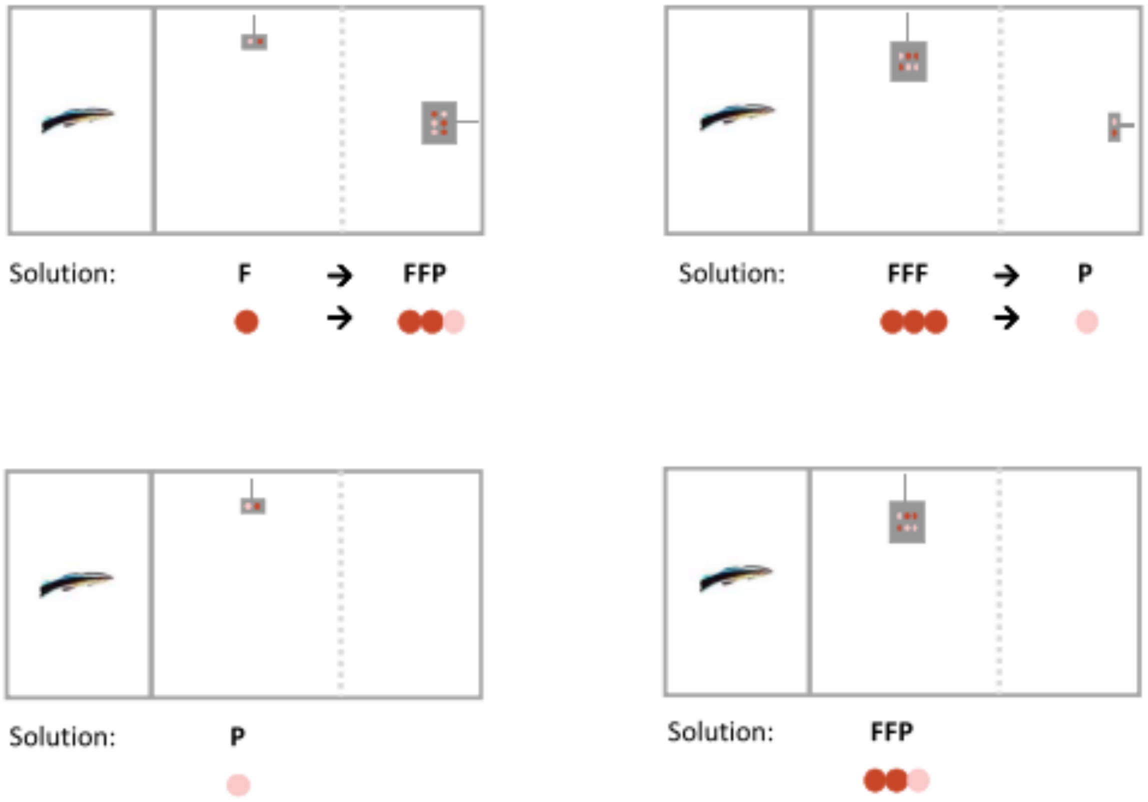

Figure 1 | Experimental Setup to Test Fine-Tuned Audience Effects with Plexiglas Plates Two Plexiglas plates of different size with differing amounts of food on them were presented to cleaners either alone (control) or in the presence of the other plate (treatment). Plates contained preferred prawn items (mimicking client mucus) and less preferred flake items (mimicking client ectoparasites) and were removed immediately in response to cleaners eating prawn. In the "image scoring treatment", cleaners could gain access to the "bystander plate" by eating only the less preferred food off the accessible plate. Below each situation there is the optimal solution for a maximal foraging success.

Cleaners and clients were placed in their experimental aquaria 2 days before the experiment, with cleaners added to the client aquaria but physically separated from clients by a clear partition. An opaque partition between the aquaria prevented visual contact between cleaner/client and bystander. All aquaria (90 x 38 x 38 cm) had running seawater, and fish were provided with a PVC tube (16 cm diameter by 20 cm long for clients and bystanders, and 1 cm diameter by 10 cm long for cleaners) for shelter placed in the middle of each aquarium. On the morning of an experiment, a second opaque partition was introduced in the middle of the bystander aquarium. The side facing the cleaner-client aquarium always contained a shelter but contained a bystander in only half of experiments. For the experiment, both the clear partition that separated client and cleaner and the other opaque partition between the aquaria were removed. Thus, cleaner and client always saw half of the neighbouring aquarium and the shelter inside but only saw a bystander in half of the trials (Figure 2). The experimenter then left the room while two digital cameras filmed the interactions. After 10 min, the experimenter reentered the room and put all partitions back into place. The bystander was then moved to the other compartment. After 60 min, the cleaner-client pair was then exposed to this new condition. The sequence of conditions was counterbalanced over all pairs. We quantified the total time that fish spent interacting and clients' jolts in both conditions in a matched-pair design. In addition, we quantified the visible number of times that a cleaner touched the client with its mouth (when the cleaner was in front or on the side of the client rather than behind from the camera's perspective) and determined the percentage of mouth contacts that caused jolts, again in a matched-pair design. All tests were performed with R version 3.1.1. and all results are two-tailed.

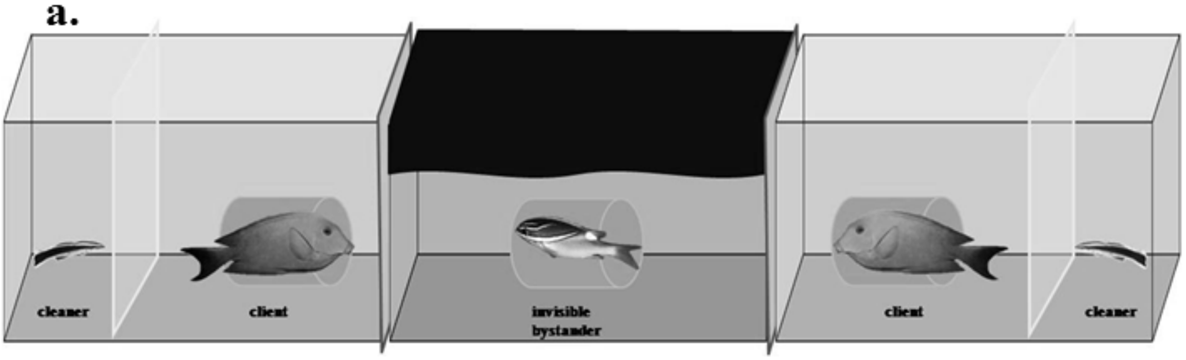

Figure 2 | Experimental Setup to Test for Image Scoring Behavior of Bystanders A bystander in the central aquarium could observe and approach, behind one-way mirrors, a cleaner-parasitized client pair and a cleaner-unparasitized client pair in adjacent aquaria with the same dimensions (90 x 38 x 38 cm). PVC tubes were provided for fish to use as shelters. The clear partitions in the cleaner-client aquaria depicted in the figure were removed during trials.

Results

Cleaners' fine-tuned audience effects with Plexiglas plates

In the first day of experiments the cleaners treated the small plate and respectively the large plate very similar no matter whether presented with a bystander plate or when presented alone (Figure 3). In the last session however, results show that the cleaners learned to discriminate the different treatments (Friedman test, $n = 21$, $X^2 = 26.8$, $p < 0.001$). Cleaners ate more against their preference on the small plate if there was a large bystander plate than if the small plate was offered alone (post-hoc multiple comparisons, $p < 0.001$, Figure 3). In addition, cleaners ate more against their preference in the image scoring situations if this allowed them to gain access to a large bystander plate than if this allowed them to gain access to a small bystander plate (post-hoc multiple comparisons, $p < 0.001$, Figure 3).

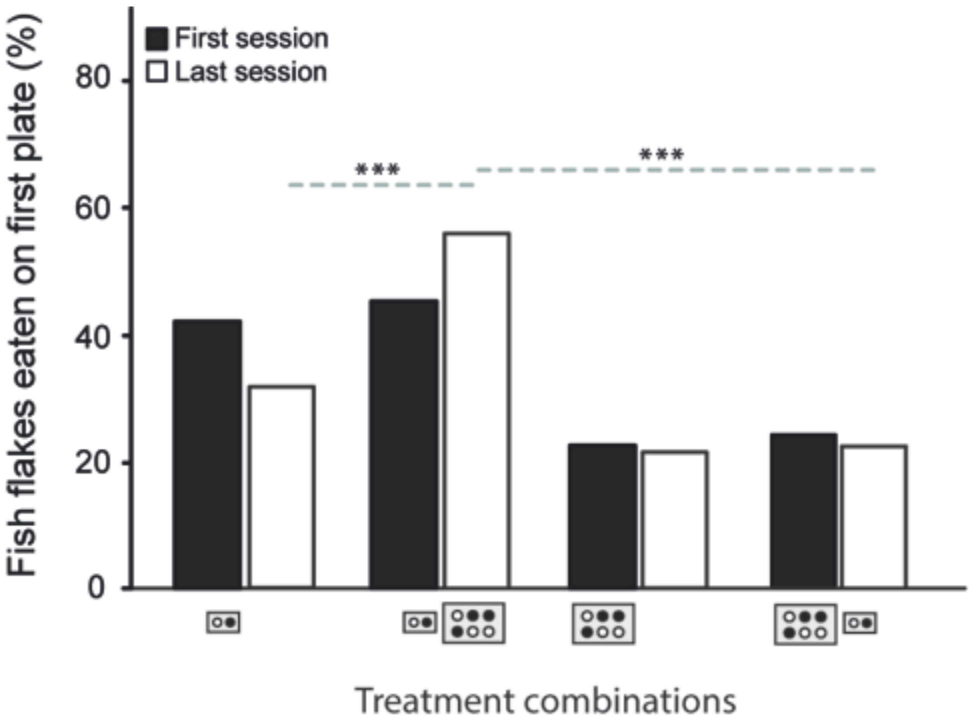

Figure 3 | Results of Fine-Tuned Audience Effects with Plexiglas Plates. Percentage of fish flakes (less preferred food) eaten on the first plate (central compartment) by the cleaner fish in four different treatments (with or without "bystander Plexiglas plate". Black colour represents the first session (6 trials) of experiments and white represents the last session of the experiments.

Cleaners' fine-tuned audience effects with real clients

In the second experiment, cleaners were tested in the presence or absence of different bystanders in an adjacent aquarium (Figure 2) to test whether different audiences affected the levels of cooperation of the cleaner fish. We calculated generalised linear mixed models with repeated measures to assess the effects of our treatment on client jolt rate, duration of the interactions and on cleaners providing tactile stimulation. Data were Poisson-transformed to meet assumptions of a normal distribution. There was no significant difference on the duration of the interactions according to whether cleaners were in the presence or absence of bystanders ($F_{2,113}$ = 0.58, P = 0.56; Figure 4), no matter the clients' or bystanders' identity. In contrast, the client jolt frequency was lower when bystanders were present than when they were absent ($F_{1,94}$ = 7.93 P = 0.0059; Figure 5). This result was mostly driven by the situation in which the breams were bystanders of surgeonfish as this combination yielded the only significant effect by itself (Wilcoxon-Test: n=14, z=2.73, p=0.006, see Pinto et al. 2011). Of the other combinations, the surgeons as bystanders of damsels yielded a trend (Wilcoxon Test: n=9, z=1.69, p=0.091) while all other combinations were far from being significant (Wilcoxon Tests: surgeons with damsels: n=15, z=0.34, p=0.73; breams with surgeons: n=15, z=0.60, p=0.55; thicklips with breams: n=13, z=1.41, p=0.16; breams with thicklips: n=12, z=1.02, p=0.31). The variation between combinations was such that we found significant effects of both client species and of bystander species on the cleaners' levels of cooperation (client species: $F_{3,36}$ = 7.35, P = 0.0006; bystander species: $F_{2,102}$ = 4.56, P = 0.013).

The observed lower jolt frequency was due to the cleaners feeding more cooperatively rather than feeding less frequently, because the number of jolts relative to all mouth contacts was significantly lower when bystanders were present than when they were absent ($F_{1,101}$ = 7.34, P = 0.008). The model also confirmed the effects of client species and bystander species identity on the cleaners' level of cooperation (client species: $F_{3,38}$ = 8.66, P = 0.0002; bystander species: $F_{2,109}$ = 4.26, P = 0.017).

The second generalised linear mixed model on tactile stimulation also yielded an overall effect of treatment: cleaners provided more tactile stimulation when an audience was present ($F_{1,95}$ = 8.88, p = 0.004, Figure 6). Client species had a significant effect as well ($F_{3,35}$ = 3.07, P = 0.04), while the effect of the bystander species only yielded a non-significant trend ($F_{2,99}$ = 2.76, p = 0.068).

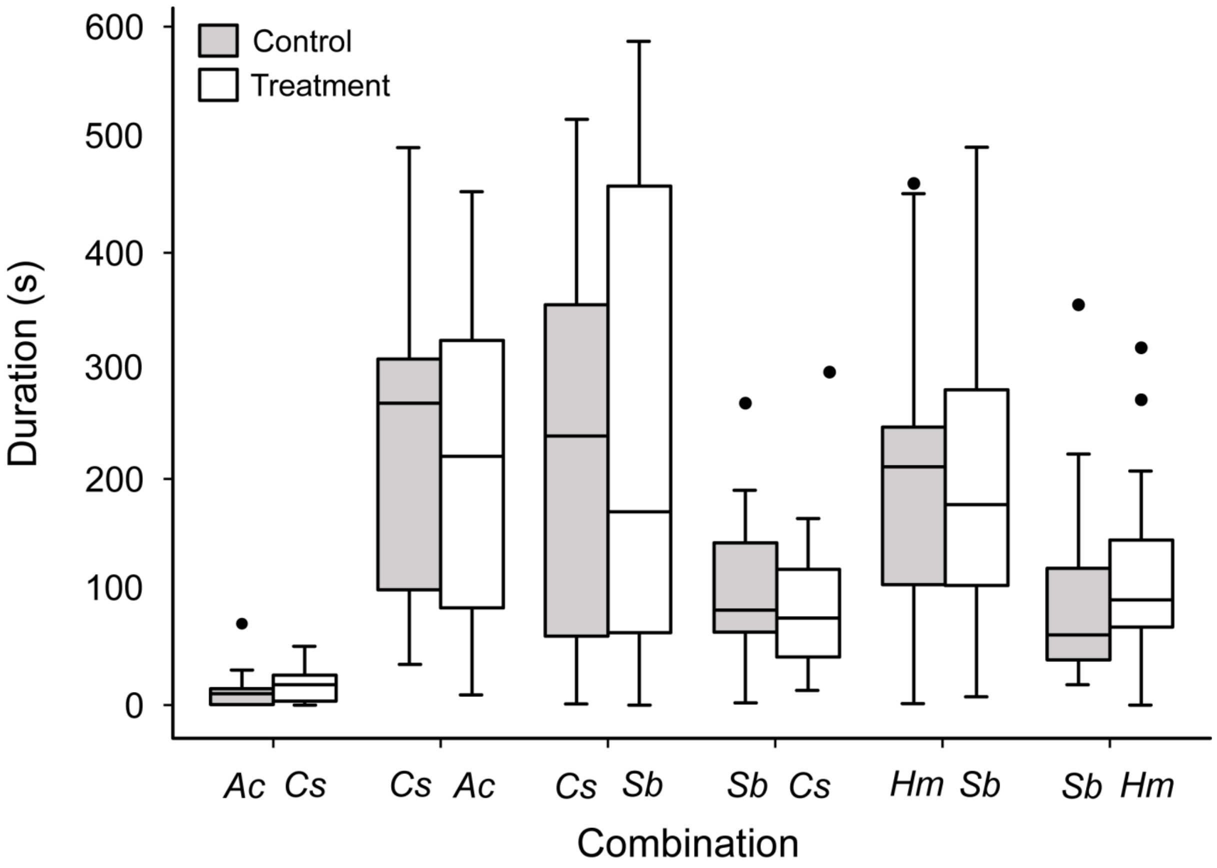

Figure 4 | Cleaners Interacting Time with Different Combinations of Clients Duration (in seconds) that the cleaner fish *Labroides dimidiatus* spent interacting with their clients in two different situations: without a “bystander client” (control) or with a “bystander client” observing the interactions (treatment). Ac: damsel *Amblyglyphidodon curacao*, Cs: surgeon *Ctenochaetus striatus*, Sb: bream *Scolopsis bilineatus* and Hm: wrasse *Hemigymnus melapterus*. Depicted are boxplots with medians and interquartiles.

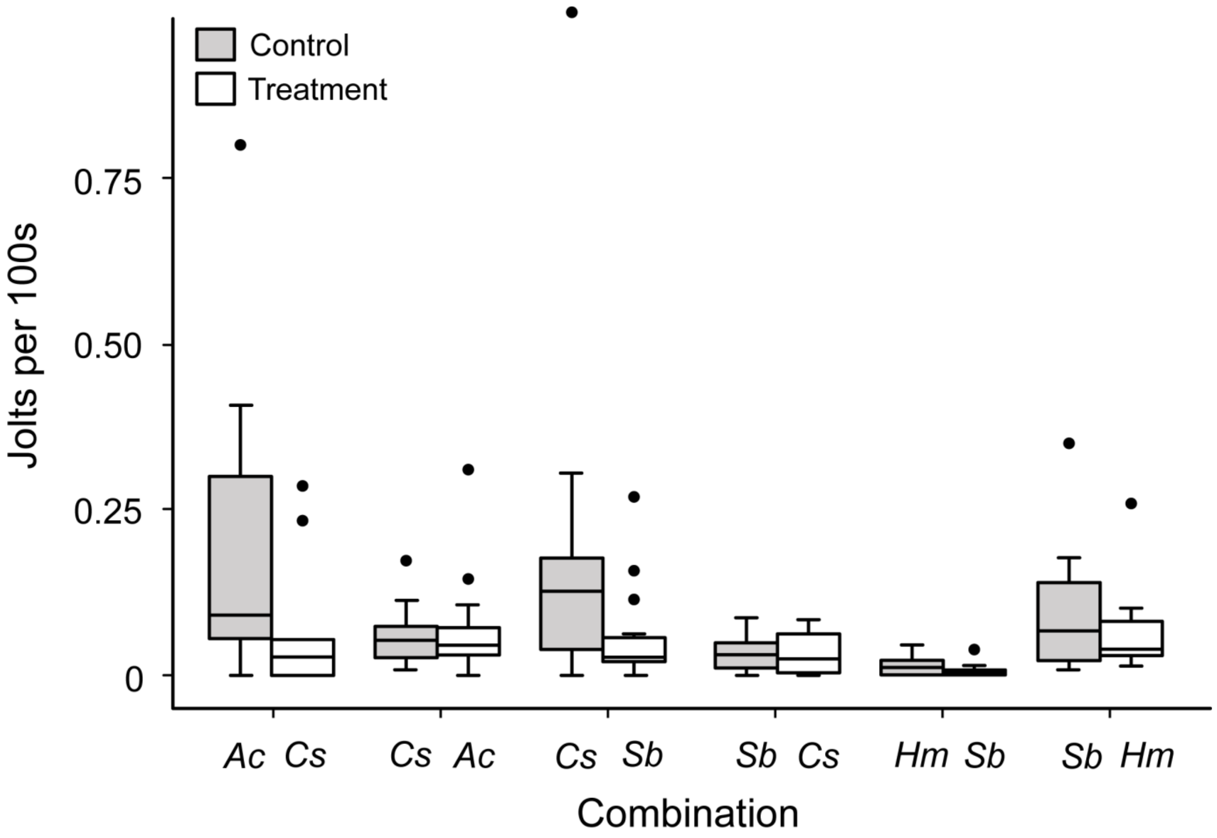

Figure 5 | Number of Jolts Caused by the Cleaners to Different Combinations of Clients Number of jolts (per 100s) performed by the cleaner fish *Labroides dimidiatus* with their clients in two different situations: without a "bystander client" (control) or with a "bystander client" observing the interactions (treatment). Ac: damsel *Amblyglyphidodon curacao*, Cs: surgeon *Ctenochaetus striatus*, Sb: bream *Scolopsis bilineatus* and Hm: wrasse *Hemigymnus melapterus*. Depicted are boxplots with medians and interquartiles.

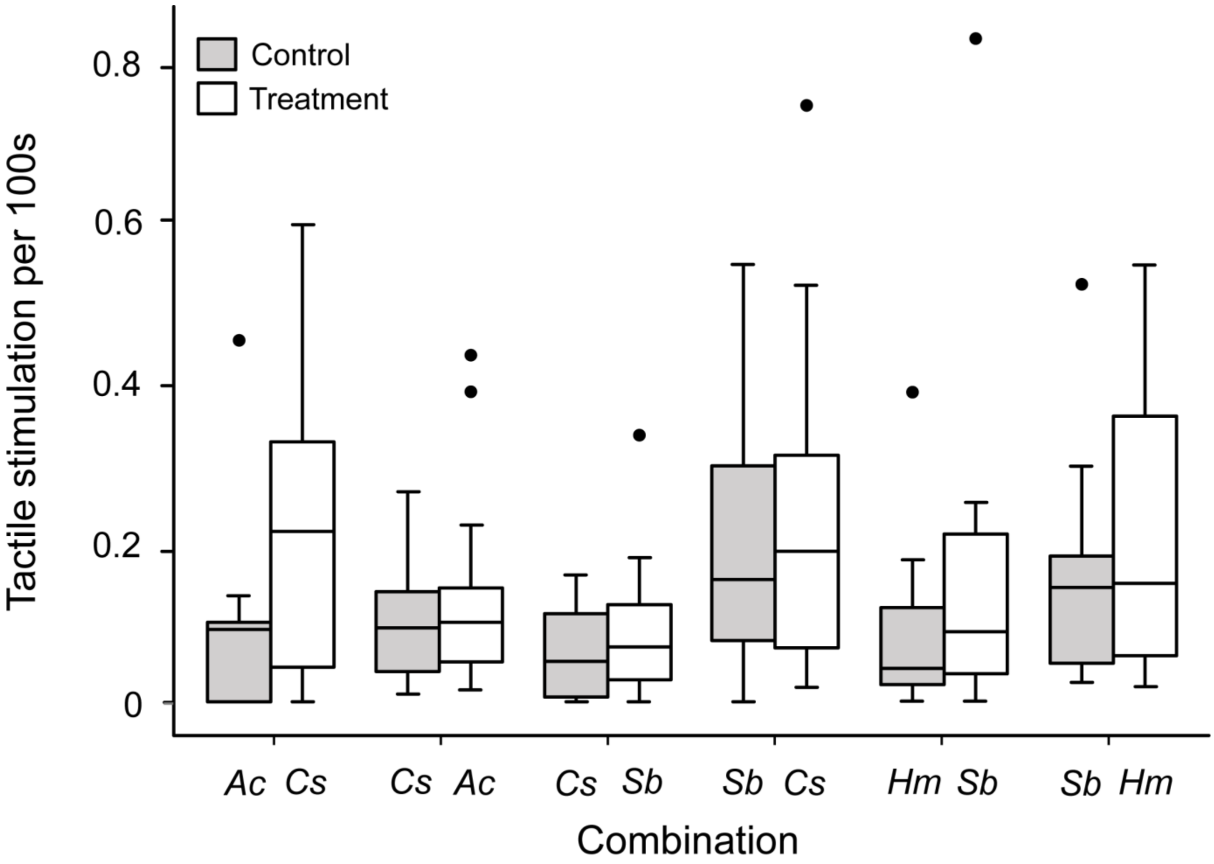

Figure 6 | Tactile Stimulation Provided by the Cleaners to Different Combinations of Clients Tactile stimulation (per 100s) provided by the cleaner fish *Labroides dimidiatus* with their clients in two different situations: without a "bystander client" (control) or with a "bystander client" observing the interactions (treatment). Ac: damsel *Amblyglyphidodon curacao*, Cs: surgeon *Ctenochaetus striatus*, Sb: bream *Scolopsis bilineatus* and Hm: wrasse *Hemigymnus melapterus*. Depicted are boxplots with medians and interquartiles.

Discussion

Both experiments consistently show that cleaners were more cooperative by eating more against their preference when in the presence of an audience compared to absence of bystanders. In the experiment with real clients, the presence of an audience selectively led to a lower client jolt ratio and an increase in the amount of tactile stimulation provided; while time spent interacting was not affected. In addition, identity of current client versus bystander matters as cleaners adjusted service quality to differing combinations. The experiments with artificial Plexiglas plates clearly fit our hypothesis that cleaners are particularly cooperative if the bystander is of higher value than the current client, while the opposite combination had no detectable effect. The combination between the damsels and surgeonfish (small versus large food patch) qualitatively fit the results from the plate. However, it appears that other variables may play a role as well. For example, we observed that the damsels became more and more territorial in captivity, and hence they showed by far the most spontaneous aggression towards cleaners (damsels: mean 12.2 +/- 9.7SD; surgeons: 1.03 +/- 2.7SD; breams: 1.43 +/- 2.5SD; thicklips: 0.29 +/- 0.83SD; Mann-Whitney-U Tests, damsels against other species: n = 45, 43 or 29, all $z > 4.34$, all $p < 0.001$). Such unprovoked territorial aggression seems to promote cooperation in cleaners (Pinto et al chapter 3). The strongest effect was observed with a resident client and a visitor bystander (see also Pinto et al 2011). Thus, partner choice options seem to be of major importance for cleaner decisions on how to adjust to an audience. This result mirrors observations for butterflyfish that the switch from access to one to access a few cleaning stations increases service quality provided by cleaners (Adam 2010), as predicted by biological market theory (Noë & Hammerstein 1995). In contrast, a resident bystander that is similarly attractive to the current visitor client does not seem to affect cleaner service quality. This suggests that partner choice may have a stronger effect on cleaner audience effects than the bystander’s quality as a food patch. Contrary to our predictions, our experiments involving two visitor species did not yield strong audience effects, and this was particularly true for the most attractive client species, the thicklip wrasse. The wrasses as bystanders were constantly swimming around in their aquarium (Ana Pinto, pers. obs.). Thus, there is the possibility that they may not have behaved in a way that cleaners felt observed. As clients, thicklips as clients jolted rarely even in the absence of bystanders, so the presence of *Scolopsis* could hardly improve cleaner service quality further. Additional species combinations and increased sample sizes may shed light on the relative importance of various variables like client and bystander absolute versus relative quality as a food patch as well as the effect of client/bystander partner choice options. Ideally, such research will be complemented by further modelling of image scoring and audience effects. What is clear from our data is that current models of image scoring (Nowak & Sigmund 1998; Roberts 1998; Leimar & Hammerstein 2001; Lotem et al. 2003; Ghang & Nowak 2015) do not capture the sophistication of cleaner wrasse decision rules.

In conclusion, cleaners increased their levels of cooperation either with artificial and real clients and demonstrated that they were able to fine tune service quality on the attractiveness of their bystanders. Thus, their behaviour is very much fine-tuned and not a fixed action pattern confirming and extending the previous study by Pinto and colleagues (2011). This study adds to increasing literature showing sophisticated cognitive rules in fish (Bshary et al. 2002; Warburton 2003; Grosenick et al. 2007; Brown et al. 2011; Vail et al. 2013, 2014; Bshary & Brown 2014; Bshary et al. 2014) and corresponding neuronal substrates (O'Connell & Hofmann 2011, 2012). The phenomenon should also be studied in other species.

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Chapter Three | Chocolate for dinner, veggies for dessert: satiated cleaner fish, *Labroides dimidiatus*, invest in their future relationships with clients

Chapter to be published in *Current Biology*

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Abstract

Supply and demand affects the values of goods exchanged in cooperative trades. All hitherto reported cases in animals describe the standard scenario that high demand leads to a higher price. Intriguingly, in humans, a temporary high demand may sometimes lead to a decrease in price, based on detailed knowledge of the market situation. Here we report that cleaner wrasse (*Labroides dimidiatus*) increase service quality during periods of high demand by client reef fishes, without such detailed knowledge. Clients visit cleaners to have ectoparasites removed but cleaners prefer client mucus, which constitutes cheating. In two experiments involving either real clients or an abstraction of interactions by using Plexiglas plates, cleaners ate more against their preference and hence behaved more cooperatively when satiated. Thus, cleaners are nicer when their own dependency on a transaction is low than when it is high. This ability to invest in relationships with clients when the marginal benefits of cheating are low yields benefits in future interactions. The strategic adjustment is caused by a motivational rather than cognitive adjustment, namely a reversal of the well-known 'dessert effect': when satiated, humans and other animals eat preferred and/or rare food while cleaners eat less preferred standard food.

Introduction

The values of goods exchanged in human markets follow the rules of supply and demand (Smith 1776). Typically, goods in high demand become more expensive but under certain circumstances, a temporarily high demand can cause a decrease in price (Rotemberg & Saloner 1986; Maskin & Tirole 1988; Tirole 1988; Lin & Sibdari 2009). Such a scenario assumes competition among traders who have detailed information about each other's price strategies as well as knowledge about the timing of fluctuations in demand. An empirical example involves anti-cyclic prices for cement (Rotemberg & Saloner 1986).

The human market idea of supply and demand to predict payoff distributions among cooperating individuals has been successfully applied to other species, as well within the framework of biological market theory and its emphasis on partner choice (Noë et al. 1991; Noë & Hammerstein 1994). Models of biological market theory invariably predicted that as demand goes up, so does the price (Noë & Hammerstein 1994; Noë & Hammerstein 1995; Johnstone & Bshary 2008; De Mazancourt & Schwartz 2010; Akçay et al. 2012; Grman et al. 2012), and empirical studies currently conform to this view. Examples include the interspecific mutualisms involving mycorrhiza and plant interactions (Schwartz & Hoeksema 1998; Kiers et al. 2011), fig trees and their pollinators (Herre & West 1997), ant–lycaenid larvae mutualisms (Leimar & Axén 1993), marine cleaning mutualism (Adam 2010), as well as intraspecific interactions between meerkats (Kutsukake & Clutton-Brock 2010), hyenas (Smith et al. 2007), mating markets (Metz et al. 2007) and primate grooming markets (Barrett & Henzi 2001; Fruteau et al. 2009). For recent reviews on partner choice in cooperative interactions, see (Jones et al. 2012; Barclay 2013).

So why is there no evidence in non-human species that a temporarily high demand may lead to a reduction in prices? As the emphasis of biological market theory is on partner choice and competition among service providers, some assumptions for a reversed effect of demand on prices seems to be fulfilled in the many cooperative examples cited above. A suspected key difference between biological and human markets concerns cognition. Humans certainly have the cognitive abilities to monitor market developments and to plan for the future. In contrast, non-human animals are more limited, as they typically lack the ability to plan for the future (Tulving 2005; Raby et al. 2007) and also generally show high rates of discounting future benefits in favour of current benefits (Stevens & Hauser 2005; Dufour et al. 2007; Roberts 2012). However, this view concerning cognitive limitations hindering cooperation in non-human species has been challenged repeatedly, as alternative, less cognitively-demanding mechanisms may offer functionally almost equivalent outcomes (De Waal 2000; Schino & Aureli 2009; Brosnan et al. 2010; Brandenburg et al. 2012; Fruteau et al. 2013). Even in humans, it becomes increasingly clear that prosocial behaviour is modulated by hormones and neurotransmitters (Kogan et al. 2011; Eisenegger et al. 2011), emphasizing the importance of emotions in addition to rationality. Potentially, specific physiological mechanisms could also underlie cases of sophisticated cooperation in other species that lack our cognitive abilities.

Here, we report laboratory experiments showing that i) the cleaner wrasse *Labroides dimidiatus* increases its service quality towards client reef fish during periods of low personal demand for cleaning interactions (simulating high recent demands of clients for cleaning), and ii) that a physiological adaptation underlies this reversal of the market law of supply and demand. Cleaning mutualism fits the assumption well of a biological market in which two classes of traders exchange goods or services. The cleaners obtain almost 100 % of their diet from engaging in more than 2,000 interactions per day (Grutter 1996) removing ectoparasites from the surface of their so-called reef fish clients, who benefit from this removal (Waldie et al. 2011; Ros et al. 2011). Conflict arises because cleaners prefer the clients’ protective layer of mucus to ectoparasites (Grutter & Bshary 2003). Therefore, clients have to make cleaners feed against their preference in order to get a good service. Clients with access to several cleaning stations do so by exerting partner choice: they come back if service was good but switch when the service was bad (Bshary & Schäffer 2002). Thus, cleaners compete with each other over access to these clients, and competition takes place through outbidding. The ratio of cleaners to clients is rather stable over periods of days or weeks but may change eventually due to demographic changes (mortality and recruitment). From a cleaner’s perspective, fluctuations in client demand for cleaning occur on a short time scale, i.e. within minutes, due to a mixture of recent own service quality and chance fluctuations in client visitation. As a consequence, a cleaner’s level of satiation varies over short time periods; it is high if by chance a few large clients have visited in short succession and relatively low if larger clients were largely absent in the past 15-30 minutes. Furthermore, rather predictable fluctuations in visitation rates occur over the course of a day (Grutter 1995; Bshary 2001). Thus, as cleaners' satiety state varies continuously in nature due to short term fluctuation in demand by clients, we experimentally manipulated their levels of satiety (providing them with extra food) to test how current need for interactions affects the cleaners' service quality.

In the first experiment, we substituted clients, ectoparasites and mucus with Plexiglas plates offering small items of preferred prawn and less preferred fish flakes mixed with prawn (further called 'flakes'). Each plate was attached to a lever so that the experimenter could remove it as soon as a subject ate a prawn item. The experiment thus measured the cleaners' willingness to feed against their preference, which corresponds to eating ectoparasites rather than mucus in nature. This experimental system has been repeatedly used successfully and seemingly captures key features of real cleaner-client interactions (Bshary & Grutter 2005, 2006; Pinto et al. 2011; Salwiczek et al. 2012; Gingins et al. 2013). In the second experiment, we let cleaners interact with clients, where client jolt rates can be used as a correlate of cheating by cleaners (Bshary & Grutter 2002). A jolt is an involuntary short twitch of the body in response to cleaner fish mouth contacts. In both experiments, we also quantified the occurrence of tactile stimulation per trial by cleaners. During tactile stimulation, a cleaner touches a Plexiglas plate or client with its pelvic and pectoral fins, a behaviour that benefits clients as it lowers stress levels (Soares et al. 2011) and is considered a measure of service quality.

The standard prediction based on market theory is that the service quality provided by cleaners varies as a function of their current dependency on cleaning interactions: if they are satiated, their current dependency is low and hence, service quality should decrease. In contrast, when cleaners are hungry, their current dependency on cleaning interaction is high and service quality should therefore increase. However, the opposite pattern may also be predicted once the specificities of cleaning mutualism are taken into account. As opposed to many other cooperative systems, a cleaner's decision about cooperating and cheating are foraging decisions. Optimal foraging theory highlights trade-offs between foraging benefits and risk of predation as well as between current and future benefits/risks (Cuthill & Houston 1997). According to this logic, satiated cleaners have little to gain from cheating as benefits would be marginal. However, cheating involves the risk of clients punishing cleaners or switching to another cleaner for their next inspection (Bshary & Schäffer 2002; Bshary & Grutter 2002), which will cause future losses either through the effort of reconciliation (Bshary & Würth 2001) or through opportunity costs. Thus, instead of exploiting the current market situation, cleaners could use phases of high satiation levels to show high service quality when the opportunity costs of forsaking mucus feeding are marginal. The resulting investment in relationship quality with clients will then lead future benefits when the caloric value of cheating is high because the cleaner is hungry. The general logic of this scenario has been mathematically demonstrated (Johnstone & Bshary 2007). Thus, our aim was to test which of the two scenarios applies to the cleaning mutualism: standard market theory or investment in relationships when times are good. Our results will also allow a discussion on the mechanisms underlying the cleaners' decisions.

Experimental Procedures

Experiments on satiation using Plexiglas plates and real clients were conducted, respectively, in June 2004 and from July to September 2010 at the Lizard Island Research Station, Great Barrier Reef, Australia. All fish were wild caught from the surrounding reefs and released at their site of capture after the experiments. In experiment 1 and 2, we used 16 (6.2-8.9 cm total length (TL) and 15 (6.1-7.9 cm TL) adult bluestreak cleaner wrasse (*Labroides dimidiatus*), respectively. For clients, we used 15 adult lined bristletooth surgeonfish (*Ctenochaetus striatus*: 18.2-11.8 cm TL), 15 adult bridled monocle bream (*Scolopsis bilineatus*: 11.5-14.8 cm TL) and 15 adult staghorn damsel (*Amblyglyphidodon curacao*: 7.4-3.2 cm TL), caught with barrier nets and hand nets. Cleaners did not always interact with clients during our ten minute trials. Due to the matched pair design of our study (see below), this led to the removal of some data points, leaving us with 11 surgeonfish, 13 breams and 15 damsels. Fish were fed daily with mashed prawn and a mixture of mashed prawn and fish flakes ("flakes"). The later was prepared every day with two third volume of prawn and one third volume of flakes and was then spread on Plexiglas plates (44). Cleaners learned to feed from the plates within one to three days of exposure. The cleaners used for experiment 1 were kept individually in aquaria of varying sizes (minimum size: 69 x 25 x 30 cm) and had been in captivity for at least 30 days. The other cleaners, bridled monocle breams and staghorn damsels clients, were kept in aquaria of varying sizes (minimum size: 50 x 30 x 25 cm) for a minimum of 7 days prior to experiments. Lined bristletooth surgeonfish clients were kept for a minimum of 30 days in 1 m diameter plastic tanks.

Cleaners' feeding against a preference

Cleaners had already completed the initial training sessions that involved the basic principles of the current study, i.e. that eating flakes was allowed but eating prawn would lead to the removal of the plate. More specifically, they had already been exposed to plates attached to levers so that they could be introduced and removed from the aquarium without major disturbance. The Plexiglas plates (12 x 7 cm) had a variety of uniform colours: beige, grey or white. Each cleaner was exposed to all different colours to become accustomed to the presentation of unfamiliar stimuli (otherwise cleaners may become neophobic, Ana Pinto pers. obs.). We offered three small items of each (prawn and flakes) on the plates. Cleaners prefer client mucus over ectoparasites (Grutter & Bshary 2003) and prawn over flakes (Bshary & Grutter 2005). Therefore, prawn items were used as the equivalent of mucus, and a cleaner that ate a prawn item was considered as having cheated, leading to the immediate removal of the plate by the experimenter. Here, we conducted further trials and manipulated the cleaners' level of satiety. On two consecutive days, cleaners were exposed to seven experimental trials per day, one trial every 60 min, starting at 8 am. On one day, all the food they obtained was linked to their performance during the trails. On the other day, they had access to plates offering only flakes items five minutes prior to each trial. Cleaners could eat as many flake items as they wished; if they did not eat another item for 15 s the plate was removed. The number of flake items eaten per round varied between 2 and 23. As a consequence of our manipulation, each cleaner was one day either always satiated or non-satiated when confronted with the experimental plate. Treatment order was counterbalanced between the 16 subjects.

The data for cleaner foraging behaviour per trial, i.e. the number of flake and prawn items eaten, were not normally distributed. Most importantly, we were interested in the flake to prawn ratio eaten by cleaners, and this value could be infinite in some trials where a cleaner never ate a prawn item within the maximum exposure duration of 60

s. As a consequence of data distribution, we summed the behaviour for each cleaner and situation to calculate the average number of fish flake items eaten per trial, the average number of prawn items eaten per round, and the overall fish flake to prawn ratio eaten. Furthermore, we repeatedly observed that cleaners provided tactile stimulation to the plate and hence, quantified for each cleaner and situation the number of trials this occurred (note that it had to occur before the cleaner ate a prawn item because that led to the immediate removal of the plate). For each variable of interest, Wilcoxon matched pair tests were used to evaluate whether satiation had a significant effect.

Cleaners' satiation effect on clients

All the species of fish used in this experiment are common clients of *L. dimidiatus* (Grutter 1995; Bshary 2001). The staghorn damsel (*Amblyglyphidodon curacao*) was chosen as a small resident (a species with access to only one cleaning station). Lined bristletooth surgeonfish (*Ctenochaetus striatus*) was chosen as a large resident client but it turned out that information from the Red Sea (Bshary 2001) does not apply to Lizard Island, where they have access to a few cleaning stations (Ana Pinto, pers. obs.). Bridle monocle bream (*Scolopsis bilineatus*) is of intermediate size with larger home ranges (visitor client) and hence, access to several cleaning stations. Each cleaner was exposed to all three client species in a counterbalanced way. Cleaners and clients were placed in their experimental aquaria 4 days before the experiment, with cleaners added to the client aquaria but physically separated from clients by a clear partition. On day 3, cleaners and clients had interacted twice for 10 min in another experiment focusing on audience effects (Pinto et al., in prep.). All aquaria (90 x 38 x 38 cm) had running seawater, and fish were provided with a PVC tube (16 cm diameter by 20 cm for clients and bystanders, and 1 cm diameter by 10 cm for cleaners) for shelter placed in the middle of each aquarium. To generate differences in the cleaners' level of satiety, half of the cleaners were allowed to feed for 15 min prior to the start of the experiment on a plain grey Plexiglas plate (15 x 10 cm) fully covered with prawn until satiation (satiated cleaners) whereas the Plexiglas plate of the other half of the cleaners remained empty (non-satiated cleaners). We ran four parallel setups, with the position of the aquaria containing satiated versus non-satiated cleaners, all counterbalanced. A trial started with the experimenter removing the clear partition in the side aquaria that allowed cleaners and clients to interact with each other. The experimenter then left the room while a digital camera recorded the behaviour of cleaners and clients for 10 min. Subsequently, the experimenter re-entered the room, separated cleaners and clients by placing the clear partition back into place. We performed one trial in the morning and another in the afternoon for each cleaner at 9 am and 1 pm, respectively. On the following day, all the cleaners were exposed to the reverse condition. For each trial, we noted every 10 s whether the cleaner and client interacted at that particular point in time in order to estimate total interaction duration (video analysis). As a measure for the time the cleaners devoted to tactile stimulation of clients we noted for each 10 s interval whether tactile stimulation had occurred at least once during that period. In addition, all client jolts were quantified. From these data we calculated a client jolt ratio (jolts/100s interaction) and a tactile stimulation index (n intervals with tactile stimulation occurring divided by total interaction duration). For the analysis we used linear mixed models with repeated measures (each cleaner with several clients in two matched situations) (IBM SPSS statistics 21).

Results

Cleaners' feeding against a preference

When satiated due to extra flake feeding prior to trials, cleaners ate significantly more against their preference as expressed by a higher flake to prawn ratio consumed than when non-satiated (Wilcoxon test, $n = 16$, $T = 15$, $P < 0.01$, Figure 1a). The change in ratio was not due to satiated cleaners eating more flake items than non-satiated ones did; indeed we found the opposite (Wilcoxon test, $n = 16$, 1 tie, resulting $n = 15$, $T = 10$, $P < 0.01$, Figure 1b). Instead, satiated cleaners often refrained from eating a prawn item while non-satiated cleaners invariably ate one per trial (Wilcoxon test, $n = 16$, $T = 0$, $P < 0.0001$, Figure 1b). Only satiated cleaners ever provided tactile stimulation (Wilcoxon test, $n = 16$, 4 ties, resulting $n = 12$, $T = 0$, $P < 0.0001$, Figure 2).

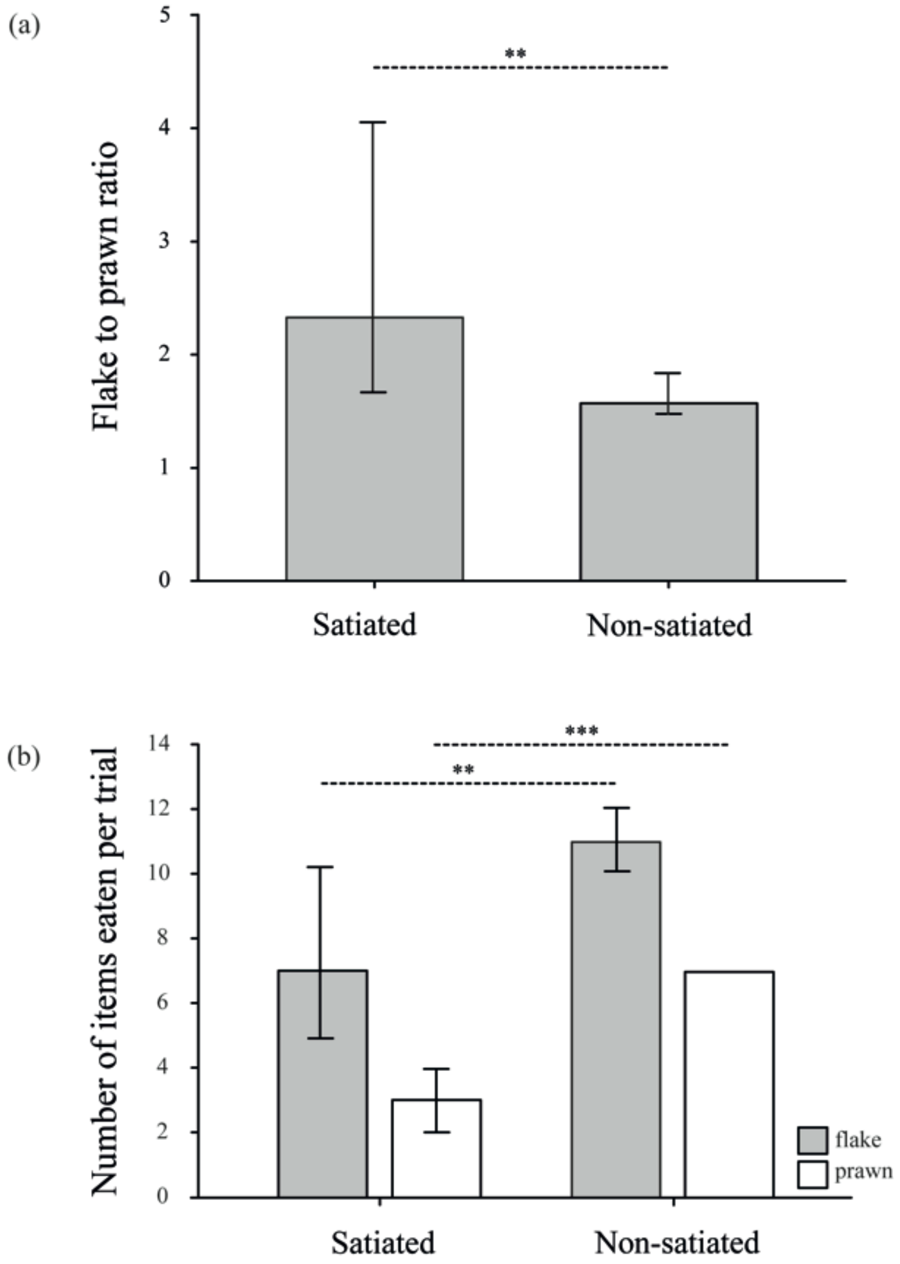

Figure 1 | Foraging of satiated and non-satiated cleaners during trials involving Plexiglas plates, prawn (preferred food) and flakes (less preferred food). a) The ratio of flake to prawn eaten per trial. b) Average number of flake and prawn items eaten per trial. Figures show median and interquartiles of the mean values of each cleaner. The asterisks indicate significant differences: ** *P* < 0.01; *** *P* < 0.001.

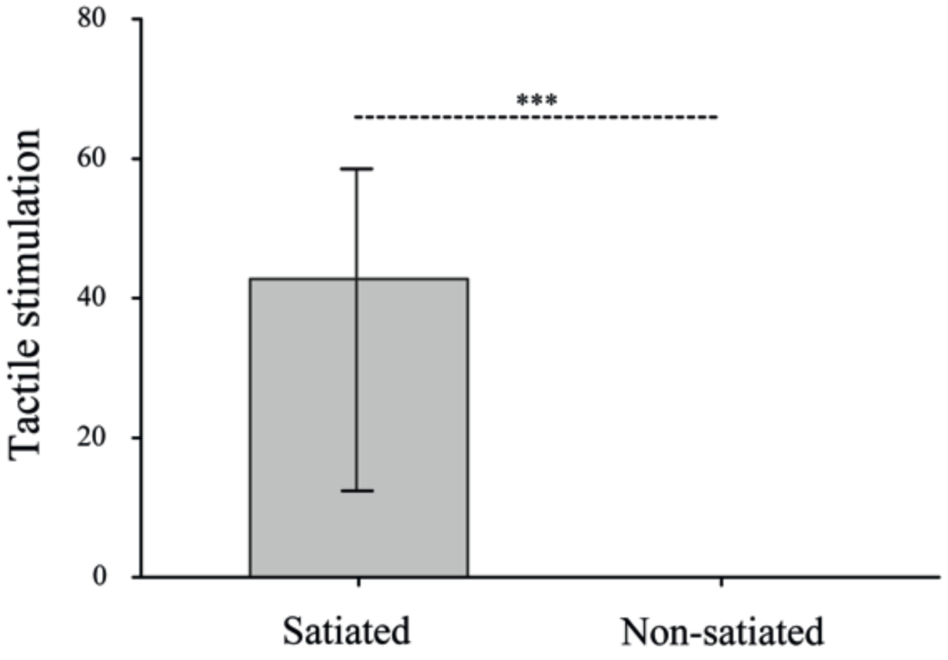

Figure 2 | The percentage of clients in which satiated and non-satiated cleaners provided tactile stimulation with their pelvic and pectoral fins to the Plexiglas plate. Figure shows median and interquartiles of the mean values of each cleaner. The asterisk indicates significant differences: *** $P < 0.001$.

Cleaners' satiation effect on clients

We calculated two generalised linear mixed models with repeated measures to assess the effects of our treatment on client jolt rate and on cleaners providing tactile stimulation. Data were Poisson-transformed to meet assumptions of a normal distribution. There was no significant interaction between species and feeding treatment or time of day (feeding treatment: $F = 1.35$, $P = 0.26$; time of day: $F = 0.34$, $P = 0.71$). Instead, we found a significant effect of feeding treatment on client jolt rate; satiated cleaners caused less jolts than non-satiated cleaners ($F = 17.40$, $P < 0.001$, Figure 3). We also found a significant effect of species identity on jolt rate, with damsels jolting least frequently ($F = 11.6$, $P < 0.001$, Figure 3).

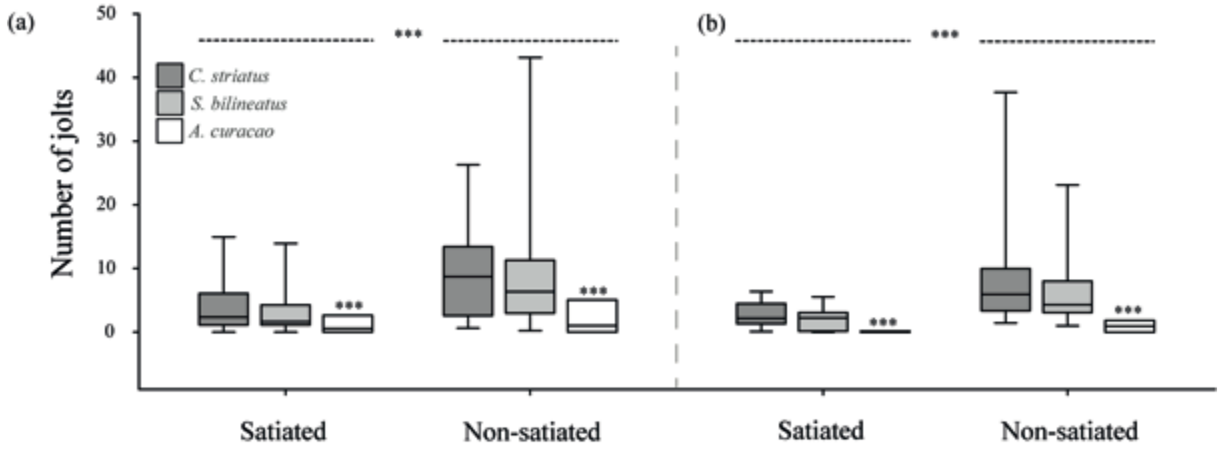

Figure 3 | Number of jolts per 100s interaction for the three different client species during interactions with satiated and non-satiated cleaners, once early a) morning and once in the b) afternoon of the same day (i.e. non-satiated cleaners would be more hungry). Figure shows median and interquartiles of the mean values of each species. The asterisk indicates significant differences: *** *P* < 0.001.

The second generalised linear mixed model on tactile stimulation did not yield any significant result with respect to treatment, species identity and interactions on the occurrence of tactile stimulation (0.17 ≤ *F* ≤ 2.13; 0.12 ≤ *P* ≤ 0.84, Figure 4). We only found an effect of a control variable, namely time of day/first versus second encounter: cleaners provided more tactile stimulation during the first encounter/the morning session (*F* = 19.9, *P* < 0.001, Figure 4).

Discussion

Both experiments consistently show that cleaners eat more against their preference when satiated, leading to a lower client jolt ratio in the second experiment. Hence, cleaners are more cooperative when satiated, i.e. when their current well-being depends little on a demand for further interactions with clients, than when non-satiated. While we manipulated satiation with extra food, in nature a temporarily high level of satiation would be caused by a temporarily high demand by clients to interact with the focal cleaner. Regardless, a temporarily low demand by cleaners or a temporarily high demand by clients should have led to a decrease in service quality, according to models of biological market theory (Noë & Hammerstein 1994, 1995; Johnstone & Bshary 2008; De Mazancourt & Schwartz 2010; Akçai et al. 2012; Grman et al. 2012).

Instead, we found that a simulated temporarily high demand for cleaning increases service quality, i.e. leads to lower prices. This result may be predicted when we apply optimal foraging theory (Cuthill & Houston 1997) and the logic of variable investment in repeated games (Johnstone & Bshary 2007) to our study system. Satiated cleaner fish gain delayed benefits from investing in their relationships with clients, by both increasing the probability of their return for the next inspection (Bshary & Schäffer 2002) and avoiding current conflicts that would cause future costs due to reconciliation (Bshary & Würth 2001). Marginal benefits of cheating are therefore low when cleaners are in a satiated state and high when in a non-satiated state. Thus, being cooperative when satiated increases the possibility to be more exploitative during future interactions when more in need. In other words, cleaners in a better state invest in relationships with their clients in order to reap the benefits in the future.

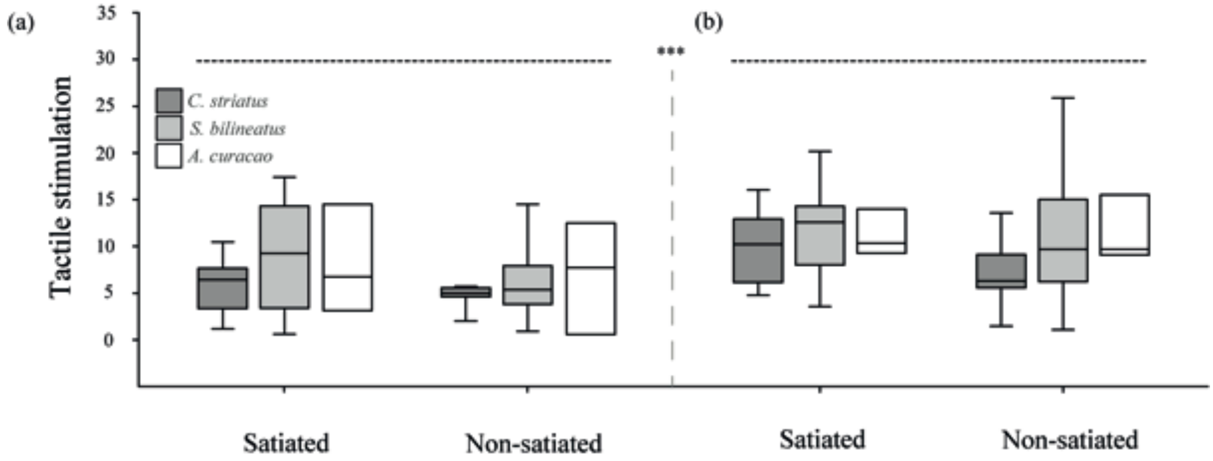

Figure 4 | An index of the occurrence of tactile stimulation provided by cleaners for the three different client species during interactions with satiated and non-satiated cleaners, once a) early morning and once in the b) afternoon of the same day (i.e. non-satiated cleaners would be more hungry). Figure shows median and interquartiles of the mean values of each species. There was no significant effect of our treatment (non-satiated/satiated) on tactile stimulation.

Providing more tactile stimulation when satiated would complement the lower cheating rates, though our evidence is restricted to the plate experiments. A possibility is that as cleaners were, overall, more satiated during the plate experiments due to a more regular exposure to unlimited food, an increase in the provisioning of tactile stimulation may only be easily detectable under conditions of extreme satiation. Tactile stimulation provides a means to give clients a sought-after service (Soares et al. 2011) without eating ectoparasites. Not interacting when satiated is not a viable option with visiting clients as these would be likely to switch to a different cleaning station for their next inspection (Bshary & Schäffer 2002).

Mechanisms underlying high service quality from satiated cleaners

Our functional explanation seems to raise concerns regarding the necessary cognitive mechanisms that are typical of humans. For example, there is no evidence that cleaners are able to plan for their future. Also, it is difficult to see how cleaners could obtain information about how other cleaners perform with respect to service quality and client visit rate, while such knowledge would be a prerequisite for a reversed market effect in models on human markets (Rotemberg & Saloner 1986; Maskin & Tirole 1988; Tirole 1988; Lin & Sibdari 2009). Instead, we propose that cleaners do not need to plan the future as a prerequisite to adjust their behaviour in a functional way. Rather, according to optimal foraging theory, satiated individuals should take fewer risks than non-satiated ones. If the analogy to general optimal foraging theory holds in our case, then the prediction would indeed be that, as a consequence of a temporarily high demand for cleaning by clients, cleaners become more satiated and hence, reduce the risk associated with cheating by temporarily increasing service quality.

In this context, we note that the motivational apparatus appears to be specifically adapted to the ecological challenge (Kamil & Mauldin 1988; Shettleworth 1993). In the plate experiment, cleaners became satiated due to the provisioning of ad libitum flake items. In terms of motivation, this should have led to the phenomenon called sensory-specific satiety also known as the ‘dessert effect’ (Rolls et al. 1981) which consists of a decrease in pleasure with continuous consumption of the same food or flavour compared with an unconsumed food or flavour (Havermans et al. 2009). Accordingly, a satiated cleaner would still be willing to eat from a different food source, which in our study was the preferred type. However, satiated cleaners specifically avoided eating the unconsumed preferred food during the plate experiments. Also in nature, a temporarily high demand by clients for cleaning would lead to a high consumption rate of ectoparasites. According to our results, the resulting satiation then decreases the probability that the cleaner eats mucus though the latter is preferred (Grutter & Bshary 2003). It is thus the reversal of the dessert effect that allows cleaners to invest into future relationships with clients when satiated. This is already the second reported case of a motivational adaptation that seems to be quite specific for cleaning mutualism: in another study, it was documented that cleaning gobies preferentially approach predatory clients despite being stressed by their presence (Soares et al. 2012).

Cleaning markets versus human markets

There is a potentially important difference between the conditions studied in human markets and the ones in our study that both lead to lower prices during periods of high demand. In the human markets, the fluctuations are global; i.e. there is for example a temporary overall increase in demand for cement that affects all sellers (Rotemberg & Saloner 1986). In our case, the overall market (the cleaner to client ratio and parasite infection rates) remains stable and fluctuations in demand are local and due to chance events, i.e. the sum of individual clients' decisions. Individual visitor clients know the overall market situation, i.e. number and distance between cleaning stations in their home range and average service quality, and would respond negatively to a cleaner that cheats above average. Therefore, an important future experiment will be to increase global demand by clients for cleaning services by removing cleaners from the system, thereby increasing the client to cleaner ratio. Under these conditions, the optimal response of clients would be to accept a lower service quality due to the increased competition between clients over access to cleaners and the higher costs of switching between cleaners. As a consequence, cleaners could benefit from becoming more exploitative in response, as also predicted for human markets (Smith 1776). On the other hand, human economic theory often assumes a property of non-satiation where the benefits of earning are linear rather than an asymptotic function of diminishing returns. As the benefits of food consumption in the cleaner fish system are clearly asymptotical, it appears that counterintuitive effects of a manipulation of cleaner to client ratios are a viable possibility.

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General Discussion

In this thesis, I addressed the general question in how far cleaner wrasse are able to adjust service quality to current conditions. The first two chapters deal with the presence of an audience while the third chapter deals with the effects of satiation.
Furthermore, I have been involved in various side projects that were all linked to aspects of the cognitive abilities that may underlie cleaner wrasse behaviour during interactions with clients. Therefore, I will use the general discussion to wrap up the progress/open questions linked to game theoretic aspects of my research but also the progress/open questions related to cleaner wrasse cognition.

Game theoretic aspects on image scoring and audience effects

The results on image scoring from the first chapter demonstrated that using Plexiglas plates as surrogates of real clients as in the previous study (Bshary & Grutter 2006) was not adequate to demonstrate what criterions clients use in nature to choose a cooperative cleaner. Similarly, results confirm that indeed reef clients pay attention to cleaners' service quality. Bystanders avoid cleaners that show cheating behaviour and hence do not appear to choose interacting over non-interacting cleaners per se. As bystanders typically only observe the end of an on-going interaction and would rather leave if not inspected immediately (Bshary & Schäffer 2002), such a decision rule is plausible as it is an easy cue to evaluate a cleaners' level of cooperation. Cleaners revealed a spontaneous increase in service quality when observed, which excludes any learning during the experiments, in contrast to the previous plate experiments by Bshary & Grutter (2006). However, it is possible that cleaners learned this decision rule in nature before they were caught, as under natural circumstances there are sufficient occasions for associative learning (Thorndike 1917) during their daily and numerous interactions with clients (Grutter 1995, 1996). Thus, it remains an open question in how far the audience effects shown by cleaners represent evolved adaptations rather than basic individual learning that could also be achieved by individuals belonging to other species. Independently of the underlying mechanism, the experiment on audience effects shows convincing evidence that as a result of the presence of bystanders an animal increases levels of cooperation.

An important question that was raised after this experiment was whether cleaners may be able to fine tune their service quality reliant on the bystanders' identity and whether bystanders integrate the current client's identity in their image scoring. For instance, important information about cleaner service quality can be obtained from observations with non-predatory clients but not from observations of interactions with predatory clients. This is because cleaners are commonly very cooperative with predators, including those that are temporarily in a biting mode (Bshary 2001, 2002). Cleaners, on the other hand, should likewise care about their image score particularly if bystanders are an attractive food source, that is, if they are large, highly parasitized and covered with high-quality mucus. Under such circumstances, gaining access to the bystander would outweigh the immediate decrease in payoffs due to the cleaner increasing its level of cooperation for the current client.

The set of experiments performed for the chapter 2 of my thesis tested for flexible audience effects in the cleaners. Both experiments confirmed previous results (Bshary & Grutter 2006; Pinto et al. 2011) that cleaners ate more against their preference and thus behaved more cooperatively when in the presence of an audience in comparison to the absence of bystanders. Even though the duration of the interactions were not affected, the presence of bystanders caused a decrease in clients' jolt ratio. Moreover, the larger sample size revealed an increase on amount of tactile stimulation provided, an effect that had not been significant in Pinto et al. (2011). Most importantly, as cleaners adapted service quality to varying identity combinations of current clients and bystander species shows that the identity of both participants matters.

The experiments involving artificial Plexiglas plates definitely confirm the hypothesis that cleaners become promptly cooperative if the value of the bystander is higher than the value of the current client, although the reverse combination had no visible effect. In addition, the combination between the damsels as current clients and the surgeonfish as bystanders (small versus large food patch) fit the results from the Plexiglas plate experiment. According to the biological market theory (Noë & Hammerstein 1995), the fact that some classes of bystanders are able to switch and access other cleaning stations promotes increased cooperative behaviour by cleaners and hence partner choice options appear to be of major prominence for cleaners decisions on how to adjust an audience. Therefore, a resident client and a visitor bystander yielded the strongest effect while a resident bystander that as similar attractiveness to the current visitor did not affect cleaner service quality.

In conclusion, cleaners demonstrated that they were able to fine tune service quality depending on the attractiveness of their bystanders as they augmented their levels of cooperation either with real clients and artificial Plexiglas plates. Therefore, results confirm and extend the previous study by Pinto and colleagues (2011) as cleaners' behaviour is indeed very much fine-tuned and not a fixed action pattern.

Interestingly, such evidence from chapter 2 not only goes beyond game theory but even beyond evidence provided for humans. Indeed, I am not aware of any experiment in humans that demonstrates similar results. Based on introspection there is no doubt that humans would adjust levels of cooperation to the value of current interaction partners as well as to the value of bystanders. Nevertheless, it is nice to have for once performed an experiment that links cooperation and cognition in a non-human animal first.

Despite the encouraging results from these studies, there were a few unexpected and challenging conditions with respect to some of the species used in this study. For example, damsels (*Amblyglyphidodon curacao*) developed territories in captivity and consequently were extremely aggressive towards the cleaners, which may have incited cooperative behaviour in cleaners. Similarly, the most attractive client species, the thicklip wrasse *Hemigymnus melapterus,* were continually swimming around in their aquarium as bystanders (Ana Pinto, pers. obs.) and hence not behaving as if they were paying attention to cleaners' behaviour. In addition, thicklip wrasses as clients rarely jolted no matter if there were in the presence or absence of breams *Scolopsis bilineatus*. Therefore, the experiments relating two visitor species did not produce strong audience effects.

Undoubtedly, current models of image scoring (Nowak & Sigmund 1998; Roberts 1998; Leimar & Hammerstein 2001; Lotem et al. 2003; Ghang & Nowak 2015) are not sufficient to explain the sophisticated decision rules from cleaner wrasses. Thus, while additional studies with an increased sample size and supplementary species combinations (for example, small unattractive resident/big attractive visitor) may elucidate the relative importance of numerous variables such as the effect of client/bystander partner choice options as well as client and bystander absolute versus relative quality as a food patch, ideally such empirical progress should be combined with further theoretical modelling of image scoring and audience effects.

On biological market theory

Biological market theory has provided a powerful tool to study cleaning mutualism (Noë et al. 1991; Noë & Hammerstein 1994, 1995; Bshary 2001; Noë 2001; Bshary & Grutter 2002; Bshary & Schäffer 2002; Bshary & Noë 2003). For example, client species with access to several cleaning stations switch cleaners if cheated (Bshary & Schäffer 2002), a response that promotes cooperative behaviour by cleaners (Bshary & Grutter 2005). Furthermore, clients with access to several cleaner stations have priority of access over resident species (Bshary 2001), apparently because of their choice options (Bshary & Grutter 2002). Also my chapter 2 provided evidence for the importance of biological market theory by the observation that bystander choice options seem to be more important than their value as a food source. It is therefore particularly interesting that the most basic prediction from market theory was not met in my study on the effects of satiation. Models of biological market theory (Noë & Hammerstein 1994, 1995; Johnstone & Bshary 2008; De Mazancourt & Schwartz 2010; Grman et al. 2012) would predict that either a temporarily high demand by clients or a low demand by cleaners would lead to a diminution in service quality. Nonetheless, the results of both experiments with artificial Plexiglas plates and real clients demonstrate the opposite: that satiated cleaners ate more against their preference and hence behaved more cooperatively then when they were not satiated. In the second experiment this was translated by a lower client jolt ratio. Furthermore, data mining of old field observations suggested that it is not satiation per se but satiation based on a temporarily high demand for cleaning that leaves cleaners more cooperative. Apparently satiated cleaners become averse to the risk of being punished or to loose clients in a repeated game, thereby increasing potential future benefits. Optimal foraging theory (Cuthill & Houston 1997) as well as flexible investments in iterated games (Johnstone & Bshary 2007) can explain these results. Nevertheless, it is important to note that the current data all deal with temporary “random” variation in supply and demand and NOT with a change in the global market situation. Therefore, an important follow-up study would be to alter cleaner to client ratios in the field and test whether the resulting increased demand following a reduction in cleaner density leads indeed to increased service quality or not.

The results of chapter 3 demonstrate that the payoffs of behavioural alternatives may be potentially affected by individual's internal state, which consequently affects the decision to either cooperate or defect. However, little is known about the physiology underlying condition dependent cooperation. In parallel to my studies on satiation, Soares and colleagues (2014) injected cortisol in wild cleaner wrasse *L. dimidiatus*. Cortisol is a stress hormone, where increased concentrations suggest to the animal the need for increased energy requirements. As a consequence of cortisol injections, cleaners provided more tactile stimulation to the small resident clients in order to get access to the larger visitor clients and bite them, a pattern that had been previously observed in the field by Bshary (2002). The blocking of cortisol led to more tactile stimulation to the large visitor clients without affecting client jolt frequencies (Soares et al. 2014) Thus, being hungry/satiated is apparently not the physiological equivalent of being stressed/relaxed. However, the two physiological processes must be linked in some ways as continued hunger should lead to stress/energy deficits. Thus, there seems to be potential for future studies to investigate in more detail the physiology of cooperation in cleaner wrasse as well as in other species.

Plexiglas plate paradigm versus the use of real clients

One key conclusion from my three chapters is that the Plexiglas paradigm introduced by Bshary & Grutter (2002) yields results that can be replicated with real clients. Thus, the Plexiglas plate design offers indeed a highly valid approach to study this system from the cleaners' perspective. At the same time it is important to realise that interactions with real clients yield additional insights. An obvious case is how bystanders decide whether to invite inspection. Also, the use of tactile stimulation is more relevant in interactions with real clients. Finally, using real clients allows us to test "spontaneous" decision rules of cleaners while the Plexiglas plate design needs to involve repeated exposure and learning by cleaners. Chapters 2 and 3 indicate that beyond simple predictions based on key concepts that are tested with the Plexiglas plate paradigm, additional variables need to be taken into account, like client aggressiveness to fully understand the cleaners' decisions concerning service quality.

Cleaner wrasse cognition

During my PhD I was involved in various side projects that largely addressed the cognitive adaptations of my study species. First, a comparative approach involving experiments on chimpanzees, orang-utans and capuchin monkeys (a collaboration with Prof. Dr. Sarah Brosnan from Atlanta) revealed that cleaners from continuous reefs outperform the primates in cognitive tasks that capture some of the complexity of cleaner client interactions (Salwiczek et al. 2012). Such specific superiority of small brained species over large brained species is predicted by an ecological/evolutionary approach to cognition, which proposes that the cognitive abilities of any species closely reflect the cognitive challenges it faces under natural conditions (Kamil 1998; Shettleworth 2010).

In another side project led by Nichola Raihani, I contributed to experiments that showed that male cleaner wrasse adjust the level of punishment of their female partner according to the stakes. In biology, punishment has been functionally defined by Clutton-Brock & Parker (1995) as a response to being cheated that causes a reduction in current payoffs to both punisher and target. As a consequence, the target should behave more cooperatively during future interactions with the punisher, thereby more than compensating the initial costs of punishment. Cleaner wrasse often inspect in stable pairs of a male and a female. Males are larger and hence dominant over females. Previous research had shown that this asymmetry leads to disproportional gains by males during joint inspections (Bshary et al. 2008), and that the disproportional gains were due to males punishing cheating females with aggressive chasing (Raihani et al. 2010). I was involved in an experiment that demonstrated that males adjust the level of punishment to "fit the crime": if the female's cheating causes a large still unexploited food source to swim off the male responds more aggressively than if the female's cheating causes only small losses of foraging opportunities (Raihani et al. 2012). These results mirror my observations from chapter 2 that cleaners are highly able to fine-tune their behaviour to the specifics of a social situation.

The starting point of a major involvement in the study of cleaner wrasse cognition was a series of experiments that aimed at testing how between-individual variation in levels of cooperation under natural and laboratory conditions and variation in cognitive abilities may affect body condition as a correlate of survival and reproductive success. For repeated field observation I picked cleaners that lived on reef patches rather than on a continuous reef. As I was only interested in variation, published experiments in which cleaners had shown sophisticated behaviour were repeated. It turned out that all individuals failed to complete the tasks. Together with Sharon Wismer, a Master student, I could demonstrate systematic differences between cleaners from patch reefs and from continuous reefs with respect to levels of cooperation and cognitive abilities. Most strikingly in the light of my thesis chapters, cleaners from the patch reefs were unable to solve an audience effect task involving two image scoring Plexiglas plates, as previously demonstrated for nearby cleaners from continuous reefs (Bshary & Grutter 2006). These differences are likely due to differences in ecological conditions: we found that patch reef cleaners have only about 800 interactions per day instead of 2’000, they interact with a lower diversity of client species and they experience less competition with other cleaners over access to clients (please see appendix, Wismer et al. 2014). An interesting future study would be to determine how different habitats (patch versus continuous reef) influence underlying neural correlates of sophisticated decision making rules.

In conclusion, my studies contribute to an increasing literature showing sophisticated cognitive rules in fish (Bshary et al. 2002; Warburton 2003; Grosenick et al. 2007; Kendall et al. 2009; Brown et al. 2011; Vail et al. 2013, 2014; Bshary & Brown 2014). For example, cooperative and complex hunting strategies can be found in fish, including the association of coral trouts with moray eels (Vail et al. 2014), collaborative hunting in the yellow saddle goatfish (Strübin et al. 2011) and lionfish (Lönnstedt et al. 2014) as well as in mammals such as orcas (Baird 2000) and lions (Stander 1992). Moreover, other examples include cooperative predator inspection in sticklebacks (Milinski 1987; Milinski et al. 1990a, 1990b; Külling & Milinski 1992; Huntingford et al. 1994; Milinski et al. 1997; Walling et al. 2004). The recent results on complex decision rules match well recent advances in the study of decision making in the brain, where it was shown that the social decision making network is highly conserved among vertebrates (O’Connell & Hofmann 2011, 2012). Based on these brain studies, we may expect more sophisticated cognitive processes to be discovered in fish. In line with this prediction, I have co-authored a book chapter that proposes that it seems more likely that species differences in (relative) brain sizes are due to quantitative differences rather than differences in the repertoire of cognitive processes (Bshary et al. 2011). Clearly, linking cooperation and cognition in fishes will provide further insights on their cognitive abilities as well as on the evolution of cooperation between unrelated individuals.

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Appendix | Variation in Cleaner Wrasse Cooperation and Cognition: Influence of the Developmental Environment?

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Abstract

Deviations from model-based predictions of strategies leading to stable cooperation between unrelated individuals have raised considerable debate in regards to decision-making processes in humans. Here, we present data on cleaner wrasse (*Labroides dimidiatus*) that emphasize the importance of generalizing this discussion to other species, with the aim to develop a coherent theoretical framework. Cleaners eat ectoparasites and mucus off client fishes and vary their service quality based on a clients' strategic behaviour. Hitherto, cognitive tasks designed to replicate such behaviour have revealed a strong link between cooperative behaviour and game theoretic predictions. However, we show that individuals from a specific location within our study site repeatedly failed to conform to the published evidence. We started exploring potential functional and mechanistic causes for this unexpected result, focusing on client composition, cleaner standard personality measures and ontogeny. We found that failing individuals lived in a socially simple environment. Decision rules of these cleaners ignored existing information in their environment ("bounded rationality"), in contrast to cleaners living in a socially complex area. With respect to potential mechanisms, we found no correlations between differences in performance and differences in aggressiveness or boldness, in contrast to results on other cooperative species. Furthermore, juveniles from the two habitat types performed similarly, and better than the adults from the socially simple environment. We propose that variation in the costs and benefits of knowledge may affect a cleaners' information acquisition and storage, which may explain our observed variation in cooperation and cognition.

Introduction

Evolutionary game theory and empirical evidence provide a variety of mechanisms for stable cooperation between unrelated individuals (Axelrod & Hamilton 1981; Conner 1986; Clutton-Brock & Parker 1995; Milinski & Wedekind 1998; Nowak & Sigmund 1998; Wedekind & Milinski 2000; Kiers et al. 2003; Bshary & Grutter 2005). Deviations from model-based predictions of strategies leading to cooperative behaviour have, however, raised considerable debate in regards to decision-making processes in humans (Gigerenzer & Selten 2002; Boyd et al. 2003; Lehmann et al. 2007; Kümmerli et al. 2010; Baumard et al. 2013). For example, in humans, some individuals behave more cooperatively (Fehr & Fischbacher 2003; Haley & Fessler 2005) or less cooperatively (Kümmerli et al. 2010), as well as less precise (Milinski et al. 2001) or more sophisticated (Milinski & Wedekind 1998), than predicted cooperative strategies in models. This mismatch has raised questions, sparked debate, and produce new concepts such as cultural group selection (Boyd et al. 2003; Lehmann et al. 2007). Most importantly, it has spurred research and debates regarding decision-making processes (Hagen & Hammerstein 2006; Baumard et al. 2013). For example, "bounded rationality" proposes that humans develop simple heuristics, by constantly looking for environmental cues that would trigger a response that has worked well under previous similar circumstances (Gigerenzer & Selten 2002). This allows humans to by-pass information processing of any single situation and its unique complexity, and instead, apply a general rule of thumb strategy that is likely to result in the desired outcome. These general rules of thumb work well, yet are less precise and potentially even wrong in a different context (Gigerenzer & Selten 2002). An alternative proposal is that humans generally begin at intermediate cooperative levels and initiate extreme strategies only if feedback indicates their appropriateness (Kümmerli et al. 2010).

In non-human animals, research on decision-making is on the rise (Hammerstein & Stevens 2012), but few studies have focused on the decision rules underlying cooperative behaviour. As an exception, experimental research using the iterated prisoner's dilemma framework to study reciprocity, typically describes cooperative outcomes that are based on "Tit-for-Tat-like" decision rules (start cooperatively and then match the partner's behaviour in the previous interaction) (Milinski 1987; Krams et al. 2008; Rutte & Taborski 2008; St-Pierre et al. 2009; Raihani & Bshary 2011).

However, in primatology, it has been recognized that precise counting reciprocal strategies, like Tit-for-Tat, do not typically fit observed interaction patterns (de Waal 2000). Unfortunately, alternative propositions, such as reciprocity based on emotional book-keeping ("I help as long as I like you"; Schino & Aureli 2009) have not been experimentally tested. Here, we demonstrate important mismatches between standard theoretical predictions regarding animal decisions during cooperative interactions and experimental data. We further present evidence that variation in the social environment may be of paramount importance in explaining deviations. Collectively, our results highlight the need for an interactive approach between empiricists and theoreticians to build a cooperation theory based on the mechanistics of decision-making.

The widely published cleaning mutualism of the bluestreak cleaner wrasse, *Labroides dimidiatus*, has provided strong experimental evidence for the usefulness of evolutionary game theory for predicting cooperative behaviour (Bshary 2011). Cleaner wrasse cooperate by eating ectoparasites off visiting client reef fishes. Conflict arises, however, as cleaner wrasse essentially prefer to eat client mucus, which constitutes cheating (Bshary 2011). The resolution of the resulting conflict depends on the clients' strategic options and may involve the threat of reciprocity by predatory clients, partner switching by visitor clients with access to several cleaning stations, and punishment by resident clients that lack cleaner choice options (Bshary 2011). Cleaner wrasse have shown to fine-tune service quality and priority to the clients' strategic options (Bshary 2011). Furthermore, cleaner wrasse behave more cooperatively in the presence of bystanders to raise their image score and hence, increase the probability of subsequently accessing bystanders (Pinto et al. 2011).

In a 4 month project conducted in 2009, however, focusing on intraspecific variation, we failed to reproduce the results of published studies. The laboratory experiments involved the use of Plexiglas plates, prawn and fish flakes as substitutes for clients, mucus and ectoparasites, respectively. These substitutions have been used repeatedly before to successfully test game theoretic predictions on cooperation (Bshary & Grutter 2002, 2005, 2006; Bshary et al. 2008; Raihani et al. 2010, 2012), and the experimental design captures the essence of cleaning interactions, as key results can be reproduced in experiments using real cleaner – client interactions (Pinto et al. 2011) and because cleaners succeed in these tasks where both closely related non-cleaning species and otherwise cooperative primate species fail (Salwiczek et al. 2012; Gingins et al. 2013). In our 4 month project, cleaner wrasse failed to eat selectively against their preference to prolong interactions. This contrasts with results published by Bshary & Grutter (2005) and various models that predict that partner switching or punishment/sanctions should promote cooperative behaviour (Bull & Rice 1991; Clutton-Brock & Parker 1995; Ferriere et al. 2002), that is, feeding on the less preferred food in our particular case. Cleaners also failed to learn to eat more against their preference to gain access to an "image scoring bystander" plate as shown in Bshary & Grutter (2006) and predicted by image scoring theory (i.e. Nowak & Sigmund 1998). Finally, the cleaners failed to learn to prefer a "visitor" plate unwilling to wait for inspection over a "resident" plate that would only be removed once depleted. Such an ability would be predicted by biological market theory, where partner choice options determine a player's leverage, and hence, the amount or quality of services that it can obtain due to the partner's adjustment in behaviour (i.e. Noë 2001). For cleaners, this ability had been shown previously in the study described by Salwiczek et al. (2012) using the same methods, and field observations suggest likewise (Adam 2010).

In contrast to all previously published studies, these cleaner wrasse were caught on small, isolated reef patches rather than from nearby continuous fringing reefs. In parallel, an experimental study on cleaner pair inspections using cleaners from a continuous fringing reef produced results as expected from previous studies (Raihani et al. 2010). We therefore repeated the study with cleaner wrasse caught simultaneously from the isolated reef patches and from a continuous fringing reef to explicitly test the possibility that individuals from one specific location fail to conform to game theoretic predictions against the alternative that some hidden variable concerning animal housing or experimental procedure had caused the failure. Given repeatability of the previous results, we asked what factors may be linked to the differences. Therefore, at both sites, we quantified cleaner wrasse density, client fish density and diversity and observed natural interspecific interactions. Taken together, these data allow an assessment of the social environmental complexity. As patch reefs were small and sparsely distributed, we predicted that we would document a lower client density and diversity there.

Differences in social environmental complexity may potentially yield a functional explanation for any observed differences between cleaners from the two habitat types, but we decided to also start investigating potential mechanisms underlying the differences. On a phenotypic level, we asked whether cleaners from the two sites differ in aggressiveness and boldness, as these personality traits may be linked to cooperation and cognition (Milinski 1987; Mathieu et al. 2012). For example, if habitats differed in predator density that may affect boldness (cleaners exposed to fewer predators being bolder; see Dingemanse et al. 2007 for a study on sticklebacks) and differences in cleaner density may affect aggressiveness (i.e. starlings: Nephew & Romero 2003; salmon: Blanchet et al. 2006). Finally, we captured juveniles from the two habitats types (two locations from each type) and repeated the same laboratory experiments to assess whether there is any evidence for the importance of ontogenetic effects on cooperation and cognition. A lack of difference in performance between juveniles from the two habitats would suggest that the observed differences between adults are due to experience.

Study Area

Our study was conducted at Lizard Island, Great Barrier Reef, Australia. Adult cleaner wrasse were observed and collected from two habitats: the continuous fringing reef at Mermaid Cove and the small patch reefs adjacent to Corner Beach (Figure 1). The fringing reef at Mermaid Cove measures approximately 20 000 m^2 (depth 1 – 7 m) and is located in a small bay on the northern side of the island. Corner Beach patch reefs consist of approximately 50 small and isolated reef patches (depth 5 – 7 m), measuring 1 – 15 m in diameter and separated by at least 4 m of open sand. All laboratory experiments were conducted at Lizard Island Research Station. Due to the explorative nature of the study, we progressed step-by-step, collecting data on three different field trips. The first one in 2010 focused on laboratory experiments with adult cleaners. During the second in 2011, we collected information in the field, while the decision to test juveniles during the third trip 2012 was based on the results of the first two trips.

Figure 1 | Lizard Island Group. Study reef locations are indicated by filled circles: Mermaid Cove continuous reef (MCCR), Corner Beach patch reefs (CBPR), Bird Island continuous reef (BICR) and Bird Island patch reefs (BIPR).

Experimental Procedures

Cognitive cooperation experiments (July – September 2010)

Twenty adult female cleaner wrasse, 10 from each habitat (Mermaid Cove and Corner Beach), were caught using hand and barrier nets (2 m x 1 m, 5 mm mesh) and individually housed in aquaria (62 cm x 27 cm x 37 cm) for 7 days prior to the commencement of experiments. All experiments on game theory followed established protocols involving Plexiglas plates as surrogates for clients (Bshary & Grutter 2005), using mashed prawn and fish flakes as food items to mimic preferred mucus (i.e. cheating) and less-preferred ectoparasites (i.e. cooperating), respectively. We first confirmed that cleaner wrasse preferred to feed on mashed prawn significantly over fish flakes mixed with equal volume of prawn, termed “flake” (Bshary & Grutter 2005), and subsequently, exposed them to the opportunity to learn that eating a prawn item would lead to the removal of the plate. Each cleaner was exposed six times to a plate containing 12 flake items and 2 prawn items, where eating prawn led to the immediate removal of the plate. Due to the skewed ratio, cleaners were more likely to consume a flake item, prior to consuming a prawn item, and hence, experienced that eating flake is accepted while eating prawn is not.

“Feeding against a preference” experiment

We measured the willingness of cleaner wrasse to feed against their preference to prolong an interaction (Bshary & Grutter 2005). The willingness to feed against their food preference was tested by offering each cleaner a novel Plexiglas plate containing three prawn and three flake items. Cleaner wrasse were allowed to forage until a prawn item was consumed; thereafter, the plate was removed until the next test trial, 60 minutes later. Thirty rounds were conducted over 3 days.

“Bystander effect” experiment

In a simplified version of Bshary & Grutter (2006), we tested whether cleaner wrasse are able to eat more against their preference in the presence of an “image scoring bystander” plate that only became accessible if the cleaner avoided prawn on the first plate. Cleaner wrasse had to avoid eating any prawn item on a current plate in the presence of a “bystander” plate, to subsequently, gain access to the “bystander” plate. If prawn was consumed on the first plate, both plates were removed. If only flake items were consumed on the first plate, the second plate remained in the aquarium. If a prawn item was consumed on the bystander plate, both plates were removed. Cleaner wrasse were alternatively offered a single Plexiglas plate containing two flake and two prawn items (control: as in the “feeding against a preference” experiment) or two differently coloured Plexiglas plates, each containing two flake and two prawn items (treatment). The ratio of flake to prawn items eaten and the total number of times a cleaner succeeded to the bystander plate were recorded. A total of 30 control and 30 treatment trials were conducted over 6 days, the order of presentation being counterbalanced over each four consecutive trials. No pre-training was offered, apart from the knowledge cleaners had obtained in experiment 1. To test for a change in the response of cleaners over feeding trial session depending on which habitat they came from, we carried out a general linear mixed-effects model (glmmPQL function in R3.02 on response data [binomial family] with factors habitat, treatment and trial and fish identity as a random factor in the error term. Fixed effects: FIResponse ~ Group + Trial + Habitat + Group * Habitat + Trial * Habitat + Group * Trial + Group * Trial * Habitat). Bshary & Grutter (2006) had tested cleaners also in a third situation, namely offering two places that were retrieved independently of each other, that is, each one only once the cleaner had eaten a prawn item off it. This control was important to demonstrate that the increased feeding against preference on the first plate was due to the "image scoring" of the second plate. As cleaners from the continuous reef did not adjust their likelihood to feed against their preference when offered one or two independent plates, we saw no need to replicate these results in the current study.

"Biological market" experiment

We tested the cleaner wrasse' ability to learn to prefer an ephemeral plate over a plate which offered an equal value of food and was always accessible (initial learning and learning after role reversal) (Salwiczek et al. 2012). Cleaner wrasse were presented simultaneously with two different Plexiglas plates, each containing one prawn item. One represented a resident client, which was willing to wait to be inspected, while the other plate represented a visitor client, which was removed from the aquarium if the cleaner fed on the "resident" plate first. The optimal solution was to always feed from the "visitor" plate first. The status of each plate was predetermined and plate positions were counterbalanced. The number of trials that a cleaner required to develop a significant preference (9/10 trials or two consecutive 8/10) for the "visitor" plate was recorded. To control for plate preferences, the status and behaviour simulated by each plate was subsequently reversed, and the experiment was repeated. The task was reversed after the initial treatment was learned. A maximum of two-hundred trials were conducted over 10 days per cleaner.

Personality experiments (July – September 2010)

Cleaner wrasse aggression was measured by placing a mirror inside the aquarium against a wall and recording the number of mirror "mouth fights" within the subsequent 2 minutes. Boldness was measured by offering the cleaner wrasse food on a Plexiglas plate with novel colour patterns, and recording the time required to touch it. Two sessions were performed, one prior to and one after cognitive cooperation experiments, 25 days apart.

Fish censuses and field observations (July – August 2011)

The abundance and diversity of client reef fishes and cleaner wrasse was estimated using ten replicate 30 m transects within each reef environment, which were haphazardly placed either parallel to the reef crest (Mermaid Cove) or parallel to the shoreline across a patch reef (Corner Beach patches). SCUBA divers recorded all visible fish clients and cleaner wrasse in either a 5 m (client individuals > 10 cm total length (TL)) or 1 m (client individuals < 10 cm TL) wide area along the 30 m transect. All fishes were identified to species level when possible and census methods followed Wismer et al. (2009).

Natural cleaning interactions were recorded for 16 randomly selected adult female cleaner wrasse (8 from each reef environment), which were filmed (Cannon G9, Lumix TZ3) on SCUBA for 30 minutes, between 09:00 and 10:30 h, at a distance of 2 m. For each cleaner-client interaction, we recorded client species (including “visitors” with access to several cleaning stations) and the duration of cleaning interaction.

Juvenile cleaner wrasse (January 2012)

All aforementioned plate experiments were repeated on juvenile cleaner wrasse (measuring < 2.5 cm TL). In total, sixteen juvenile cleaner wrasse were caught from both habitat types (i.e. continuous reef and patch reefs). Due to the low availability of juveniles at Corner Beach patch reefs, we captured juvenile cleaner wrasse at two locations for each habitat type, including the patch reefs and the fringing continuous reef adjacent to Bird Island on the exposed side of Lizard Island (i.e. four individuals were collected per site) (Figure 1). Collection and experimental protocols followed that of adults.

Results

Adult cleaner wrasse in the cognitive / cooperative laboratory experiments

Adult female cleaner wrasse caught from the continuous reef performed better across all laboratory learning tasks compared with their patch reef counterparts. In the “feeding against a preference” experiment, continuous reef cleaner wrasse ate a significantly higher ratio of flake to prawn items in comparison with patch cleaner wrasse (Mann-Whitney U test, $m = 10$, $n = 10$, $z = -2.95$, $p = 0.003$, Figure 2a). In fact, continuous reef cleaners ate significantly against their preference, i.e. more than the 0.75 flake items per round expected if cleaners eat randomly (Gingins et al. 2013) (Wilcoxon one sample test, $n= 10$, $t = 7.5$, $p < 0.05$), while patch reef cleaners ate significantly according to their preference, that is, < 0.75 flake items per round (Wilcoxon one sample test, $n = 10$, $t = 3$, $p < 0.01$. In the “bystander effect” experiment, the Repeated Measures ANOVA revealed a significant difference with respect to the interaction between feeding against preference between the “single” plate and the “first” plate in the image scoring situation and location ($F_{1,17} = 27.9$, $p < 0.001$). Only individuals from the continuous reef significantly increased feeding against their preference in the image scoring situation (Figure 2b). As patch reef cleaner wrasse largely failed to adjust their behaviour to the image scoring situation, they succeeded to the second plate less often than continuous reef cleaner wrasse (Mann-Whitney U test, $m = 10$, $n = 9$, $z = 2.20$, $p = 0.027$, Figure 2c). Interestingly, continuous reef cleaner wrasse responded to “bystander” plates from the onset of feeding trials. In our full model, the effects of situation (one plate or two plates) and the cleaners’ habitat (continuous reef or patch reef) were both significant ($p = 0.024$ and $p = 0.0006$, respectively), while neither treatment group improved during the experiment (General linear mixed-effects model, $df = 1115$, $t = 1.23$, $p = 0.22$), and none of the interactions were significant either (all $df = 1115$, all $t < 1.2$, all $p > 0.24$) (Figure 2d, e). Lastly, continuous reef cleaner wrasse completed the “biological market” experiment (involving the choice of an ephemeral food source over a permanent one) in a fewer number of trials than patch reef cleaner wrasse, which generally failed to complete the task within the maximum of 200 trials (Mann-Whitney U test, $m = 10$, $n = 9$, $z = 2.20$, $p = 0.026$, Figure 2f).

Laboratory experiments on aggressiveness and boldness in adult cleaner wrasse

In contrast to the experimental findings on cooperation and cognition, cleaners of the two sites did not differ significantly with respect to aggressiveness or exploration, in either of two experimental sessions each (Mann-Whitney U tests, m = 10, n = 10, z = -1.36-1.17, p = 0.174-0.364) (Figure 3). Individual performance correlated significantly between experimental sessions (Spearman Rank correlations, all n = 20; aggressiveness: $r_s = 0.689$; exploration: $r_s = 0.759$, both $p < 0.05$).

Fish censuses and field observations

The continuous reef site, compared with patch reefs, had significantly higher client abundance and diversity estimates, as well as cleaner densities (unpaired t-tests, all n = 10; client abundance: t = 5.25, p < 0.001; diversity: t = 4.59, p < 0.001; cleaner density: t = 3.61, p = 0.002, Figure 4). This resulted in a higher cleaner to client ratio, as an indicator of between-cleaner competition, at the continuous reef (1.14 cleaner wrasse per 100 clients) versus the patch reef location (0.64 cleaner wrasse per 100 clients) (Mann-Whitney U test, m = 10, n = 10, z = 2.57, p = 0.010).

Cleaner wrasse from the continuous reef, compared with patch reefs, had significantly more interactions, a higher diversity of client species, and a larger number of clients classified as visitors (Mann-Whitney U tests, all m = 8, n = 8; total interactions: z = -3.20, p = 0.001; diversity: z = -2.73, p = 0.006; visitors: z = -2.52, p = 0.011, Figure 5). Nonetheless, the duration of individual client interactions and the proportion of time spent cleaning did not differ significantly between cleaner wrasse of the two reef environments (Mann-Whitney U tests, all m = 8, n = 8; duration: z = 1.31, p = 0.189; cleaning proportion: z = -1.36, p = 0.172, Figure 5).

Juvenile cleaner wrasse

In contrast to adult cleaner wrasse, the performance of juveniles from the two contrasting habitats did not differ significantly from one another in any of the three laboratory tasks (Figure 6). In the initial “feeding against a preference” experiment, both continuous and patch reef juveniles fed against their preference at a relatively similar ratios (i.e. median of 1.4 and 1.33, respectively) (Mann-Whitney U test, m = 8, n = 8, z = -0.21, p = 0.833) (Figure 6a). In the “bystander effect” experiment, both continuous and patch reef juveniles fed more against their preference on the “first” plate in the “two-plate image scoring” scenario than when interacting with the “single” plate (Figure 6b), with no significant interaction between plate identity and location (Repeated Measures ANOVA: plate identity: $F_{1,14}$ = 8.5, p = 0.011; location: $F_{1,14}$ = 0.4, p = 0.53; interaction: $F_{1,14}$ = 1.7, p = 0.22). All individuals from both location managed to access the second plate in the image scoring situation and at similar rates (Mann-Whitney U test, m = 8, n = 8, z = 0.0, p = 1.0) (Figure 6c). Like adults from the continuous reef location, they fed less against their preference on the “second” plate compared with the “first” plate in the image scoring situation (Wilcoxon test, n = 16, z = -2.25, p = 0.024).Like the adults, juveniles responded to “bystander” plates from the onset of feeding trials, and neither treatment group improved during the experiment (General linear mixed-effects model, df = 302, t = -0.834, p = 405) (Figure 6d, e). Lastly, both continuous and patch reef cleaner wrasse failed to complete the “biological market theory” experiment in 200 trials, and hence, the performance between the two juvenile groups did not differ significantly from one another (Mann-Whitney U test, m = 8, n = 8, z = 0.420, p = 0.674) (Figure 6f).

The juveniles were collected from four locations rather than from two like the adults, and we did not quantify cleaner and client densities as well as client diversity and interaction patterns at the two added sites. As the addition might have caused uncontrolled variance, we decided to calculate explicit comparisons of performances by individuals collected only at the adult reef patch system. In experiment 1, the four juveniles ate significantly more against the preference than the ten adults from the same location (mean juveniles = 2.01 flake items per trial; mean adults = 0.51 flake items per trial; Mann-Whitney U test, m = 10, n = 4, z = -2.70, p = 0.004). In experiment 2, the four juveniles altered their foraging behaviour between single plate and first plate in the image scoring situation significantly more so than the 10 adults did (mean increase juveniles = 2.78 flake items per trial equalling 180% increase; mean adults = 0.091 flake items per trial equalling 16% increase; Mann-Whitney U test, m = 9, n = 4, z = -2.47, p = 0.011). As a consequence, juveniles were significantly more likely than adults to gain access to the second plate during image scoring trials (mean 57% of trials for juveniles and 2.2 % of trials for adults; Mann-Whitney U test, m = 9, n = 4, z = -2.92, p = 0.003).

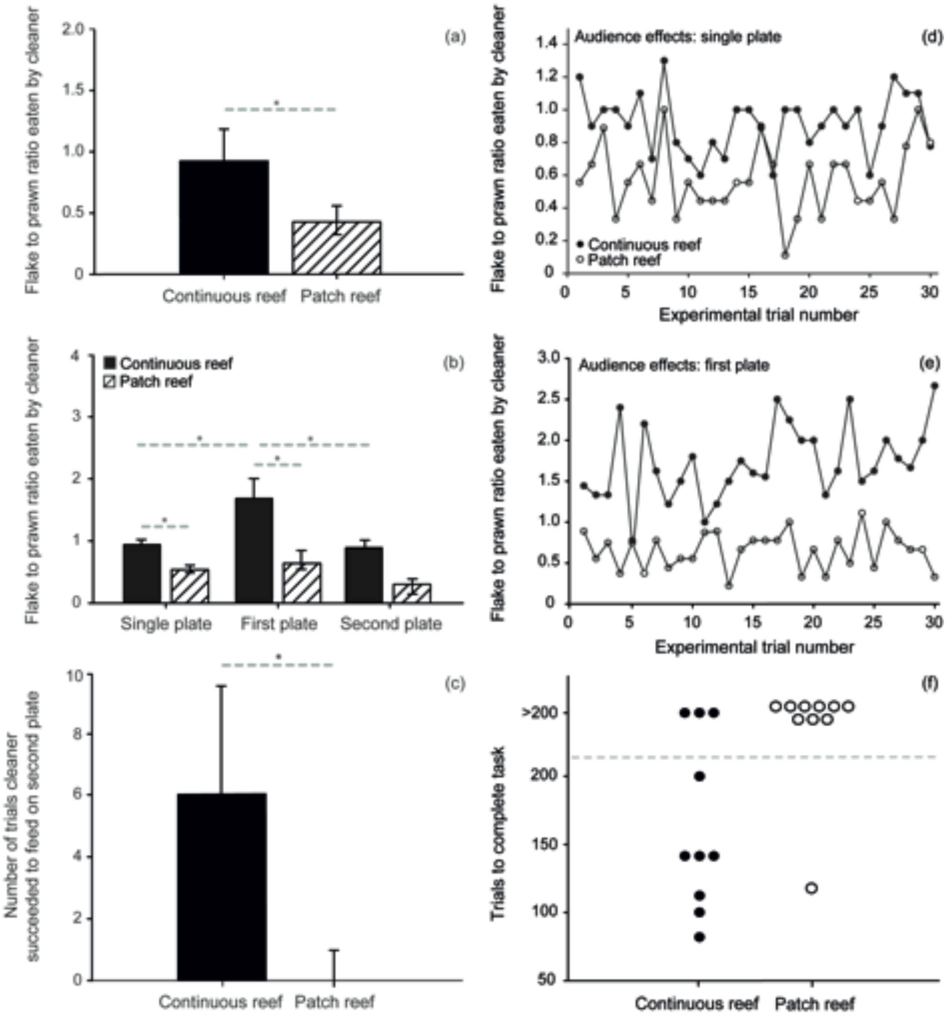

Figure 2 | Behaviour of Adult Cleaner Wrasse in the Laboratory
"Feeding against a preference" experiment, a) median flake to prawn ratio consumed. "Bystander effect" experiment, b) median flake to prawn ratio consumed per plate type, c) median number of times cleaner succeeded to feeding on second plate in the "two-plate, image scoring" scenario, d) median flake to prawn ratio consumed over 30 trials in "single" plate control and e) "first" plate treatment scenario. "Biological market" experiment, f) number of trials needed to complete both initial and reversal component (maximum 200 trials). Error bar: interquartiles. *: significant differences between cleaner wrasse of the two reef environments (all $p < 0.03$).

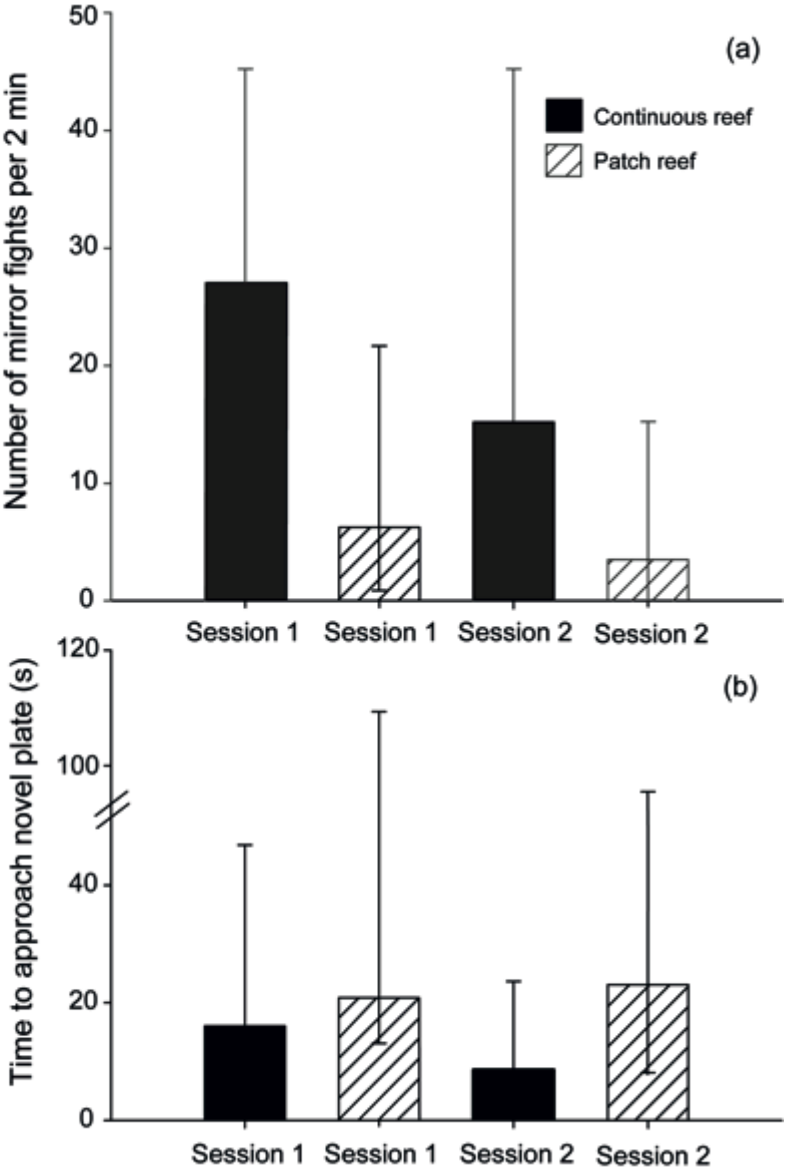

Figure 3 | Boldness and Aggression do not Differ Between Continuous and Patch Reef Cleaner Wrasse a) Number of mirror fights per 2 minutes as a measure of aggressiveness. b) Duration (seconds) to approach a plate with novel colour patterns as a measure of boldness (or exploration). Values are median and interquartile (error bars).

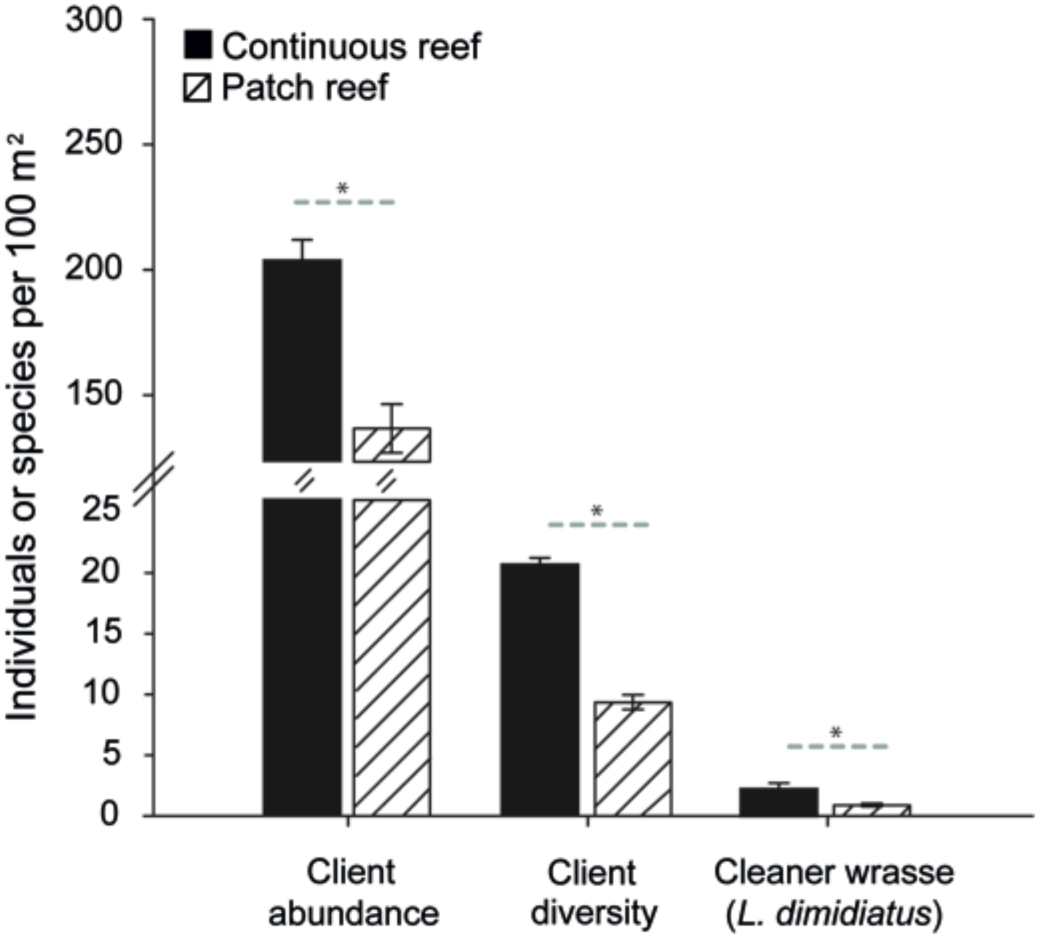

Figure 4 | Fish Estimates on Continuous and Patch Reefs Abundance and diversity of reef fish clients and abundance of cleaner wrasse at the continuous fringing reef at Mermaid Cove and Corner Beach patch reefs, Lizard Island, Great Barrier Reef. Values are mean and standard error (error bar). *: significant differences between the two reef environments (all $p \leq 0.002$).

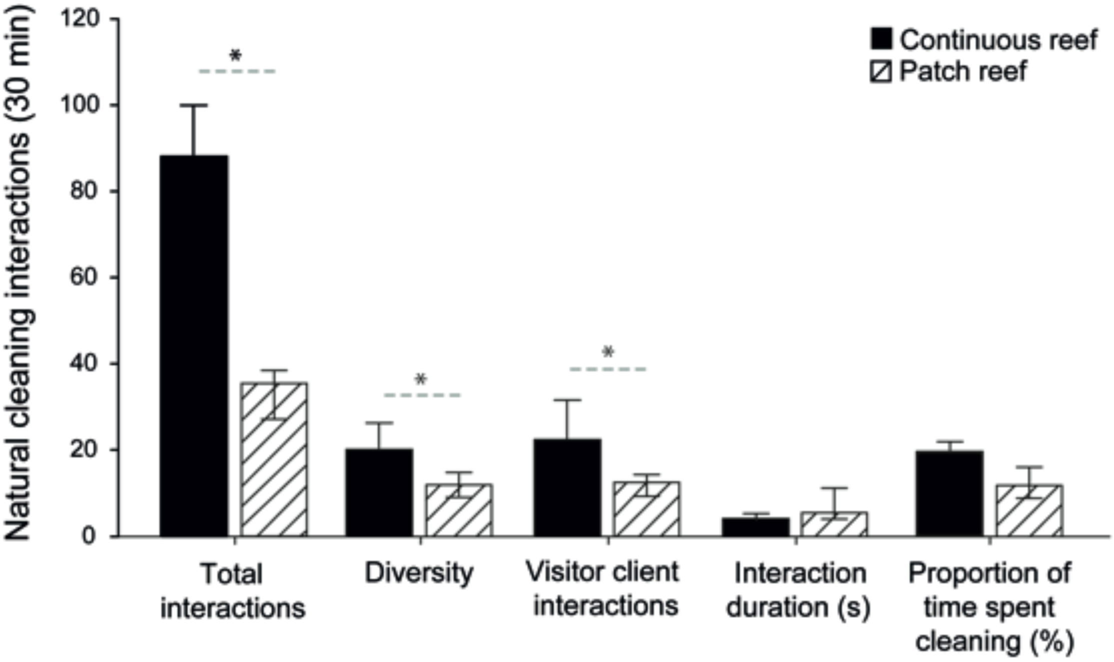

Figure 5 | Behaviour of Cleaner Wrasse on Continuous and Patch Reefs
Characteristics of natural cleaning interactions at Corner Beach patch reefs and Mermaid Cove continuous reef. Values are median and interquartiles (error bars). *: significant differences between cleaner wrasse of the two reef environments (all $p \leq 0.011$).

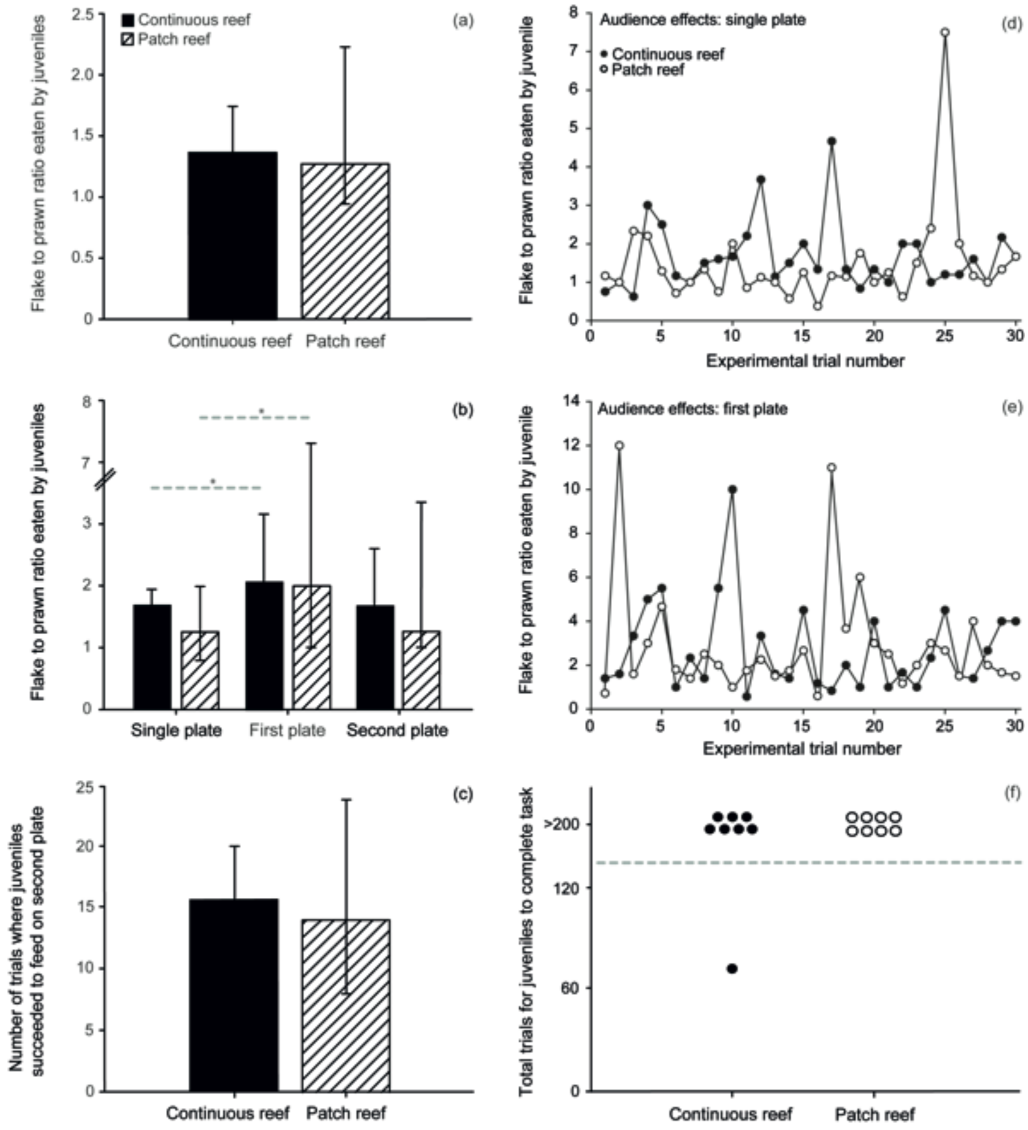

Figure 6 | Behaviour of Juvenile Cleaner Wrasse in the Laboratory
"Feeding against a preference" experiment, a) median flake to prawn ratio consumed. "Bystander effect" experiment, b) median flake to prawn ratio consumed per plate type, c) median number of times juvenile cleaner succeeded to feeding on second plate in the "two-plate, image scoring" scenario, d) median flake to prawn ratio consumed over 30 trials in "single" plate control and e) "first" plate treatment scenario. "Biological market" experiment, f) number of trials needed to complete both initial and reversal component. Error bar: interquartiles. *: significant differences of flake to prawn ratio eaten by juvenile cleaner wrasse between the single and treatment plates ($p < 0.05$).

Discussion

The cooperation experiments demonstrate an important mismatch between the behaviour of adult cleaner wrasse from a particular reef location, consisting of patch reefs, and published evidence linking cleaning strategies with game theoretic predictions regarding audience effects (Nowak & Sigmund 1998) and biological markets (Noë 2001; Pinto et al. 2011; Salwiczek et al. 2012). Feeding against their preference, incorporating image scoring by “food sources”, and preferring an ephemeral food source would have yielded more food and hence would have been superior decisions. Indeed, individuals from the continuous reef appeared to assimilate the necessary detailed information regarding client strategies and applied their decision rules quickly to our laboratory experiment. As such, results from the patch reefs correspond to various results on human cooperation where mismatches between predictions and observations have been documented, leading to discussions about decisions rules underlying behaviour (Gigerenzer & Selten 2002; Fehr & Fischbacher 2003; Haley & Fessler 2005; Kümmerli et al. 2010).

So why did we observe such a mismatch between theory and the data from patch reef cleaners? Our ecological data suggest that the mismatch is linked to living in a comparatively simple social environment. First, cleaners on the patch reefs have an estimated 800 cleaning interactions per day, compared to 2’000 on the continuous reef site. This means that image scoring situations or resident and visiting clients seeking cleaning simultaneously will occur at lower frequencies at the patch reef site. This reduces the frequency in which benefits of detailed knowledge may be obtained and at the same time longer time intervals and less frequent exposure probably make learning more difficult. Second, the lower cleaner density together with the lower cleaner to client ratio at the patch reefs means that it is more costly for visiting clients to exert partner choice in a biological market (Noë 2001; **Johnstone & Bshary 2008**), lowering the potential costs for cleaners of ignoring visitors or cheating in their presence. Taken together, these effects of a comparatively simple social environment may make it advantageous to ignore the available detailed information in nature, which leads to failure in our cognitive laboratory experiments. The experiments test for rather diverse abilities. Feeding against preference is not so much a learning experiment but a test for restraint (a psychological parameter). The image scoring experiments apparently tapped into existing decision rules: cleaners from the complex social environment spontaneously fed more against their preference in the presence of a second plate and did not improve over the course of the experiment. Finally, the market experiment tested learning abilities directly. Nevertheless, it could be that cleaners from the complex social environment had knowledge from interactions with real clients they could apply to the task, while cleaners from the simple social environment may have lacked the knowledge. In conclusion, the differences in social composition between the two locations are striking and provide a good working hypothesis for the explanation of the documented differences.

Cooperation, cognition and personality

We found no evidence that differences in performance between the cleaners caught at the two sites can be explained with a personality syndrome that would link the two standard axes tested in animal behaviour, aggressiveness and/or boldness (i.e. Wilson et al. 1994), to cooperation and cognition. This contrasts with the limited research on the link between cooperation and personality in animals, which has hitherto provided some evidence for the importance of behavioural syndromes as explanation for individual variation (Bergmüller et al. 2010**)**. In a classic study on predator inspection in sticklebacks, cooperative behaviour was linked to boldness (Milinski 1987). Furthermore, helpers in cooperatively breeding cichlids fall into two broad life history classes: bold individuals help in aggressive tasks (territory defense, predator harassment) and are likely to migrate, while shy individuals help in maintenance tasks (egg fanning, sand digging) and are likely to queue for breeding positions within the territory (Bergmüller 2010**)**. Other studies also found correlations between aggressiveness and or boldness/exploration and cognitive performance (Boogert et al. 2006; Guillette et al. 2009; Sih & Del Giudice 2012). Thus, our results differ from previous studies in providing evidence for an environment-linked cooperative personality and cognitive ability in cleaner wrasse, which is independent of the two personality traits we tested.

On the potential role of ontogeny

A major challenge is to test how the differences come about. Genetic variation that is maintained by differential selection in the two habitats offers one possible explanation, while ontogenetic effects provide an alternative. Though a pelagic egg and larval stage, as found in *L. dimidiatus*, results in a lack of genetic population structure (Avise & Shapiro 1986), it could still be that an initial mixture of more/less genetically cooperative and cognitive juveniles shows different survival depending on the local conditions, or that different types of juveniles select the habitat to which their genetic levels of cooperation/cognition fits. Our results on the juveniles certainly contradict the latter hypothesis as juveniles generally performed well in the first two experiments, independently of location. The results were not due to our sampling of four sites for juveniles in contrast to only two sites for the adults as the direct comparison between adults and juveniles from our main reef patch location yielded the same significant differences. Furthermore, we find it difficult to reconcile the data with the differential survival hypothesis. As it stands, adult cleaners from the patches could not show audience effects while juveniles from the same habitat could, and only adults from the continuous reef solved the full partner choice experiments while juveniles did not. The latter results conform to an earlier study (Salwiczek et al. 2012) and could be due to juveniles interacting relatively infrequently with visitors (Barbu et al. 2011). It thus appears that cleaners living in a socially simple environment may lose the ability to respond spontaneously to image scoring by clients, while cleaners living in a complex social environment acquire the ability to learn to prefer visiting client species. Note that these changes may well be adaptive in each environment. Possibly, clients in the marginal habitat do not image score and hence cleaners learned to stop caring, which would explain why they do not respond in the experiment either. in any case, such results seem to be more parsimoniously explained with ontogentic effects due to learning/forgetting than with differential selection on genetic strategies. In line with this view, evolutionary developmental studies have demonstrated the profound effects that rearing environments can have on an animal's learning abilities (van Praag et al. 2000; Kotrschal & Taborsky 2010; Thornton & Lukas 2012). In particular for fishes it has been demonstrated that their brains are highly plastic, and variation can be linked to cognitive performance (Ebbesson & Braithwaite 2012; Gonda et al. 2012). Indeed, our results indicate that natural variation in complexity may present promising experimental opportunities to investigate links between development and cognition. In our view, the ‘simple’ reefs still boasting an estimated 800 (versus 2000 for complex reef) social interactions per 11-12 hour day, make the cleaners’ failure in our experiments even more surprising.

Nevertheless, we note that a potential causal link between low client abundance, low client diversity, low interaction frequency and the poor performance of the patch reef cleaner wrasse is amenable to further experimental examination. Translocation experiments would resolve the current shortcoming of our data. As it stands, our current evidence is correlative, and the two locations studied in detail for the comparison between adults potentially differ with respect to various factors other than client fish community. Increasing the number of locations is unlikely to provide a solution as we predict that low client density and diversity will invariably be associated with locations containing reef patches with low coral cover and poor visibility. Translocation experiments would also overcome the problems inherent in our explorative step-by-step approach, where laboratory experiments on adults, field measures and experiments on juveniles were conducted in consecutive years. While this approach was necessary due to the surprising nature of our results that are not supported by theory and previous studies, the consequence is that there is the possibility of unexplained variance due to unmeasured ecological variation between years. Another important future direction will be to test whether cleaners exposed to complex social environments are also better at solving tasks that are not specifically linked to cleaning interactions. As it stands, our results could be largely due to prior experience, leaving open the question whether complex social environments cause a general improvement in cognitive abilities.

Our results have several important implications for cooperation theory and decision making theory in general. Most notably, our results seem to oppose the bounded rationality hypothesis (Gigerenzer & Selten 2002), which focuses on the advantage of simplification in a complex environment. According to this framework, we would have expected that cleaners from the simple social environment are more precise in their actions, instead of the opposite. We think that future empirical and theoretical research on cooperation would greatly benefit from more detailed analyses of costs and benefits underlying different decision rules. Evolutionary theory has proven useful in predicting behaviour when trade-offs are specified and mechanisms underlying behaviour are incorporated into models (Davies et al. 2012). However, this has rarely been applied to evolutionary game theory on cooperation and is currently not listed as a priority (Nowak 2012). Nevertheless, we need a theory that makes predictions about learned decision making strategies in both animals and humans. With respect to cooperation, we need a theory that can better explain learned decision making strategies in both animals and humans. For example, intelligence or executing precise decisions induces a cost on an individual in the form of investment of detailed learning. For patch reef cleaners, the investment and benefit of acting precise may not be worth the associated cost, and decision rules which work well in complex environments may not be applicable or even necessary in more simple environments. In contrast, cleaner wrasse from complex environments may invest in precise strategies since the net benefit may be worth the cost. Ideally, game theory should integrate assumptions about the costs and benefits of information gathering and storage, as well as, learned decision making mechanisms (Mery & Kawecki 2003; Heyes 2010; Lotem & Halpern 2012). With such an approach we are likely to gain further insight into realistic decision rules to possibly understand when deviations from seemingly optimal strategies are adaptive and how that affects the evolution and stability of cooperation.

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