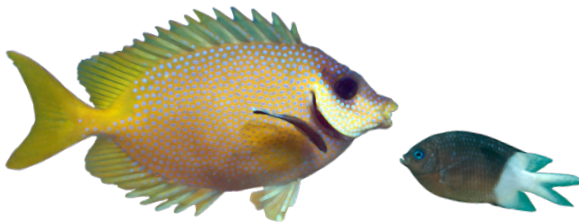


THE ECOLOGY UNDERLYING DECISION RULES
OF BLUESTREAK CLEANER WRASSE
DURING CLIENT INTERACTIONS

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PhD Thesis submitted to:

The Faculty of Sciences, Institute of Biology
University of Neuchâtel, SWITZERLAND

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Defended on the 30th of January 2017

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Titre:

**“The Ecology underlying Decision Rules
of Bluestreak Cleaner Wrasse
during Client Interactions”**

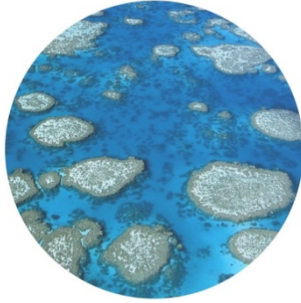
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Neuchâtel, le 16.05.2017

Le Doyen, Prof. R. Bshary





The cure for anything is salt water. Sweat, tears, or the sea.

- ISAK DINESEN

An understanding of the natural world and what's in it, is a source of not only a great curiosity, but great fulfillment.

- SIR DAVID ATTENBOROUGH

ACKNOWLEDGEMENTS

The undertaking of my PhD was a highly rewarding and challenging experience. It would not have been possible without the support, encouragement and guidance of the following people.

First and foremost, I would like to thank Redouan Bshary, for his supervision, patience and investment in me over the past years. I am eternally grateful for his scientific insight, our thought provoking conversations and his unwavering support throughout this thesis. He has grown into a wonderful friend and a great role model! I am so very grateful for being given the opportunity to work on such an interesting topic and being a part his lab.

My field trips ran efficiently and smoothly as a result of the hard work of the staff at Lizard Island Research Station. I kindly thank: Anne & Lyle, Lance & Marianne, Bob & Tania, and Bruce & Cassy, for their continuous support and friendship throughout the years. They truly helped make Lizard Island feel like my home away from home during my field trips that often lasted several months. I leave with a lifetime of memories and close friendships.

Field studies are a team effort! I thank the following people for their eager help while on Lizard Island: S. Binning, A. Bshary, R. Bshary, A. Derendinger, S. Gingins, C. Lefevre, F. Marcadier, J. McAfoose, R. Mendonca, A. Pinto, N. Raihani, O. Rey, D. Roche, A. Ros, D. Sun, A. Vail, P. Vulllioud, J. Welsh and E. Wismer. From endless hours of field observations, to catching and marking fish, to mashing prawn and helping perform experiments. I could not have completed my PhD without their help. Their happy faces and enthusiasm also made for a lot of fun along the way!

I kindly thank D. Bellwood, for guidance on conducting field surveys, D. Roche and A. Ros for statistical advice, D. Roche for French translations and A. Grutter for critical insight into the ecology of cleaner wrasse. My manuscripts were greatly improved due to their help. I also thank my lab

colleagues in Neuchâtel for wonderful times in the office, amazing friendships and critical discussions: M. Aellen, R. Bergmüller, S. Binning, C. Borgeaud, F. Di Lascio, T. Fagundes, S. Gingins, R. Mazzei, J. McClung, A. Pinto, D. Roche, A. Ros, M. Steinegger, M. Wubs and Z. Triki. I am very appreciative for the wonderful support I received from the friendly administrative and IT staff within the Institute of Biology, at the University of Neuchâtel, Switzerland.

I am grateful to the following funding bodies for their generous support: The Swiss National Science Foundation (R. Bshary); The Commission for Travel Grants of the Swiss Academy of Sciences (S. Wismer); La Commission Egalité - Promoting Women in Science (S. Wismer), and The Lizard Island Reef Research Foundation (2012 Doctoral Fellow, S. Wismer) - who were particularly supportive during and after my maternity leave.

And finally, I would like to thank my friends and family. I thank Jordan, my husband, for his continued love and support, not just during the course of this thesis, but throughout the past 14 years. I am especially thankful for his constant encouragement to follow my dreams, making me laugh in the darker moments of life and always believing in me. My daughter Sophie, I thank for inspiring me to be the best person I can be. She made my last field season unbelievably enjoyable by being able to experience Lizard Island through her eyes. I hope that I pass on my love for the ocean to her. To Julien, our little boy on the way, you have been the biggest motivation to finish writing up in a timely manner! Very much looking forward to meeting you! I thank my parents Edwina and Chris, and my brother Kevin, for their keen interest, love and support throughout another degree. I promise, I will get a 'real' job soon! A special mention to my mother Edwina and my mother-in-law Lois, for providing loving care to my daughter Sophie while I was conducting many months of field studies. To Ana, thank you for your sisterhood throughout the years and loving support throughout my PhD on every possible level! Lastly, a big thank you to my friends all over the world for their love, friendship and support from both near and far.

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GENERAL SUMMARY

Cooperation is defined as a ‘helping’ behaviour that provides direct fitness benefits to other individuals. Such behaviours have long intrigued biologists, as it poses a problem for classic evolutionary theory, i.e. why should an individual perform a behaviour that is beneficial to other individuals? Indeed, an expansive body of work on evolutionary game theory, as well as, empirical studies, have provided many mechanisms for promoting stable cooperation between unrelated individuals. Humans, however, often deviate from the optimal strategies predicted by theoretical models, which has emphasized the need to understand decision making processes. For example, the use of decision short cuts, known heuristics (or rules of thumb in non-human animals), allows individuals to make decisions quickly and accurately in frequently occurring situations, but may lead to less than optimal behaviour in novel contexts. Additionally, cognitive constraints, such as learning capabilities or failure to identify relevant environmental or social cues, may also cause deviations from predicated behaviour.

Using bluestreak ‘cleaner’ wrasse (*Labroides dimidiatus*) as a model system, the primary aims of this PhD thesis were 1) to investigate important mismatches between standard theoretical predictions regarding animal decisions during cooperative interactions and experimental data, as well as, 2) to explore how well cleaners are able to readily identify and use relevant cues for decision making. Cleaners engage in mutualistic relationships with so-called reef fish ‘clients’, which visit cleaner territories for ectoparasite removal. Cleaners, however, prefer feeding on nitrogen-rich client mucus, which constitutes cheating. Hence, to help ensure a cooperative cleaner, clients employ various partner control mechanisms, including punishment and partner switching. This dynamic cleaning mutualism has hitherto provided strong empirical evidence in support of evolutionary game theory for predicting cooperative behaviour.

In Chapter 2, however, I demonstrate that cleaners from socially complex reef environments largely outperform cleaners from socially simple reefs in classic cooperation- and cognition-based experiments. A lower abundance and diversity of reef fish clients, as well as, a lower density of cleaners, characterize socially simple reefs. Cleaners from these simple environments generally failed to: 1) feed against their preference, 2) adjust their cooperative behaviour in the presence of an audience, and 3) consistently provide service priority to a temporary food source over a permanent food source. These findings strongly contrast published evidence on cleaner foraging behaviour in laboratory-based experiments. To further understand these inconsistencies, in Chapter 3, I investigated whether the two cleaner groups used different cues when making foraging decisions; specifically, in regards to client service priority. Cleaners from the socially complex reef environment were found to use a precise cue when making decisions, leading to higher accuracy in the laboratory, whereas cleaners from the socially simple reef environment used a correlated cue, or a rule of thumb, which lead to an overall poorer performance.

In Chapter 4, I determined that the rules applied by the two cleaner groups in nature appear to be locally adaptive and that the cognitive constraints displayed by cleaners from the socially simple reef environment were context specific, as both cleaner body condition and cognitive performance in an abstract task did not differ between reef environments. Finally, in Chapter 5, I investigated how well cleaners are able to extract relevant cues for decisions involving cheating and refuge-seeking. Here, I demonstrated the ability of cleaners to generalize predator species in a social tool context; yet this ability disappeared when cleaners were tested in an abstract context.

Collectively, these results have important implications for both cognition and evolutionary game theory. The results are discussed with an emphasis placed on the importance of the ecological approach to cognition, as well as, suggestions for future modifications to theoretical models.

RÉSUMÉ GENERAL

La coopération est définie comme un «comportement d'aide» qui offre des avantages directs à d'autres individus. Un tel comportement a longtemps intrigué les biologistes car il pose un problème pour la théorie évolutive classique : pourquoi un individu devrait-il effectuer un comportement qui bénéficie un autre individu plutôt que lui-même? En effet, un vaste ensemble de travaux sur la théorie des jeux évolutifs ainsi que des études empiriques ont depuis identifié de nombreux mécanismes qui expliquent le maintien d'une coopération stable entre des individus non apparentés. Cependant, le comportement humain ne correspond souvent pas aux stratégies optimales prédites par les modèles théoriques, d'où la nécessité de comprendre les processus de prises de décisions. Par exemple, l'utilisation de raccourcis de décision, correspondant à une heuristique connue (ou d'une règle empirique dans le cas des animaux non-humains), permet aux individus de prendre des décisions rapides et précises dans des situations auxquels ils sont fréquemment confrontés. Par contre, ces raccourcis peuvent conduire à des comportements sub-optimaux dans des contextes nouveaux. Les contraintes cognitives, telles que les capacités d'apprentissage ou l'incapacité à identifier les indices environnementaux ou sociaux pertinents, peuvent également entraîner des différences par rapport au comportement prédit.

En étudiant le labre nettoyeur (*Labroides dimidiatus*) comme modèle, cette thèse avait pour objectifs : 1) d'étudier les importantes disparités entre les données expérimentales et les prévisions théoriques standard concernant les décisions animales lors d'interactions coopératives; et 2) d'explorer la façon dont les nettoyeurs sont en mesure de facilement identifier et utiliser des repères pertinents pour la prise de décision. Les nettoyeurs participent à des interactions mutualistes avec des poissons de récifs coralliens appelés «clients» qui viennent les visiter dans leur territoire afin de se faire déparasiter. Cependant les nettoyeurs préfèrent se comporter en parasites et tricher en se nourrissant du mucus des

client qui est riche en azote plutôt que de leurs parasites. Par conséquent, pour encourager les nettoyeurs à être coopératifs, les clients utilisent divers mécanismes de contrôle tels que la punition et le changement de partenaire. Ce mutualisme entre nettoyeurs et clients a jusqu'ici fourni de solides preuves empiriques soutenant l'usage de la théorie des jeux évolutifs pour prédire le comportement coopératif.

Dans le chapitre 2, je démontre que les nettoyeurs qui proviennent de récifs caractérisés par une structure sociale complexe surpassent largement les nettoyeurs provenant de récifs caractérisés par une structure sociale simple lors d'expériences classiques de coopération et de cognition. Les récifs « simples » sont caractérisés par une abondance et une diversité de clients moindre ainsi qu'une plus faible densité de nettoyeurs par rapport aux récifs « complexes ». Mes expériences démontrent que les nettoyeurs provenant d'environnements simples ne réussissent généralement pas à : 1) se nourrir contre leur préférence, 2) adapter leur comportement coopératif en présence d'un observateur et 3) offrir systématiquement la priorité à une source de nourriture temporaire plutôt qu'à une source de nourriture permanente. Ces résultats contrastent fortement avec les données publiées sur des comportements de recherche de nourriture dans des expériences en laboratoire traditionnelles. Pour mieux comprendre ces disparités, j'ai étudié dans le chapitre 3 si les deux groupes de nettoyeurs utilisent des indices différents lors de la prise de décisions au moment où ils vont se nourrir, particulièrement en ce qui concerne la priorité offerte aux clients. Les nettoyeurs provenant d'environnements socialement complexes sont capables de trouver un repère précis lors de la prise de décision, conduisant à une plus grande précision dans les tâches en laboratoire. Par contre, les nettoyeurs provenant d'environnements socialement simples utilisent une règle de base qui conduit à une performance plus faible lors de la même tâche.

Dans le chapitre 4, j'ai déterminé que les règles appliquées par les deux groupes de nettoyeurs en milieu naturel semblent être adaptées à leur habitat respectifs et que les contraintes cognitives des nettoyeurs de l'environnement socialement simple étaient spécifiques au contexte

dans lequel ils vivent et dues au fait que la santé des nettoyeurs et leur performance cognitive dans un tâche abstraite ne diffèrent pas entre les deux groupes. Finalement, dans le chapitre 5, j'ai étudié la façon dont les nettoyeurs sont en mesure d'extraire des indices pertinents pour les décisions impliquant la tricherie et la recherche de refuge. J'ai démontré que la capacité des nettoyeurs à généraliser la reconnaissance de différentes espèces de prédateurs dans un contexte d'outil social. Cependant, cette capacité disparaît lorsque les nettoyeurs sont testés dans un contexte abstrait.

Les résultats de cette thèse ont des retombées importantes pour faire avancer notre compréhension de la cognition chez les animaux et de la théorie des jeux évolutifs. Les résultats sont discutés en soulignant l'importance de l'approche écologique de la cognition et en suggérant des possibilités d'amélioration des modèles théoriques sur la question.

Key words

Cleaner fish; *Labroides dimidiatus*; cognition; cooperation; evolutionary game theory; decision rules; coral reef; social complexity.

Mots clés

Poisson nettoyeur; *Labroides dimidiatus*; cognition; coopération; théorie des jeux; règles de décision; récifs coralliens ; complexité sociale.

1

GENERAL INTRODUCTION

1.1 THE EVOLUTION OF HELPING

Cooperation and altruism, collectively referred to as ‘helping’, can be defined as behaviours that provide a direct fitness benefit to another individual (Lehmann and Keller 2006). In nature, such helping behaviours are widespread, ranging from single-celled organisms to higher vertebrates and includes both intraspecific and interspecific relationships (Brosnan et al. 2010). Examples span from group feeding in social insects (Ward and Enders 1985), mutualistic relationships between legume plants and Rhizobia (Kiers et al. 2003), and cooperative hunting strategies in mammals, including lions (Stander 1992) and orcas (Baird 2000).

Helping has long intrigued evolutionary biologists, as it poses a problem for classic evolutionary theory. Why should an individual perform a behaviour that is beneficial to other individuals? Altruistic helping, i.e. a behaviour which results in a decrease in direct fitness of the individual providing help and an increase in the direct fitness of the recipient (Lehmann and Keller 2006), is generally studied in the context of kin selection (e.g. Hamilton 1964; Maynard Smith 1964). Kin selection is a strategy whereby traits are favoured due to their beneficial effect on close relatives (West et al. 2007), and according to Hamilton’s rule ($rB > C$), can cause genes to increase in frequency *when* the relatedness of the recipient to the actor, multiplied by the benefit to the recipient, is greater than the reproductive cost incurred to the actor (Hamilton 1964). In this context, altruistic behaviour can be favourable, as the individual providing the help would increase its *inclusive* fitness, which includes both the reproductive success of its kin, as well as its own reproductive success (West et al. 2007).

However, when individuals are unrelated, inclusive fitness benefits derived from kin selection are not possible. Hence, explanations for ‘cooperation’ between unrelated individuals within the same species (or termed ‘mutualism’ between species; West et al. 2007) must be linked to direct fitness benefits (Lehmann and Keller 2006). For example, individuals which hunt or build nests cooperatively benefit directly

from their cooperative actions (Lehmann and Keller 2006). Cooperation may also evolve between unrelated individuals if the actor acquires direct or indirect information during interactions, which subsequently allows for a better than random guess whether a given individual will behave cooperatively in repeated reciprocal interactions (Lehmann and Keller 2006). For example, to avoid being exploited, experimental evidence suggests that humans are more likely to cooperate with individuals that have been cooperative in previous interactions (Fehr and Fischbacher 2003).

To date, many studies using evolutionary game theory (Dugatkin and Reeve 1998), as well as, empirical evidence, have demonstrated conditions or strategies which promote stable cooperation between unrelated individuals, both in humans and animals (e.g. Axelrod and Hamilton 1981; Connor 1986; Milinski 1987; Clutton-Brock and Parker 1995; Nowak and Sigmund 1998; Wedekind and Milinski 2000; Bshary and Grutter 2005). These include models of cooperation based on the Prisoner's Dilemma game (e.g. Axelrod and Hamilton 1981) and partner control mechanisms to deter defectors (i.e. cheating individuals). Such mechanisms include punishment (a behaviour in response to cheating that reduces the fitness of the instigator and discourages or prevents the cheater from repeating the action; Clutton-Brock and Parker 1995) (e.g. Bshary and Grutter 2005; Raihani et al. 2010), image-scoring (based on an individual's reputation of cooperativeness) (e.g. Wedekind and Milinski 2000; Pinto et al. 2011) and partner switching (e.g. Bshary and Grutter 2005; Fu et al. 2009).

1.2 COOPERATION AND COGNITIVE ASPECTS

Cognition often plays a key role in cooperation, without it, many of the strategies and mechanisms listed above would not be possible. The two cognitive components which appear to be particularly important are memory and individual recognition (Dugatkin 2002). This is true for humans playing experimental Prisoner's Dilemma games (e.g. Axelrod 1980; Nowak and Sigmund 1993; Gracia-Lázaro et al. 2012), where it is beneficial to remember a partner's previous moves, but also for

animals. For example, most organisms that engage in advanced cooperative relationships are mobile, which causes spatial and temporal separations between interactions (Brosnan et al. 2010). Hence, the ability to remember and recognize individuals becomes very important, as decisions can be based on past interactions, allowing for the recognition of both cooperative and non-cooperative partners (Brosnan and de Waal 2002). Hence, individuals can invest (cooperate) without being repeatedly exploited by potential cheaters (Brosnan et al. 2010). This is particularly true for certain social species, e.g. primates, where strategic behaviour, including the ability to cooperate, defect and manipulate partners, is thought to promote an individual's fitness (Byrne and Whiten 1988; Brosnan et al. 2010).

When personal experiences cannot be recalled or if no previous interactions have occurred, it is also useful to be able to use and understand 'public' information, which can help individuals make better predictions in regards to future interactions (Brosnan et al. 2010). This requires cognitive abilities that allow individuals to make decisions based on observed information, by acquiring and evaluating third party behaviour, rather than by personal experience (Brosnan et al. 2003, 2010). Hence, in such instances, cooperation is strongly linked to cognition.

1.3 THE DECISION RULES UNDERLYING COOPERATION

Even with strong links between cooperation and cognition, research has repeatedly shown that the decision rules used by humans during social interactions do not always fit the optimal strategies predicted by game theoretic models (El Mouden et al. 2012), which assume organisms behave as fitness-maximizing agents (Davies et al. 2012). For example, individuals behave more cooperatively (Feh and Fischbacher 2003; Haley and Fessler 2005) or less cooperatively (Kümmerli et al. 2010), as well as less precise (Milinski et al. 2001) or more sophisticated (Milinski and Wedekind 1998), than predicted cooperative strategies in models. El Mouden et al. (2012) discusses six explanations for such apparent 'maladaptive' behaviour. This includes: 1) natural selection

acts upon the average consequences of particular traits, and hence, the trait can be favoured if the cost/benefit analysis is positive; 2) there is variation in the solutions to fitness trade-offs across time and circumstances; 3) non-optimal behaviour can be a result of a novel environment, e.g. experimental setting; 4) perfect proxies of fitness are expected to result in a better fit between the model and the observed behaviours, yet are difficult to obtain or measure; 5) not all traits are the target of selection and can be understood using economic games; and finally, 6) natural selection favours cheap solutions, e.g. decision strategies like heuristics or rules of thumb.

Many studies focus on the heuristics explanation. In particular, deviations from models have raised considerable debate in regards to decision-making processes in humans (e.g. Gigerenzer and Selten 2002; Boyd et al. 2003; Lehmann et al. 2007; Kümmerli et al. 2010; Baumard et al. 2013). For example, ‘bounded rationality’ proposes that humans develop simple heuristics, or rules of thumb, by constantly looking for environmental cues that would trigger a response that has worked well under previous similar circumstances (Gigerenzer and Selten 2002). This allows individuals to by-pass information processing of any single situation and its unique complexity, and instead, applies a simple and frugal strategy that is likely to result in the desired outcome. Humans use heuristics in a wide range of situations, such as, when making estimates of relatedness (e.g. dialects, facial resemblance), which allows for helping behaviours to be directed towards relatives (El Mouden et al. 2012). These decision rules generally work very well, yet are less precise and potentially wrong in a novel or infrequently encountered context (Gigerenzer and Selten 2002).

1.4 PHD THESIS TOPIC

The primary aim of my PhD thesis is to explore the interactions between these three components: cooperation, cognition and decision strategies, by investigating the fine-tuned behavioural strategies employed by bluestreak cleaner wrasse (*Labroides dimidiatus*). Specifically, I investigate important mismatches between standard

theoretical predictions regarding animal decisions during cooperative interactions and experimental data. Bluestreak cleaner wrasse, or simply ‘cleaners’, engage in a mutualistic relationship with various reef fish species, by removing ectoparasites, as well as, dead and diseased tissue from these so-called ‘clients’ (Losey et al. 1999; Grutter 1995, 1996). Although each cleaner removes approximately 1200 parasites per day (Grutter 1995), their preferred food choice is client mucus, which is energetically costly to produce, and where its removal, therefore constitutes cheating (Grutter and Bshary 2003). Hence, clients have to employ various partner control mechanisms to ensure a cooperative cleaner (e.g. Bshary and Grutter 2002a, 2002b, 2005, 2006; Pinto et al. 2011). These dynamics make this marine cleaning mutualism an ideal model system to study cooperation between unrelated individuals. Indeed, it has hitherto provided strong empirical evidence in support of evolutionary game theory for predicting cooperative behaviour (Bshary 2010, 2011). (Further details are provided in the subsequent section, ‘1.7 Study species’).

Recent evidence, however, suggests that not all cleaners follow game theoretic predications. In fact, the decisions by some cleaners appear to be not particularly adaptive. In 2009, during a four-month field study investigating intraspecific variation in cleaner behaviour, our colleagues were unable to replicate published results from ‘classic’ cleaner foraging experiments (i.e. ‘cooperating’ and feeding against a food preference, audience effects and biological market theory – experiments are summarized in the general methodology below). The single differentiating factor in this study was that all cleaners were caught from small, isolated patch reefs, instead of the ‘standard’ larger, continuous fringing reefs. This was done to help identify individual subjects in the future, i.e. if a cleaner’s territory is restricted to a single patch reef, the cleaner is less likely to move to another location, making recapture of a specific individual more probable and also less time consuming. The results from this ‘failed’ project was the primary motivation for my PhD thesis, as it sparked numerous intriguing questions in regards to the decision strategies employed by cleaner wrasse in the various contexts of their cleaning mutualism. The primary

aims of this thesis were to 1) compare the performance of bluestreak cleaner wrasse from two socially contrasting reef environments in published laboratory-based cognition and cooperation experiments, 2) explore factors that could explain variation in decision rules between the two cleaner groups and 3) investigate how well different cleaners are able to identify and use relevant cues in their decision making.

1.5 GENERAL METHODOLOGY

I used established, as well as novel, laboratory-based cognition and cooperation experiments to investigate my aims, which required cleaners to feed from Plexiglas plates that representing reef fish clients. Plexiglas plates contained 1) only preferred (prawn) or 2) both preferred (prawn) and less-preferred (flake) food items, mimicking client mucus and ectoparasites, respectively. Hence, feeding on flake items simulated *cooperating* and feeding on client ectoparasites, where feeding on prawn items simulated *cheating* and feeding on client mucus. Using this methodology, I was able to test 1) how well cleaners cooperate and feed against their food preference in the laboratory (i.e. flake to prawn ratio consumed), 2) the ability of cleaners to adjust their feeding ratios when in the presence of an audience (i.e. cooperate more and consume a higher ratio of flake to prawn items when an image-scoring bystander Plexiglas plate was present), 3) the ability of cleaners to consistently give service priority to an ephemeral Plexiglas plate over a permanent Plexiglas plate (i.e. based on biological market theory, where clients have choice options in deciding on who to cooperate with – Noë and Hammerstein 1994) and 4) the ability of cleaners to evade punishment from a cheated Plexiglas plate by seeking refuge with a predator model (i.e. testing for generalization abilities among predator models). Furthermore, I used underwater video observations to quantify natural cleaning interactions and fish censuses to survey the availability of reef fish clients, as well as, to assess the densities of cleaners across the various reef environments.

1.6 THESIS CHAPTERS

The primary aim of **Chapter 2** was to first explicitly test whether variation in cleaner performance indeed exists between individuals from small, isolated patch reefs and large, continuous fringing reefs. Hence, I conducted a direct comparison of both adult and juvenile cleaners from both reef environments. As in 2009, experiments were based on cleaner foraging decisions and tested an individual's willingness or ability to 1) cooperate and feed against a food preference, 2) adjust to an image-scoring bystander and 3) repeatedly choose an ephemeral food source over a permanent food source. Secondly, in order to determine whether differences in cleaner behaviour are also apparent under natural conditions, detailed underwater observations were performed on individuals from both reef environments. This allowed me to determine, for example, whether cleaners from one reef environment engaged in a higher number of cleaning interactions or caused a higher frequency of client jolts, e.g. cheated more often. Finally, in order to correlate possible factors influencing variation in cleaner performance, the social environment was quantified by estimating client abundance and diversity, as well as cleaner density, in each reef environment. Cleaners are exceptionally social, engaging in over 2000 client interactions per day (Grutter 1995). Given that all foraging interactions are also social interactions, it is likely that social factors play a key role in a cleaner's foraging decisions. Collectively, the results from this chapter allowed me to determine exactly how cleaner performance differs between the various cooperation- and cognition-based laboratory experiments, depending on cleaner age and environment, and to speculate on the potential factors influencing such variation.

Chapter 3 builds on the results obtained from the first data chapter and investigated the decision rules applied by cleaners in regards to client service priority. In Chapter 2, cleaners from patch reefs, or 'socially simple reef environment', were indeed largely unable to solve the biological market theory experiment. In this task, cleaners were presented with a scenario where service priority had to be given to an ephemeral food source (i.e. a Plexiglas plate representing a visitor fish client) over a permanent food source (i.e. Plexiglas plate representing a resident fish client). In nature, clients categorized as visitor clients have

the option of switching cleaning stations, as they have a large home range, and will therefore leave if not immediately inspected by a cleaner (Bshary 2001). Resident clients, however, are restricted to a single cleaner territory and will queue for service (Bshary 2001). Hence, cleaners should always give service priority to visiting clients. In the laboratory, this strategy allowed for the consumption of two food items (versus one) per trial: feed on the ephemeral visitor client plate first, followed by the permanent resident client plate. The two Plexiglas plates differed only in colour (as different client species would) and were equal in size. It appeared that cleaners from the socially poor patch reef environments, as well as juvenile cleaners, are unable to differentiate between visitor and resident clients and their associated strategies in the laboratory using only colour cues. This is in contrast to cleaners from the continuous reef, which represented a socially complex reef environment. The first aim of Chapter 3, therefore, was to test whether cleaners are capable of solving this task when the only differentiating factor between the two client categories is *size*, and not colour. In nature, visitor clients are often larger than residents, and applying the rule of thumb ‘service the larger client first’ would therefore generally yield adequate foraging results. All cleaners easily solved this task when size was the differentiating factor. Subsequently, in a follow-up experiment, both plate size and colour were modified, where plate colour was a correct cue for client category and plate size a correlated cue. This allowed me to determine what cues cleaners use to make decisions when deciding on which client to give service priority to. For example, do they service the visitor Plexiglas plate first regardless of size, i.e. the ‘best strategy’, or do they apply a more general rule of thumb, i.e. service the larger plate first, regardless of colour. Once again, I compared cleaners from both reef environments in these tasks.

Major questions arising from the first two data chapters were addressed in **Chapter 4**. First, why are cleaners from the socially poor patch reef environments unable to solve our laboratory experiments? Perhaps the decision rules applied by cleaners from this reef environment are locally adaptive and our experiments simply lack ecological relevance?

Second, are the decision rules employed by cleaners flexible? For example, can individuals from either reef environment adjust their decision rules when placed in the alternative reef environment for a duration of time? These questions were investigated by 1) measuring the initial body condition of cleaners from both reef environments as a (likely) index of fitness, 2) performing a large-scale translocation experiment, where cleaners were relocated to the opposing reef environment for a 21-day period and 3) testing the cognitive abilities of cleaners from both reef environments in an abstract spatial cognitive task in order to determine whether patch reef cleaners are generally cognitively constrained or whether they excel at a task which is not linked to their ecology. The collective results from these methods are discussed in the context of adaptive behaviour and future improvements to cleaner translocation studies.

In **Chapter 5**, I further investigated how well cleaners are able to extract relevant cues when making decisions; however, unlike the experiment conducted in Chapter 3, there were no correlated cue options available. Here, I investigated the ability of cleaners to generalize in a social tool context. In nature, when cleaners cheat and consume client mucus, versus ectoparasites, it often provokes punishment in the form of aggressive chasing. Field observations have repeatedly documented cleaners to flee a chasing client and to seek shelter with a nearby predator. Hence, in such situations, the cleaner uses the predator fish as a ‘social tool’ to evade punishment from the cheated client. The aim of Chapter 5 was to determine whether cleaners use generalized rule application to identify potential social tools. To address this question, I experimentally simulated the described social tool scenario in the laboratory using a variety of predator and harmless species fish models. For example, can cleaners generalize predators to minimize punishment or do they learn the usefulness of each predator species independently? The results of this study emphasize the importance of ecologically relevant experiments to uncover complex cognitive processes in non-human animals. Given the high failure rates of patch cleaners in laboratory-based cognitive tasks, this experiment was only conducted on cleaners from the socially complex continuous

reef environment.

1.7 STUDY SPECIES – CLEANING MUTUALISM

1.7.1 Distribution and biological characteristics

Bluestreak cleaner wrasse, *Labroides dimidiatus* ('cleaners'), are a territorial, reef-associated marine fish belonging to the family Labridae (Randall et al. 1997; Figure 1). They are widely distributed across the Indo-Pacific Ocean, ranging from the Red Sea and East African reefs, to the Great Barrier Reef and the South Pacific, often inhabiting coral-rich reefs in a depth range of 1 - 40 m (Randall 1986; Lieske and Myers 1994). In these regions, cleaners are known as a keystone species that promote reef fish abundance and biodiversity (Amal et al. 1999; Bshary 2003; Grutter et al. 2003; Waldie et al. 2011). These important fish can reach a total length (TL) of 14.0 cm (Allsop and West 2003) and have been documented to live up to 4 years (Robertson 1974); although, a lifespan of 2 years is much more common. All cleaners are born female and are considered protogynous hermaphrodites living within a harem mating system, which generally consists of a single dominant male, 4 to 6 adult females and 1 and 2 juveniles (Heron Island: Robertson 1972; Robertson and Choat 1974). When the dominant male disappears or dies, the largest female of the harem transitions, often around 9 cm in TL, with total sex reversal complete within 18 days (Robertson and Choat 1974; Allsop and West 2003). There is very little dimorphism between the two sexes, with size (particularly length) being the only distinguishing feature; although, the colouration patterns of juveniles varies considerably (Figure 1).

1.7.2 Cleaning ecology

As the name suggests, cleaners 'clean' by removing ectoparasites and dead or diseased tissue from the body surface, gills and oral cavity of other reef fish (Losey et al. 1999; Côté 2000). They do so within their small territories termed 'cleaning stations', which are generally confined to a few cubic meters of reef and often contain a distinctive

feature, e.g. cave or large coral bommie, that acts as a visual cue for so-called returning reef fish ‘clients’ (Kuitert and Tonzuka 2001; Bshary and Noë 2003). Clients, which consist largely of teleost fish, visit cleaner stations between 5 to 30 times per day and may spend up to 30 min per day being inspected (e.g. *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, with each cleaner engaging in approximately 2000 daily client interactions and consuming more than 1200 parasites per day (predominantly gnathiid isopod larvae; Grutter 1996) (Grutter 1995). Cleaners inspect and clean clients either as singletons or working in a pair, e.g. a male and the largest female in his harem, with recent evidence suggesting that the latter option provides superior service quality to visiting reef fish clients (Bshary et al. 2008).

The overall relationship between cleaners and their reef fish clients is mutualistic (Grutter 1999; Grutter and Bshary 2003), where in the process of removing large amounts of parasites (Grutter 1996), cleaners benefit from a rich and relatively untapped food source (Poulin and Grutter 1996). Consequently, client parasite load is significantly reduced (Grutter 1999), that otherwise may lead to a reduction in growth, and eventual reproductive output (Poulin and Grutter 1996; Clague et al. 2011; Waldie et al. 2011). However, there is still a degree of conflict within this mutualistic relationship. Experimental evidence suggests that cleaners significantly prefer to feed directly on the nitrogen rich, protective mucus layer of client fish, instead of the gnathiid isopod ectoparasites (Grutter and Bshary 2003). Such an act constitutes cheating, as mucus is energetically costly for a client to produce (Grutter and Bshary 2003). Given that cleaners remove a significant proportion of ectoparasites per day, this suggests that cleaners often feed against their food preference under natural conditions and face a daily conflict over what to feed on (Grutter and Bshary 2003).

1.7.3 A model system for the study of cooperation

The cleaning mutualism between cleaners and clients is an ideal model system to study cooperation between unrelated individuals, for several reasons. Primarily, the dynamic balance between cheating versus cooperation of cleaner wrasse, e.g. feeding on mucus versus ectoparasites, allows for the in depth study of partner control mechanisms, while their social nature, which includes 2000 social interactions per day, allows for abundant opportunities. Furthermore, cleaners are easily caught, habituated and taught laboratory-based cooperation tasks, which has led to several key findings with broader cooperation implications, as summarized below.

Partner control mechanisms are integral in this cleaning mutualism, as client reef fish have to counteract and avoid cheating cleaners. This can be accomplished several ways, including punishment (Bshary and Grutter 2005), partner switching (Bshary and Grutter 2002a, 2002b) and image scoring (Bshary and Grutter 2006; Pinto et al. 2011). For example, cheating behaviour by cleaners can be witnessed during natural (or laboratory) cleaning interactions and is generally indicated by a client body 'jolt' in response to cleaner fish mouth contact, where the jolt is not associated with parasite removal (Bshary and Grutter 2002a; Bshary and Noë 2003). Since non-predatory clients lack the option of reciprocal cheating, i.e. an asymmetrical relationship with respect to strategic options, they use one of two basic options to help ensure they interact with a cooperative cleaner: 1) punishment or 2) fleeing, depending on their status as either a 'resident' or 'visitor' client (Bshary 2001; Bshary and Grutter 2002a). Visitor clients refer to fish with large home ranges that encompass several cleaner stations, whereas resident clients are site attached or have small home ranges that only allow access to a single cleaner station (Bshary 2001, 2003; Bshary and Grutter 2002b). Since resident fish are spatially restricted, they generally punish a defecting cleaner by chasing them aggressively, which imposes an immediate energetic cost on the cleaner, where a visitor will simply swim away as they have access to other cleaner stations, e.g. switch partners (Bshary and Grutter 2002a). Both of these strategies, punishment and partner switching, have shown to increase

the frequency of cooperative behaviour during subsequent interactions between the same individuals (Bshary and Grutter 2005).

In contrast, as one would expect, interactions between cleaners and predatory clients are highly cooperative (Bshary 2001). In this scenario, both partners have symmetrical strategic options, i.e. each can choose to either cooperate or defect (e.g. eating mucus or eating the cleaner), yet the outcome of such cheating would be highly asymmetrical, as the predator is relatively unaffected, yet the cleaner loses its life (Bshary 2001; Bshary and Grutter 2002a). Therefore, such interactions cannot be controlled by reciprocity, as cleaners will not have the option of cheating in subsequent rounds if a predatory client were to cheat first (Bshary and Grutter 2002a). Instead, unconditional cooperation is maintained as it is in the cleaner's self-interest to do so (Bshary 2001; Bshary and Grutter 2005). As for predatory clients, cooperative behaviour is likely maintained as the long-term benefits of repeated parasite removal likely outweigh the short-term advantage of cheating (Trivers 1971; Bshary and Grutter 2002a), and cooperative interactions are therefore sustained with little conflict.

Furthermore, clients may ensure cooperative cleaner behaviour by using image scoring. For example, potential clients in the vicinity of a cleaner's territory, known as bystanders, observe current cleaning interactions, whilst creating an 'image score' of the particular cleaner (Bshary and Grutter 2006). If the cleaner cooperates and there is no conflict in observed interactions, the bystander will generate a positive image score and will likely invite the cleaner for inspection (expecting cooperative behaviour) (Bshary and Grutter 2006; Bshary and Bergmüller 2008). In contrast, defecting behaviour causes a negative image score, which causes clients, if able, to move on to other stations (Bshary and Grutter 2006). Image scoring by the bystander is therefore self-serving, yet the cleaner also benefits as it will be chosen as a future 'cooperative' partner (Bshary and Bergmüller 2008). Consequently, cleaner wrasse behave more cooperatively in the presence of an audience (e.g. Pinto et al. 2011).



Figure 1. Images of cleaners: a) adult phase, b) juvenile phase, c) batfish and sea turtle visiting a cleaning station and d) a pair of cleaners servicing and providing tactile stimulation to a harlequin sweetlips. Photographs: João Paulo Krajewski (abd) and Colin Robson (c).

1.7.4 Cleaners and the study of cognition

The strategies employed by cleaner wrasse during client interactions also allows for a unique opportunity to study fish cognition, and has to date, revealed surprising facts. As a result of the daily cleaner-client interactions and associated social conflicts discussed above, it is clear that cleaners use an array of sophisticated behavioural strategies to obtain their food, which includes the ability to know when to cooperate, cheat or manipulate clients for their personal benefit (Bshary et al. 2002; Bshary 2011). To do so, cleaners distinguish between three client categories: (1) predatory and non-predatory, (2) resident and visitor and

(3) familiar and non-familiar (Bshary 2001; Tebbich et al. 2002). In fact, their ability to respond to punishment with increased service quality in follow-up interaction with the punisher suggests that they may even be capable of recognizing up to 100 clients individually (Bshary and Grutter 2002a), while remembering their last interaction with each of them (Bshary 2011), although this remains to be fully tested. Using ‘tactile stimulation’, cleaners are capable of manipulating predatory clients as a form of ‘pre-conflict management’, and reconciling with non-predatory clients after a cheating event. Tactile stimulation is defined as ‘a cleaner hovering above a client while touching the client’s dorsal fin area with its pectoral and pelvic fins’ (Bshary and Würth 2001; Bshary in 2011). This ‘massaging’ behaviour is not associated with foraging, but rather an ‘extra service’ provided by cleaners that has shown to decrease stress levels in clients (Soares et al. 2011).

Furthermore, cleaners also understand the effects of an image scoring scenario and use may these situations to exploit their clients. For instance, cleaners may cooperate with and give tactile stimulation to a ‘current’ client, to subsequently deceive and cheat a larger, more desirable bystander client (i.e. tactical deception, Hauser 1998) (Bshary 2011). As final examples, during cognitively demanding laboratory tasks, based on biological market theory (Salwiczek et al. 2012) and the ability to solve a reverse-reward contingency task (Danisman et al. 2010), cleaners easily show reversal learning abilities (Salwiczek et al. 2012), and a tendency towards self-control (Danisman et al. 2010) and the ability to feed against their food preference (e.g. Bshary and Grutter 2005). The aforementioned examples of fine-tuned behavioural tactics employed by cleaners may be parsimoniously explained by associative learning, as they receive almost constant feedback throughout their 2000 daily interactions (Bshary 2011).

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2

VARIATION IN BLUESTREAK CLEANER WRASSE (*LABROIDES DIMIDIATUS*) 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 bluestreak cleaner wrasse (*Labroides dimidiatus*) ('cleaners') 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 yielded a tight 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 reef environment. Decision rules of these cleaners ignored existing information in their environment ('bounded rationality'), in contrast to cleaners living in a socially complex reef environment. 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 reef environments performed similarly, and better than the adults from the socially simple reef 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.

2.1 INTRODUCTION

Evolutionary game theory and empirical evidence provide a variety of mechanisms for stable cooperation between unrelated individuals (Axelrod and Hamilton 1981; Conner 1986; Clutton-Brock and Parker 1995; Milinski and Wedekind 1998; Nowak and Sigmund 1998; Wedekind and Milinski 2000; Kiers et al. 2003; Bshary and 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 and 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 (Feh and Fischbacher 2003; Haley and Fessler 2005) or less cooperatively (Kümmerli et al. 2010), as well as less precise (Milinski et al. 2001) or more sophisticated (Milinski and Wedekind 1998), than predicted cooperative strategies in models. This mismatch has raised questions, sparked debate and produced 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 and 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 and 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 and 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 and Stevens 2012), yet 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 and Taborsky 2008; St-Pierre et al. 2009; Raihani and 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 and 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* (hereafter 'cleaner') has provided strong experimental evidence for the usefulness of evolutionary game theory for predicting cooperative behaviour (Bshary 2011). Cleaners cooperate by eating ectoparasites off visiting client reef fishes. Conflict arises, however, as cleaners 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). Cleaners have shown to fine-tune service quality and priority to the clients' strategic options (Bshary 2011). Furthermore, cleaners behave more cooperatively in the presence of bystanders in order to raise their image score and hence, increase the probability of subsequently accessing bystanders (Pinto et al. 2011).

In a four-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 and 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; Gingsins et al. 2013). In our four month project, cleaners failed to eat selectively against their preference in order 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; Ferrière et al. 2002), i.e. 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 and Grutter (2006) and predicted by image scoring theory (i.e. Nowak and 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 it can obtain due to the partner’s adjustment in behaviour (i.e. Noë 2001). For cleaners this ability had been shown previously in Salwiczek et al. (2012) using the same methods, and field observations suggest likewise (Adam 2010).

In contrast to all previously published studies, these cleaners 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 cleaners 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 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 reef 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 reefs 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 and Romero 2003; salmon: Blanchet et al. 2006). Finally, we captured juveniles from the two reef types (two locations from each type) and repeated the same laboratory experiments in order 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 reefs would suggest that the observed differences between adults are due to experience.

2.2 STUDY SITE

Our study was conducted at Lizard Island, Great Barrier Reef, Australia. Adult cleaners were observed and collected from two reefs: 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 m² (depth 1 to 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 to 7 m), measuring 1 to 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.

2.3 MATERIALS AND METHODS

2.3.1 Cognition- and cooperation-based laboratory experiments (July-September 2010)

Twenty adult female cleaners, 10 from each reef type (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 and 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 cleaners preferred to feed on mashed prawn significantly over fish flakes mixed with equal volume of prawn (Bshary and 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 13 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.

2.3.1.1 Experiment 1: Feeding against a preference

We measured the willingness of cleaners to feed against their preference in order to prolong an interaction (Bshary and Grutter 2005). The willingness to feed against their food preference was tested by offering each cleaner a novel Plexiglas plate containing 3 prawn and 3 flake items. Cleaners 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.

2.3.1.2 Experiment 2: Audience effects

In a simplified version of Bshary & Grutter (2006), we tested whether cleaners 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. Cleaners had to avoid eating any prawn item on a current plate in the presence of a ‘bystander’ plate, in order 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. Cleaners were alternatively offered a single Plexiglas plate containing 2 flake and 2 prawn items (control: as in the ‘feeding against a preference’ experiment) or two differently coloured Plexiglas plates, each containing 2 flake and 2 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, with the order of presentation being counterbalanced over the consecutive trials. No pre-training was offered apart from the knowledge cleaners had

obtained in experiment 1. In order to test for a change in the response of cleaners over feeding trial session depending on which reef they came from we carried out a general linear mixed-effects model (glmmPQL function in R3.02 on response data [binomial family] with factors reef, treatment and trial, and fish identity as random factor in the error term. Fixed effects: $FIResponse \sim Group + Trial + Reef + Group * Reef + Trial * Reef + Group * Trial + Group * Trial * Reef$.

Bshary and Grutter (2006) had tested cleaners also in a third situation, namely offering two plates that were retrieved independently of each other, i.e. 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 preference when offered one or two independent plates we saw no need to replicate these results in the current study.

2.3.1.3 Experiment 3: Biological market theory

We tested the cleaner’s 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). Cleaners 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 ten days per

cleaner.

2.3.2 Personality experiments (July-September 2010)

Cleaner aggression was measured by placing a mirror inside the aquarium against a wall and recording the number of mirror ‘mouth fights’ within the subsequent two minutes. Boldness was measured by offering the cleaner 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.

2.3.3 Fish censuses and field observations (July-August 2011)

The abundance and diversity of client reef fishes and cleaners 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 cleaners in either a 5 m (individuals > 10 cm total length (TL)) or 1 m (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 cleaners (8 from each reef environment), which were filmed (Cannon G9, Lumix TZ3) on SCUBA for 30 minutes, between 09:00 and 10:30 hours, 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.

2.3.4 Juvenile cleaners (January 2012)

All aforementioned plate experiments were repeated on juvenile cleaners (measuring less than 2.5 cm TL). In total, sixteen juvenile cleaners were caught from both reef types (i.e. continuous reef and

patch reefs). Due to the low availability of recruits at the Corner Beach patch reefs we captured at two locations for each reef type. We included the patch reefs and 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.

2.4 RESULTS

2.4.1 Adult cleaners in the cognition- and cooperation-based laboratory experiments

Adult female cleaners caught from the continuous reef performed superior across all laboratory learning tasks compared to their patch reef counterparts. In the ‘feeding against a preference’ experiment, continuous reef cleaners ate a significantly higher ratio of flake to prawn items in comparison to patch cleaners (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, i.e. less than 0.75 flake items per round (Wilcoxon one sample test, $n = 10$, $T = 3$, $P < 0.01$). In the ‘audience effects’ 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 preference in the image scoring situation (Figure 2b). As patch reef cleaners largely failed to adjust their behaviour to the image scoring situation, they succeeded to the second plate less often than continuous reef cleaners (Mann-Whitney-U-test, $m = 10$, $n = 9$, $z = 2.20$, $P = 0.027$, Figure 2c). Interestingly, continuous reef cleaners 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’ reef type (continuous reef or patch reef) were both

significant ($P = 0.024$ and $P > 0.001$, 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 2de). Lastly, continuous reef cleaners completed the ‘biological market theory’ experiment (involving the choice of an ephemeral food source over a permanent one) in a fewer number of trials than patch reef cleaners, 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).

2.4.2 Laboratory experiments on aggressiveness and boldness in adult cleaners

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, $m = 10$, $n = 10$, $z = -1.36$ to 1.17 , $P = 0.174$ to 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 $P < 0.05$).

2.4.3 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 (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 cleaners per 100 clients) versus the patch reef location (0.64 cleaners per 100 clients) (Mann-Whitney-U-test, $m = 10$, $n = 10$, $z = 2.57$, $P = 0.010$).

Cleaners 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 cleaners of the two reef environments (Mann-Whitney-U-tests, all $m = 8$, $n = 8$, duration: $z = 1.31$, $P = 0.189$; cleaning proportion: $z = -1.36$, $P = 0.172$).

2.4.4 Juvenile cleaners

In contrast to adult cleaners, the performance of juveniles from the two contrasting reefs 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 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 ‘audience effects’ 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 to 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, $df = 302$, $t = -0.834$, $P = 0.405$) (Figures 6de). Lastly, both continuous and patch reef cleaners 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 ten 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, $m = 9$, $n = 4$, $z = -2.92$, $P = 0.003$).

2.5 DISCUSSION

The cooperation experiments demonstrate an important mismatch between the behaviour of adult cleaners from a particular reef location, consisting of patch reefs, and published evidence linking cleaning strategies with game theoretic predictions regarding audience effects (Nowak and Sigmund 1998) and biological markets (Noë 2001) (Pinto et al. 2011; Salwiczek et al. 2012). Feeding against preference, incorporating image scoring by ‘food sources’, and preferring an ephemeral food source, would have yielded more food and hence 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 experiments. 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 decision rules underlying behaviour (Gigerenzer and Selten 2002; Fehr and Fischbacher 2003; Haley and 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 2000 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 and 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 biological market theory 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.

2.5.1 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 and Del Giudice 2012). Thus, our results differ from previous studies in providing evidence for an environment-linked cooperative personality and cognitive ability in cleaners, which is independent of the two personality traits we tested.

2.5.2 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 reefs 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 (Awise and 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 reef 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 reef type 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 reef environment 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 ontogenetic 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 and Taborsky 2010; Thornton and 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 cleaners 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.

2.5.3 Conclusion

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 and 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, cleaners 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 and Kawecki 2003; Heyes 2010; Lotem and 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.

2.6 ACKNOWLEDGEMENTS

We kindly thank the staff of Lizard Island Research Station - a facility of the Australian Museum, for their continuous support and friendship. Likewise, D. Bellwood, K. Cheney, C. Lefèvre, J. Welsh, N. Raihani, A. Derendinger, P. Vullioud and A. Ros for field support. M. Manser for external supervision and A Ros for statistical advice. Funding was provided by the Swiss National Science Foundation (grant to Redouan Bshary) and the Commission for Travel Grants, Swiss Academy of Sciences (grant to Sharon Wismer).

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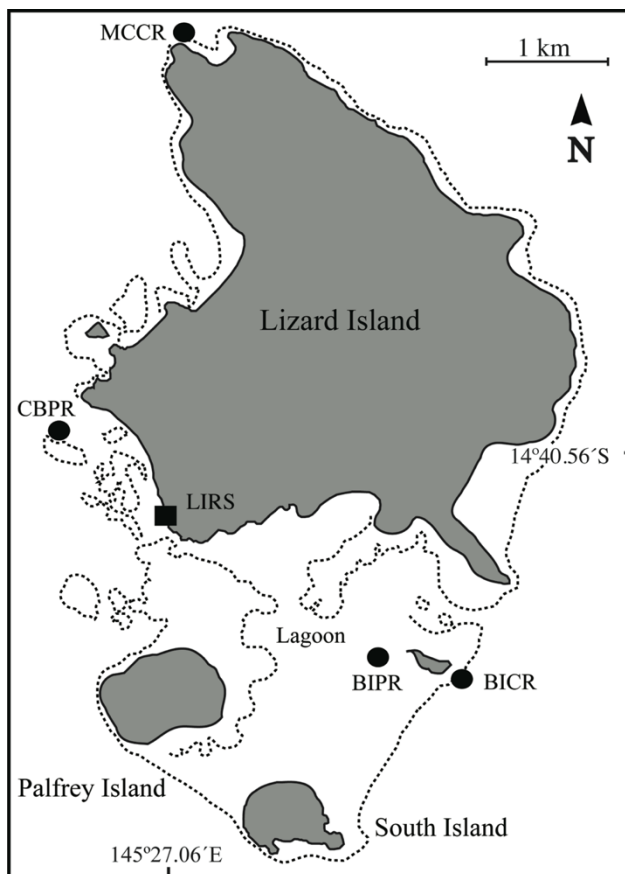


Figure 1. Lizard Island Group. Study reef locations are indicated by filled circles: Mermaid Cove continuous reef (MCCR), Corner Beach patch reefs (CBPR), Bird Island continuous reef (BICR) and Bird Island patch reefs (BIPR).

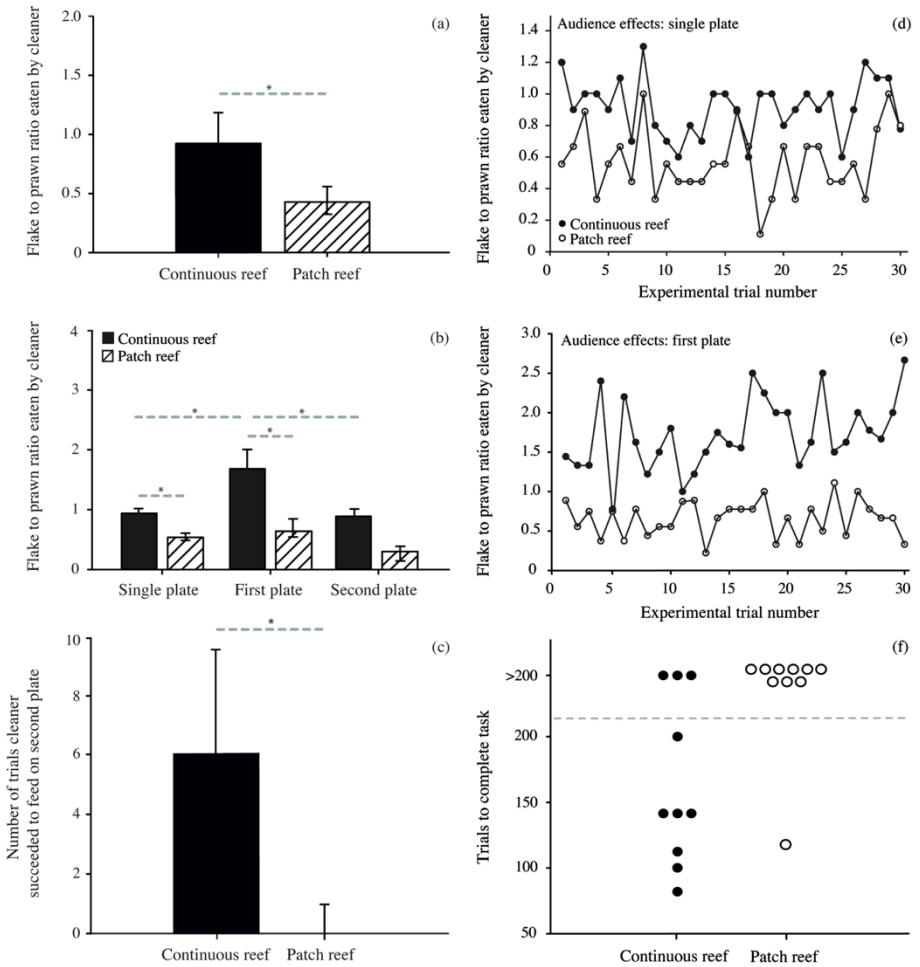


Figure 2. Behaviour of adult cleaners in the laboratory. ‘Feeding against a preference’ experiment, a) median flake to prawn ratio consumed. ‘Audience effects’ experiment, b) median flake to prawn ratio consumed per plate type, c) median number of times cleaner succeeded to feeding on second plate in 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 theory’ experiment, f) number of trials needed to complete both initial and reversal component (maximum 200 trials). Error bars: interquartile values. *: significant differences between cleaners of the two reef environments (all $P < 0.03$).

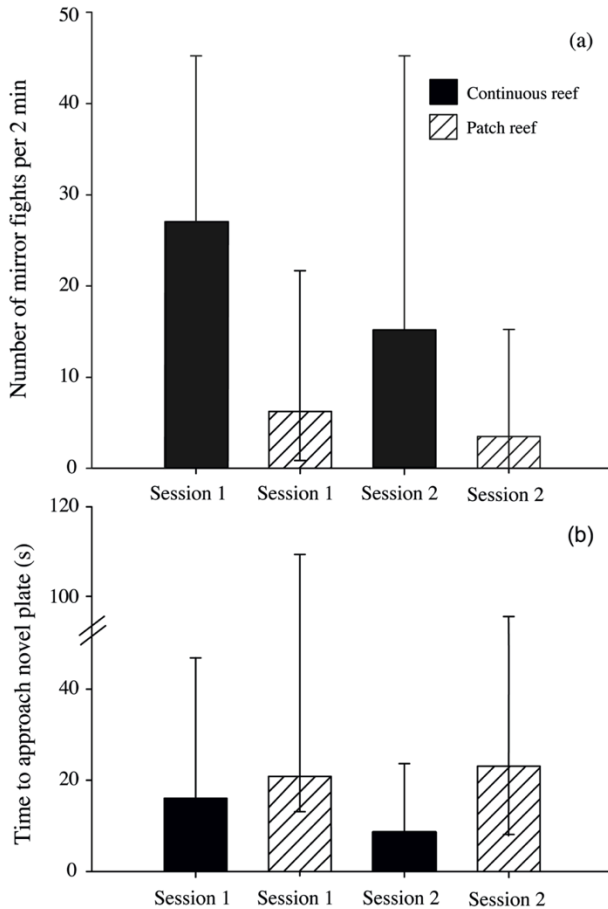


Figure 3. Boldness and aggression do not differ between continuous and patch reef cleaners. a) Number of mirror fights per two minutes as a measure of aggressiveness. b) Duration (seconds) to approach a plate with novel colour patterns as a measure of boldness (or exploration). Values are median and interquartiles (error bars).

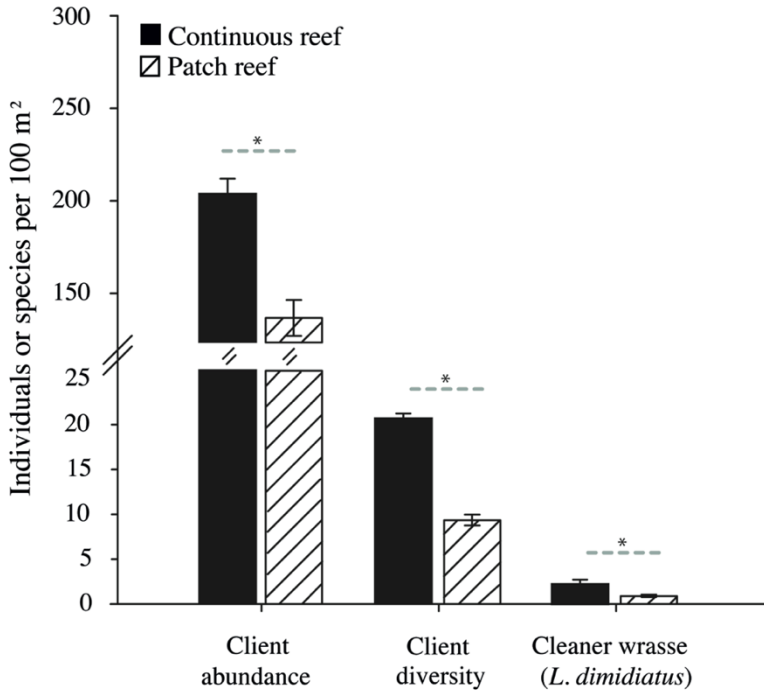


Figure 4. Fish estimates on continuous and patch reefs. Abundance and diversity of reef fish clients and abundance of cleaners at the continuous fringing reef at Mermaid Cove and Corner Beach patch reefs, Lizard Island, Great Barrier Reef. Values are mean and standard error (error bar). *: significant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

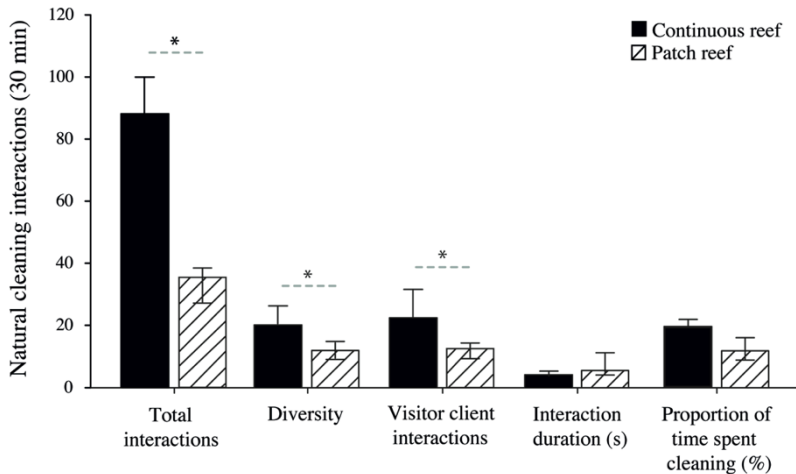


Figure 5. Behaviour of cleaners on continuous and patch reefs. Characteristics of natural cleaning interactions at Corner Beach patch reefs and Mermaid Cove continuous reef. Values are median and interquartiles (error bars). *: significant differences between cleaners of the two reef environments (all $P \leq 0.011$).

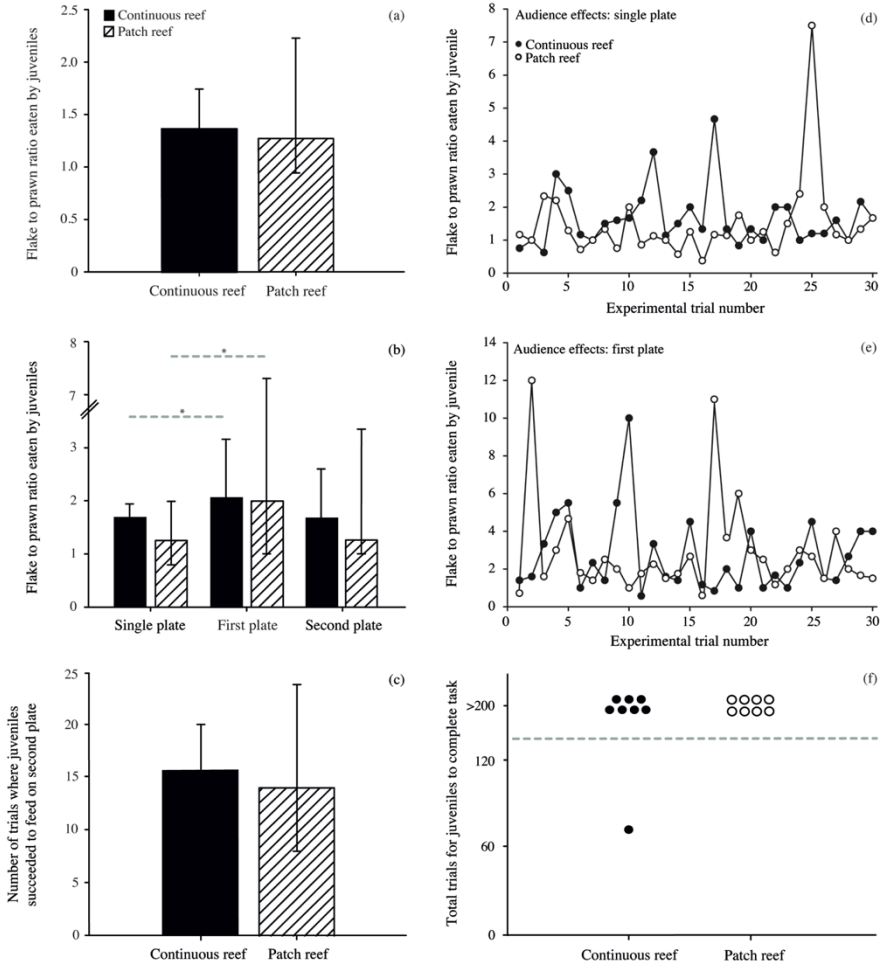


Figure 6. Behaviour of juvenile cleaners in the laboratory. ‘Feeding against a preference’ experiment, a) median flake to prawn ratio consumed. ‘Audience effects’ experiment, b) median flake to prawn ratio consumed per plate type, c) median number of times juvenile cleaner succeeded to feeding on second 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 theory’ experiment, f) number of trials needed to complete both initial and reversal component. Error bar: interquartile values.

3

SOCIAL COMPLEXITY DRIVES DECISION RULES IN BLUESTREAK CLEANER WRASSE (*LABROIDES DIMIDIATUS*)

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ABSTRACT

Heuristics, or rules of thumb, are decision shortcuts that often result in accurate outcomes without significant computational effort. However, they may lead to suboptimal decisions when the context is misidentified, infrequent or novel. Here, we investigate the decision rules of bluestreak ‘cleaner’ wrasse (*Labroides dimidiatus*) when selecting one of two reef fish ‘clients’ that invite inspection simultaneously. Cleaners remove ectoparasites from visitor and resident clients. Priority should always be given to visitors, which have access to multiple cleaners, while residents will queue for service. Published evidence, however, indicates that juvenile cleaners and cleaners from socially simple reef environments fail at this task in a laboratory setting (clients: two equal-sized Plexiglas plates that differed in colour), in contrast to cleaners from socially complex reef environments. Our aim, therefore, was to determine whether cleaners from the two reef environments, as well as juvenile cleaners, use alternate cues when making decisions in regards to client service priority. Specifically, we tested to what extent cleaners use *size* cues to distinguish between the two client categories, and how they integrate both *size* and *colour* cues into their decisions. In nature, visitor clients are generally larger than resident clients, and therefore, giving service priority to the larger client would often result in the better foraging decision, i.e. servicing two clients over one. We found that all cleaners showed a marked preference for the larger plate in the absence of colour cues, irrespective of client category. However, when both plate size and colour were incorporated into the experiment, cleaners from the socially complex reef environment switched to using colour cues that specifically differentiated between client categories, while cleaners from the socially reef simple environment continued to use size cues. Hence, the former used a precise decision rule, while the latter used a rule of thumb, leading to an overall inferior performance. Differences may be driven by experience and learning, in particular, the ability to identify and use relevant cues in the environment.

3.1 INTRODUCTION

Cooperation is often considered an evolutionary puzzle. However, evolutionary game theory and empirical studies have both provided a variety of mechanisms for explaining stable cooperation between unrelated individuals (i.e. Axelrod and Hamilton 1981; Conner 1986; Milinski 1987; Clutton-Brock and Parker 1995; Nowak and Sigmund 1998; Wedekind and Milinski 2000; Kiers et al. 2003; Bshary and Grutter 2005; Pinto et al. 2011). These include models of cooperation based on the Prisoner's Dilemma (e.g. Axelrod & Hamilton 1981; Doebeli and Hauert 2005) and partner control mechanisms such as punishment (e.g. Clutton-Brock and Parker 1995; Bshary and Grutter 2005; Raihani et al. 2010) and image-scoring (e.g. Wedekind and Milinski 2000; Pinto et al. 2011). Recently, however, empirical research on humans has shown that decision rules used in social interactions do not always fit the simple strategies predicted by game theoretic models (e.g. Fehr & Fischbacher 2003; Haley and Fessler 2005; Kümmerli et al. 2010); which has raised considerable debate in regards to decision-making processes in humans (Hagen and Hammerstein 2006; El Mouden et al. 2012; Baumard et al. 2013).

Humans are clearly capable of reflexion and analytical thought, but often use short cuts. For example, heuristics (or rules of thumb in non-human animals) are a simple strategy in which decisions are reached quickly and relatively accurately by ignoring a portion of the available information (Barnard 2004; Hutchinson and Gigerenzer 2005). Heuristics are based on the concept of 'bounded rationality': when individuals make decisions, their rationality is limited by knowledge, time, and cognitive/computational abilities (Gigerenzer and Todd 1999; Gigerenzer and Selten 2002). A repertoire of heuristics ('the adaptive toolbox') typically consists of simple 'search', 'stop' and 'decision' rules, that are well adapted to an individual's physical and social environment. Heuristics can lead to optimal results with little effort, but users also run the risk of misidentifying the correct context, and hence, making mistakes (Gigerenzer and Selten 2002). A classic example in human cooperation is one where subjects become more cooperative

when they subconsciously feel that they are being observed (Haley and Fessler 2005; Bateson et al. 2007). This raises the question: under which conditions is it favourable to use heuristics versus a precise decision rule?

Here, we examined the decision rules applied by the bluestreak cleaner wrasse *Labroides dimidiatus* – a classic model system of cooperation, when selecting one of two reef fish ‘clients’ that invite inspection simultaneously. Bluestreak cleaner wrasse (hereafter ‘cleaners’) remove ectoparasites from reef fish (Grutter 1999) and engage in approximately 2000 client interactions per day inside their small territories called ‘cleaning stations’ (Grutter 1995; Wismer et al. 2014). Hence, two or more clients commonly seek a cleaner’s service simultaneously at a cleaning station. Clients can be categorised according to their strategic choice options. Resident clients have small home ranges and access to a single cleaning station, and will therefore queue for service (Bshary 2001; Bshary and Schäffer 2002). In contrast, visitor clients have larger home ranges that encompass several cleaning stations and leave the cleaning station if not serviced immediately (Bshary 2001). Therefore, in order to maximize food intake, cleaners should give service priority to visitor clients.

This scenario (deciding to service a visitor or resident client first) can be mimicked in the lab with Plexiglas plates: an experimenter simultaneously presents a cleaner with two plates containing equal amounts of food. Both plates are equal in size but differ in colour. The ‘visitor plate’ is removed if the cleaner feeds on the ‘resident plate’ first. This experimental approach is based on biological market theory (see Noë 2001) and has previously been used by Bshary and Grutter (2002) and Salwiczek et al. (2012). Given the ecological relevance of this experiment, cleaners quickly learn to develop a preference for the visitor plate, and have been shown to outcompete several primate species (Bshary and Grutter 2002; Salwiczek et al 2012). However, a recent study showed that juvenile cleaners in general, and adult cleaners from small, isolated patch reefs are unable to solve this task in a laboratory setting. Patch reefs are considered a ‘socially simple reef

environment’ because they have a low abundance and diversity of reef fish clients and a low cleaner to client ratio. In contrast, continuous reefs are considered ‘socially complex reef environments’ (Wismer et al. 2014). These results raise the questions: Are some adult and juvenile cleaners generally unable to give priority to visitor clients, or do they use rules of thumb that allow them to solve the task in nature, but not in the lab?

In nature, visitor clients are often larger than resident clients (Bshary 2001) and cleaners preferentially clean larger fish (Grutter et al. 2005). Servicing larger clients first would frequently produce optimal results, whereby cleaners obtain food from the visitor *and* resident clients rather than just the resident, i.e. cleaning the visitor first, without having to recognize individual client species and categorizing them based on a cleaner’s strategic options (e.g. partner switching versus queuing for service). Thus, it may be that juvenile and adult cleaners from socially simple reef environments consistently apply the rule ‘give priority to the larger client’. This rule of thumb might work well in nature but will lead to a suboptimal performance in laboratory experiments where client plates differ in colour and not in size (see for e.g. Salwiczek et al. 2012; Wismer et al. 2014).

Here, we aimed to determine whether cleaners from both the socially simple and socially complex reef environments, as well as, juvenile cleaners, use size cues to decide which client to service first. To do so, we modified the biological market theory experiment (Salwiczek et al. 2012) so that the resident and visitor plates differed only in size but not in colour. Secondly, in order to determine to what extent cleaners use size as a cue when making decisions in regards to client service priority, the experiment was further modified to include variation in plate size and colour. Here, the colour-based cue correctly differentiated visitor clients from resident clients (a precise cue), whereas the size-based cue only differentiated visitor clients from resident clients correctly 76% of the time (a correlated cue), similar as it would be in nature. This allowed us to determine whether cleaners from the two reef environments continue to use the size-based cue, e.g. rule of thumb:

service larger plate first irrespective of client status, or the colour-based cue, e.g. precise rule: always service the visitor plate first, even if it is the smaller plate, when making decisions regarding client service priority.

3.2 STUDY SITE

This study was conducted over three years (January - February 2012; March - April 2013; July - September 2014) at the Lizard Island Research Station (LIRS), Great Barrier Reef, Queensland, Australia (14°40'S, 145°28'E). During all three field seasons, cleaners were collected from two reef types: 1) a large continuous tract of fringing reef representing a socially complex reef environment and 2) small isolated reef patches representing a socially simple reef environment (description of variation in social complexity among sites provided in Wismer et al. 2014). Study sites and capture locations are shown in Figure 1.

3.3 MATERIALS AND METHODS

3.3.1 Subjects and housing

In total, 50 cleaners were caught from reefs surrounding Lizard Island: 16 juveniles in 2012, 18 adult females in 2013 and 16 adult females in 2014. An equal number of cleaners were collected from both environments across all field seasons. Cleaners were caught using barrier nets (2 m x 1 m, 5 mm stretched mesh) and placed in sealed plastic bags containing ample oxygen supply for transport to LIRS. All fish were individually housed in glass (adults: 62 cm x 26 cm x 37 cm) or plastic (juveniles: 43 cm x 32 cm x 30 cm) aquaria with a continuous flow of fresh sea water directly from the reef. Fish were supplied with two polyvinylchloride tubes (approx. 2 cm x 15 cm) for shelter. From day one in captivity, all fish were fed mashed prawn once daily on grey Plexiglas plates (10 x 5 cm) and were habituated to their captive environment for one week prior to the experiments.

3.3.2 Laboratory experiments

3.3.2.1 Experiment 1: Modified biological market theory: size variation

Our experimental design is a modification of the aforementioned biological market theory experiment published by Bshary and Grutter (2002) and Salwiczek et al. (2012). Cleaners were tested in their individual aquarium, which was divided into two compartments with an opaque partition placed 20 cm from the wall of the aquarium and along the long edge (Figure 2). The smaller compartment was the cleaner fish 'holding' area, while the larger compartment was the experimental area containing the experimental Plexiglas plates. During experimental trials, the partition was lifted and a cleaner was simultaneously presented with two Plexiglas plates, each containing one item of mashed prawn (~ 0.001 g). One plate always represented a visitor client; this plate was removed from the aquaria if the cleaner did not feed on it first. The other plate represented a resident client, which remained in the aquarium regardless of a cleaner's foraging choice. In this modified design, the experimental visitor and resident plates differed only in size (large plate: 10 x 5 cm; small plate: 3 x 2 cm).

All cleaners participated in two experimental sessions per day (am/pm; 10 trials per session), with a maximum of 120 trials per cleaner. The position of the visitor plate (left or right) in the aquaria was balanced and randomized across the 10 trials: always 5 on each side, but no more than 3 in a row per side. We recorded the number of trials required for individual cleaners to learn to preferentially inspect the visitor plate first, i.e. at least 9 out of 10 trials or 8 out of 10 trials twice in a row. Once this criterion was reached, the status (i.e. size) of the visitor and resident plates was switched to test reversal learning abilities. A cleaner which was first tested with a large visitor plate was now tested with a small visitor plate and vice versa. Initial plate status was counterbalanced between treatment groups (i.e. for each environment, half the cleaners were first presented with a large visitor plate and the other half, with a small visitor plate). We tested juvenile cleaners in 2012 ($n = 16$) and adult females in 2013 ($n = 18$).

3.3.2.2 Experiment 2: Modified biological market theory: size and colour variation

To determine which rules of thumb cleaners from different social complexity environments use when confronted with multiple species of different sizes, this experiment included variation in both plate colour (i.e. ‘species’) and size. Here, cleaners were once again simultaneously presented with a visitor and a resident plate, each containing one item of mashed prawn. The same protocol was applied as in the previous experiment: the visitor plate was removed from the aquarium if the cleaner fed on the resident plate first. In this experiment, however, the cleaner was presented with a series of Plexiglas plate combinations of varying sizes, consisting always of one green and one pink plate. Plate pairings were randomized from 7 different size possibilities (Figure 1 Appendix) and included the following proportions: visitor plate larger than the resident plate (76 % of the experimental trials; ‘low’ degree of learning difficulty), visitor plate same size as the resident plate (12 %, ‘intermediate’ degree of learning difficulty) and visitor plate smaller than the resident plate (12 %, ‘high’ degree of learning difficulty) (Table 1 Appendix). These values represent approximate size ratios between resident and visitor clients observed in nature interacting with cleaners (Bshary 2001). In total, 7 Plexiglas plates were used for each colour, ranging in size from 1.5 cm² to 12 cm² (Figure 1 Appendix). Each cleaner was exposed to 210 trials over 11 days (the last 10 trials consisted of only ‘intermediate’ and ‘high’ learning difficulty trials to explicitly test which rules cleaners apply in infrequent client pairings, e.g. V = R and V < R). Visitor plate colour was counterbalanced between treatment groups. For each trial, we recorded the plate that the cleaner inspected first. This experiment was conducted on 16 adult females in 2014. There was no reversal learning component.

3.3.3 Data analysis

We used survival analysis (Cox proportional hazards regression model; ‘coxph’ function in the R package ‘survival’) to evaluate differences in the success rate and the number of trials required by cleaners to reach

the learning criterion in both experiments. We tested for the effects of reef environment (Experiment 1, 2), life stage (Experiment 1) and visitor plate size (Experiment 1) on learning. Model assumptions (i.e. proportional hazards) were checked with the function `cox.zph`. We tested the effect of life stage, environment and visitor plate size on the number of choices for the larger plate in the first 10 trials of Experiment 1 using a general linear model (LM), to investigate spontaneous behaviour/choices. The proportion of choices for the larger plate was logit transformed (Warton and Hui 2011). Model assumptions for this and other linear models were checked with plots of residuals vs. fitted values and qqplots of residuals. A LM was also used to test for the effect of environment and task difficulty (specified as an ordered factor) on the (logit transformed) proportion of correct choices by cleaners in Experiment 2. For Experiment 2, we used three general linear mixed-effects models (LMM) to test the effect of reef environment and time (i.e. number of trials) on the proportion of correct choices for the visitor plate (logit transformed) for situations where the visitor plate was 1) larger than the resident plate (low difficulty), 2) the same size as the resident plate (intermediate difficulty) and 3) smaller than the resident plate (high difficulty). Cleaner identity was included as a random factor to account for the non-independence of observations on the same individuals. We calculated the marginal R^2 (variance explained by the fixed factors; $R^2_{\text{GLMM}(m)}$) and conditional R^2 (variance explained by the fixed and random factors; $R^2_{\text{GLMM}(c)}$) following Nakagawa and Schielzeth (2013). Finally, we used three LMs to test for the effect of environment on the (logit transformed) proportion of correct choices by cleaners in the last 50 trials of Experiment 2, i.e. after the learning criterion was reached at 150 trials, in all categories (low, intermediate, high). All analyses were done in R 3.1.2 (R Development Core Team 2014).

3.4 RESULTS

3.4.1 Laboratory experiments

3.4.1.1 Experiment 1: Modified biological market theory: size variation

There was no effect of reef environment or life stage (P for both main effects > 0.12) and no two- or three- way interactions between environment, life stage and visitor plate size on the number of trials required for cleaners to solve the task (Figure 3a). However, visitor plate size significantly influenced learning time ($\chi^2 = 12.3$, $P = 0.024$; Figure 3a): cleaners required more trials to reach the learning criterion when the visitor plate was smaller than the resident plate, indicating that this is a high difficulty learning task (coef \pm s.e.m = -1.74 ± 0.53 , $z = -3.28$, $P = 0.001$). The majority (71.4 %) of adult cleaners solved the task in less than 120 trials when presented with a large visitor plate, whereas most (63.6 %) failed to solve the task when the visitor plate was small (Figure 3a). Juveniles performed similarly: 100% of individuals solved the task in less than 120 trials when the visitor plate was large, whereas 75 % failed to solve the task when the visitor plate was small (Figure 3a).

Life stage, environment or visitor plate size had no effect on the proportion of choices for the larger plate in the first 10 trials (all P s > 0.25 , $R^2 = 0.08$; Figure 3b). However, all cleaners exhibited a significant preference for the larger Plexiglas plate, irrespective of plate status (resident or visitor), i.e. cleaners chose the larger plate more than expected by chance (Helmert contrasts intercept estimate \pm s.e.m = 1.00 ± 0.23 , $t = 4.384$, $P < 0.001$; Figure 3b, see Box 1 in the Appendix).

3.4.1.2 Experiment 2: Modified biological market theory: size and colour variation

When both size and colour variation were incorporated into the experimental design, environment had a significant effect on cleaner learning time ($\chi^2 = -28.5$, $P = 0.037$; Figure 4). Fish from the socially simple reef environment required marginally more time to reach the learning criterion (coef \pm s.e.m = -1.36 ± 0.70 , $z = -1.95$, $P = 0.05$). Cleaners from the socially complex reef environment learned to solve the task (i.e. inspect the visitor plate first) in approximately 40 trials (median), whereas cleaners from the socially simple reef environment required approximately 105 trials (median) (Figure 4).

The performance of cleaners across all 210 experimental trials also varied significantly between environments ($F_{1,39} = 4.21, P = 0.047$), with cleaners from the socially simple reef environment exhibiting a lower overall percentage of correct choices for the visitor plate compared to cleaners from the socially complex reef environment (estimate \pm s.e.m = $-0.38 \pm 0.19, t = -2.05, P < 0.047$). There was also a significant overall effect of ‘difficulty’ (i.e. visitor plate larger, equal or smaller than the resident plate) on learning (i.e., number of trials required to complete task; $F_{2,39} = 9.61, P < 0.001$). Both groups of cleaners performed significantly better in ‘low’ versus ‘high’ difficulty plate pairings (environment-difficulty interaction, $F_{2,39} = 0.21, P = 0.810$) (Figure 5). The overall percentage of correct choices for cleaners from socially simple and complex reef environments in ‘low’ difficulty trials ranged from 69-73 % (median), in comparison to 52-53 % (median) in ‘high’ difficulty trials, respectively.

There was an overall significant effect of time ($F_{3,33} = 12.5, P < 0.001$), but not environment ($F_{1,11} = 1.8, P = 0.205$; interaction ns, $P = 0.764$) on the proportion of correct choices for the visitor plate when the task difficulty was ‘low’ (i.e. visitor larger than resident, Figure 6a), with the percentage of correct choices increasing as the experiment progressed (Figure 6a). The proportion of correct choices also varied with time when the task difficulty was ‘intermediate’ (visitor same size as resident, $F_{1,33} = 10.6, P < 0.001$; Figure 6b) and when the task difficulty was ‘high’ (visitor smaller than resident), although the effect of time in this third situation was weaker ($F_{3,33} = 3.4, P = 0.029$), indicating that cleaners learned more slowly (Figure 6c). There was no overall effect of environment in both ‘intermediate’ ($F_{1,11} = 1.2, P = 0.304$) and ‘high’ ($F_{1,11} = 2.1, P = 0.179$) difficulty plate combinations. However, after all cleaners reached the learning criterion (i.e. after 150 trials), there were significant differences in the performance of cleaners from the two reef environments in both ‘intermediate’ ($F_{1,11} = 8.3, P = 0.015$) and ‘high’ ($F_{1,11} = 8.3, P = 0.015$) difficulty plate combinations (Figure 6b, c). Cleaners from the socially complex reef environment chose correctly in over 80 % (median) of the plate combinations in both ‘intermediate’ and ‘high’ difficulty combinations, whereas cleaners from the socially

simple reef environment did not perform above chance (50 %, median) in either category (Figure 6b, c). In contrast, no significant difference was found between the two environments in ‘low’ difficulty plate combinations (trials 151-200), both groups of cleaners chose correctly in over 80 % (median) of the trials ($F_{1,11} = 0.24$, $P = 0.633$).

3.5 DISCUSSION

Our aim was to determine the extent that cleaners from two different social reef environments and age classes use size as a cue to distinguish between visitor and resident clients. Furthermore, we aimed to determine how both groups of cleaners integrate plate colour and size cues, where colour provides precise client status information, while size is only a correlate. We found that, in the absence of colour cues, adult and juvenile cleaners from both reef environments showed a marked preference for the larger plate, irrespective of whether the plate was a resident or a visitor. Therefore, cleaners typically solved our biological market theory experiment as long as the visitor plate was larger than the resident plate. However, cleaners of both age and environment classes had difficulty in solving the task when the visitor plate was smaller than the resident plate. When both plate size and colour were altered in the second experiment, cleaners from the socially complex reef environment used plate colour as a key cue, e.g. client status, while cleaners from the socially simple reef environment generally used plate size as a cue. In other words, cleaners from the socially complex reef environment used a precise decision rule, while cleaners from the comparatively simple social environment ones used a rule of thumb, leading to both slower learning and lower overall poorer performance.

3.5.1 Conditions favouring precise knowledge vs. rules of thumb in a biological market

Here, we demonstrate the presence of two different decision rules used by cleaners when deciding on client service priority. Nevertheless, it is clear that the colour-pattern based rule yields optimal results, not just in our experiment, but also in nature, as it allows for the identification of

client species and their corresponding strategic options. Thus, the key question arising here is why some cleaners use a size-based rule. Collectively, along with the results by Salwiczek et al. (2012) and Wismer et al. (2014), our first experiment shows that the size-based rule is likely the starting point, as it is used by juvenile cleaners from both socially simple and complex reef environments. As larger clients typically have more ectoparasites in comparison to smaller clients (Grutter 1995; Grutter and Poulin 1998), client size largely predicts its quality as a ‘food patch’, and hence, the preference for larger clients makes sense in the absence of client choice option considerations. For juvenile cleaners, this rule would work particularly well, as they rarely interact with visitor clients (Potts 1973, RB and SW unpublished observations).

Adult cleaners from socially simple reef environments also have fewer interactions with visitors than adult cleaners from the socially complex reef environments (Wismer et al. 2014). Thus, one possible explanation for our results could be that only high exposure to visitors and thus, the corresponding decisions in regards to who to give priority to, may trigger a re-evaluation and the development of the colour-based (i.e. species) rule. This interpretation is hence based on opportunities, and assumes that all cleaners would benefit from the precise decision rule. An alternative possibility is that cleaners from both environments use the rule that is locally optimal. This hypothesis is based on cost-benefits of obtaining information. Obtaining information can incur various costs, including acquisition, storage and retrieval (Mery and Kawecki 2003) as well as delays in decision making (Gigerenzer & Selten 2002). Thus, acquiring precise information on the strategic options of each client species is likely to induce some physiological costs in cleaners. These costs may either be similar for all cleaners or higher for individuals from the socially simple reef environment as they will be less frequently exposed to a situation where a resident and visitor simultaneously invite an inspection. At the same time, the benefits of being able to identify fishes will be higher for cleaners from the socially complex environment as they will make more correct decisions per time unit due to being more frequently exposed to this situation. As a

consequence, there is the possibility that the precise decision rule only yields net benefits to cleaners from the socially complex environment. A possible way to test between the two hypotheses would be to assess the body condition of cleaners from the two environments as an indicator of their relative performance, followed by a second measure of body condition after translocations between such environments to assess the outcomes of cleaners' decision rules in both environments.

Independently of the considerations mentioned above, another challenge will be to understand how individuals decide to re-assess their size-based rule and what processes lead to the adoption of a colour (i.e. species) based rule. The ability to identify relevant cues from the environment is the first aspect of cognitive processing of information (Shettleworth 2010). Differences in species performance in cognitive tasks may often be based on their ability to identify such cues, even if their cognitive processes are the same (Lotem and Halpern 2012). For example, the observation that adult cleaners from socially complex environments learn the solution to a biological market experiment faster than chimpanzees, orang-utans and capuchins (Salwiczek et al. 2012) could potentially be explained by ecological differences between the two groups leading to differences in cue assessment: cleaner ecology makes plate colour equivalent to client colour, where the identification of clients allows assessment of their strategic options through associative learning. In contrast, since primates never identify foraging options based on the colour of the background; the relevant information for them might instead be in the colour of food items. Thus, despite certain study species being able to learn by associative learning, the difference in their performance may be due to differences in their cue identification resulting from their different ecologies.

3.5.2 Psychological constraints and optimal decisions

Variation in cleaner performance may also be influenced by psychological mechanisms, which have often shown to constrain an animal's behaviour, resulting in less than optimal behaviour across various contexts (Fawcett et al. 2013; Lotem 2012). For example, when

faced with alternate decisions in the laboratory, animals often choose options which do not fit the standard optimality approach (Fawcett et al. 2013; 2014). This could be for several reasons, including impulsiveness (e.g. immediate reward versus delayed reward: Henly et al. 2008) or having difficulty valuing options in a rational way (e.g. making state dependent not rational decisions: Marsh et al. 2004) (Fawcett et al. 2013). Learning constraints may further play a key role in explaining suboptimal behaviour (e.g. brood host acceptance: Lotem 1993). For our study system, it may also be that cleaners from the socially simple reef environment may simply be slower learners, i.e. they learn the correct sequence but require more trials, or have psychological constraints which prevent them from learning the appropriate sequence all together. This once again may be a reflection of experience. Without ample opportunity to learn, cleaners from the socially simple reef environment may lack the experience needed to perform in an optimal manner.

3.5.3. Domain specificity of cognitive performance

Our results support the view that the differences between cleaners from the two environments are tightly linked to relevant local conditions during interactions with clients. The majority of adult cleaners from the socially complex reef environment were unable to develop a preference for the small visitor plate in the first experiment. It appears that they are not able to apply the rule ‘give priority to the plate which is not willing to wait’, which would have been a more general ability. One possible ecological explanation is that the possibility of the smaller of two identical clients being the visitor does not occur in nature. Identical appearance in nature would imply that the two clients belong to the same species. Within a reef fish species, home range often increases with age (juvenile to adult) (e.g. Welsh et al. 2013). It would therefore not make ecological sense that the smaller individual of the same species had a large home range that allowed for choice options, whereas the larger individual would be willing to wait due to home range restrictions. Hence, cleaners from the socially complex reef environment may simply default to the ecologically relevant rule of

servicing the larger individual first, rather than using a more abstract rule that would allow them to identify a rule which would maximize their food intake. The results provide strong support to the ecological approach to cognition (see Kamil 1998; Shettleworth 2010) applied to the lives of individuals, while cleaners do not show good evidence for general intelligence (e.g. primate examples reviewed in Deaner et al. 2006).

3.5.4. Conclusion

The cleaning mutualism of cleaners and their clients has hitherto provided strong empirical evidence in support of evolutionary game theory for predicting cooperative behaviour (Bshary 2011). Our current findings, however, show that the published evidence linking optimal cleaning strategies with game theoretic predictions relating to biological markets (Noë 2001; Salwiczek et al. 2012) needs to be extended in order to explain variation in decision rules between different social reef environments within the same population. Similarly, human decision rules have been shown to deviate from strategies predicted by models: individuals may behave more cooperatively (Fehr and Fischbacher 2003; Haley and Fessler 2005) or less cooperatively (Kümmerli et al. 2010), as well as less precisely (Milinski et al. 2001) or in a more sophisticated way (Milinski and Wedekind 1998). This emphasizes the importance of decision making processes underlying cooperative behaviour (Gigerenzer and Selten 2002; Fehr and Fischbacher 2003). Decisions should be adaptive and maximize an individual's fitness (Dill 1987), therefore, in order to understand how such deviations from seemingly optimal strategies are adaptive, future theoretic models should aim to integrate both assumptions regarding the costs and benefit of information acquisition and storage, as well as learned decision-making mechanisms (Mery and Kawecki 2003; Heyes 10; Lotem and Halpern 2012).

3.6 ACKNOWLEDGEMENTS

We kindly thank the staff at Lizard Island Research Station - a facility

of the Australian Museum, for continued field support and friendship; A. Vail for field assistance; A. Lotem for insightful comments on the manuscript; Funding was provided by a Lizard Island Reef Research Foundation doctoral fellowship (SW) and the Swiss Science Foundation (RB).

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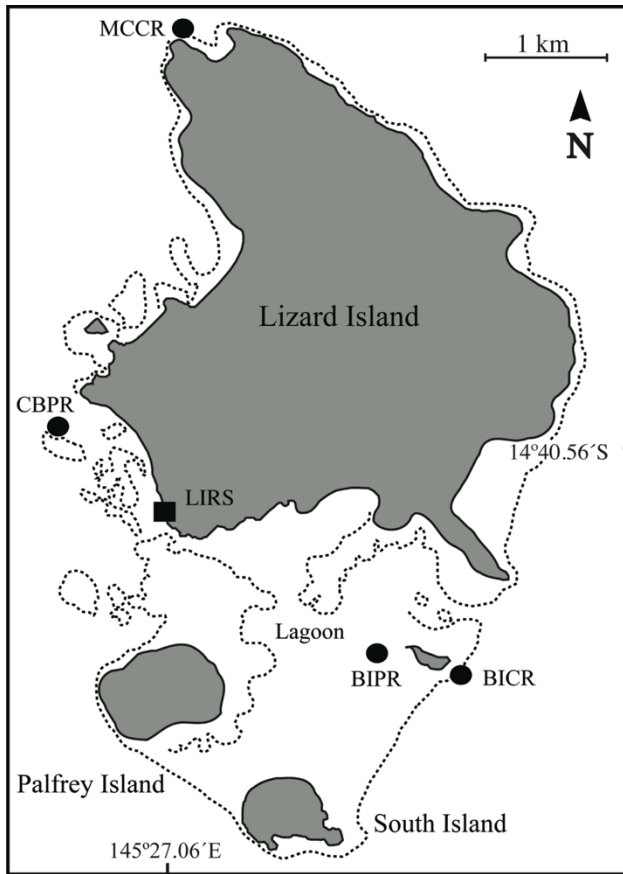


Figure 1. Lizard Island Group. Capture locations of cleaners are indicated by filled circles. Socially ‘complex’ sites: Mermaid Cove continuous reef (MCCR) and Bird Island continuous reef (BICR). Socially ‘poor’ sites: Corner Beach patch reefs (CBPR) and Bird Island patch reefs (BIPR). Juvenile cleaners were collected from all sites (2012: $n = 4$ from each site in 2012), while adults were only collected from Corner Beach and Mermaid Cove (2013: $n = 9$ from each site; 2014: $n = 8$ from each site). Lizard Island Research Station (LIRS).

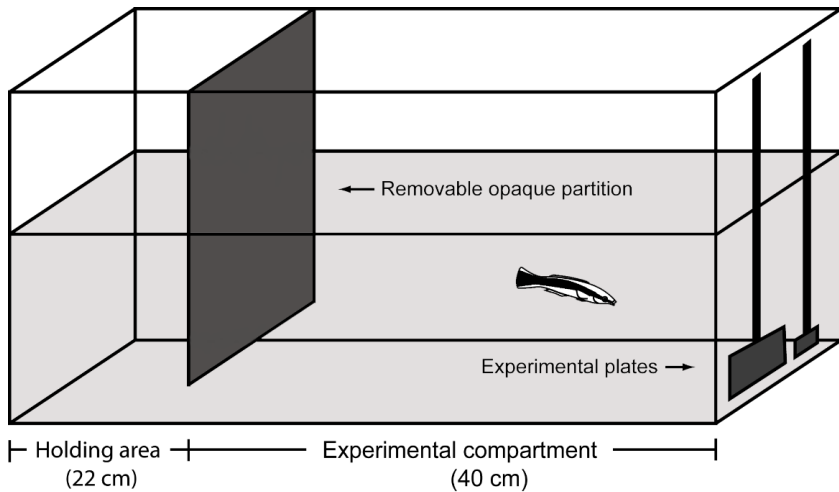


Figure 2. Experimental set-up used for adult cleaners. All three experiments use a similar aquarium arrangement: a cleaner holding compartment, an opaque removable partition and an experimental compartment containing the removable Plexiglas feeding plates. The illustration shows the size variation of Plexiglas plates utilized in Experiment 1.

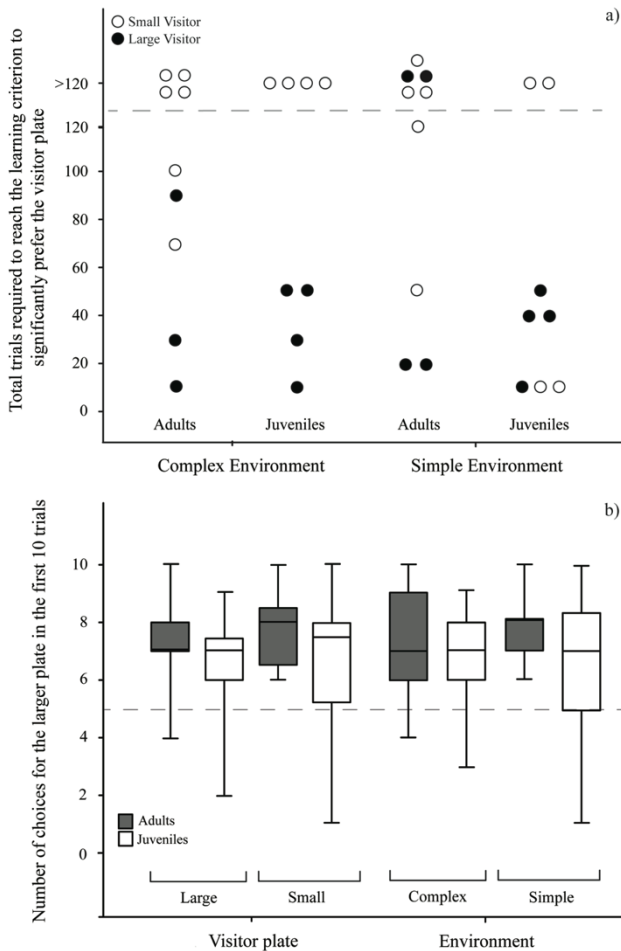


Figure 3. Performance of cleaners in Experiment 1. a) The number of trials required for cleaners to develop a preference for the visitor plate. Each circle represents one individual. Individuals above the dashed line did not complete the task in the maximum number of trials allowed (120). b) Box plots showing the number of choices for the larger plate in the first 10 experimental trials. Error bars represent maximum and minimum values. The dashed line indicates random choice, i.e. a 50% chance of choosing the correct plate.

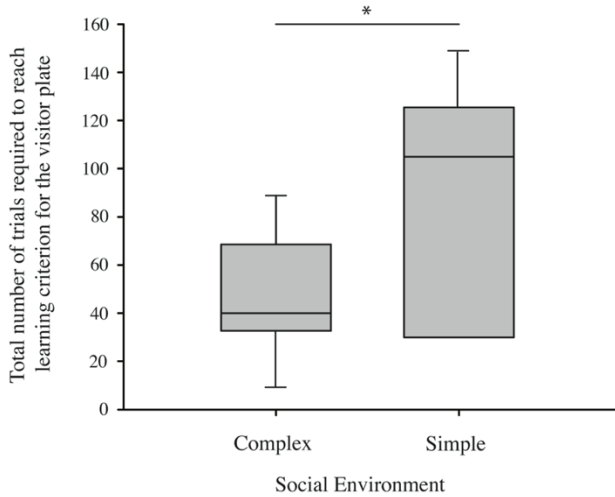


Figure 4. Performance of cleaners in Experiment 2: time to reach the learning criterion. Boxplots showing the number of trials required for cleaners from the two environments to develop a significant preference for the visitor plate (i.e. 9 out of 10 trials). Asterisks indicate significant differences at $P < 0.05$. Error bars represent maximum and minimum values

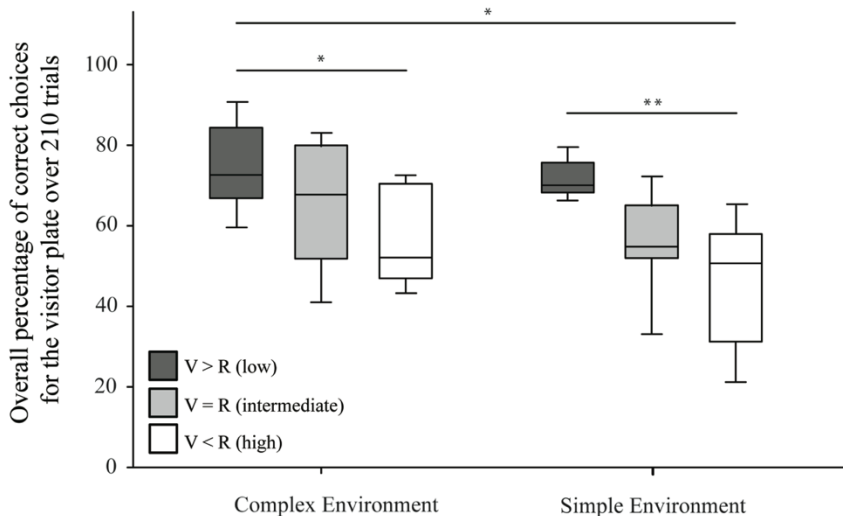


Figure 5. Performance of cleaner wrasse in Experiment 2: correct choices in 210 trials. Boxplots showing the overall percentage of correct choices of cleaner wrasse by environment (complex versus simple) and task learning difficulty (low: $V > R$, intermediate: $V = R$ and high: $V < R$). Error bars represent maximum and minimum values. Asterisks indicate significant differences at $* = P < 0.05$ and $** = P < 0.01$.

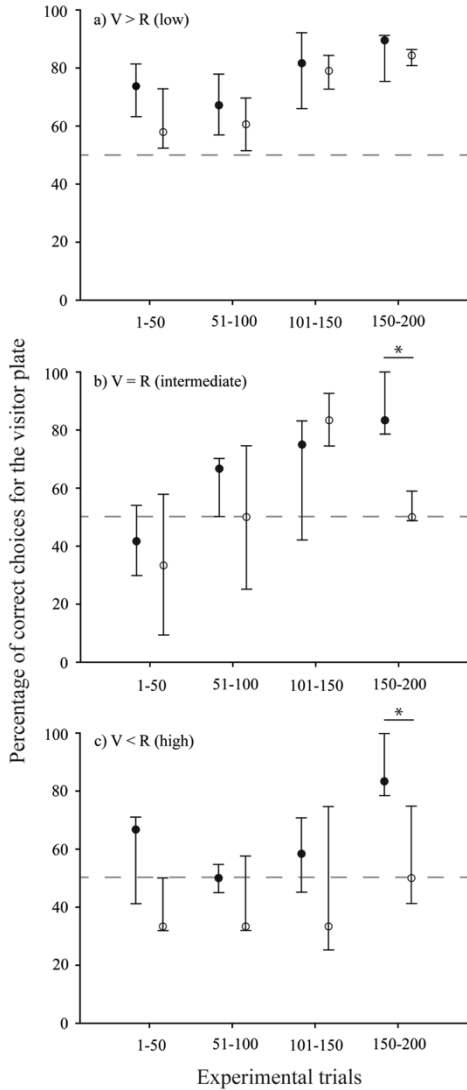


Figure 6. Performance of cleaner wrasse in Experiment 2: variation across time. The percentage of correct choices for the visitor plate across three levels of task learning difficulty: a) low ($V > R$), b) intermediate ($V = R$) and c) high ($V < R$). Median and interquartile values are displayed. Filled circles: complex social reef environment, open circles: simple social reef environment. The proportion of variance explained by fixed and random factors in our models ($R^2_{GLMM(c)}$) was a) 0.43, b) 0.68 and c) 0.22.

4

VARIATION IN SOCIAL COGNITIVE PERFORMANCE IN BLUESTREAK CLEANER WRASSE (*LABROIDES* *DIMIDIATUS*) APPEARS TO BE CONTEXT SPECIFIC

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ABSTRACT

Complex environments are known to enhance both brain development and cognition across a range of taxa, leading to flexible behavioural strategies. This may be driven by repeated neural stimulation in such variable environments, which can result in better and faster learning. Bluestreak cleaner wrasse (*Labroides dimidiatus*, ‘cleaners’) from socially complex reef environments have repeatedly shown to outcompete cleaners from socially simple reef environments in multiple laboratory-based cognitive experiments designed to capture the social aspects of cleaning interactions with ‘client’ reef fish. Here, in order to determine whether differences are context specific, we tested for differences in cleaner 1) initial body condition, 2) behavioural flexibility, by performing a large-scale translocation experiment, and 3) cognitive abilities in an abstract spatial discrimination task. We found no differences in both initial body condition and cognition, which suggests that the decision rules applied by cleaners in their own reef environment are compatible to local reef conditions and our published laboratory experiments may lack ecological and social relevance in socially simple reef environments. The full scope of the translocation study could not be completed as only one translocated treatment individual was recaptured after 21 days. Control individuals, which were released at their territory, were all identified or recaptured. The single translocated fish was also the only individual to decrease in length during the release period, suggesting it did not adjust well to its new reef environment. Future improvements to translocation studies and ethical considerations are discussed.

4.1 INTRODUCTION

Behavioural flexibility can be defined as the capacity to adapt ones' behaviour in response to environmental cues (Coppens et al. 2010; Wright et al. 2010). Flexible or 'plastic' strategies are particularly important when coping with spatial and temporal environmental variability, both predictable and unpredictable (Day and McPhail 1996; Sol and Lefebvre 2000; Richerson and Boyd 2000). This includes seasonal, local and diel changes in food availability, risk of predation in certain localities and invasion of other species (Dill 1983). Hence, flexible behavioural strategies allow for rapid responses in such situations, increasing an individual's foraging success, survival and ultimately, its fitness (Niemelä et al. 2013). The ability, however, to apply flexible strategies may be limited by brain size (Sol and Lefebvre 2000), while the associated costs of plastic behaviour, which includes maintenance, production, information acquisition and genetic costs, places further constraints on individuals (reviewed in: DeWitt et al. 1998).

Cognition and the ability to behave flexibly is also strongly influenced by ontogenetic experiences within a variable environment (Kotrschal and Taborsky 2010). In fish, for example, individuals which experience environmental fluctuations early in life have shown enhanced behavioural flexibility in regards to 1) predator avoidance strategies (e.g. increased shoaling response: Salvanes et al. 2007), 2) feeding performance (e.g. increased consumption of live prey: Braithwaite and Salvanes 2005) (Kotrschal and Taborsky 2010) and 3) social behaviour (e.g. discrimination in aggressive acts: Salvanes and Braithwaite 2005). Such responses may be a result of repeated neural stimulation in such variable environments, which can result in better and faster learning (Braithwaite and Salvanes 2005). Hence, enrichment studies have repeatedly shown that animals raised in enriched and or variable environments have enhanced brain development, leading to improved learning and memory (e.g. Van Praag et al. 2000; Frick and Fernandez 2003; Botero et al. 2009). Nevertheless, it is important to realise that such studies are typically conducted under laboratory conditions using

lab reared subjects (but see Wismer et al. 2014; Chapter 2), where standard holding conditions and even the enriched conditions arguably provide much simpler environments than the animal would encounter in nature. Thus, it remains largely unclear how far variation found in natural environments would cause similar results, and how far such variation is due to variation in local suitable behaviour or due to constraints.

Here we test for differences in cognition and flexibility due to environmental variability in bluestreak cleaner wrasse *Labroides dimidiatus* (hereafter ‘cleaners’). Cleaners cooperate with visiting reef fish clients by removing ectoparasites, but also frequently ‘cheat’ their clients by feeding directly on their protective mucus layer, which is costly to produce (Bshary 2011). To maximize their daily food intake across repeated cleaning interactions, cleaners have to cooperate and cheat their clients in a strategic manner. This requires the ability to be aware of image scoring individuals (i.e. behave more cooperatively in the presence of an audience) and categorize clients based on their strategic options (i.e. queue for service versus partner switching) and (Bshary 2011). Recent evidence, however, suggests that such abilities may not be consistent across all individuals (Wismer et al. 2014; Chapter 2 data). As demonstrated in previous chapters in this thesis, cleaners from socially complex reef environments (e.g. continuous reefs) perform significantly better in standard laboratory-based cooperation tasks compared to cleaners from socially simple reef environments (e.g. patch reefs) (Wismer et al. 2014; Chapter 2). In particular, cleaners from complex reefs appear to use detailed and precise knowledge when making foraging decisions in the laboratory, in contrast to cleaners from more simple reefs, which use general rules of thumb (Chapters 2 and 3). Such differences in decision rules appear to be strongly influenced by the complexity of the social environment, which varies in regards to client abundance and diversity, as well as cleaner to client ratios (Wismer et al. 2014).

Based on these findings, several key questions remain to be fully understood. First, do cleaners behave optimally in the context of their

own habitat or is applying precise knowledge a superior strategy that leads to increased foraging success, independently of habitat? As cleaners are broadcast spawners with a pelagic egg and larval phase, reproductive success cannot be measured directly. We therefore opted for body condition as a likely correlate of fitness, evaluating whether or not cleaners from the socially complex reef environment are in better physical condition. This was tested by comparing the initial length-weight relationships of cleaners from socially simple and complex reef environments. Second, we aimed to determine whether the behavioural strategies employed by cleaners from the two reef environments are flexible. For example, can adult cleaners readily adjust their decision rules to the local conditions, or alternatively, are cryptic genetic differences between cleaners driving their decision strategies? We aimed to test this possibility by translocating cleaners from the two reef environments in order to determine whether they are able to adjust their behaviour to suit their new social conditions, i.e. making decisions that are similar to those individuals that grew up under the local conditions. In particular, are cleaners from socially simple reef environments able to adjust to new, more complex social conditions present in socially complex reef environments, and therefore, also perform better in subsequent cognitive tasks, and do cleaners from socially complex environments lose their strong performance in cognitive tasks when translocated to a socially simple environment? Finally, variation in cleaner performance during laboratory-based cooperation tasks may be a result of general cognitive capabilities or due to the specific social aspects tested in experiments. In other words, cleaners from socially simple reef environments may have cognitive limitations or our experiments may lack ecological relevance for them. Therefore, we tested cleaners from both reef environments on an ‘abstract’ spatial discrimination experiment to test for variation in cognitive abilities between the two groups in a context that is not relevant for interactions with client reef fish.

4.2 STUDY SITE

This study was conducted at Lizard Island Research Station, on the northern Great Barrier Reef, Queensland, Australia (14°40'S, 145°28'E), between July and September 2014. Our specific study sites, as discussed above, were 1) Mermaid Cove, a continuous reef at the northern tip of Lizard Island which represented the socially complex reef environment and 2) Corner Beach patch reefs, situated on the south-western side of Lizard Island, which represented the socially simple reef environment (Figure 1a). Within each of these sites, 16 cleaner territories were identified (haphazardly) and their locations were individually marked with submerged polypropylene tubes. Cleaner territories at Corner Beach were additionally marked with buoys at the surface, as poor visibility and strong currents at this site made underwater navigation between reef patches challenging.

4.3 MATERIALS AND METHODS

4.3.1 Fish capture, tagging and release

We subsequently caught 32 adult female cleaners, one from each marked territory, using hand and barrier nets (2 m x 1 m, 5 mm mesh). Captured fish were placed in sealed plastic bags containing ample oxygen supply and transported to LIRS. All fish were immediately weighed, measured (total length in mm) and 'marked' using visible implant elastomer tags (from Northwest Marine Technology, Shaw Island, WA, USA) (Figure 2). Three elastomer tags were injected into the dorsal muscle of each fish using a 0.3 cc insulin syringe with a 29 gauge/1.27cm needle (Becton Dickinson, New Jersey, USA). All cleaners caught from Mermaid Cove were tagged with fluorescent yellow elastomer, while cleaners caught from Corner Beach patches were injected with fluorescent blue elastomer. Tagged fish were subsequently placed in individual glass aquaria (62 cm x 26 cm x 37 cm) with a continuous flow of fresh sea water and two polyvinylchloride tubes (approx. 2 cm x 15 cm) for shelter. All fish were allowed to recover and fed mashed prawn once daily on grey Plexiglas plates (10 x 5 cm).

All cleaners were released back to the reef once all fish were marked and fed from feeding plates in captivity (approximately 3-5 days). Individuals were assigned into either a control or treatment group: 8 control fish and 8 treatment fish per reef environment. Control fish were placed back to their individual territories within their respective reef environment, while treatment fish were translocated to ‘empty’ territories within the alternate reef environment (one fish per territory). For example, cleaners from the complex reef environment were released to the simple reef environment and vice versa. Control fish were released close to the reef substratum by slowly opening the sealed plastic bag and allowing the fish to re-enter its territory. In contrast, in order to help entice translocated fish to remain within their new site, treatment fish were initially released into a mesh cage (approx. size: 70 cm³) within their new territory, which was placed over sand adjacent to the reef and contained rubble/small coral bommies. The aim of this procedure was to moderately habituate cleaners to their new sites and to prevent potential aggression from resident reef fish or cleaners from adjacent territories. Mesh cages were removed from all translocation sites the following morning.

After a 21-day release period, we attempted to recapture all tagged cleaners. If marked fish were not found at the site of release/within the marked territories, we used a systematic underwater search pattern to search adjacent areas for tagged individuals. Recaptured fish were subsequently transported to LIRS, individually housed in glass aquaria and fed mashed prawn once daily.

4.3.2. Laboratory experiments

4.3.2.1 Experiment 1: Audience effects

The aim of this experiment is to test whether cleaners adjust their levels of cooperation (i.e. feeding against their preference) in the presence of an image scoring bystander client. Cleaners were alternatively offered a single Plexiglas plate containing 2 flake and 2 prawn items (control) and two differently coloured Plexiglas plates, each containing 2 flake

and 2 prawn items (treatment). Cleaners had to avoid eating prawn items on the first plate in the presence of a bystander plate, in order to subsequently gain access to the food items on the bystander plate. If a prawn item 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. The ratio of flake to prawn items eaten and the total number of times a cleaner succeeded to the bystander plate were recorded. Full methodology provided in Chapter 2.

4.3.2.2 Experiment 2: Biological market theory

Here we tested the cleaner's 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). Cleaners were presented simultaneously with two different coloured 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. The task was reversed after the initial treatment was learned. A maximum of two-hundred trials were conducted over ten days per cleaner. *Full methodology provided in Chapter 2.

Since only one translocated treatment individual (out of 16) was located and recaptured after the release period, the full experimental comparison between control and treatment individuals was not possible. However, to gain preliminary data, the two experiments above were conducted on the one recaptured translocated individual.

4.3.2.3 Experiment 3: Abstract spatial discrimination task

This experiment tested the cognitive capabilities of cleaners in an abstract context. All cleaners were presented with two identical grey Plexiglas plates (each 4.5 cm x 4.5 cm), marked with either a 3 mm white or yellow band at the top or left side of the plate, respectively. Plates were constructed from two pieces of Plexiglas glued on either side of a handle, which created a 4 mm space in between the two pieces, hereafter referred to as the ‘hidden compartment’ (Figure 3). The two plates were placed at the back of the experimental aquarium and were separated by a 9 cm clear Plexiglas partition. Both plates contained a mashed prawn food item, which was placed either 1) on the back surface of the plate, making it accessible to the cleaner, or 2) in the hidden compartment between the two pieces of Plexiglas, making it inaccessible to the cleaner. Inserting prawn into the hidden compartment controlled for olfactory cues. The plate containing the accessible food reward was always placed on the same side of the aquarium, either on the left or right side. We recorded the number of trials needed for the cleaner to learn the location of the plate with the accessible food reward. In each trial, we removed the ‘unchosen’ plate when the head of the cleaner moved beyond the edge of the 9 cm clear partition, indicating that it had chosen to inspect the plate on that side of the partition. Once a preference for the correct plate was achieved (we used the same learning criterion as in the biological market theory experiment in Chapters 2 and 3), the task was reversed, i.e. cleaners which commenced the experiment with a left-side (and white bar) reward plate were now presented with a right-side (and yellow bar) reward plate. Each cleaner was given a maximum of 100 trials to learn the task (20 trials per day split between am and pm). Reward plate position and colour were counterbalanced between environments. This experiment was conducted on a total of 16 adult cleaners (8 from each reef environment, that including control individuals from both reef environments).

4.3.3 Data analysis

We used a general linear model (ANCOVA) to compare the length – weight relationships of cleaners from the two reef environments.

Survival analysis (Cox proportional hazards regression model; ‘coxph’ function in the R package ‘survival’) was used to evaluate differences in the success rate and the number of trials required by cleaners to reach the learning criterion in the abstract spatial discrimination experiment, testing for the effect of reef environment and experimental phase (i.e. initial vs. reversal) on learning. All analyses were done in R 3.1.2 (R Development Core Team 2014).

4.4 RESULTS

4.4.1 Cleaner body condition: initial length – weight relationships

There was no significant effect of reef type on the initial body condition (i.e. length – weight relationship) of cleaners ($F_{1,28} = 3.25$, $P = 0.082$; Figure 4). For example, cleaners with an initial total length of 7.7 cm weighed 4.34g at the socially complex reef and 4.23g at the socially simple reef (Figure 4). The overall length – weight relationship of cleaners was highly significant ($F_{2,29} = 124.3$, $P < 0.0001$).

4.4.2 Cleaner body condition: post-release comparisons

After the 21-day release period, all control fish (i.e. tagged and released at their site of capture) were either caught, or if recapture was not possible, identified and counted in the field. However, only a single treatment fish (i.e. translocated to opposing reef site) was identified and recaptured: a Mermaid Cove cleaner (socially complex reef) released at the socially simple reef. In contrast to control fish, which on average, increased 1.8 mm in length, the treatment fish was the only individual to decrease in length (2 mm) (Figure 5). Likewise, the translocated individual lost weight (0.2 g) during the release period, while most control fish gained weight (Figure 5).

4.4.3 Laboratory experiments

4.4.3.1 Experiment 1 and 2: Audience effects and biological market theory

The performance of the single translocated cleaner in the cognitive foraging experiments was generally in the range of published evidence for cleaners captured at the socially complex reef environment (Wismer et al. 2014). In the audience effects experiment, the translocated individual ate more against its preference on the first plate (1.1) (in the bystander scenario) versus the single plate (1.28), comparable to published evidence (Figure 6). Although, it showed the highest levels of ‘cooperation’ (feeding against its preference) on the second plate (in the bystander scenario) it only succeeded to feeding on the second plate a total of 2 times (Figure 6). This is in contrast to published results on cleaners captured from socially complex reef environments, which treat the second plate (in the bystander scenario) similarly to the single plate and succeed to feeding on the second plate 6 times (median) (Figure 6). However, similar to published evidence on socially complex reef cleaners, the translocated individual solved the biological market theory experiment in 140 trials, by learning to give service priority to the visitor plate, both in the initial phase and the reversal learning component.

4.4.3.2 Experiment 3: Abstract spatial discrimination task

Learning time in the abstract spatial discrimination experiment depended on the experimental phase (initial vs. reversal experiment; $\chi^2 = -70.5$, $P = 0.024$; Figure 7), with cleaners requiring more trials to develop a preference for the reward plate in the reversal experiment (coef \pm s.e.m = -1.28 ± 0.62 , $z = -2.05$, $P = 0.040$), irrespective of reef environment ($P = 0.98$; interaction ns, $P = 0.435$). Only one individual failed to complete the initial phase of the experiment, whereas 6 individuals failed to complete the reversal phase.

4.5 DISCUSSION

The first two aims of this study were 1) to determine whether cleaners behave optimally in the context of their own environment and 2) to investigate whether there are differences in the cognitive abilities between the two cleaner groups when tested on an abstract cognitive

experiment. We found that there are no differences in both initial body condition and cognitive performance on the spatial discrimination experiment between cleaners from socially simple and complex reef environments. This suggests that the decision rules applied by cleaners within their own environments are well suited to the specific conditions present within each reef environment, as discussed in further detail in the next section. These results place an emphasis on our third aim, which was to determine whether the behavioural strategies employed by cleaners are flexible. We translocated cleaners from opposing reef environments to determine how this affects their body condition, as well as, their performance in published laboratory-based experiments (audience effects and biological market theory). However, given that only one treatment fish was recaptured, the full scope of this study could not be realized. These results are therefore discussed in the context of future improvements to translocation studies, as well as, ethical considerations.

4.5.1 Cleaner initial body condition

In fisheries science, fish body condition is commonly estimated using length - weight relationships (Bolger and Connolly 1989). Such analyses assume that the heavier fish of a given length is in a better physiological state, which can be related to its past foraging success, its ability to cope with environmental stressors, and ultimately, its evolutionary fitness (Bolger and Connolly 1989; Jakob et al. 1996). Indeed, the fecundity of female fish is positively correlated with their body condition (e.g. Atlantic cod: Kjesbu et al. 1991; eastern Scotian Shelf haddock: Blanchard et al. 2003; bullhead: Abdoli et al. 2005). Body condition, based on length – weight relationships, is however, only an index of fitness and has its limitations. Although it is considered good indicator of ‘well-being or fitness’, ultimately, a ‘growth’ index (i.e. combination of increase in body length, body condition and tissue energy concentration) represents a more accurate fitness expression (Bolger and Connolly 1989). Other indices of estimating fish fitness include the ‘gut’ index (i.e. energy content of the intestines) and the ‘liver’ index (i.e. liver weight as percentage of body

weight) (Jensen 1980). However, obtaining such alternative indices is often a time consuming process, requiring advanced laboratory techniques that are generally not well-suited for field work. Hence, we used the more standard index of estimating fish fitness, i.e. body condition measured as length – weight relationships, while recognizing the associated limitations.

The initial body condition of cleaners did not differ significantly between the two types of reefs; hence, both groups of cleaners appear to be succeeding in their own environment. This suggests that the natural foraging strategies applied by cleaners from the socially simple reef environment well-suited, even though their performance in the laboratory was subpar (i.e. experiments: feeding against preference, audience effects, biological market theory; Wismer et al. 2014; Chapters 2 and 3). Hence, the rules applicable in the laboratory-based cognitive experiments may simply not suit the ecology of socially simple patch reef environments. For example, given the low abundance of cleaners at these sites, clients have fewer choice options and may therefore be willing to wait for service and not image score. This removes the need for cleaners to be aware of audience effects. Furthermore, precise solutions are time consuming and costly, as they require an individual to invest in detailed learning; whereas, rules of thumb are ‘quick and cheap’ and result in good outcomes in commonly encountered situations (Gigerenzer and Selten 2002; El Mouden et al. 2012). For cleaners from socially simple reef environments, the investment and benefit of acting precise may not be worth the associated cost. In contrast, cleaners from socially complex reef environments may invest in precise strategies since the net benefit may be worth the cost. Hence, the decision rules applied by cleaners from socially simple reefs appear to work well in their own environment, and therefore, there may be no pressure to evolve more sophisticated or precise strategies.

There are, however, other factors to consider. For example, based on simple length – weight data, we are unable to determine the age of our focal individuals. It could be that cleaners from the socially simple reef

environment display a similar body condition, yet grow at slower rates. Therefore, the individuals we measured from the socially simple reef environment could be older in comparison to cleaners from the socially complex reef environment and actually in ‘worse’ body condition given possible age differences. Secondly, there is still the possibility that one reef environment is superior to the other, but differences in body condition are not detected for other reasons. For example, cleaners from the socially simple reef environment experience less conspecific competition as these reef environments are characterized by a lower cleaner to client ratio. However, given their cognitive constraints in a social context, they are inhibited from attaining a better body condition. Furthermore, in this context, it is also unknown why lower cleaner to client ratios in the socially simple reef environment result in fewer client interactions. Thirdly, cleaners from the socially simple patch reef environments may save energy during cleaning interactions as the nature of these reefs requires them to swim less, thereby, attaining similar body condition to that of cleaner from the socially simple reef environment. Hence, although our data on initial body condition suggests that living within socially simple reef environments is a valid alternative to the more complex reef, our findings are preliminary and require further investigation.

4.5.2 Translocation experiment and ethical considerations

Unfortunately, only a single translocated cleaner (out of 16) was recaptured, in comparison to the majority of the control fish. This is after taking extra measures for the treatment fish which included: 1) first releasing cleaners into a mesh cage adjacent to the release site, which allowed for habituation to the new reef, while being protected from potential predators, 2) releasing cleaners into areas where another cleaner had been removed (i.e. to avoid territorial aggression from conspecifics), and 3) releasing cleaners at reefs where ample shelter sites were available, e.g. hard coral and crevices. However, for unknown reasons, all other treatment fish were not located. All release sites and adjacent reefs were thoroughly inspected over several days using a systematic search pattern by two or more divers.

Where did they go? Although it is not possible to determine the precise fate of the treatment fish, we speculate that these individuals were predated on. This could have happened when swimming over unprotected sandy areas in an attempt to return to their own territory (e.g. homing behaviour in reef fish: Warner 1995; Marnane 2000; Kaunda-Arara and Rose 2004) or by being forced off the reef by aggressive individuals, both conspecifics and other fish species (i.e. Myrberg and Thresher 1974; previous cleaner release: pers. obs. S Wismer 2014). Alternatively, predation is also likely if cleaners failed to secure a resting site during the night (competition for shelter sites in reef fish: Shulman 1985), or if their behaviour upon release indicated a weakness or vulnerability to predators (e.g. lizard fish predation: pers. obs. Z Triki 2015), such as excessive ‘dancing’ movements (e.g. juvenile cleaner predation: pers. obs. S Wismer 2012). Nonetheless, these results highlight the importance of specific territories to individual cleaners, which should promote modifications of future cleaner release practices. For example, cleaners are used annually for a plethora of laboratory experiments, and are subsequently often released back to the reef. However, care should be given to release cleaners at their precise site of capture, and not simply to reefs which are advantageous to the researcher, in order to decrease potential mortality rates.

4.5.3 Single recaptured treatment individual: body condition and cognitive performance

Although the sample size of treatment individuals is significantly limited, it is interesting to note that the single recaptured treatment individual was the only fish to decrease in length over the 21-day release period. Although rarely documented, various species have been reported to decrease in body length during harsh environmental conditions. For example, this ‘shrinking’ phenomenon has been documented in shrews, which can shrink up to 7 % during harsh winters (Saure and Hyvärinen 1965), marine iguanas, which can reduce their body length up to 20% during El Nino events when food availability becomes limited (Wikelski and Thom 2000) and in salmonids, which

have shown to shrink up to 10 % during winter months (Huusko et al. 2011). Such decreases in body length is thought to be an adaptive survival strategy to combat unfavorable environmental conditions (Huusko et al. 2011).

The translocated cleaner may have been negatively impacted by the novel socially simple patch reef environment for several reasons. For example, 1) while habituating to the new site, the cleaner may have gone several days with reduced cleaning interactions/reduced food intake, 2) socially simple patch reefs have a lower abundance of clients, resulting in fewer total cleaning interactions per day for cleaners, 3) aggression by resident cleaners may have driven the translocated cleaner to unfavourable reef areas with fewer clients, and or 4) translocated cleaners have had limited access to novel resident clients, which require a slow build-up of a cleaning relationship (i.e. Bshary 2002). Moreover, the single translocated cleaner did not apply the decision rules applicable to the socially simple reef environment in subsequent cognitive experiments, i.e. it feed on the second plate in the audience effects experiment and still solved the biological market theory experiment, indicating the socially simple patch reef environment did not influence its decision making processes within the 21-day release period. Hence, there is also a possibility that the decision rules applied by the single translocated cleaner were not suited for the socially simple reef environment, and hence, we documented a reduction in both length and weight in this individual. However, it remains very difficult to draw such conclusions based on a very limited sample size of 1, and further investigations are needed. For example, a *within* reef translocation control would help clarify this question. If such control individuals also decreased in length and weight, it would be clear that it is not the ‘false’ decision rules driving this result, but rather an alternative explanation.

4.5.4 Performance in the abstract spatial discrimination experiment

We found no significant differences in the performance of cleaners in the abstract spatial discrimination experiment, a task which provided no

plausible ecological advantage for either cleaner group. This further suggests that cleaners from socially complex reef environments may only excel in tasks that reflect the ecological challenges linked to their own social environment and that published differences are not simply due to variation in cognitive abilities.

Environmental enrichment studies on other fish species, however, often suggest the opposite. For example, fish reared in enriched environments, versus standard or barren environments, have shown 1) superior performance in a cognitive spatial task (e.g. juvenile Atlantic salmon, *Salmo salar*, Salvanes et al. 2013) and 2) an improved ability to learn to consume live prey by ‘social learning’ (e.g. juvenile cod, *Gadus morhua*, Strand et al. 2010). Such experiments, however, only incorporate physical enrichment, not social, and compared barren environments to enriched environments. This is in stark contrast to our socially simple reef environment, where cleaners live on three dimensional patch reefs and engage in approximately 800 client interactions per day (Wismer et al. 2014). Hence, it is *comparatively* simple to our complex reef environment, and shares few similarities to a barren hatchery as used in experiments above. Such characteristics may further explain the lack of cognitive variation in the abstract spatial task between the two reef environments.

4.5.5 Conclusion

In summary, the decision rules employed by the two cleaner groups appear to be well-suited to their environment, as cleaner body condition did not differ significantly between the two reef environments. Furthermore, the translocated individual showed both a decrease in length and a reduction in weight after the 21-day release period and it continued to perform well in the standard laboratory experiments (audience effects and biological market theory). Hence, the decision rules applicable in one reef environment may not be suitable in the opposing reef environment, yet this conclusion requires further investigation. Finally, the cognitive constraints shown by cleaners from the socially simple reef environment in the standard laboratory

experiments appear to be context specific, since their performance in the abstract spatial discrimination task was comparable to cleaners from the socially complex reef environment.

Our results, collectively, suggest that the ontogenetic reef environment is integral in shaping a cleaner's decision rules, which may (or may not) be flexible. However, cryptic genetic differentiation between the two cleaner groups may also strongly influence cleaner decision strategies, and therefore, cannot be excluded as an alternative explanation. Hence, a next step would be to analyse the genetic distance (e.g. FST values) between the two cleaner populations. Several other key elements are lacking from the current study, for example, 1) increasing the sample size of translocated-treatment individuals, in order to obtain a better understanding of the cognitive limitations of cleaners and 2) incorporating a within-reef translocation control, to determine if cleaner body condition also decreases when translocated to a different territory within the same social reef environment.

Due to the high failure rates in recapturing treatment individuals, future translocation studies should also adopt a modified experimental design. In order to reduce stress, fish should be caught, measured, marked and released as quickly as possible. The 3-5 days spent in the lab prior to release in our study may have further negatively influenced the cleaners. Secondly, in order to increase recapture rates of these fish, translocated fish should be either continuously monitored by SCUBA divers or tracked with acoustic tags. Findings from such a modified translocation experiment will provide novel and important insight into the decision strategies and cognitive limitations of cleaners.

4.6 ACKNOWLEDGMENTS

We kindly thank the staff at Lizard Island Research Station - a facility of the Australian Museum, for continued field support and friendship; S. Binning, A. Bshary, A. Pinto, O. Rey, D. Roche and E. Wismer for field assistance; Funding was provided by a Lizard Island Reef

Research Foundation 2012 Doctoral Fellowship (SW) and the Swiss Science Foundation (RB).

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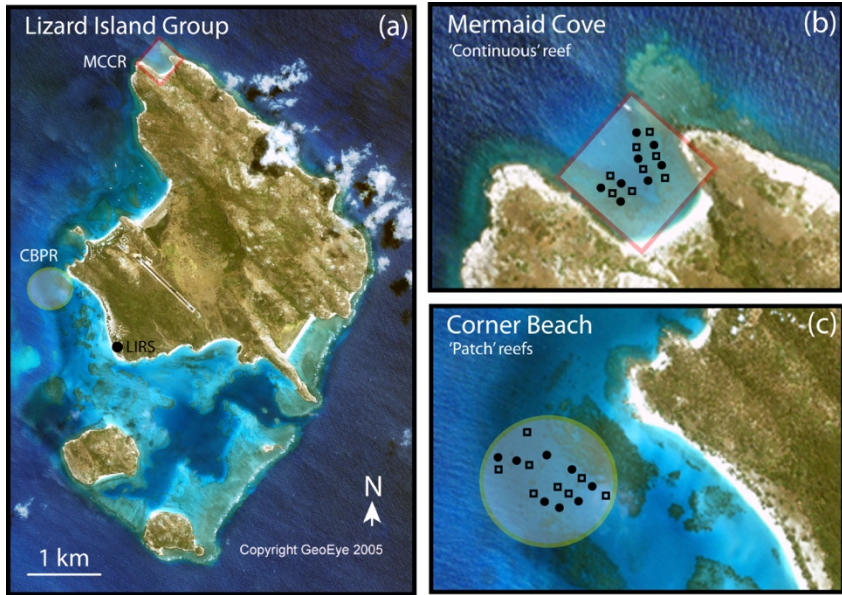


Figure 1. Aerial photograph of the study sites. a) Lizard Island Group, showing the locations of: Lizard Island Research Station (LIRS), Mermaid Cove continuous reef (MCCR) (socially complex) and Corner Beach patch reefs (CBPR) (socially simple). Detailed photographs of b) Mermaid Cove continuous reef and c) Corner Beach patch reefs, showing the approximate release sites of tagged cleaners. Circles represent patch reef cleaners; squares represent continuous reef cleaners. Each site contained 8 control fish and 8 treatment fish.



Figure 2. Cleaner marked with a visible implant elastomer tag. A photograph of a recaptured control fish from Mermaid Cove, which was injected with fluorescent yellow elastomer. All fish were given three tags, each approx. 1-2mm in length. This allowed for identification in cases (such as this one) where a tag(s) was lost. Photograph courtesy of Simon Gingins.

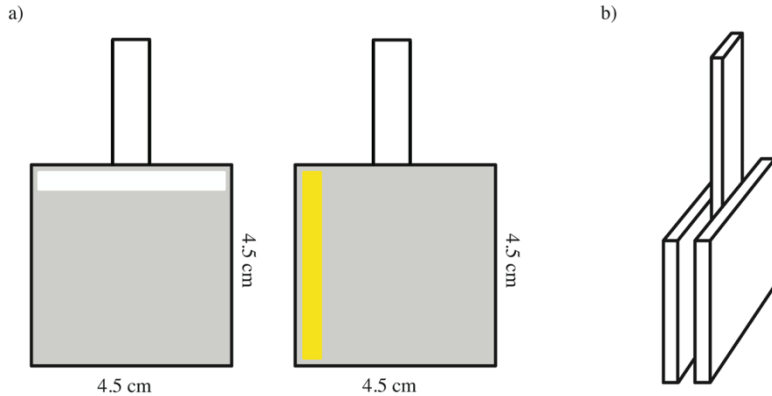


Figure 3. Plexiglas feeding plates utilized in the abstract spatial discrimination experiment. a) Illustration shows the two types of experimental plates presented to cleaners in either the initial or reversal component of this experiment (counterbalanced, i.e. two white or two yellow starting plates). Food items were placed centrally on the reverse side of the accessible reward plate. b) The hidden compartment between the two plates where inaccessible food items were placed to control for olfactory cues.

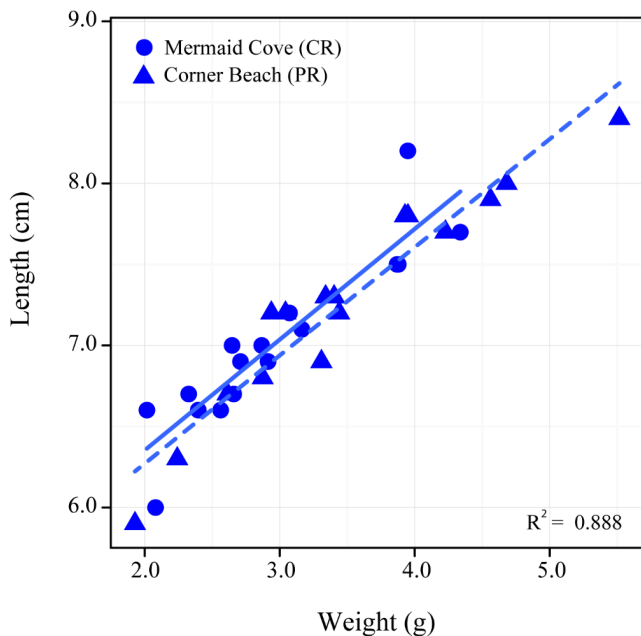


Figure 4. Initial body condition. The length – weight relationship of cleaners from the two reef sites prior to tag and release. The slope for the path reef (PR) cleaners is shown as a dashed line and the slope of the continuous reef (CR) cleaners is shown as a solid line. The R^2 value represents the overall proportion of variance explained by our model.

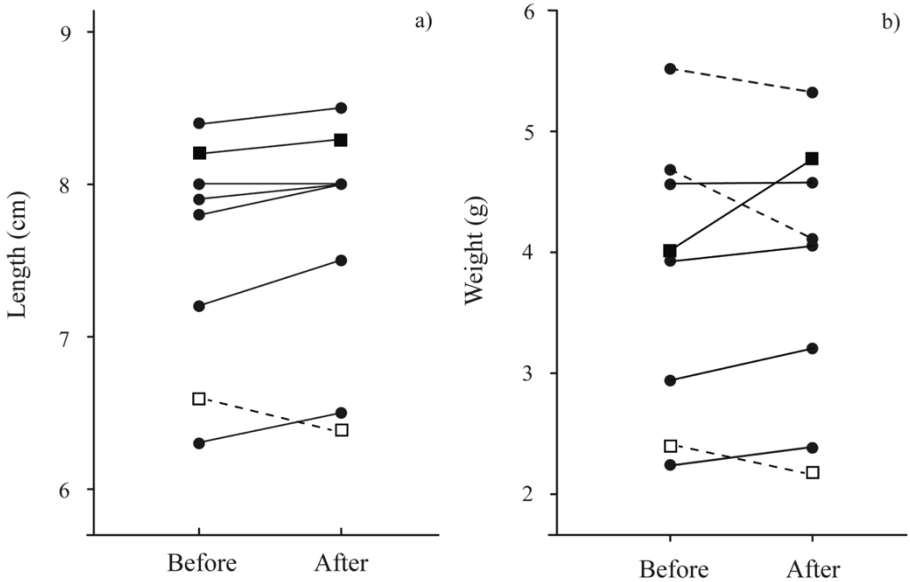


Figure 5. Initial and post-capture body condition. a) The lengths and b) weights of cleaners before after the 21-day release period. Filled circles represent control fish from the patch reef site and the open square represents the single treatment individual which was translocated. The filled square represents a single control fish from the continuous reef site, which was individually recognizable due to its large size. Individual identification of control fish from the continuous site was generally not possible due to the nature of this habitat, which allowed for easy movement of cleaners between sites.

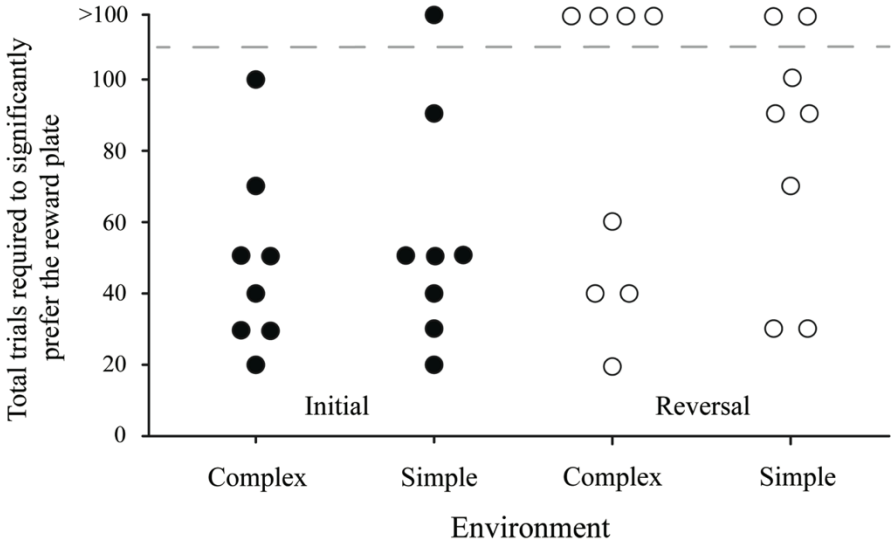


Figure 7. Performance of cleaners in the abstract spatial discrimination experiment. The number of trials required for cleaners to develop a preference for the reward plate. Each circle represents one individual. Individuals above the dashed line did not complete the task in the maximum number of trials allowed (100). Filled circles: initial phase of the experiment; open circles: reversal phase of the experiment.

5

GENERALIZED RULE APPLICATION IN BLUESTREAK CLEANER WRASSE (*LABROIDES DIMIDIATUS*): USING PREDATOR SPECIES AS SOCIAL TOOLS TO REDUCE PUNISHMENT

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ABSTRACT

Generalized rule application promotes flexible behaviour by allowing individuals to adjust quickly to environmental changes through generalization of previous learning. Here, we show that bluestreak ‘cleaner’ wrasse (*Labroides dimidiatus*) use generalized rule application in their use of predators as social tools against punishing reef fish clients. Punishment occurs as cleaners do not only remove ectoparasites from clients, but prefer to feed on client mucus (constituting cheating). We tested for generalized rule application in a series of experiments, starting by training cleaners to approach one of two fish models in order to evade punishment (by chasing) from a ‘cheated’ client model. Cleaners learned this task only if the safe haven was a predator model. During consecutive exposure to pairs of novel species, including exotic models, cleaners demonstrated generalization of the ‘predators-are-safe-havens’ rule by rapidly satisfying learning criteria. However, cleaners were not able to generalize to a ‘one-of-two-stimuli-presents-a-safe-haven’ rule, as they failed to solve the task when confronted with either two harmless fish models or two predator models. Our results emphasize the importance of ecologically relevant experiments to uncover complex cognitive processes in non-human animals like generalized rule learning in the context of social tool use in a fish.

5.1 INTRODUCTION

The ability to generalize rules and apply them to novel situations is a fundamental aspect underlying flexible cognitive behaviour (Emery and Clayton 2004). This allows individuals to adjust to environmental changes through the generalization of previous learning, without having to learn specific contingencies ‘from scratch’ (de Mendonça-Furtado and Ottoni 2008). In cognitive learning experiments, animals which develop such ‘learning sets’ (Harlow 1949) decrease error rates in subsequent contextually similar problems (Wynne 2001). But they only do so if they are capable of mastering the original problem, by extracting and applying the key principle that all of the problems had in common (Wynne 2001). In contrast, rote learners often solve tasks at a slower rate by learning each problem individually (e.g. pigeons: Wilson et al. 1985; Emery and Clayton 2004). It has been argued that the social environment, in particular, is variable which leads to selection for increased brain sizes in social species to enable individuals to behave more flexibly (Deaner et al. 2007). This view thus links behavioural flexibility to the social brain and Machiavellian intelligence hypotheses (Byrne and Whiten 1988; Dunbar 1998). The ability to extract and apply generalized rules is also considered an attribute of human higher cognitive functioning (i.e. language: Pinker 1991), yet more simple rules have been documented in non-human animal taxa (i.e. rats: Murphy et al. 2008).

Despite the conceptual focus of the importance of generalized rule learning in a social environment, experiments have generally been conducted in an abstract context (i.e. ‘match to sample’ experiments using symbols, sounds or colours as the stimulus sample and with food as the reward). Experiments on generalized rule learning typically involve the following rule: two stimuli are presented, one consistently yields a food reward, while the other one does not. Animals that are able to apply this rule to new tasks are unable to know the correct choice during the first presentation of two novel stimuli, but can deduce the correct choice for the second presentation (i.e. if it received food during the first trial, then it should repeat the choice, and if it was not

rewarded, then it should shift). Comparing the performance of various mammals in the crucial ‘trial 2’ of novel tasks, the number of stimuli pairs learned through operant conditioning before evidence for generalized rule learning emerged, increased considerably from rhesus monkeys to squirrel monkeys, marmosets and cats, while rats and squirrels exhibited no evidence for generalization, even over 1800 problems (Passingham 1981; Wynne 2001; Shettleworth 2010). Although these results correlate to relative brain size, this is not necessarily the driving force (Wynne 2001). An independent study on dunnarts, using visual stimuli, demonstrated the performance of these marsupials exceeded even that of rhesus monkeys, despite their small brain size (cephalization index 0.07, smaller than that of low-performing rats and squirrels) (Darlington et al. 1999; Wynne 2001). It is suggested that the foraging habitat of dunnarts drives this exceptional performance, as catching fast moving prey in an open, high predation risk environment may select for quick learning (Wynne 2001). Thus, while evidence for generalized rule learning exists in a variety of animal taxa, including also marine mammals (i.e. Herman et al. 1994), rodents (i.e. Murphy et al. 2008) and birds (i.e. de Mendonça-Furtado and Ottoni 2008), the lack of / variation in ecological validity may explain why the variation in species’ performance is large and not necessarily linked to brain size (Wynne 2001). As there is clear evidence that many cognitive abilities are tightly linked to a species’ ecology (i.e. ecological approach to cognition: Kamil 1998; Shettleworth 2010), generalized rule learning capabilities may span across a more diverse range of animal taxa and context than what is currently documented, and be more readily demonstrated as long as the ability to generalize is ecologically relevant to the species.

Testing such generalization abilities in a purely social context provides novel experimental opportunities. For example, generalization learning, a behaviour documented repeatedly in primates (i.e. Byrne and Whiten 1988), is yet to be explicitly tested in a social tool use scenario. Social tools differ from physical tools in that an individual or social agent is utilized or manipulated to achieve personal goals (Bard 1990), i.e. an “agent” uses a “social tool” to affect a “target” for the agent’s benefit;

as defined by Whiten and Byrne (1988a). Efficient social tool use requires that the tool is socially dominant over the target. This is indeed the case in early descriptions involving baboons (i.e. protected threat: Kummer et al. 1990), and Slocombe and Zuberbühler (2007) found that chimpanzees that have been attacked exaggerate their distress calls systematically in the presence of third parties that are dominant to the aggressor. However, such data do not allow for distinguishing between generalized rule application and the possibility that subjects learned each combination from scratch through operant conditioning.

Here, we test experimentally whether bluestreak cleaner wrasse (*Labroides dimidiatus*), a species which utilizes social tools under natural conditions, as discussed below, uses generalized rule application to identify potential tools. Cleaner wrasse (hereafter simply ‘cleaners’) are a reef-associated fish species which maintain territories termed ‘cleaning stations’ where they remove ectoparasites from visiting reef fish clients (Côté 2000). Although a mutualistic relationship (Ros et al. 2011; Waldie et al. 2011), conflict arises as cleaners prefer feeding directly on protective client mucus, which is considered an act of cheating (Grutter and Bshary 2003). In order to promote cooperative cleaning interactions, clients counter such cheating behaviour by employing various control mechanisms (Bshary and Grutter 2005; Pinto et al. 2011), including punishment in the form of aggressive chasing following a cheating event (Bshary and Grutter 2005). Cleaners sometimes reduce the amount of punishment by exploiting the presence of predatory clients as a third party (Bshary et al. 2002). Predators are approached and given tactile stimulation (socio-positive behaviour: Soares et al. 2011), while the punisher is deterred (Bshary et al. 2002). Hence, cleaners (agent) use predator species (social tool) to minimize the degree of punishment they receive from the cheated client fish (target). Preliminary field observations suggest that social tool use in cleaners is a relatively frequently occurring phenomenon (approximately once per eight hours, Ras Mohammed National Park, Egypt; Bshary et al. 2002). The observed tool use interactions invariably involved locally abundant grouper species (*Cephalopholis miniata* and *C. hemistiktos*) serving as the social tool (R.B. unpublished

data; Bshary et al. 2002). Thus, the question arises how readily cleaners would be able to use other, less common predator species as social tools. I.e., are cleaners capable of generalizing between predator species, and hence, able to recognize potential social tools? Also, would this ability be linked to the safe haven being a predator or could cleaners also readily learn and generalize that a harmless species may provide a safe haven?

To address these questions, we experimentally simulated the described cleaner social tool scenario in the laboratory, in order to test whether cleaners are able to apply generalized rule learning to minimize punishment (chasing), or whether they have to learn the usefulness of each predator species independently. Our first study, conducted in 2011, was exploratory in nature and tested whether cleaners are able to generalize a series of predator species, both local and exotic, when presented with a series of predator-harmless fish model combinations. Additionally, we tested whether cleaners could generalize when offered two harmless client models. These results were preliminary for the latter, in that tasks were presented in a fixed sequence. Therefore, we subsequently tested specific predictions derived from our initial results. First, we tested whether cleaners are able to distinguish ‘safe havens’ more quickly when represented by a predator model, in comparison to a harmless fish model. Second, using a counterbalanced design, we tested whether cleaners can generalize the concept of ‘predator fish represent a safe haven’ to other predator species, which vary in morphology, i.e. grouper versus moray eel, and location, i.e. from locally abundant species to species exotic to our study site, and hence, unknown to the subjects. And finally, we explored whether generalization abilities of cleaners are linked to always being presented with two clients belonging to different categories (i.e. predator-harmless) or whether cleaners can also generalize when facing a same category task (predator-predator or harmless-harmless). If cleaners were able to solve any of these problems, we predicted that the number of trials needed to reach individual learning criterion would be less in generalization tasks than during the learning of the initial task.

5.2 STUDY SITE

Our study was conducted between July and August 2011 and July and September 2014 at Lizard Island Research Station (LIRS), in the northern Great Barrier Reef, Queensland, Australia (14°40'S, 145°28'E).

5.3 MATERIALS AND METHODS

5.3.1 Subjects and housing

Adult female cleaners (n = 12 in 2011; n = 16 in 2014) were caught using barrier nets (2 m x 1 m, 5 mm mesh) from continuous fringing reefs surrounding Lizard Island. Post capture, all fish were placed in sealed plastic bags containing ample oxygen supply for transport to LIRS. Cleaners were individually housed in glass aquaria (62 cm x 26 cm x 37 cm) with a continuous flow of fresh sea water and were supplied with two polyvinylchloride tubes (2 cm x 15 cm) for shelter. Fish were fed mashed prawn once daily on grey Plexiglas plates (5 x 10 cm) from day one in captivity and were allowed to adjust to their captive environment for one week prior to commencing experiments.

5.3.2 Laboratory experiments

Prior to the commencement of experiments, all cleaners, both in 2011 and 2014, were taught that cheating behaviour would lead to punishment. We simulated this frequently observed natural behaviour by offering each cleaner a Plexiglas plate (7 x 12 cm) that contained both a preferred (mashed prawn; hereafter 'prawn') and a less preferred food item (fish flake mixed with mashed prawn; hereafter 'flake'), as equivalents of client mucus and ectoparasites, respectively. The Plexiglas plate mirrored the behaviour of a client reef fish: There was no consequence if the cleaner fed on the less-preferred flake item (i.e. cooperate), but it was immediately chased with the plate in a straight line for a distance of 20 cm if a prawn item was consumed (i.e. cheat) (detailed description of feeding against preference methods used in the

cleaner system are provided in: Wismer et al. (2014). All individuals were subjected to eight learning trials.

5.3.2.1 Experiment 1: Generalization of predatory species

The goal of the first experiment was to simulate the described social tool scenario, and to determine whether cleaners are capable of generalizing among predatory species in this context. For experimental trials, all aquaria were subsequently separated lengthwise (partially) using a transparent Plexiglas partition (42 cm), creating three compartments (Figure 1). The smaller compartment contained the client Plexiglas feeding plate, while the two elongated sections contained a model of a predator (peacock cod, *Cephalopholis argus*) or a harmless (two-lined monacle bream, *Scolopsis bilineatus*) fish (made from laminated coloured pictures). The models' positions were counterbalanced in sessions of ten trials. Cleaners were now once again offered the client feeding plate. When a prawn item was consumed, as happened in each trial, the plate was swiftly moved in a 20 cm straight line towards the partition (Figure 1). If the cleaner subsequently swam into the compartment containing the harmless species, the cleaner was chased for 40 cm towards the harmless model (Figure 1). In contrast, if the cleaner swam into the compartment toward the predator model, all chasing ceased (Figure 1). The goal was for cleaners to learn that the predator model (i.e. *C. argus*) represents a 'safe' area which would reduce the energetic cost of cheating.

All cleaners were subjected to two experimental sessions per day, each consisting of 10 consecutive trials, which commenced at 9:00 and 14:30 hrs. The total number of sessions conducted per cleaner for each model combination varied according to performance, i.e. the time it took to reach our criterion for learning. The learning criterion was based on cleaners developing a significant preference for the predator model in the 'Initial Treatment' model combination, as described above, either by performing correctly in at least 9/10 trials or two times 8/10 in a row. Once the criterion was reached, a cleaner was presented with a novel model combination, comprising in total of five consecutive

treatments and 1 control (Table 1). However, individuals which did not satisfy the learning criterion in a total of 200 trials for the Initial Treatment, were not presented with a subsequent novel model combination. All cleaners were subjected to identical sequence of treatments.

‘Treatment 1’ to ‘Treatment 4’ consisted of locally occurring species whereas ‘Treatment 5’ consisted of two Caribbean endemics, the nassau grouper (*Epinephelus striatus*) and the queen angelfish (*Holocanthus ciliaris*). The purpose of the latter model combination was to take into account that cleaners may (although unlikely) have had direct natural social tool use experience with every predator species we used as models (cleaners may classify the Caribbean species as the local species they resemble). The ‘Control Treatment’ model combination consisted of two harmless species (coral rabbitfish, *Siganus corallinus*, acting as the harmless species and the blackeye thicklip, *Hemigymnus melapterus*, acting as the predator), in order to determine whether cleaners generalize between predators or apply new rule learning in every model combination separately.

5.3.2.2 Experiment 2: Generalization in an abstract context

In order to explicitly test which rules cleaners are capable of generalizing and to avoid potential sequence effects, in 2014, we repeated a modified version of Experiment 1. The set-up of aquaria, the learning phase and the performance of experimental trials remained the same. However, in this field season, we independently tested whether cleaners are able to generalize a rule which is not ecologically relevant. If cleaners are capable of generalizing the “predators-are-safe-havens” rule, are they also able to generalize the “one-of-two-stimuli-is-a-safe-haven” rule (i.e. by using two harmless fish models)? We tested this question using a counterbalanced design. In total, 16 cleaners were used, eight of which were first exposed to an initial combination consisting of one predator and one harmless model (PH) (ID No. 1-8), whereas the other eight (ID No. 9-16), were first exposed to a model combination consisting of two harmless species fish models (HH)

(Tables 2-3 in the Appendix). Cleaner ID No. 1-8, who learned to prefer the predator model in the Initial Treatment in 200 or less trials, were subsequently exposed to 5 other treatment combinations, consisting of one predator and one harmless fish model (i.e. including Caribbean endemics or species restricted to the outer Great Barrier Reef), as well as same-status models (i.e. two predator and two harmless models, PP, HH) (Table 2 in the Appendix). All cleaners were exposed to unique model combinations comprised of at least one moray eel, one grouper and one exotic fish model, and were always shown a given model no more than once.

Cleaner ID No. 9-16 tested the latter rule, “one-of-two-stimuli-is-a-safe-haven”, whose first model combination consisted of two harmless fish species representing the two stimuli (i.e. one of the harmless fish models represented a safe haven) (Table 3 in the Appendix). If they achieved in preferring the correct harmless model in this Initial Treatment, they would have been tested on additional HH model combinations. However, given that this was not the case, cleaners (ID No. 9-16) were subsequently also tested on a similar sequence as cleaners (ID No. 1-8) (Table 4 in the Appendix). This allowed us to determine whether fish which could not learn the ecologically irrelevant rule of “one-of-two-stimuli-is-a-safe-haven”, could still generalize the simpler rule of “predators-are-safe-havens”. Images of fish models are provided in Figure 2.

5.3.3 Data analysis

Variation in cleaner performance among model combinations was investigated using two general linear mixed-effects models (LMM) (Experiments 1 and 2). Data were log transformed and model assumptions were checked with plots of residuals vs. fitted values and qqplots of residuals. Furthermore, a Fisher exact probability test was used to analyse variation in performance between the two cleaner groups of Experiment 2, while a Sign test was used to determine whether a significant proportion of the total number of cleaners tested were capable of generalizing. As a further exploratory measure, one-

sample Wilcoxon signed rank tests were used to determine after how many trials cleaners, as a group across both experiments, performed above random probability. Data were analysed in Statistica and R 3.1.2 (R Development Core Team 2014). Individuals which failed to solve the task were not included in the analyses and shown as outliers on the figures.

5.4 RESULTS

5.4.1 Laboratory experiments

5.4.1.2 Experiment 1: Generalization of predatory species

Cleaners were able to generalize between predator fish models. A comparison of the speed of learning (e.g. number of trials to prefer the predator model) during the Initial Treatment combination and the five treatment trials (Table 1) yielded overall significant differences ($F_{5,30} = 12.66$, $P < 0.001$); the performance of cleaners in the five treatments were all significantly different from the Initial Treatment (all P 's < 0.001) and did not differ from each other (Figure 2; Figure 2a in the Appendix). Cleaners completed the Initial Treatment combination in 85 trials (median) (Initial, Figure 3), while all subsequent treatment combinations were completed much faster, ranging from 20 to 30 trials (median) (T1 to T5, Figure 3). However, during the maximal 120 trials of the Control Treatment (HH), none of the cleaners were able to develop a significant preference for the harmless fish model that resulted in a refuge from chasing (HH, Figure 3). These results are based on six individuals from a total sample size of 12 cleaners (three individuals were unable to learn the initial combination in 200 trials and three individuals failed to participate in the experiment by remaining in shelter tubes during experimental trials).

Note, while the results above show that cleaners are able to generalize between predator-harmless client combinations, the conclusion remains unclear, in regards to their failure to learn that a harmless client could also represent a safe haven. This may be driven by a sequence effect

and / or that cleaners were previously exposed to the harmless client models in a scenario where they did not provide a safe haven. Additionally, sample size for the generalization trials was small, since half of the twelve cleaners dropped out of the experiment. Therefore, we conducted a second series of experiments with the aim to address sequence effects and to increase sample size to address more specific questions.

5.4.1.3 Experiment 2: Generalization in an abstract context

First, a comparison between cleaner groups (i.e. ID No. 1-8 vs. 9-16) on the speed of learning during the Initial Treatment, consisting of either PH or HH combinations respectively, yielded significant differences (Fisher Exact Test: $P = 0.026$) (Figure 4). Five out of eight cleaners (ID No. 1-8), which were first exposed to a PH model combination in the Initial Treatment, learned to prefer the correct predator model in less than 200 trials, ranging from 70-190 trials, with a median of 130 trials (Figure 4). In contrast, all 8 cleaners that were given the Initial Treatment combination consisting of two harmless fish models (e.g. HH) failed to prefer the non-chasing model within the maximum of 200 trials. However, when subsequently tested on an ‘Initial’ model combination consisting of one predator and one harmless fish model (e.g. PH), most cleaners ($n = 5$) were able to prefer the predator model within 200 trials, ranging from 50 to 100 trials, with a median of 80 trials (Figure 4).

In subsequent treatment combinations (T1-3), consisting of one predator and one harmless fish model, cleaners consistently preferred the predator model in fewer number of trials in comparison to the Initial Treatment (Figure 5), thus producing comparable results obtained in Experiment 1. A comparison of the speed of learning during the Initial PH and the three treatment combinations yielded overall significant differences ($F_{3,32} = 16.35$, $P < 0.001$); the performance of cleaners in the three treatments were all significantly different from the Initial PH (All P 's < 0.001) and did not differ from each other (Figure 5; Figure 2b in the Appendix). Collectively, cleaners solved T1-3 in 40, 40 and 35

(mean) trials (Figure 5). Both groups of cleaners contained individuals which were unable to generalize and prefer the predator model in T1-3 in less than 120 trials, shown as outliers in Figure 5. In contrast to T1-3, all cleaners (ID No. 1-16) failed to significantly prefer the correct, non-chasing model in Treatments 4 and 5, which consisted of two same-status fish modes (e.g. HH, PP), during the maximal 120 trials (Figure 5).

Collectively, out of the 25 cleaners tested between Experiments 1 (n = 9 in 2011) and 2 (n = 16 in 2014), 16 cleaners were able to learn the Initial Treatment (PH) in less than 200 trials. Fourteen out of these 16 cleaners learned all subsequent combinations faster than the original combination, while only two cleaners did not provide evidence for generalization (Sign Test: n = 16, x = 2, $P = 0.004$)

5.4.1.4 Group performance

In order to test the collective performance of cleaners, we first investigated whether ‘predator species’ (i.e. model morphology; grouper versus moray eel) and ‘abundance’ (local versus exotic) had a significant effect on the generalization abilities of cleaners. Both in 2011 (Friedman two-way analysis of variance: $\chi^2 = 4.0$, $df = 2$, n = 6, $P = 0.135$) and 2014 (Friedman two-way analysis of variance: $\chi^2 = 0.7$, $df = 2$, n = 5, $P = 0.691$), these categories had no significant effect on cleaner performance. We hence combined all data to calculate the percentage of correct choices in the first session (i.e. first ten trials) of each treatment (i.e. T1-5 in Experiment 1 and T1-3 in Experiment 2) (Figure 6). As a group, cleaners obtained a mean value of 60.1 % of correct model choices for the first ten trials, ranging from 40 to 76 %, which is significantly above chance (one-sample Wilcoxon signed rank test: n = 14, T = 7.0, $P = 0.004$). A trial by trial analysis revealed that individuals performed significantly above random probability (50 %) in their predator choices by the sixth, eighth and tenth trial (One-Sample Wilcoxon Signed Rank Test: n = 14, T = 21, 18, 5, $P = 0.049$, 0.030, 0.002) (Figure 6), where the 10th trial remains significant even after a Bonferroni correction.

5.5 DISCUSSION

Our aim was to investigate whether cleaners can learn and subsequently generalize that a client model provides a safe haven from punishment, and to explore to what degree ecological relevance affects their performance. As predicted, based on interactions observed in nature, the majority of cleaners in our study learned to use predator species as social tools to minimize the amount of punishment they received after a cheating event. The few individuals that failed to learn the task may have either experienced a lack of exposure to social tool use situations under natural conditions or alternatively lacked the cognitive ability to exploit the situation. Such results are not surprising, given that there is often great variation in individual performance in cognitive experiments (reviewed in: Thornton and Lukas 2012). Cleaners that mastered the initial learning task generalized to novel models in subsequent tasks, independently of familiarity with the species and species body colour or shape. For example, although the profiles of grouper species are similar to one another (but differ considerably in colour), the morphology of moray eels differs significantly from groupers, precluding generalization based purely on predator shape. This differs from a study on spontaneous predator recognition in minnows, where generalization appears to be restricted to similar shaped species (Ferrari et al. 2010). Furthermore, the exotic fish combinations (Caribbean fish and outer barrier fish models) demonstrate that cleaners can generalize between predators, even when exposed to species which they could not have encountered before. Our results demonstrate that cleaners clearly understood the differences and consequences between predator and harmless fish models as they were able to apply a generalized rule associated with a natural reef environment.

Cleaners, however, failed to choose the model which provided a refuge from punishment when presented with two harmless species during the Initial treatment. As non-predatory clients do not provide safe havens against chasing client in nature, it appears that this lack of ecological validity impaired the cleaners' learning ability in our experiment. Their failure to learn the initial discrimination task prevented us from the

ideal test whether cleaners can generalize in a new species pair combination. Nevertheless, the data from both experiments show that cleaners fail to generalize ‘a predator is a safe haven’ to a situation in which both models belong to the same category, i.e. two harmless fish species but also two predatory species. These results strongly suggest that cleaners indeed have the categories ‘predator’ and ‘harmless’ in their mind and that these units allow the generalization between tasks. The distinction between predators and harmless clients is of fundamental ecological importance for cleaners as the former may potentially try to eat them (Côté 2000). As a counterstrategy, cleaners provide predators with the best service quality, i.e. low cheating rates and high rates of tactile stimulation (Bshary 2011), a behaviour that reduces stress in clients (Soares et al. 2011). Having client categories for service quality may then help cleaners to learn to use predators as social tools. As it stands, the combination of punishment by clients and the presence of a predator is generally very low, except for small resident grouper species. These conditions seem to largely preclude the option to learn the usefulness of each species as social tool through trial and error, while a generalized rule allows the efficient exploitation of rare events. Given the somewhat surprising result that cleaners cannot generalize to the condition with two predator models, an interesting future study would be to test their ability to discriminate between other important client categories: various studies provide evidence that cleaners discriminate between resident clients with access to the local cleaner only, and visitor clients with access to several cleaning stations (Bshary 2011). Maybe species combinations from these two categories would facilitate the initial learning as well as the generalization even if both species are either predatory or harmless. Such a study would allow distinguishing between the importance of pre-existing categories versus the ecological relevance of the task for cleaner performance.

Generalization abilities and the quick application of learning sets is considered an attribute of higher cognitive functioning (Murphy et al. 2008), and was once thought to be a correlate of brain size (Wynne 2001). However, our results support the view that the ecological validity is of key importance for a species’ performance, as put forward

as explanation why a desert marsupial performs so well in the standard generalization paradigm (Darlington et al. 1999). Our results are not directly comparable with previous studies that focussed on subjects' performance in trial 2. As it stands, within the 3-5 test combinations our cleaners did not perform above chance in trial 2 (Fig. 5). However, the fact that they performed above chance in the first 10 trials with so few test combinations shows very fast learning that was clearly absent during the Initial test. The results fit recent evidence that cleaners are an excellent example of a species capable of solving complex cognitive tasks if placed in the context of their own ecology (Salwiczek et al. 2012; Gingins et al. 2013). For example, cleaners have shown to outcompete three primate species (capuchin monkeys, chimpanzees and orang-utans) in a laboratory-based cognitive foraging experiment relevant to client selection under natural conditions but not to primate foraging strategies, where individuals were given a choice between two actions, both of which yielded identical immediate rewards, yet only one an additional delayed reward (Salwiczek et al. 2012).

The ability to use and manipulate social tools for a personal benefit is also considered a unique cognitive ability, and to date, primarily documented in anecdotes in primates (Whiten and Byrne 1988a). In olive baboons (*Papio anubis*), for example, a female has been observed, after unsuccessfully obtaining meat from an antelope carcass guarded by a male, to attack another female (social tool), until the male (target) came to the attacked female's rescue, leaving his carcass behind, which was subsequently stolen by the original female (agent) (Observer: Strum; Byrne and Whiten 1990). Such observations have led to the Machiavellian intelligence or social brain hypotheses, which propose that the emergence of higher cognitive functions and expansion of neocortical regions in primates is the consequence of intense social complexity as a selective factor through evolution (Whiten and Byrne 1988b; Dunbar 1998). A socially complex environment likely plays a key role in cleaner cognition as well. For example, on a given day, cleaners are involved in over 2000 cleaning interactions (Grutter 1995), and have to continuously engage in fine-tuned social strategies (that counter client strategies) to maximize their daily food intake. It is

therefore not surprising that they have evolved the ability to use sophisticated decision rules in a social context, including the ability to use social tools for their personal benefit.

In conclusion, we have demonstrated generalized rule learning in a fish species in the context of social tool use. Minnows have been shown previously to generalize between predatory fish odour cues (Ferrari et al. 2007). As there is also recent evidence for transitive inference in a cichlid (Grosenick et al. 2007), as well as the documentation of referential gestures and sophisticated decisions about when to collaborate with whom in a grouper (Vail et al. 2013, 2014), our study adds to the growing evidence that the cognitive abilities of fishes are much more advanced than previously appreciated (further examples of fish intelligence in Brown et al. 2011; Bshary et al. 2014). Such evidence as well as the cognitive performance of some invertebrate species (Chittka and Niven 2009) show that we are still far from understanding why mammals and birds evolved larger brains than other taxa.

5.6 ACKNOWLEDGEMENTS

We kindly thank the staff at Lizard Island Research Station - a facility of the Australian Museum, for continued field support and friendship; S. Binnig, A. Pinto, O. Rey and A. Vail for field assistance; D. Roche for statistical help; Funding was provided by a Lizard Island Reef Research Foundation doctoral fellowship (SW) and the Swiss Science Foundation (RB).

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Table 1. Predator and harmless fish model combinations utilized in Experiment 1: ‘Generalization of predatory species’. All cleaners were subjected to treatment-control order presented below. 1 = only the head was used due o length restrictions; 2 = terminal phase parrotfish; 3 = Caribbean endemics. Ceph. = Cephalopholis.

	Abbrev.	Predator model	Harmless model
Initial Treatment	(Initial)	Peacock cod (<i>Ceph. argus</i>)	Two-lined monocle bream (<i>Scolopsis bilineatus</i>)
Treatment 1	(T1)	Honeycomb grouper (<i>Epinephelus merra</i>)	Dash-dot goatfish (<i>Parupeneus barberinus</i>)
Treatment 2	(T2)	Blue-spotted rockcod (<i>Ceph. cyanostigma</i>)	Blackeye thicklip (<i>Hemigymnus melapterus</i>)
Treatment 3	(T3)	Dothead rockcod (<i>Ceph. microprion</i>)	Coral rabbitfish (<i>Siganus corallinus</i>)
Treatment 4	(T4)	Giant moray ¹ (<i>Gymnothorax javanicus</i>)	Bullethead parrotfish ² (<i>Chlorurus sordidus</i>)
Treatment 5	(T5)	Nassau grouper ³ (<i>Epinephelus striatus</i>)	Queen angelfish ³ (<i>Holocanthus ciliaris</i>)
Control	(C)	Blackeye thicklip (<i>Hemigymnus melapterus</i>)	Coral rabbitfish (<i>Siganus corallinus</i>)

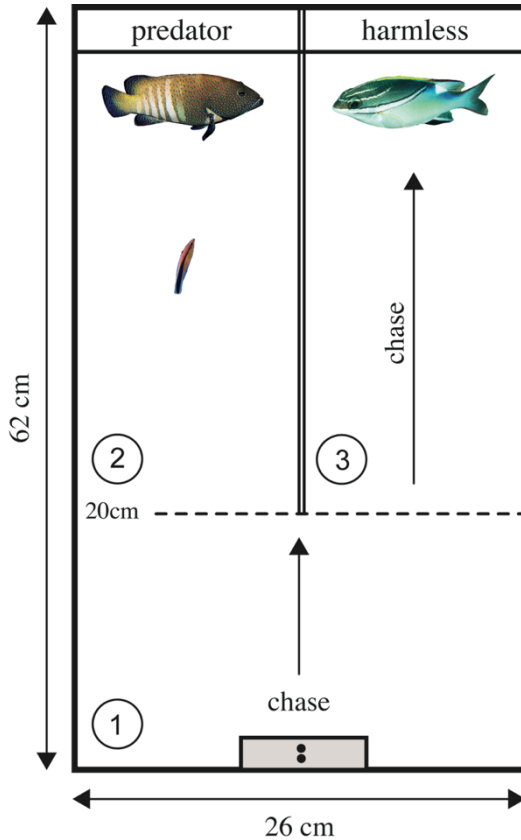


Figure 1. Experimental set-up used to test cleaner generalization abilities in both 2011 and 2014. Aquaria divisions, model placements and chasing directions used in Experiments 1-2. Cleaners were systematically chased upon consuming a prawn item when in compartments 1 (feeding plate) and 3 (harmless model; right). Chasing ceased at a distance of 20 cm if they entered compartment 2 (predator model; left). Model positions (right, left) were counterbalanced across experimental sessions.



Balistoides conspicillum
Clown triggerfish



Chlorurus sordidus
Bullethead parrotfish



Cephalopholis argus
Peacock rockcod



Cephalopholis cyanostigma
Bluespotted rockcod



Ctenochaetus striatus
Lined bristletooth



Hemigymmus melapterus
Blackeye thicklip



Cephalopholis microprion
Dothead rockcod



Cephalopholis miniata
Coral cod



Hologacanthus ciliaris
Queen angelfish



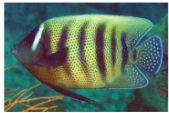
Parupeneus barberinus
Dash-dot goatfish



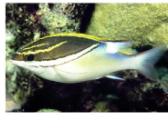
Epinephelus merra
Dwarf spotted rockcod



Epinephelus striatus
Nassau grouper



Pomacanthus sexstriatus
Six banded angelfish



Scolopsis bilineatus
Bridled monocle bream



Epinephelus tukula
Potato cod



Gymnothorax undulatus
Undulated moray



Siganus coralimms
Coral rabbitfish



Stegastes nigricans
Dusky gregory



Gymnothorax javanicus
Giant moray



Variola louti
Coronation cod

Figure 2. Fish models used in Experiments conducted in both 2011 and 2014. Harmless fish models are on the left (2 columns) and predator models are on the right (2 columns). Images from fishbase.org.

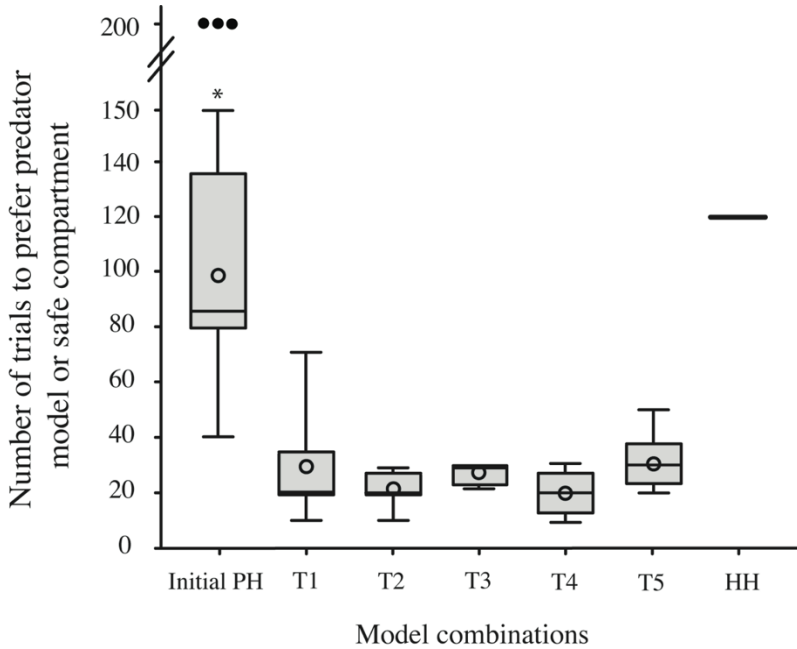


Figure 3. Performance of cleaners in generalization Experiment 1. Boxplots of the number of trials required for cleaners to develop a significant preference for the predator model in initial (Initial PH) and subsequent treatment (T1-5) and control model combinations (HH = C). Boxplots show median (bar), mean (open circle), interquartile (rectangle), and maximum and minimum values (error bars). Small filled circles represent the 3 individuals which did not successfully complete the initial treatment in 200 trials. Asterisks: $P < 0.001$. The proportion of variance explained by our model (R^2) was 0.57.

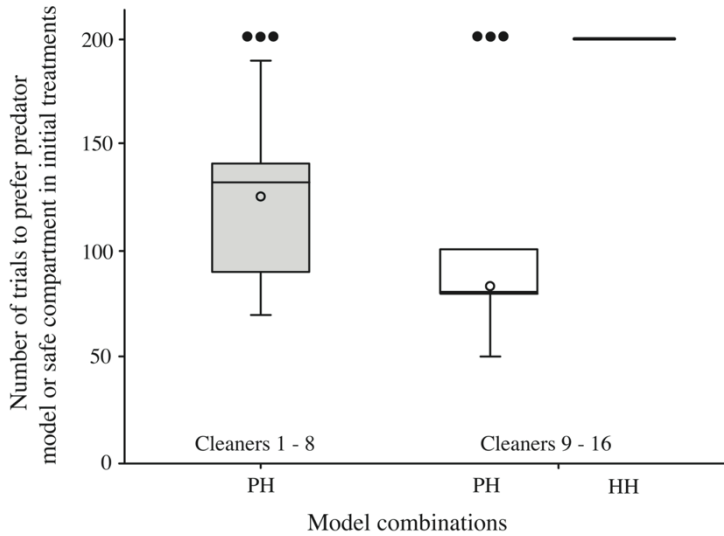


Figure 4. Performance of cleaners in the initial model combination of Experiment 2. The grey boxplot symbolizes cleaners (1-8), which started Experiment 2 with one predator and one harmless species fish model (PH), while the white boxplot and the bar represents cleaners (ID No. 9-16) which started with two harmless models (HH), and were subsequently exposed to one predator and one harmless species model (PH). Boxplots show median (bar), mean (open circle), interquartile (rectangle), and maximum and minimum values (error bars). Filled circles represent individuals which did not learn the initial treatment in 200 trials.

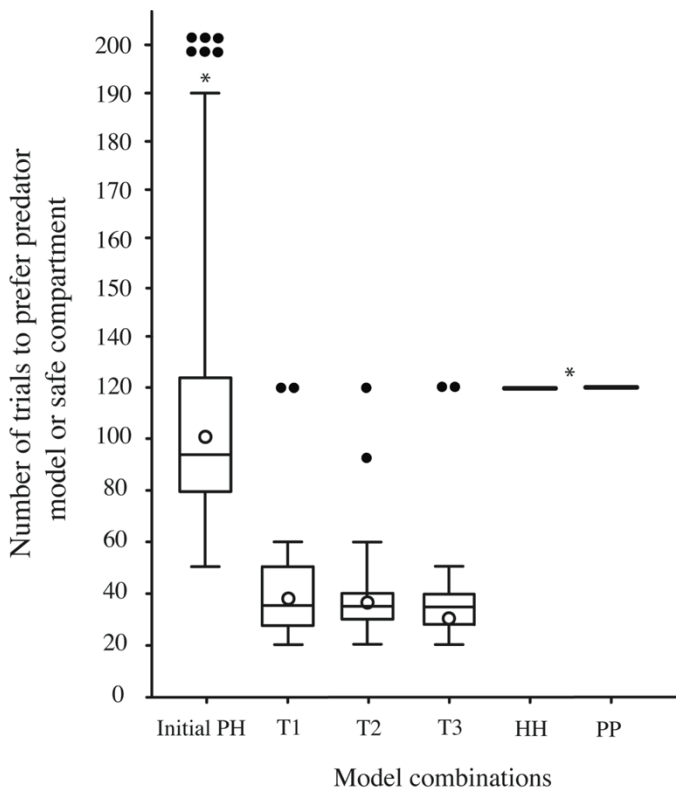


Figure 5. Performance of cleaners in initial and treatment model combinations of Experiment 2. Boxplot show median (bar), mean (open circle), interquartile (rectangle), and maximum and minimum values (error bars), using pooled data between the two cleaner groups, ID No. 1-8 and 9-16. Filled circles represent outliers or individuals which did not generalize the treatment in 120 trials. Treatments 4 and 5 (T4, T5) are grouped here as HH and PP (see Tables 2 and 4 in the Appendix). Asterisks: $P < 0.001$. The proportion of variance explained by our model (R^2) was 0.58.

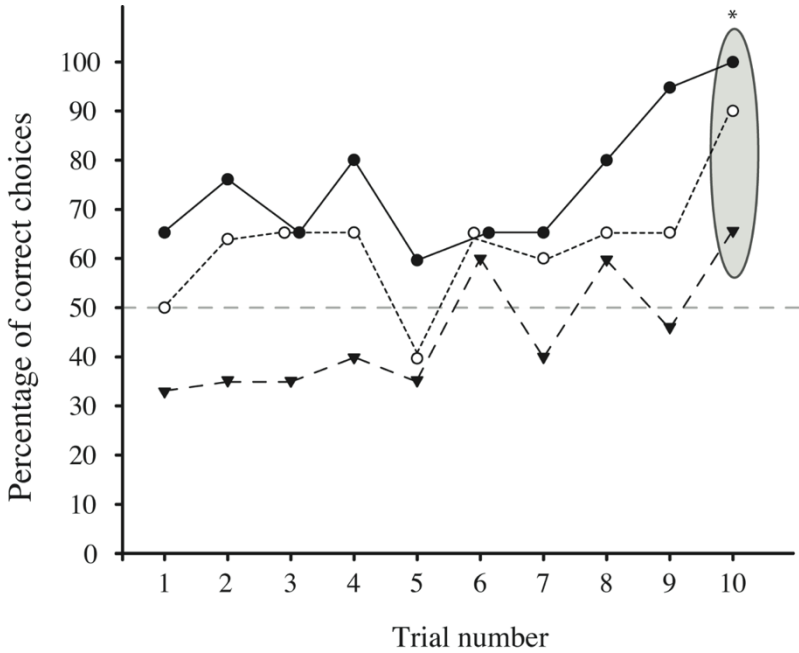


Figure 6. Cleaner group performance. The percentage of correct choices by cleaners in the first experimental session (trials 1-10) of treatments 1-5 (Experiment 1; 2011) and treatments 1-3 (Experiment 2; 2014). Symbols represent median (open circles) and interquartile values (filled circles and triangles). Asterisks indicate when cleaners as a group performed above chance, i.e. 50%.

6

GENERAL DISCUSSION

6.1 SUMMARY

The first aim of this thesis was to compare the performance of bluestreak cleaner wrasse ('cleaners') from two socially contrasting reef environments in published laboratory-based cognition and cooperation experiments, and to subsequently explore factors that could explain variation between the two cleaner groups. Cleaners from socially complex reef environments outcompeted cleaners from socially simple reef environments across all laboratory tasks. A higher abundance and diversity of reef fish clients, as well as, a higher density of cleaners themselves, characterized socially complex reef environments. As a consequence, cleaners from these reefs engaged in a higher number of cleaning interactions, with a higher diversity of clients. Such differences in the social environment are thought to be the driving force behind the disparity documented between the two cleaner groups. Cleaners from socially complex reef environments showed 1) higher levels of cooperation across experimental trials (ratio of flake to prawn consumed), 2) the ability to adjust to image scoring bystanders and 3) the capability of consistently choosing a temporary food source over a permanent option. This led to a higher number of consumed food items across all experimental trials. In contrast, cleaners from the socially simple reef environment showed 1) lower levels of cooperation (often only consuming prawn items) and 2) the inability to both adjust to image scoring bystander clients and to give service priority to a temporary visitor client over a permanent resident client. Hence, cleaners from the two reef environments develop alternate decision strategies in how they service their clients, with cleaners from the socially complex reef environment applying more sophisticated decision rules, which lead to an overall better performance.

To investigate this finding in further detail, the second aim of this thesis was to determine whether the two cleaner groups use different cues when making decisions; specifically, in regards to client service priority. Here, I found that in the absence of colour cues, all cleaners, including juveniles, gave service priority to a larger (of two) Plexiglas plate, irrespective of plate status. In nature, fish classified as visitors are

often larger than residents, and therefore, the decision rule ‘service the larger client first’ often results in cleaning two clients over just one. However, when both colour and size were incorporated into a follow-up experiment, cleaners from the socially complex reef environment showed a tendency to use a precise colour-based rule, which explicitly differentiated visitors from residents. Their counterparts, however, continued to use the size-based rule, a correlated cue that did not specifically differentiate visitors from residents, and which consequently, led to fewer food items consumed and an overall poorer performance.

Such a decision rule appears to be ‘less optimal’; however, they may be well suited for the socially simple reef environment. Hence, the third aim of this thesis was to determine whether there are fitness differences between the two cleaner groups and how well cleaners adjust to the opposing reef environment. I found that there are no (likely) fitness differences between the two cleaner groups. Moreover, the single (located) translocated individual showed a decrease in both weight and length, while control fish showed a tendency to increase in both. Although the sample size was limited, these results suggest that the rules applied by cleaners from the socially simple reef environment appear to be locally adaptive. It remains unclear, however, whether there is a genetic component affecting variation in cleaner performance, as well as, how well cleaners adjust their decision rules when placed in an alternate reef environment. Questions to be investigated in future experiments (details provided in the last section of this discussion).

The final aim of this thesis was to investigate in further detail how well cleaners are able to extract relevant cues (when no correlated cues are available) for decisions involving cheating and refuge-seeking. Here, I investigated the ability of cleaners to generalize in a social tool context, by comparing the performance of individuals collected from socially complex reef environments. I demonstrated that cleaners can use generalized rule application to identify potential social tools in simulated laboratory experiments; however, that this ability rapidly disappears when individuals are tested in a context which is

ecologically irrelevant. The results emphasize the importance of ecologically relevant experiments to uncover complex cognitive processes in non-human animals.

6.2 BIG PICTURE

6.2.1 The ecological approach to cognition

Collectively, the findings from this dissertation have broader implications for 1) cognition and 2) evolutionary game theory. First, these results, are particularly important in the context of the *ecological* approach to cognition (e.g. Kamil 1998; Bshary et al. 2007; Shettleworth 2010; Salwiczek et al. 2012). In contrast to the *anthropocentric* approach to cognition, which studies animal behaviour in the context of human behaviour (Shettleworth 2012), the ecological approach to cognition suggests that an animal's cognitive ability is tightly linked to its evolutionary history, and hence, also its ecology (Kamil and Mauldin 1988; Kamil 1998; Bshary et al. 2007; Shettleworth 2010; Salwiczek et al. 2012). This approach to cognition is often applied in comparative studies between different species (Shettleworth 2010). For example, Salwiczek et al. (2012) demonstrated that cleaners are able to outcompete capuchin monkeys, chimpanzees and orang-utans in a complex foraging experiment, which was more ecologically relevant to cleaners.

The ecological approach to cognition, however, is also used to compare the cognitive abilities of close relatives, or between sub-populations of the same species, who due to variation in their ecological conditions, face different cognitive demands, and hence, may therefore have diverged in their cognitive abilities (Shettleworth 2010; Thornton and Lukas 2012). A classic example of such a comparison is provided in Pravosudov and Clayton (2002), a study that tested for differences in caching, memory and brain volume of two sub-populations of black-capped chickadees (*Poecile atricapilla*). The Alaskan population, which experiences a harsher climate in comparison to the Colorado population, 1) cached significantly more food, 2) were more efficient at

cache recovery, (3) had higher hippocampal volumes and 4) performed better in a spatial memory task (crucially, populations did not differ when tested on a non-spatial task). Therefore, in line with the ecological approach to cognition, the Alaskan population excelled in areas that one would expect for surviving in a comparatively harsher climate, yet showed no differences in cognition, in comparison to the Colorado population, when tested in a more ecologically-irrelevant context. Surprisingly, such ‘contrasting’ cognitive comparisons within the ecological approach to cognition are rarely preformed, and to date, are restricted to food caching (e.g. Pravosudov and Clayton 2002).

Data from my thesis provides two additional examples of ‘contrasting’ cognitive comparisons in the context of the ecological approach to cognition. First, the two cleaner groups demonstrated clear differences in their cognitive performance in the various laboratory tasks, with cleaners from the socially complex reef environment consistently applying sophisticated decision rules to the tasks they encountered. Such advanced strategies appear to be well suited to the social ecology of their reef environment. However, when the two groups of cleaners were tested on an abstract cognitive task (e.g. spatial discrimination task, Chapter 4), I found no differences in the cognitive performance between the two cleaner groups. Hence, as in the chickadee example, cleaners from the socially complex reef environment excel in tasks linked to their ecology, as to be expected, yet showed no enhanced cognitive skills when tested on an ecologically irrelevant task. A second example is demonstrated in Chapter 5, where cleaners were able to generalize predators in a social tool use context to evade punishment. Yet, when the identical problem was placed in a more abstract context, e.g. generalizing harmless fish species, their ability to generalize disappeared. Once again, cleaners excelled in an experiment which was ecologically relevant, yet failed when tested in an abstract context with no ecological significance.

My results highlight the importance of incorporating a ‘contrasting’ cognitive experiment (control) when testing for adaptive and unique cognitive skills within the ecological approach to cognition. Secondly,

when designing future laboratory experiments specifically on cleaners, the ecological approach should be strongly considered, as subtle variation in reef environments can have significant impact on cleaner cognitive performance. And finally, the ecological approach to cognition is useful method for uncovering hidden, surprisingly advanced and or novel cognitive abilities in individuals, when experiments are placed in an ecologically relevant context, as demonstrated in Chapter 5 (generalization and social tool use in cleaners).

6.2.2 First evidence for generalized rule learning in a fish species

Fish have often been viewed throughout scientific history as “automatons”, with their behaviour controlled by unlearned predispositions (Brown et al. 2006). However, more recently, particularly over the last decade, there has been an increase in studies documenting the surprisingly ‘advanced’ cognitive abilities displayed by various fish species, and that learning indeed plays a key role in their behavioural development (e.g. Brown and Laland 2003; Brown et al. 2006; Bshary et al. 2002, 2014). Examples include: long-term memories comparable to vertebrates (rainbowfish: Brown 2001), social learning abilities (archer fish: Schuster et al. 2006), the use of referential gestures (groupers: Vail et al. 2013), counting abilities (mosquitofish: Agrillo et al. 2008; Dadda et al. 2009), tool use (wrasse: Brown 2012) and awareness of audience effects (cleaner wrasse: Pinto et al. 2011).

To the best of my knowledge, data presented in Chapter 5, i.e. on generalized rule application in the context of social tool use, is the first documented evidence for such behaviours in a fish species. Social tool use, specifically, has only been observed in primates (e.g. Whiten and Byrne 1988; Kummer et al. 1990). This adds to an impressive list of recently discovered cognitive processes in fishes. The ability to generalize rules and apply them to novel situations is a fundamental aspect underlying flexible cognitive behaviour and is considered a complex cognitive process (Emery and Clayton 2004; Murphy et al. 2008). The fine-tuned decision rules applied by cleaners during natural

cleaning interactions, such as, giving service priority to visitor clients, can be explained by simple associative learning (e.g. operant conditioning: learning through consequences). However, the ability to abstract general rules appears to surpasses such basic learning mechanisms. My results further support the notion that higher cognitive processes are not exclusive to larger brained vertebrates, such as mammals and birds (Jerison 1973), and that differences in brain sizes between large-brained species and other vertebrates may be due to quantitative and additive differences in performance. For example, even though cleaners may display similar cognitive processes to those found in primates, the complexity of situations, as well as, the number of different contexts primates encounter, is likely far greater: e.g. tool use, complex diet, coalition formation, social learning etc.

6.2.3 Cue learning

The ability to identify relevant cues from the environment is the first aspect of cognitive processing of information (Shettleworth 2010) and considered a fundamental aspect underlining good decision making (Vickers 2007; Gigerenzer 2008). Hence, variation in the performance of different species in identical cognitive tasks may be linked to their ability to identify and use relevant cues, even if their cognitive processes are the same (Lotem and Halpern 2012). I tested how well cleaners are capable of both cue recognition and cue use in two different laboratory-based contexts: 1) what cues do cleaners use for decisions regarding client service priority, i.e. a *precise* colour-based cue or a size-based *correlated* cue, and 2) how well can cleaners extract an ecologically relevant cue versus an ecologically irrelevant cue to evade punishment, i.e. generalize predators but not harmless fish models. The results from these two experiments suggests that some individuals are superior in both identifying and using the correct or relevant cue to make the best decision. Such abilities are, in part, linked to an individual's cognitive abilities, and how it perceives its environment. Indeed, cognition is defined as 'the mechanisms by which animals acquire, process, store, and act on information from the environment, which includes *perception*, learning, memory and

decision-making (Shettleworth 2010). Some individuals may have a better perception of their environment, due to enhanced social learning abilities or a genetic predisposition, and will therefore be able to more readily identify and use relevant cues for decision making. Recognizing that individuals may vary in their ability to perceive, identify and use a relevant cue is an important consideration when comparing both species and individuals from different environments, as it may help explain variation in their behaviour and decision strategies.

6.2.4 Evolutionary game theory

Finally, my results are important in the context of evolutionary game theory. Although game theory, as well as empirical evidence, have provided a variety of mechanisms for stable cooperation between unrelated individuals (e.g. Axelrod and Hamilton 1981; Conner 1986; Kandori 1992; Clutton-Brock and Parker 1995; Milinski and Wedekind 1998; Bshary and Grutter 2005), humans often deviate from strategies predicted by cooperation-based models (e.g. Feh and Fischbacher 2003; Haley and Fessler 2005; Kümmerli et al. 2010). This has raised interesting questions in regards to the decision-making processes in humans (e.g. Gigerenzer and Selten 2002; Hagen and Hammerstein 2006; El Mouden et al. 2012; Kacelnik 2012; Baumard et al. 2013), including the concept of heuristics or rules of thumb, i.e. ‘cheap’ solutions to complex problems (Gigerenzer and Selten 2002; El Mouden et al. 2012). This decision strategy works well in frequently occurring situations, but may result in irrational or ‘maladaptive’ behaviour in novel contexts, leading to deviations from optimal model predictions (El Mouden et al. 2012).

To date, the widely published cleaning mutualism between cleaners and their clients has provided strong experimental evidence for the effectiveness of evolutionary game theory for predicting cooperative behaviour (examples reviewed in Bshary 2011). However, as demonstrated throughout this thesis, not all cleaners conform to model predictions, highlighting that the mismatch between model predictions and *actual* behaviour is widespread, and not limited to humans. This

further emphasizes the current shortcomings of evolutionary game theory, as it currently fails to predict consistent variation in performance between individuals, i.e. between species or between conspecifics from different environments, in respect to standard experiments where the optimal solutions are clear. Deviations may, in part, be a result of an individual's genetic (i.e. between species) or ontogenetic (i.e. within species) predisposition to a given context. For example, bonobos have shown to outperform chimpanzees in a cooperation task due to higher levels of tolerance, whereas the chimps showed constraints in this area (Hare et al. 2007). Furthermore, variation may also be influenced by psychological mechanisms, which can constrain an animal's behaviour and may result in suboptimal behaviour (Fawcett et al. 2013; Lotem 2012). For example, this may be due to impulsiveness (e.g. immediate reward versus delayed reward: Henly et al. 2008), having difficulty valuing options in a rational way (e.g. making state dependent not rational decisions: Marsh et al. 2004) or due to learning constraints (e.g. brood host acceptance: Lotem 1993).

Nevertheless, discrepancies are likely a common occurrence when studying 'brainy' animals, that have the ability to learn, adapt and subsequently, cope with their environment. In regards to cooperation theory, ideas should be developed specifically for such animals, including humans. First, models should aim to integrate 1) assumptions in regards to the costs and benefits of information gathering, storage and computation, as well as, 2) ecological information. For example, cooperative behaviour can be influenced by a great diversity of proximate factors and testing wild-caught individuals should also require prior ecological knowledge to better interpret their behaviour. Second, models should also aim to incorporate learning mechanisms, e.g. reinforcement learning: Kacelnik (2012), as well as, cognitive constraints, which appear to play a key role in the decision making process. For example, how well an individual can identify and use relevant cues will directly impact its decisions (Lotem and Halpern 2012), and given the context, also its cooperative behaviour. Collectively, this will allow us to explore more realistic decision rules for both animals and humans, as well as optimal rules for specific

circumstances, in order to better understand the causes of deviation from seemingly optimal cooperative behaviour.

6.3 OMITTED DATA

Due to journal page restrictions, two interesting experiments were omitted from the manuscripts presented in Chapters 2 and 5. These experiments and accompanying results are summarized below.

6.3.1 Chapter 2: Audience effects experiment using live fish clients

As discussed in Chapter 2, cleaners from the socially simple patch reef environment were either unwilling or unable to adjust their behaviour when in the presence of an image scoring bystander plate. This was in contrast to cleaners from the socially complex continuous reef environment, which fed more against their preference when an image scoring bystander plate was present, e.g. they ‘cheated’ less. This experiment, much like many other lab experiments involving cleaners, utilized Plexiglas plates containing both preferred (prawn) and less-preferred (flake) food items that mimicked reef fish clients, mucus and ectoparasites, respectively. A reoccurring critique of this methodology raises the question whether cleaners behave similarly with Plexiglas plates as they would with living fish clients. Hence, we repeated the audience effects (‘image scoring’) experiment in the lab using real fish clients (species: *Ctenochaetus striatus* and *Scolopsis bilineatus*), following Pinto et al. (2011), and compared the behaviour of cleaners from both reef environments using video analysis. We recorded the jolt rate of client fish *C. striatus* when cleaners and their client fish were 1) adjacent to an empty tank (control) and 2) adjacent to a tank containing a *S. bilineatus*, the image scoring bystander client (treatment). Detailed methodology is described in Box 2 of the Appendix.

Indeed, when real fish clients are used, cleaners from the socially simple reef environment do not alter their behaviour when they are being observed by another client, as client jolt rate did not differ significantly between the treatment and the control (Wilcoxon Matched

Pairs Test: $n = 13$, $T = 27.0$, $P = 0.346$). In contrast, cleaners from the socially complex reef environment caused significantly fewer client jolts, e.g. cooperated more, when an image scoring bystander client was watching (Wilcoxon Matched Pairs Test: $n = 14$, $T = 11.0$, $P = 0.015$). Cleaners from the socially simple reef environment also caused significantly more client jolts when the bystander was present, in comparison to cleaners from the socially complex reef environment (Mann-Whitney-U-test: $m = 13$, $n = 12$, $z = 2.17$, $P = 0.035$). These results are illustrated in Figure 1 at the end of the General Discussion. This additional experiment therefore supports the findings obtained from the plate experiments and suggest that cleaners behave similarly with Plexiglas plates mimicking client fish as they would with live reef fish clients.

6.3.2 Chapter 5: Feeding against preference experiment – predator vs. harmless model

The experiment that was omitted from Chapter 5 also investigated cleaner service quality. As previously discussed, cleaners have shown to utilize nearby predators to evade punishment from a recently cheated client, both during natural cleaning interactions and under simulated laboratory conditions. Hence, a logical follow-up experiment determined whether cleaners lower their service quality (e.g. cheat more) when in the presence of a predator, versus a harmless species, as the threat of being punished is considerably reduced under this circumstance. This was tested by presenting cleaners with a Plexiglas plate simulating a client fish, containing both preferred (prawn) and less-preferred (flake) food items, and recording the flake to prawn ratio eaten by cleaners over individual trials when in the presence of either a predator (*Cephalopholis argus*) or harmless species (*Scolopsis bilineatus*) fish model. Further methodological details are provided in Box 3 of the Appendix. Interestingly, feeding against preference in cleaners was not significantly affected by the identity of model bystanders (Figure 2). The median flake to prawn ratio consumed off the Plexiglas plate remained similar across the 25 trials, no matter whether predatory or harmless fish models were added to the aquarium,

despite their different effect on punishment (Wilcoxon Matched Pairs Test: $n = 6$, $T = 6.0$, $P = 0.685$).

Several non-mutually exclusive explanations are possible. First, cleaners may have to invest more into reconciliation with the client during the follow-up interaction, thereby reducing the immediate benefits of social tool use. Second, predators are also bystanders, in the sense that they are potential future clients, and while they do not seem to need image scoring as a means to avoid cheating cleaners due to the threat of ‘terminal reciprocity’ (Bshary 2002; Bshary and Bronstein 2011), the disturbance caused by the initial client chasing may increase the probability that they leave without inspection. Finally, being chased by a client makes cleaners behave like prey, which may trigger dangerous responses in the predator. Hence, this may explain why cleaners use predators as social tools when possible (e.g. availability to predator), but do not provoke the need for increased social tool use.

6.4 CURRENT AND FUTURE RESEARCH

There are currently several studies investigating unanswered questions arising from my thesis topic, which include MSc and PhD projects conducted by Olivia Rey, Camille Demairé and Zegni Triki. A major answered question was to determine whether the poor performance of patch reef cleaners across the various lab experiments is unique to cleaners caught from Corner Beach, or whether cleaners from other reefs exhibit similar failure rates? Research conducted by MSc student O. Rey indicated that there are indeed several locations around Lizard Island which are characterized by a simple social reef environment, and that individuals caught from these sites were once again largely outperformed in the lab by cleaners caught from various other socially complex reefs, this time only tested in the audience effect experiment. Irrespective of reef type, i.e. patch or continuous reef, she also found that the performance of cleaners in the audience effect experiment correlated significantly with cleaner density. For example, cleaners caught from reefs that exhibited a high density of conspecifics performed better in the lab than cleaners that were caught from a reef

environment characterized by low cleaner density. It appears, therefore, that cleaner density may be a key factor influencing the results. This may reflect, at least partially, the cost incurred by clients when switching cleaners. For example, at high cleaner density reef sites, clients can easily change between cleaners with little cost, since there are many cleaners to choose from, which may in turn lead to more sophisticated cleaner strategies. In contrast, at low cleaner density reef sites, the cost of switching for clients is much higher, as there are fewer cleaners, and clients may therefore, for example, be more tolerant of cheating cleaners (i.e. sophisticated and cooperative cleaner strategies may not be needed).

The MSc project of C. Demairé investigated this hypothesis in further detail, by experimentally reducing the density of cleaners at a socially complex reef site by 50%. Her aim is to subsequently compare the interaction patterns before and after removal at the experimental site and at a control site that was not manipulated. Linked to the removal, Zegni Triki compared the performance of the following two cleaner groups in the audience effects and market experiments: 1) cleaners which were initially removed from the reef (i.e. high density cleaners) versus 2) individuals which were left in the newly established low density reef site for a month (i.e. low density cleaners). This will give us an indication whether cleaners alter their cleaning strategies depending on the density of conspecifics in their environment. The main focus of the PhD project of Z. Triki will be to investigate potential physiological and genetic factors influencing variation in cleaner cognitive performance. For example, she will compare the social decision-making regions of the brain (description in: Lee and Harris 2013) of cleaners caught from both socially simple and socially complex reef environments, in order to determine a link between observed variation in cleaner behaviour and underlying neural correlates. She also aims to clarify whether variation in the cognitive performance of cleaners is due to ontogenetic effects, as discussed throughout this thesis, or genetic differences (e.g. due to differential selection or a genetic predisposition), by analysing the genetic distance (FST values: Nei 1973) between the two cleaner populations. Lastly,

using modified methodology, Z. Triki will continue the translocation study presented in Chapter 4, in hopes to not only understand the fate of translocated cleaners, but to also investigate whether cleaners possess cognitive flexibility in altering their decision rules. Collectively, these projects will help fill our missing knowledge gaps and further our understanding as to why cleaners from socially simple reef environments deviate from seemingly optimal cleaning strategies.

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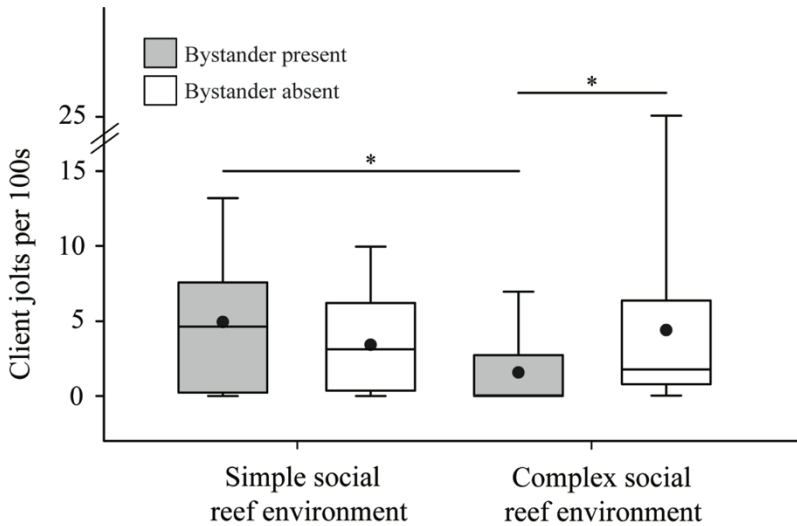


Figure 1. Body jolts caused by cleaners during interactions with *C. striatus*, both when a bystander was present and absent (Chapter 2). Boxplots show the median (horizontal line), mean (filled circle), interquartile (box) and maximum-minimum values (whiskers). Asterisks indicate a significant differences: between reef environments (bystander present) at $P > 0.04$; between treatments (complex reef environment) at $P < 0.02$.

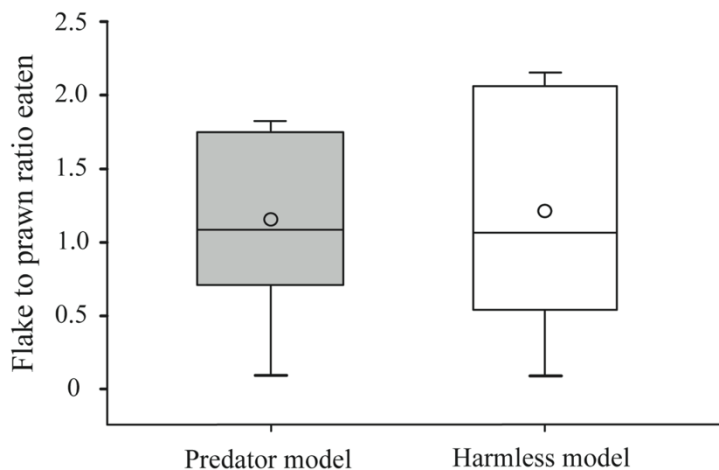
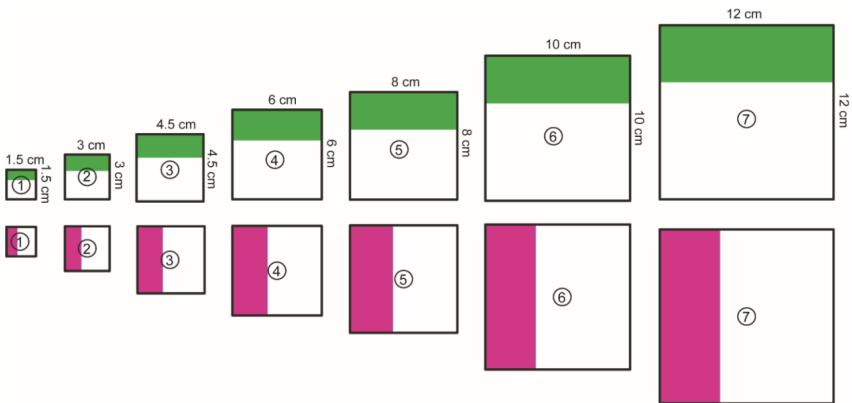


Figure 2. Performance of cleaners in the cooperation experiment (Chapter 5). Box plots show the willingness of cleaners to feed against their food preference and ‘cooperate’ (by eating flake) when in the presence of either a predator or harmless species fish model; $n = 6$.



A1. Figure 1. Plexiglas feeding plates utilized in Experiment 2 (Chapter 3). Experimental plates were numbered 1 to 7, which corresponded to increasing plate size (i.e. 1.5 cm² to 12 cm²). Cleaners were always presented with one green and one pink Plexiglas plate, which represented a visitor and resident client (visitor plate colour was counterbalanced among cleaners), in various size combinations that were in proportion to the natural environment (Table 1 in the Appendix).

A2. Table 1. An example of plate size combinations utilized in Experiment 2 (Chapter 3). Numerical values represent plate size ID's (Figure 1 Appendix). The visitor plate size is presented first. Bold-asterisk values show pairings where the visitor plate was either smaller or equal in size to resident plates (i.e. 24 %). All cleaners were exposed to a total of 210 trials, consisting of 8 x 25 unique plate pairings, and 10 trials at the end of the experiment consisting only of plate pairings where the visitor plate was equal to or smaller in size than the resident plate (i.e. 5 of each).

6 – 4	6 – 3	5 – 3	4 – 4 *	7 – 2
5 – 1	7 – 3	4 – 5 *	3 – 4 *	4 – 3
3 – 1	6 – 5	4 – 2	4 – 1	3 – 3 *
6 – 2	5 – 2	5 – 5 *	7 – 1	3 – 2
3 – 5 *	7 – 4	7 – 5	6 – 1	5 – 4

A3. Box 1. Further explanation of Figure 3b (Chapter 3). The number of choices for the larger plate exhibited by cleaners in the first ten trials of Experiment 1 is significantly greater than 5 out of 10 trials, i.e. above chance, irrespective of plate status. Back-transformation (logit) verifies that $p > \frac{1}{2}$:

$$\text{Logit} = \log(p/1-p) \geq 0$$

$$p/1-p > 1$$

$$p > 1-p$$

$$2p > 1$$

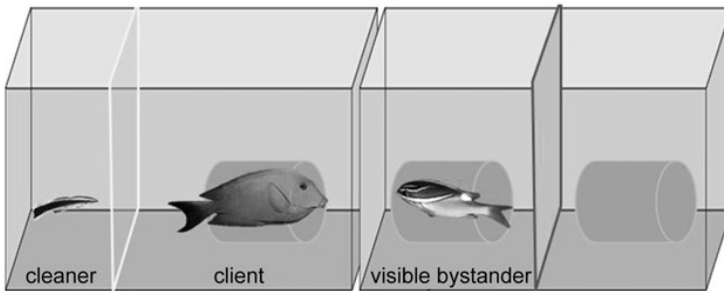
$$p > \frac{1}{2}$$

A4. Box 2. Methodological description: audience effects experiment using live fish clients (General Discussion).

This experiment tested whether cleaners adjust their behaviour during interactions with client fish (*Ctenochaetus striatus*, lined bristletooth) when in the presence or absence of an image scoring bystander (*Scolopsis bilineatus*, bridled monocle bream). For example, do cleaners cheat less and cause fewer client jolts when they have an audience? Methods followed Pinto et al. (2011).

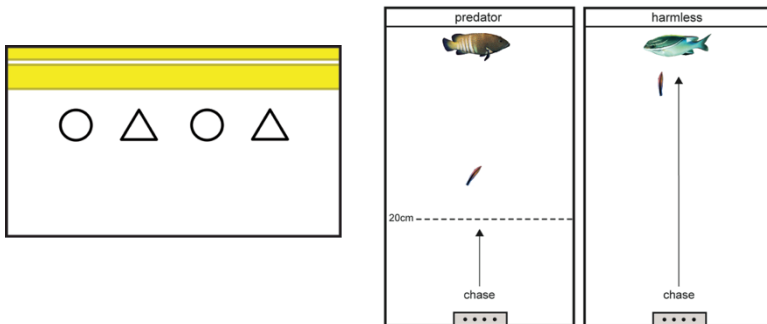
The experimental set-up is illustrated in the figure below (from Pinto et al. 2011). It consisted of two adjoining glass aquaria, each measuring 90 cm x 38 cm x 38 cm, separated by an opaque Plexiglas partition. One aquarium contained the client fish *C. striatus* and the cleaner (behind a clear Plexiglas partition), while the other aquarium contained the bystander *S. bilineatus*. The client aquarium contained a single, centrally-placed shelter tube (15 cm diameter), while the bystander aquarium contained two centrally-placed shelter tubes. All fish were placed in their aquaria two days prior to the commencement of the experiment. On the morning of an experiment, a second opaque partition was introduced into the middle of the bystander aquarium, between the two shelter tubes. During the experiment, both the clear partition separating the cleaner from the client and the opaque partition between the two aquaria was removed, which allowed for the cleaner and client to interact and to see into the front portion of the neighboring aquarium. In half of the trials, the bystander was initially placed in the front compartment of the bystander aquarium, allowing the cleaner and the client to see the bystander, while in the other half of the trials, the bystander was placed into the back portion of the aquarium, non-visible to the cleaner and the client. Two digital cameras filmed the interactions between the cleaner and the client, and after 10 min, all partitions were put back into place. The bystander was then moved to the other compartment. After 60 min, the cleaner-client pair was exposed to the new condition for another 10 min. The sequence of conditions was counterbalanced over all pairs/environments. The number of client jolts were recorded for both conditions.

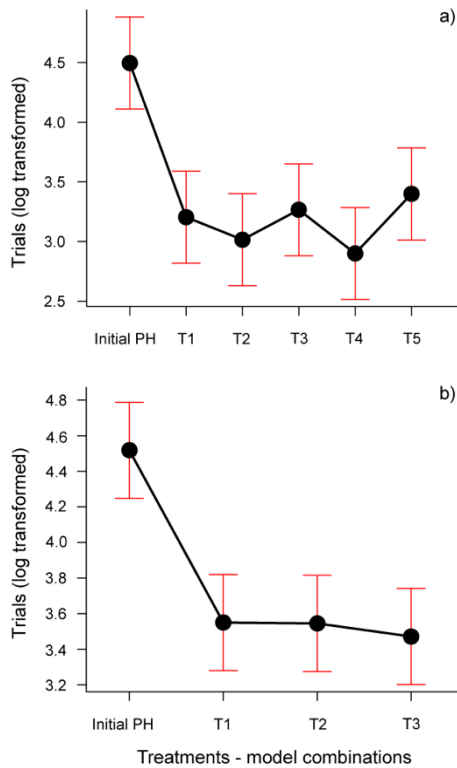
A4. Box 2 Continued. This experiment was conducted in 2010 (n = 9; 4 patch, 5 cont.) and in 2013 (n =18; 9 from each environment); statistical analyses utilized pooled data between the two years. Methodology for cleaner catching, transport and laboratory habituation followed methods as per all other data chapters. Client fish *C. striatus* were kept a minimum of 30 days in captivity prior to experiments for habituation, while bystander fish *S. bilineatus* were caught 2 days prior to experiments, a method shown to reduce mortality and stress in this species. Client fish were housed in large oval holding tanks with a 1 m diameter, and both client and bystander fish were released at site of capture after experiments.



A5. Box 3. Methodological description: feeding against preference experiment - predator vs harmless species fish model (General Discussion, Chapter 5).

This experiment tested whether cleaners adjust their cooperative behaviour, i.e. their willingness to feed against their food preference, in the presence of a predator or a harmless species fish model. Cleaners were offered a novel Plexiglas feeding plate (see Figure below), containing two prawn and two flake items, which simulated a fish client. As in the generalization/social tool experiment of Chapter 5, the same rules were applied in regards to feeding and punishment. For example, if the cleaner fed on a flake item, it was allowed to continue foraging on the plate. However, if the cleaner cheated and fed on a prawn item, it was either 1) chased for a total of 20 cm if the aquarium contained a predator model or 2) chased for the distance of the aquarium if a harmless species was present (see Figure below). Each cleaner was subjected to a total of 50 experimental trials, alternating between the predator (Peacock cod; *Cephalopholis argus*) and harmless (Two-lined monocle bream; *Scolopsis bilineatus*) species fish model. The mean ratio of flake to prawn items consumed over the total number of trials by all individuals was calculated for both fish model categories. All cleaners were tested in their own aquaria, which consisted of a single experimental compartment (62 cm x 26 cm), containing either a predator or harmless model. This experiment was conducted in 2011 and utilized the same individuals as per Experiment 1 in Chapter 5 (n = 6).





A6. Figure 2. Model predictions for Experiment 1 and 2 (Chapter 5). Mean and 95% confidence intervals for treatments computed using a mixed-effects model, accounting for repeated measures on the same individuals over time, for Experiment a) 1 and b) 2. Visualized with the R package effects.

A7. Table 2. Experimental design utilized in Experiment 2, illustrating the sequence of model combinations presented to cleaners (ID No. 1-8) (Chapter 5). Cleaners who did not learn to prefer the correct model in the Initial Treatment (ID No. 1, 4, 7) were not exposed to subsequent treatments. Predator species are listed above harmless fish species. 1 = species exotic to Lizard Island; 2 = combination where two predators were used; 3 = combination where two harmless species were used. All models were 17 cm in total length; due to length restrictions, only the head of the moray eel was used.

Cleaner 1

Initial treatment: *Gymnothorax javanicus* (giant moray) and *Parupeneus barberinus* (dash-dot goatfish).

Cleaner 2

Initial treatment: *Gymnothorax undulatus* (undulated moray) and *Stegastes nigricans* (dusky gregory). Subsequent treatments: *Cephalopholis microprion* (dothead rockcod) and *Siganus corallines* (coral rabbitfish); *Epinephelus striatus* (nassau grouper¹) and *Holacanthus ciliaris* (queen angelfish¹); (*Cephalopholis miniata* (coral cod) and *Ctenochaetus striatus* (lined bristletooth); *Pomacanthus sexstriatus* (six banded angelfish³) and *Scolopsis bilineatus* (bridled monocle bream³); *Gymnothorax javanicus* (giant moray*) and *Variola louti* (coronation cod*).

Cleaner 3

Initial treatment: *Epinephelus merra* (dwarf spotted rockcod) and *Pomacanthus sexstriatus* (six banded angelfish). Subsequent treatments: *Gymnothorax javanicus* (giant moray) and *Parupeneus barberinus* (dash-dot goatfish); *Epinephelus striatus* (nassau grouper¹) and *Holacanthus ciliaris* (queen angelfish¹); *Variola louti* (coronation cod) and *Hemigymnus melapterus* (blackeye thicklip); *Cephalopholis miniata* (coral cod²) and *Cephalopholis argus* (peacock rockcod²); *Chlorurus sordidus* (bullethead parrotfish³) and *Scolopsis bilineatus* (bridled monocle bream³).

Cleaner 4

Initial treatment: *Cephalopholis microprion* (dothead rockcod) and *Siganus corallinus* (coral rabbitfish).

Cleaner 5

Initial treatment: *Cephalopholis cyanostigma* (bluespotted rockcod) and *Scolopsis bilineatus* (bridled monocle bream). Subsequent treatments: *Epinephelus striatus* (nassau grouper¹) and *Holacanthus ciliaris* (queen angelfish¹); *Cephalopholis argus* (peacock rockcod) and *Chlorurus sordidus* (bullethead parrotfish); *Gymnothorax javanicus* (giant moray) and *Parupeneus barberinus* (dash-dot goatfish); *Ctenochaetus striatus* (lined bristletooth³) and *Siganus corallinus* (coral rabbitfish³); *Epinephelus merra* (dwarf spotted rockcod²) and *Cephalopholis microprion* (dothead rockcod²).

Cleaner 6

Initial treatment: *Cephalopholis miniata* (coral cod) and *Ctenochaetus striatus* (lined bristletooth). Subsequent treatments: *Epinephelus striatus* (nassau grouper¹) and *Holacanthus ciliaris* (queen angelfish¹); *Gymnothorax javanicus* (giant moray) and *Parupeneus barberinus* (dash-dot goatfish); *Epinephelus merra* (dwarf spotted rockcod) and *Pomacanthus sexstriatus* (six banded angelfish); *Cephalopholis cyanostigma* (bluespotted rockcod²) and *Gymnothorax undulates* (undulated moray²); *Chlorurus sordidus* (bullethead parrotfish³) and *Stegastes nigricans* (dusky gregory³).

Cleaner 7

Initial treatment: *Variola louti* (coronation cod) and *Hemigymnus melapterus* (blackeye thicklip).

Cleaner 8

Initial treatment: *Cephalopholis argus* (peacock rockcod) and *Chlorurus sordidus* (bullethead parrotfish). Subsequent treatments: *Epinephelus tukula* (potato cod¹) and *Balistoides conspicillum* (clown triggerfish¹); *Cephalopholis cyanostigma* (bluespotted rockcod) and *Scolopsis bilineatus* (bridled monocle bream); *Gymnothorax undulates* (undulated moray) and *Stegastes nigricans* (dusky gregory); *Parupeneus barberinus* (dash-dot goatfish³) and *Hemigymnus melapterus* (blackeye thicklip³); *Gymnothorax javanicus* (giant moray²) and *Epinephelus striatus* (nassau grouper²).

A8. Table 3. Experimental design utilized in Experiment 2, showing the Initial Treatment combination of two harmless fish models presented to cleaners (ID No. 9-16) (Chapter 5). Since no cleaner was able to prefer the correct model-safe haven, subsequent novel model combinations were not utilized in this experiment. Two cleaners started the experiment with identical models, but the species representing a safe haven was balanced in the design.

Cleaner 9, 10

Initial treatment: *Ctenochaetus striatus* (lined bristletooth) and *Siganus corallinus* (coral rabbitfish).

Cleaner 11, 12

Initial treatment: *Scolopsis bilineatus* (bridled monocle bream) and *Pomacanthus sexstriatus* (six banded angelfish).

Cleaner 13, 14

Initial treatment: *Parupeneus barberinus* (dash-dot goatfish) and *Hemigymnus melapterus* (blackeye thicklip).

Cleaner 15,16

Initial treatment: *Chlorurus sordidus* (bullethead parrotfish) and *Stegastes nigricans* (dusky gregory).

A9. Table 4. Experimental design utilized in Experiment 2, illustrating the sequence of model combinations presented to cleaner wrasse (ID No. 9-16), subsequent to completing the same model combinations of Experiment 2 (Chapter 5). Cleaner wrasse who did not learn to prefer the correct model in the Initial Treatment (ID No. 13-15) were not exposed to subsequent treatments. To avoid exposing cleaner wrasse to models they have already encountered, the design varies marginally from the sequences originally presented to cleaner No. 1-8. Predator species are listed above harmless fish species. 1 = species exotic to Lizard Island reefs; 2 = combination where two predators were used; 3 = combination where two harmless species were used.

Cleaner 9

Initial treatment: *Gymnothorax javanicus* (giant moray) and *Parupeneus barberinus* (dash-dot goatfish). Subsequent treatments: *Epinephelus merra* (dwarf spotted rockcod) and *Pomacanthus sexstriatus* (six banded angelfish); *Epinephelus tukula* (potato cod¹) and *Balistoides conspicillum* (clown triggerfish¹); *Cephalopholis cyanostigma* (bluespotted rockcod) and *Scolopsis bilineatus* (bridled monocle bream); *Cephalopholis cyanostigma* (bluespotted rockcod²) and *Cephalopholis argus* (peacock rockcod²); *Chlorurus sordidus* (bullethead parrotfish³) and *Stegastes nigricans* (dusky gregory³).

Cleaner 10

Initial treatment: *Gymnothorax undulatus* (undulated moray) and *Stegastes nigricans* (dusky gregory). Subsequent treatments: *Cephalopholis microprion* (dothead rockcod) and *Parupeneus barberinus* (dashdot goatfish); *Epinephelus striatus* (nassau grouper¹) and *Holacanthus ciliaris* (queen angelfish¹); *Cephalopholis miniata* (coral cod) and *Ctenochaetus striatus* (lined bristletooth); *Chlorurus sordidus* (bullethead parrotfish³) and *Scolopsis bilineatus* (bridled monocle bream³); *Epinephelus merra* (dwarf spotted rockcod²) and *Variola louti* (coronation cod²).

Cleaner 11

Initial treatment: *Epinephelus merra* (dwarf spotted rockcod) and *Chlorurus sordidus* (bullethead parrotfish). Subsequent treatments: *Gymnothorax javanicus* (giant moray) and *Parupeneus barberinus* (dash-dot goatfish); *Epinephelus striatus* (nassau grouper¹) and *Holacanthus ciliaris* (queen angelfish¹); *Variola louti* (coronation cod) and *Hemigymnus melapterus* (blackeye thicklip); *Cephalopholis miniata* (coral cod²) and *Cephalopholis argus* (peacock rockcod²).

Cleaner 12

Initial treatment: *Cephalopholis microprion* (dothead rockcod) and *Siganus corallines* (coral rabbitfish). Subsequent treatments: *Gymnothorax undulatus* (undulated moray) and *Stegastes nigricans* (dusky gregory); *Epinephelus tukula* (potato cod¹) and *Balistoides conspicillum* (clown triggerfish¹); *Cephalopholis argus* (peacock rockcod) and *Chlorurus sordidus* (bullethead parrotfish); *Parupeneus barberinus* (dash-dot goatfish³) and *Hemigymnus melapterus* (blackeye thicklip³); *Gymnothorax javanicus* (giant moray²) and *Cephalopholis cyanostigma* (bluepotted rockcod²).

Cleaner 13

Initial treatment: *Cephalopholis cyanostigma* (bluespotted rockcod) and *Scolopsis bilineatus* (bridled monocle bream).

Cleaner 14

Initial treatment: *Cephalopholis miniata* (coral cod) and *Scolopsis bilineatus* (bridled monocle bream).

Cleaner 15

Initial treatment: *Variola louti* (coronation cod) and *Hemigymnus melapterus* (blackeye thicklip).

Cleaner 16

Initial treatment: *Cephalopholis argus* (peacock rockcod) and *Siganus corallinus* (coral rabbitfish). Subsequent treatments: *Epinephelus tukula* (potato cod¹) and *Balistoides conspicillum* (clown triggerfish¹); *Cephalopholis cyanostigma* (bluespotted rockcod) and *Scolopsis bilineatus* (bridled monocle bream); *Gymnothorax undulates* (undulated moray) and *Hemigymnus melapterus* (blackeye thicklip); *Epinephelus tukula* (potato cod²) and *Cephalopholis microprion* (dothead rockcod²); *Pomacanthus sexstriatus* (six banded angelfish³) and *Scolopsis bilineatus* (bridled monocle bream³).
