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What Makes
A Cleaner
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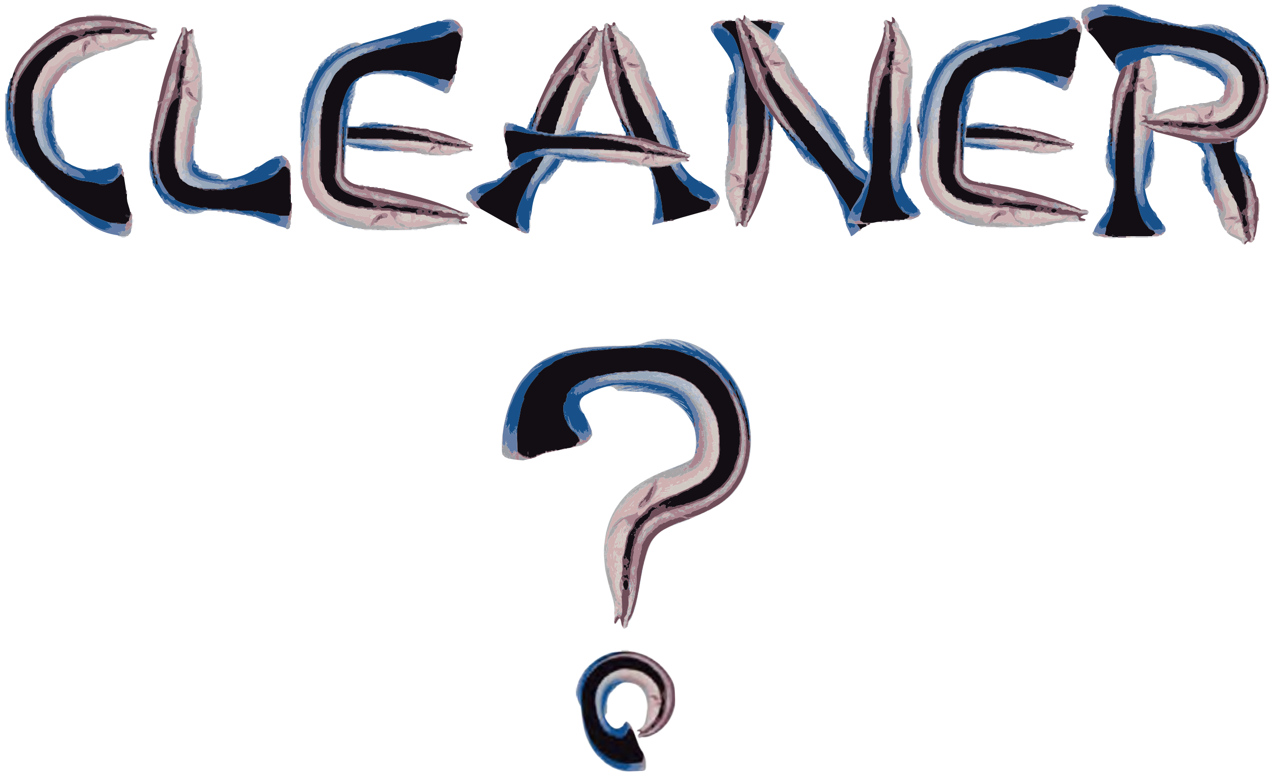

Simon Gingins - PhD Thesis - University of Neuchâtel - 2016

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autorise l'impression de la présente thèse soutenue par**

Monsieur Simon GINGINS

Titre:

“What makes a cleaner a cleaner ?”

sur le rapport des membres du jury composé comme suit:

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Le Doyen, Prof. B. Colbois

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GENERAL SUMMARY

In his last presidential address to the Royal Society in 2005, Robert M. May stated that "The most important unanswered question in evolutionary biology, and more generally in the social sciences, is how cooperative behaviour evolved and can be maintained". My thesis provides a contribution to answering this big question by investigating how one particular species evolved for an ecology that rests heavily upon cooperative interactions: the bluestreak cleaner wrasse *Labroides dimidiatus*. This species engages in up to 2000 cooperative interactions per day with dozens of other 'client' coral reef fishes, thus making it a prime system for studying cooperation between unrelated individuals. Conflicts between cleaners and clients arise because the former prefer to bite clients to eat their protective mucus rather than focusing on ectoparasites. In response to such exploitation, clients use various forms of partner control mechanisms that promote cooperative behaviour in cleaners. As a result, cleaners are known to use a diversity of strategic behaviours to determine when to cheat and when to cooperate. Cleaners' behaviour thus appears very well adapted to the demands of their peculiar ecology.

In my thesis, I used a comparative approach to investigate which traits appear to be associated with the highly social ecology of cleaners, or in other words: What makes a cleaner a cleaner? Through a series of experiments, I compared the cognitive skills, escape performance, foraging ecology and vision of *L. dimidiatus* with other species of labrids that do not engage in cleaning (or only occasionally). These comparisons allowed me to identify some of the characteristics that set cleaners apart, and thus further our understanding of how social ecology can affect the evolution of a species.

In the first chapter, I showed that *L. dimidiatus* was able to fine-tune its level of cooperation to the specifics of different cleaning-related situations, an ability that was absent in the closely related *Halichoeres melanurus.* In the second chapter, I investigated whether the demands of a highly social lifestyle led to an overall increase of cognitive performance in *L. dimidiatus*, or whether performance was tightly linked to ecological demands. *L. dimidiatus* outperformed five other species of labrids in two ecologically relevant tasks. However, all species performed similarly in a task with little ecological relevance, suggesting that cognition in cleaners is tightly linked to the challenges faced in nature. In the third chapter, results from foraging experiments suggest *that L. dimidiatus* evolved a foraging position that allows for increased efficiency in cleaning interactions. In comparison with four other species, *L. dimidiatus* adopted a significantly lower body angle with regards to the substrate when foraging. Furthermore, this species experienced almost no reduction in efficiency when searching for cryptic food items in comparison with conspicuous ones, while the other species all performed worse in the cryptic condition. It thus appears that the peculiar foraging posture of *L. dimidiatus* is well suited for cleaning interactions. Unfortunately, my current data did not allow assessing whether the visual system also adapted for a low body angle while foraging. Finally, in the last chapter, I asked whether because of the service they provide to predators, a reduction in predation pressures led to the decay of escape performance in *L. dimidiatus*. Interestingly, measures of escape performance in a controlled laboratory setup showed that *L. dimidiatus* was among the top performers in comparison with 5 other labrids. These results suggest that the risks associated with cleaning interactions are sufficient to maintain a high escape performance in cleaners, despite their privileged relationship with predatory clients.

In summary, a wide range of characteristics appear to be important for cleaning interactions, and species that specialized in this activity seem to have undergone very different selective pressures than fishes with more standard ecologies. Identifying some of the key aspects related to the ecology of cleaners provides a good example of how the evolution of a species can be affected by the demands of a highly social life. In this system, I argue that competition among service providers and conflicts of interests between cleaners and clients appear to be the major drivers of adaptation.

Keywords: Cooperation, mutualism, fish, behavioural ecology, cleaner, Labridae, cognition, comparative approach.

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A - INTRODUCTION

More than 150 years ago, Darwin established the theory of evolution through natural selection (Darwin 1859), and the concepts developed in '*on the origin of species*' have forever changed the way we reason about life on earth. A central concept in evolution is the process of adaptation, whereby organisms become gradually better suited to survive in their environment. On earth, organisms have been able to colonize almost all the environments, from the highest mountains (Swan 1992) to well below the ocean floor (Inagaki *et al.* 2015). Without specific adaptations to survive in such hard conditions, this would have been impossible. But physical conditions are not the only drivers of adaptation; species also tend to adapt to the way they feed, mate, compete, avoid predation, and interact socially. As a result, the traits we observe today in organisms are expected to reflect the selective pressures associated with the way they live. In other words: evolution leaves its footprint in the distribution of traits in living things (Ronquist 2004).

A.1 - The comparative approach

One of the most influential tools in evolutionary biology is the comparative approach, which assumes that by comparing organisms, we can understand how natural selection drove species to adapt. The fact that closely related taxa are generally more alike than distant ones has been used for decades to construct phylogenetic trees (first using morphological data, and then genetic data). However, if a trait evolved for a specific function, we can expect distant species that face the same challenges to have experienced similar selective forces and thus to share the same trait, or an analogous trait serving the same function. Conversely, we can also expect closely related organisms to differ with regards to traits associated with their respective ecologies. I use a broad definition of the term 'ecology' to describe the relationship between organisms and their environment (both physical and social). Species are thus expected to share traits either because of shared ancestry, or because they responded to similar ecological pressures. In the last several decades, improvements in the field of phylogenetic analyses have greatly improved our understanding of evolutionary processes (Felsenstein 1985; Harvey & Pagel 1991; Pennell 2012). For instance, it is now possible to investigate whether specific traits evolved faster in some taxa than in others (Butler & King 2004), to determine ancestral states (Ronquist 2004), or simply to control for phylogenetic relationships in comparative studies (Felsenstein 1985; Harvey & Pagel 1991; Martins & Hansen 1997). For the purpose of my thesis I did not perform phylogenetic analyses (see section A.6) and hence I will not expand the discussion of these techniques here, but rather focus on discussing the relative importance of ecology and phylogeny.

Occasionally, a species (or a group of species) evolves an ecology that differs markedly from its close relatives. Such cases provide prime systems for investigating adaptation because shared ancestry allows to compare species that are similar on many aspects, while each species is expected to have undergone selective pressures associated to demands of its specific ecology. One of the most striking and well-studied examples is the human animal (*Homo sapiens*) and its relatives (primates). Maybe because we are directly implicated, many researchers have been studying what is referred to as 'human uniqueness' (Premack 2007, 2010; Penn *et al.* 2008; Heyes 2012; Shettleworth 2012). The most striking difference between humans and primates is probably in terms of cognitive skills. The complexity and the paramount importance of social interactions in the ecology of humans are argued to have played a major role in the evolution of its cognitive skills (Cosmides & Tooby 1992). Already in 1973, E.O. Wilson discussed the importance of ecology in human evolution, highlighting the fact that the early man was a social carnivore and that "This fact alone […] helps to account for his convergence to the high degree of coordinated behavior and in-group altruism which early man shared with the social canids and the lion. Similarly, it helps to explain man's divergence in many details of social behavior from his closest relatives, the vegetarian chimpanzee and gorilla" (Wilson 1973). Many of the current theories now incorporate ecology, social or else, as a major driving factor for the evolution of cognition (Bolhuis & Wynne 2009; van Horik & Emery 2011; Shettleworth 2012). Other systems provide simpler examples, such as clownfishes or food-hoarding birds. Clownfishes are species of damselfishes (pomacentridae) that live in tight association with anemones. This peculiar ecology has been associated with an increase in morphological evolution and species diversification in comparison with other pomacentrids (Litsios *et al.* 2012). Engaging down this evolutionary trajectory thus appears to have been associated with stronger selective pressures in the ancestors of clownfishes than in the rest of the family. In birds, some species cache food in periods of abundance in order to retrieve it when food is scarce, thus relying to some extent on spatial memory to find the hidden food. In comparison with closely related species that do not engage in such behaviour, food-hoarding birds have better spatial memory skills and a larger hippocampus (Krebs *et al.* 1989; Healy & Krebs 1992; Pravosudov & Clayton 2002; Pravosudov & Roth II 2013). Food-hoarding thus appears to have specifically selected for increased spatial memory and the associated brain parts, while other domains of cognition might not have been affected (Olson *et al.* 1995; Pravosudov & Clayton 2002).

These few examples illustrate that in order to understand how species respond to ecological demands, a comparative approach can be of great value, particularly when closely related species facing different ecological demands are available for comparison. Phylogenetic relationships must be taken into account to control for similarities due to shared ancestry, but predictions about the direction of selective pressures in each taxa can only be made in the light of their ecology. In my thesis, I used the comparative approach to investigate adaptation in another system in which some species developed an ecology that is markedly different from their close relatives: cleaner wrasses. These species of coral reef fishes engage in up to 2000 cooperative interactions per day with other reef fishes in order to eat their ectoparasites (Grutter 1996). Most labrids do not engage in cleaning behaviour, but rather feed on small invertebrates, corals, or small fishes (Randall 1967; Côte 2000; Cowman *et al.* 2009). Comparing cleaners with other labrids thus allows investigating which traits are associated with their highly interspecifically social ecology.

A.2 – The evolution of helping

Helping is ubiquitous in nature, and occurs at all levels of biological complexity (West *et al.* 2007a). One of the big questions in evolution is thus to understand how helping emerged and is maintained (Sherratt & Wilkinson 2009). I follow Lehmann and Keller (2006) in their definition of helping as a behaviour that increases the direct fitness of another individual. Nowadays, it is generally accepted helping occurs because of two main reasons: (1) helping behaviour yields direct benefits (i.e. by-product mutualism, pseudo-reciprocity and reciprocity) (2) helping individuals sharing the same genes helps to propagate them (kin selection and greenbeard effects) (Lehmann & Keller 2006; West *et al.* 2007b; Bshary & Bergmüller 2008). Helping thus encompasses both situations, while the term 'cooperation' is limited to behaviours that yield direct benefits (Lehmann & Keller 2006). In order to understand how helping evolved, it is important to investigate the conditions that lead to its emergence and the traits that are associated with it (Brosnan *et al.* 2010). For instance, reciprocal interactions between unrelated partners illustrate how certain traits facilitate the emergence of helping. Reciprocity is quite vulnerable to exploitation because each act is an investment, hence there is a strong temptation to cheat by not returning the investment (Trivers 1971). Under these conditions, individuals are expected to monitor the behaviour of interacting partners in order to avoid interacting with potential cheaters. Memory and individual recognition is thus expected to play an important role in reciprocity (Dugatkin 2002), and several authors have argued that its emergence has been constrained by cognitive limitations (Stevens & Hauser 2004; Hauser *et al.* 2009; Connor 2010). However, cooperation between unrelated individuals can also be achieved without advanced cognitive abilities, such as in interactions between plants and bacteria (Kiers *et al.* 2003). In these systems, a lack of mobility combined with simple self-serving partner control mechanisms are argued to be the key aspects for stable cooperation (Brosnan *et al.* 2010). These few non-exhaustive examples illustrate that various conditions can lead to the emergence of diverse forms of helping.

Once helping emerged, natural selection is expected to drive species to adapt to this new component of their ecology. In vertebrates, helping often requires the use of decision rules to avoid exploitation by cheaters (Brosnan *et al.* 2010). Therefore, much attention has been paid on how individuals evolved to make the right decisions in the context of helping (Hauser *et al.* 2009; Brosnan & Bshary 2010; McNally *et al.* 2012; Fawcett *et al.* 2014). As mentioned earlier, the use of decision rules often requires learning and memory (Dugatkin 2002) and as a result, cognitive abilities are expected to be under positive selection in order to deal with the complexity of social interactions (Byrne & Whiten 1988; McNally *et al.* 2012). In a clade of cooperative breeding monkeys (callitrichids), the specific cognitive traits associated to cooperative breeding appear to be more developed in comparison with sister taxa that have different breeding systems (Burkart & van Schaik 2010). Since cognitive abilities appear to be tightly linked to brain size (Deaner *et al.* 2007), brain size has been shown to correlate with the complexity of social ecology in primates for instance (Dunbar 1992, 1995; Barton 1996). Recently, a growing interest in the role of hormones in promoting helping behaviour suggests that the endocrine system might also play a major role in cooperative interactions (Brosnan & Bshary 2010; Soares *et al.* 2010a). In order to take the right decision in cooperative interactions, species might thus evolve decision-making strategies, a different hormonal system, specific cognitive skills, or even a larger brain. Here, I investigate whether, because of the paramount importance of cooperative interactions in their ecology, cleaners' behaviour and cognition differ markedly from closely related species, which would suggest adaptation. In addition, I also investigate other aspects that are tightly linked to their peculiar ecology: their ability to escape from conflicts and the way they forage.

A.3 – Cleaning in wrasses

Labridae (wrasses) is one of the largest and most diverse family of fishes, with more than 600 species and 82 genera (Westneat & Alfaro 2005; Cowman *et al.* 2009). In this family, there is a great diversity of body shapes, colour morphs and feeding ecologies (Randall *et al.* 1997; Wainwright *et al.* 2004). The great trophic diversity present in the family is the result of trophic innovations spread throughout the last 65 million years. The majority of these innovations occurred in the Miocene (23 - 5 million years ago) (Cowman *et al.* 2009). At least 58 species have been witnessed to engage in cleaning behaviour, making labrids the family with the highest diversity of cleaners (Côté 2000; Baliga & Law 2015). Most of them only clean occasionally, often at the juvenile stage, and rely on other sources to obtain most of their food. These fishes are referred to as 'facultative cleaners', and tend to vary in their dependence on cleaning even within species (Barbu *et al.* 2011). Facultative cleaning is very likely more common than we know, as new species are constantly added to the list. During the duration of my thesis, for instance, colleagues and I have observed 4 species cleaning which were previously thought to be non-cleaners. Facultative cleaning is estimated to have emerged independently between 26 and 30 times within labrids, mostly in the late Miocene (Baliga & Law 2015). Six species, however, are 'obligate cleaners' and obtain the great majority of their food through cleaning interactions (Côté 2000; Arnal & Morand 2001). All of these species of specialists belong to the same genus (*Labroides*), with the exception of *Symphodus melanocercus*, a species from the Mediterranean. Cleaning in *Labroides* is estimated to have evolved from a coral feeding lineage approximately 9.5 million years ago (Cowman *et al.* 2009). The recurrent emergence of cleaning and the variation in dependency on this behaviour within wrasses make this family a prime system for investigating which traits are associated with cleaning.

A.4 - Adaptations to cleaning

Cleaning in wrasses appears to emerge mostly at the juvenile stage, with a few species that subsequently specialized in this ecology (Baliga & Law 2015). Juvenile traits might thus facilitate the emergence of this behaviour, and Baliga & Law (2015) argued that cleaning evolved via a heterochronic process, with obligate cleaners being under selection to carry over juvenile traits to the adult stage. Thus far, no convincing relationship between cleaning and morphological traits such as body size or shape have been found (Côté 2000; Arnal *et al.* 2006). However, cleaners tend to have a dark lateral stripe, often associated with yellow or blue, which provides a strong contrast (Arnal *et al.* 2006; Cheney *et al.* 2009). The conspicuousness of this signal thus suggests that it serves to advertise their status as a cleaner to potential client fishes. This hypothesis is furthermore supported by recent evidence showing that an aggressive mimic, the fangblenny *Plagiotremus rhinorhynchos*, benefits of reduced predation risk thanks to this coloration (Cheney 2013). Compared to other wrasses, cleaners also tend to have a smaller mouth (Wainwright *et al.* 2004). Feeding on ectoparasites appears to be associated with small and rapid movements of the jaw (Baliga & Mehta 2015), and thus require less developed cranial muscles than in species predating on large or hard shelled preys (Wainwright *et al.* 2004). Except for body coloration and potential adaptations in the feeding apparatus, morphological adaptations appear to be minimal in cleaners (Krattinger, unpublished data for Master thesis). However, their behaviour is the principal characteristic that sets them apart, and hence might be the trait that has been under the strongest selective pressures.

The most studied species, the obligate cleaner *Labroides dimidiatus*, uses a variety of strategic behaviours to deal with the complexity of cleaning interactions (see section 1.7 for details), while facultative cleaners do not appear to behave as strategically (Barbu *et al.* 2011). *L. dimidiatus* can engage into more than 2000 cleaning interactions and eat more than 1200 parasites per day (Grutter 1996). Conflicts of interests arise between *L. dimidiatus* and its clients because the former actually prefers to eat mucus than ectoparasites (Grutter & Bshary 2004). Clients visit cleaners in order to get their parasites removed. By consuming mucus cleaners thus cheat and exploit their clients, which are known to respond in various ways according to their choice options. On the one hand, clients that have large enough territories (hereafter 'visitors') and thus access to several cleaners often terminate an interaction after being cheated, and are more likely to visit another cleaner for the next interaction (Bshary & Schäffer 2002; Soares *et al.* 2013). On the other hand, clients with small territories (hereafter 'residents') often have access to only one cleaner and thus lack the option to switch partners. When cheated, residents might punish cleaners by aggressively chasing them (Bshary & Grutter 2002, 2005). In addition, there is always an inherent risk in interacting with predators (Trivers 1971) and cheating in this context might have dire consequences. Cheating by consuming mucus thus has a cost, and cleaners are known to use a variety of strategic behaviour to access their favourite food (mucus). First, they adjust their behaviour to the different categories of clients (Bshary 2001). The clients that receive the best service are predators, very likely because they can potentially eat a cheating cleaner. Among non-predators, cleaners further distinguish between visitors and residents and give priority to the former. In addition, *L. dimidiatus* is able to flexibly adjust its service quality according to several conditions, such as in the presence of bystander clients (Pinto *et al.* 2011) or when it is co-inspecting a client with another cleaner (Bshary *et al.* 2008; Gingins & Bshary 2015). Obligate cleaners are also the only ones to provide tactile stimulation (a form of massage performed with their ventral fins) (Potts 1973), a behaviour that is beneficial for clients in that it reduces their stress level (Soares *et al.* 2011). The behaviour of *L. dimidiatus* thus appears highly specialized for cleaning interactions.

The social brain hypothesis predicts that the computational demands of a highly social ecology selects for increased brain size (Dunbar 1992; Barton & Dunbar 1997). Interestingly, gross brain anatomy does not suggest that obligate cleaners of the genus *Labroides* have larger brains than other fishes (Chojnacka *et al.* 2015). However, it might be in the details of cleaners' brain anatomy that differences can be found. For instance, the diencephalon, a part involved in the social decision-making network (O'Connell & Hofmann 2012), appears to be enlarged in *L. dimidiatus* (Chojnacka *et al.* 2015). Similarly, Mendonça et al. (Mendonça *et al.* 2013) found fewer arginine vasotocin (AVT) receptors in the brain of *L. dimidiatus* in comparison with the closely related non-cleaner *L. unilineatus*. AVT injections have been shown to decrease the propensity of cleaners to interact with clients (Soares *et al.* 2012) and hence having fewer receptors might facilitate approaching and interacting with heterospecifics, which is essential in cleaning interactions. Evolving for an ecology that revolves around cleaning interaction has thus the potential to select for a variety of behavioural, cognitive, neuronal and morphological traits.

A.5 - Main question

In just a few words, “What makes a cleaner a cleaner?” is the main question of my PhD thesis. My aim is to investigate how the highly social ecology of cleaners impacted their evolutionary trajectory, and which traits are associated with their ecology. Through a series of experiments, I investigated various aspects that I expected to be of ecological relevance for cleaners: cognitive skills, escape performance, foraging ecology and vision (see section A.7 for details). Comparing species that specialized in cleaning behaviour with closely related species that do not engage in cleaning (or only occasionally) allows determining which characteristics set cleaners apart. As a result, we can understand which types of selective pressures might be associated with this highly social ecology, and gain insight on how engaging down the path of cooperation can affect the evolution of a species.

A.6 - Study species

In most of the chapters of my thesis, I am comparing six species of labrids that co-occur on the Great Barrier Reef. I chose to compare one species of obligate cleaner, *Labroides dimidiatus*, with three facultative cleaners and two non-cleaners. My aim was to perform comparisons between obligate, facultative and non-cleaners without correcting for phylogenetic relationships, thus I made sure that the species used were not clustered in the phylogeny according to their cleaning behaviour (see Fig. 1). I will give a short description of the ecology of each species involved in my thesis below, starting with the focal species and then proceeding by degree of relatedness.

The life cycle of wrasses starts with a planktonic larva. All wrasses are open water spawners and thus release eggs and sperm in the water without any type of parental care. Wrasses begin their sexually mature life as females, and can change sex once they become larger and reach the top of the hierarchy. In many species, these phase changes (juvenile, female, male) are associated with different colorations and thus facilitate the determination of the individual’s reproductive status. In the descriptions below, I will also briefly describe the characteristics I used to determine the different phases.

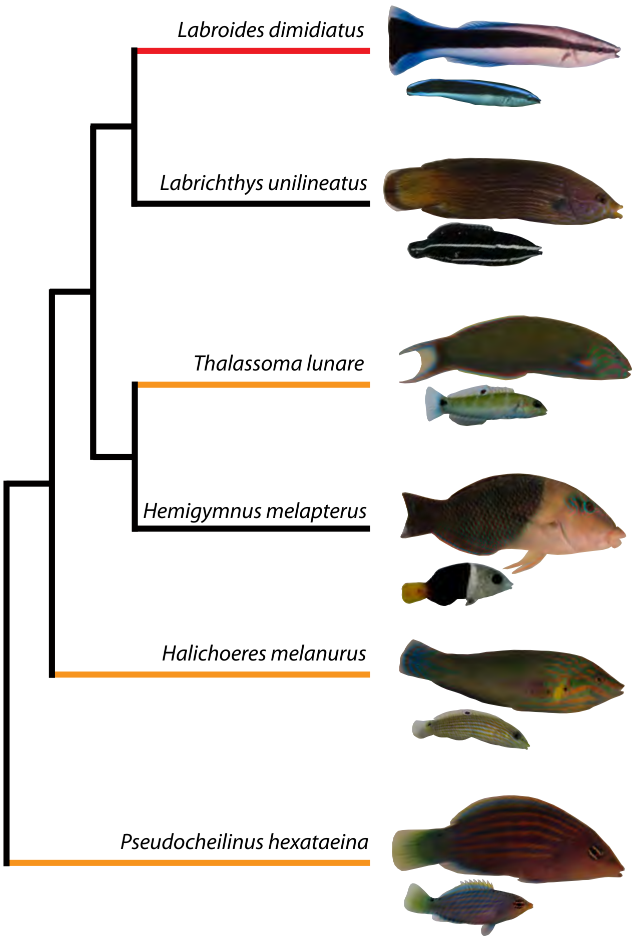

Figure 1. Schematic representation (i.e. branch lengths are not scaled) of the phylogenetic relationships between study species, based on the phylogeny of Cowmann and Bellwood (2011). The colour of the branch indicates dependency on cleaning: red = obligate cleaner; orange = facultative cleaner; black = non-cleaner. For each species, a juvenile and an adult are represented (pictures are not reproduced to scale).

A.6.1 - Labroides dimidiatus

Also known as the bluestreak cleaner wrasse, *L. dimidiatus* is the focal species of my thesis and used as the representative of obligate cleaners for comparisons. In this species, there is no sexual dimorphism that allows discriminating between males and females. Adults have a white to yellowish head and become bluish towards the tail, with a black lateral stripe across the whole body that becomes wider at the tail. Juveniles are entirely black with a thin bright blue horizontal line on the upper part of their flank. Size: up to 11.5 cm (Allen *et al.* 2005).

A.6.2 - Labrichthys unilineatus

The tubelip wrasse is the species that is most closely related to *L. dimidiatus* involved in the study. *L. unilineatus* is a strict corallivore that feeds mainly on hard corals such as *Acropora* (Cole *et al.* 2010). While the closely related tubelip *D. xanthurus* is a very active cleaner at

the juvenile stage, *L. unilineatus* has so far never been described as a cleaner, despite extensive observations (Cole 2009). In January 2016, however, my colleague Sandra Binning observed one juvenile individual cleaning a damselfish *P. moluccensis* at Lizard Island, Australia. Since this is very recent news and a single occurence, I still categorized this species as a non-cleaner for the purpose of my thesis.
Large males are dark with a series of thin blue stripes across the body and a faint yellow bar across the anterior part of the body, behind the pectoral fin. Females look very similar, but lack the yellow bar and have bright yellow lips instead. Juveniles have a thin bright blue stripe from the mouth to the tail. Size: up to 16 cm (Allen *et al.* 2005).

A.6.3 - Thalassoma lunare

The moon (or crescent) wrasse feeds mostly on small crustaceans and occasionally on small fishes (Connell 1998). Juveniles are frequently observed to engage in cleaning behaviour, yet individuals who clean only dedicate a very small proportion (<2%) of their time-budget to this activity (Barbu *et al.* 2011). I thus categorized this species as a facultative cleaner. Large males are green to bright blue, with a yellow patch in the caudal fin and clear blue patches on the pectoral fins. Females look very similar, but often more green than blue. Juveniles are green/brown with a clear dark spot on the dorsal fin and at the base of the tail. Size: up to 25 cm (Allen *et al.* 2005).

A.6.4 - Hemigymnus melapterus

The blackeye thicklip feeds by extracting small animals from mouthful of sands or by predating upon larger invertebrates found on the reef (Randall *et al.* 1997). This species has never been documented to clean. Juveniles have a yellow tail and a dark eye, while adults have the tail of the same colour as the rear half of their body. Size: up to 60cm (Allen *et al.* 2005).

A.6.5 - Halichoeres melanurus

The pinstriped wrasse feeds on small invertebrates (Randall *et al.* 1997). Thus far, it has never been described as a facultative cleaner. However, since 2012, we observed juveniles cleaning on several occasions on the reefs surrounding Lizard Island, Australia. Since this information was not available at the time I published the first chapter of my thesis, it is still described as a non-cleaner in this first chapter (Gingins *et al.* 2013). This change of status would not have affected the interpretation of the results. However, I categorized *H. melanurus* as a facultative cleaner in all subsequent chapters. Adults have a blue tail, with a dark patch for males, and bright orange and green patterns on the head. Juveniles have pale orange stripes along the body, and a dark ocellated spot on the dorsal fin and at the base of the caudal fin. Size: up to 12 cm (Allen *et al.* 2005).

A.6.6 - Pseudocheilinus hexataenia

The sixtripe wrasse spends the great majority of its time hiding among tight coral branches and has a very small territory. It feeds on small invertebrates. Juveniles have often been observed cleaning (Côté 2000), yet individuals that engage in this behaviour only spend a small proportion of their time cleaning (Barbu *et al.* 2011). I thus categorized it as a facultative cleaner. There is no notable change in coloration through ontogeny in this species. Size: up to 7.5 cm (Allen *et al.* 2005).

A.7 – Thesis outline

In order to determine how cleaners evolved for their ecology, it is important to identify the key aspects of cleaning interactions. For *L. dimidiatus*, determining when to cheat by taking a bite of mucus and when to focus on the client's ectoparasites appears to be of paramount importance. The four chapters of my thesis mainly revolve around cleaners' abilities to provide a high quality of service, to determine when service quality can be lowered, and to deal with the consequences of providing a bad service (Fig. 2). The first two chapters focus on the cognitive skills required for interacting with clients and strategically adjusting their level of cooperation. In the last two chapters, focus is shifted to more general aspects of cleaners' bauplan in relation to their ecology, namely foraging techniques and escape performance.

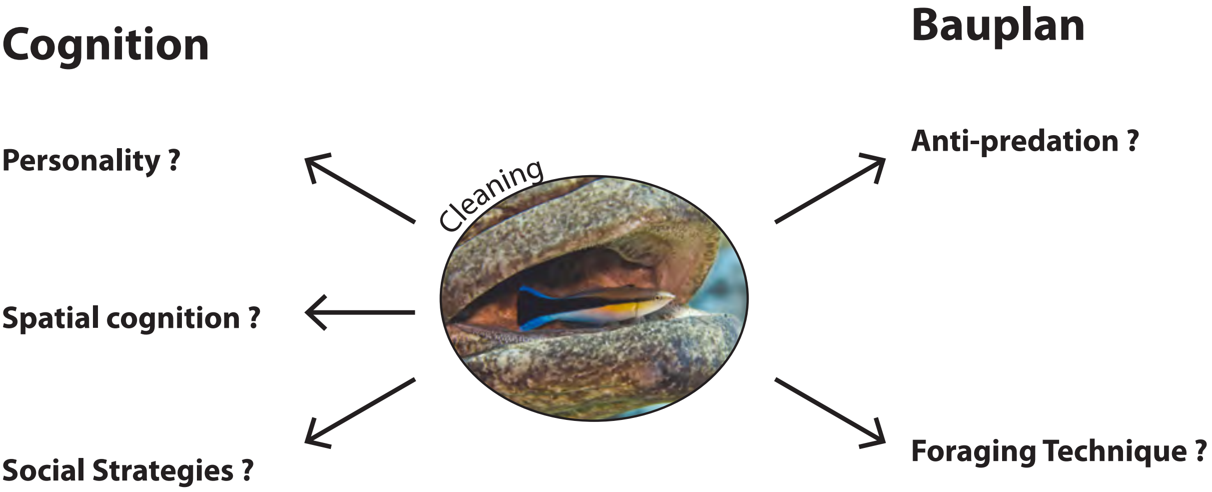

Figure 2. What makes a cleaner a cleaner? Schematic representation of the various aspects of cleaners' cognition and bauplan investigated during my thesis. I use the term 'bauplan' to describe behaviours that have a strong morphological underpinning, in contrast with cognitive traits that relate to the way animals acquire, process and act upon information.

Cleaners interact with a great diversity of clients, which diverge on many aspects including their abilities to terminate interactions and the quantity and quality of their mucus. While cleaners are constantly tempted to cheat and take a bit of mucus, most client species lack the option to cheat (i.e. they cannot eat the cleaner). In cleaning interactions, there is thus an asymmetry with regards to the options of the two interacting partners. A recent model predicts that under such conditions, variation in the temptation to cheat and in the victim's capacity to terminate the interaction lead to shifts between cooperation and exploitation (Johnstone & Bshary 2002). In the first chapter, I investigated whether this model could predict the way cleaners adjust their level of cooperation in similar situations. More specifically, I investigated how differences in clients' control over the end of an interaction and in the temptation for cleaners to cheat affected their cooperative behaviour in laboratory experiments. I also tested the closely related *H. melanurus* in the same setup in order to investigate whether a species that is not specialized in cleaning could perform as well as *L. dimidiatus* in the same task. This was the first time that another labrid species was tested in a task designed for cleaners.

Along with the abundant literature on the impressive abilities of *L. dimidiatus* to behave strategically (reviewed in Bshary 2011), the results of the fist chapter indicated that this species has developed complex decision rules to deal with the challenges of its highly social lifestyle. Furthermore, it appeared that similar abilities might not be present in fishes with a different ecology. Currently, there are two main views on the evolution of cognition. On the one hand, the ecological approach emphasises ecological validity as the main predictor of a species' performance in a given task (Shettleworth 2010). According to this view, cognition is expected to be modular and respond to the challenges faced by each species. On the other hand, a more generalist approach argues that cognitive processes require several domains of cognition to work in concert (Bressler & Menon 2010; Lefebvre 2010). As a result, selection is expected to work on cognition as a whole rather than on specific modules. In the second chapter, I investigated which of the generalist or the ecological approach explains the cognitive performance of *L. dimidiatus* best. Does *L. dimidiatus* generally perform better in cognitive tasks? Or are cognitive abilities tightly linked to the challenges faced? In order to investigate these questions, I used a larger panel of wrasses than in my first chapter, six in total (see section 1.6), and investigated their performance in lab experiments. I selected two cognitive tasks that are of particular ecological relevance for cleaners, but not for species with a different ecology, and one task that had little ecological relevance for all species. By comparing the performance of the six species across these three different contexts, it is thus possible to gain insight on whether cognitive skills differ between obligate cleaners, facultative cleaners and non-cleaners and whether the ecology of cleaners selected for general or specific cognitive abilities. In addition, since facultative cleaners predominantly clean at the juvenile stage, I conducted all the experiments on both juveniles and adults in order to investigate the potential ontogenetic effects that might favour the emergence of cleaning in certain species.

As soon as clients start selecting the cleaners they interact with according to the service they provide, we can expect competition amongst cleaners to select for increased service quality. One of the main aspects investigated in the first two chapters was the ability of cleaners to adjust their cooperative behaviour, and hence the quality of their service, in different situations. In the third chapter, I investigated another aspect of service quality: the efficiency at finding parasite during cleaning interactions. In nature, it appeared that obligate cleaners of the genus *Labroides* all search for parasites with a low body angle with regards to the client fish, while facultative cleaners and non-cleaners typically adopt a more perpendicular position with regards to the substrate when foraging. In combination with a quite elongated and relatively flat lower jaw (Wainwright *et al.* 2004), a low body angle might allow *L. dimidiatus* to scape parasite or mucus off the surface of client fishes more easily. In addition, it also allows cleaners to give tactile stimulation with their ventral fins (Potts 1973). Here, I quantified body angles during foraging in a standardised way and further posited that the particular position adopted by obligate cleaners allows for better efficiency at finding small and sometimes cryptic parasites all around the body of client fishes. In order to test this, I compared the efficiency of *L. dimidiatus* with four other species of wrasses in two foraging experiments. The first experiment aimed at testing whether a low body angle allows for better manoeuvrability in cleaning-like situations. The second experiment investigated whether the foraging posture of obligate cleaners improved the ability to find cryptic prey because their outline is more visible when looking at an angle than perpendicularly. Comparing the five species across these contexts allows understanding whether the foraging posture of *L. dimidiatus* evolved for increased efficiency at finding parasites, and thus higher service quality.

Different ways of searching for food place emphasis on different regions of the field of vision, and often the visual system of a species adapts to its ecology (Archer *et al.* 1999; Collin & Shand 2003). For instance, species foraging on the bottom tend to look for food at an angle directed downwards, while planktonic feeders use a more straightforward angle. These differences are often reflected in the arrangement of the retinal cells on the retina (Shand *et al.* 2000; Gomi & Miyazaki 2015). In the second part of this chapter, I also investigated the retinal topography of the five same species in order to assess whether the different postures they adopt when foraging selected for higher densities of photoreceptors in different areas of their retinas.

In the last chapter, I investigated how being a cleaner influenced the escape response. Fishes escape from predators with rapid swimming bursts called ‘fast-starts’ (Domenici 2011), and fast-start performance appears to be tightly linked to predation pressures in nature (Domenici *et al.* 2008; Langerhans 2009; Domenici 2010). *L. dimidiatus* regularly interacts with predators, and even inspects the inside of their mouth. In this context, attempts from predators to eat the cleaner are virtually inexistent. Cleaning stations even appear to be safe havens for reef fish as predators reduce attacks in the presence of cleaners (Cheney *et al.* 2008). Recent studies suggest that characters can be lost thanks to interspecific interactions, for instance in cases when the same function is performed by a mutualistic partner (Ellers *et al.* 2012). In the same line of thought, we could predict that the particular relationship between cleaners and predatory fishes has lead to reduced selective pressures on cleaners escape performance. However, even if it rarely occurs, the possibility that a predator attempts to eat the cleaner still exists, and some non-client predators also occasionally attack cleaners. Furthermore, cleaners frequently use escape responses to flee from punishing clients. As a result, we could also predict that *L. dimidiatus* experienced strong selective pressures on escape performance because of the risks associated with its ecology. In order to test these predictions, I filmed in high-speed the fast-start response of my six study species in a controlled laboratory setup. The results allowed investigating whether selective pressures associated with the ecology of cleaners appeared to be stronger or weaker than for closely related species that predominantly use escape in the context of predator-prey interactions.

A.8 - References

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CHAPTER 1 - Power and temptation cause shifts between exploitation and cooperation in a cleaner wrasse mutualism

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Abstract

In many instances of cooperation, only one individual has both the potential and the incentive to 'cheat' and exploit its partner. Under these asymmetric conditions, a simple model predicts that variation in the temptation to cheat and in the potential victim's capacity for partner control leads to shifts between exploitation and cooperation. Here, we show that the threat of early termination of an interaction was sufficient to induce cleaner wrasse *Labroides dimidiatus* to feed selectively against their preference (which corresponds to cooperatively eating client fish ectoparasites), provided that their preference for alternative food was weak. Under opposite conditions, cleaners fed selectively according to their own preference (which corresponds to cheating by eating client mucus). A non-cleaning fish species, *Halichoeres melanurus,* in contrast, failed to adjust its foraging behaviour under these same conditions. Thus, cleaners appear to have evolved the power to strategically adjust their levels of cooperation according to the circumstances.

Contributions: RB & ASG collected the data on *Labroides dimidiatus*. SG and JW collected the data on *Halichoeres melanurus*. SG, RB, ASG & RAJ jointly wrote the manuscript.

Introduction

In recent years, biologists have become increasingly aware that there are many possible mechanisms of partner control, all of which can hypothetically suppress cheating and hence maintain cooperation between unrelated individuals (Lehmann & Keller 2006; West *et al.* 2007; Bshary & Bergmüller 2008; Cant 2010; Leimar & Hammerstein 2010; Archetti *et al.* 2011; Bshary & Bronstein 2011; Jones *et al.* 2012). The empirical evidence suggests that many forms of cooperation, especially interspecific mutualisms, feature a marked asymmetry between interacting individuals in the scope for cheating or for partner choice (Sachs & Simms 2006; Bshary & Bronstein 2011; Jones *et al.* 2012). As a consequence, attention has shifted from reciprocity (as explored in iterated prisoner's dilemma games) to alternative control mechanisms like punishment (Clutton-Brock & Parker 1995; Raihani *et al.* 2012), sanctions (Herre *et al.* 1999), partner switching (Ferriere *et al.* 2002; Johnstone & Bshary 2008) or indirect reciprocity based on image scoring (Nowak & Sigmund 1998; Lotem *et al.* 2003). Several models of cooperation focus explicitly on such forms of partner control in asymmetric contexts (Sherratt & Roberts 2001; Johnstone & Bshary 2002, 2008; Lotem *et al.* 2003; Bshary *et al.* 2008; Holland & DeAngelis 2009; Golubski & Klausmeier 2010).

Empirical evidence for the importance of these alternative control mechanisms is also accumulating and has even driven the development of some of the theoretical papers cited above. However, while the collection of quantitative experimental data on cooperative behaviour has traditionally involved human subjects (Wedekind & Milinski 2000 among many other examples; see Fehr & Gächter 2002; Milinski *et al.* 2002), experiments on non-human cooperation have typically been only qualitative in nature (Kiers *et al.* 2003; Melis 2006; Bshary *et al.* 2008; Raihani *et al.* 2010; Pinto *et al.* 2011). Exceptions are provided by Kiers *et al.* (2011) who quantified the exchange of nutrients between plants and rhizobia, and by Raihani *et al.* (2011) who tested how male cleaners adjust levels of punishment according to the magnitude of the damage inflicted on them by cheating female partners.

A more quantitative approach is needed for at least two reasons. First, to properly understand the evolution and maintenance of cooperation we need to determine the extent to which animals adjust levels of cooperation to variable conditions. Variable conditions may affect cooperation in many ways, like through the effects of internal states on behaviour (Sherratt & Roberts 2001; Johnstone & Bshary 2007) or through the introduction of errors due to uncertainty: the 'trembling hand' (Selten 1975) is a key variable promoting cooperation and variable conditions may provide a biological basis for errors. Furthermore, variable conditions may select for increased cognitive abilities necessary to fine-tune behaviour (Brosnan *et al.* 2010) but also for the development of simple rules of thumb that do overall well while largely ignoring the complexity of the environment (Gigerenzer & Todd 1999). In both cases, decision rules of individuals have to be known to determine under which conditions cooperation is stable. A second reason to use a more quantitative approach is to identify variables that have a major effect on the potential for conflict. As it stands, it is notoriously difficult to measure the exact fitness consequences of cheating/being cheated, punishment/being punished, etc. in animals. Few laboratory studies have developed experimental designs that measure precise payoffs (St-Pierre *et al.* 2009) while the vast majority of studies may only offer informed guesses about relative payoffs. Variable behaviour according to conditions may allow us to identify situations in which conflict is minimal and cooperation maximal, and to identify conditions under which the effectiveness of partner control mechanisms like punishment may break down, leading to the end of interactions or to parasitism (Sachs & Simms 2006).

Here, we provide a quantitative study on levels of cooperation, with the intention of testing a simple model of partner control that potentially explains how the outcome of an interaction can shift from cooperation to exploitation (i.e. from mutualism to parasitism) and vice versa (Johnstone & Bshary 2002). The model assumes that an interaction between two players lasts for a variable duration that depends on the decisions of the two players. Only one player has the option to cheat, while the partner lacks any such option. Instead, the potential victim has the capacity to terminate or at least shorten the duration of their interaction. The model predicts cooperative outcomes as long as a) the payoff per time unit for cheating is sufficiently low compared to the payoff per time unit for cooperating (i.e. the temptation to cheat is low enough), and b) the potential victim has sufficient control over the duration of the interaction (i.e. the threat of termination is strong enough). As many real life interactions are characterised by asymmetries in strategic options (Sachs & Simms 2006; Bshary & Bronstein 2011) and an extended duration of action (Raihani & Bshary 2011), the model is potentially of broad relevance.

The cleaning mutualism involving the cleaner wrasse *Labroides dimidiatus* provides a model system to study cooperation in asymmetric games such as this: cleaners may cooperate with client fish by consuming their ectoparasites, leading to net benefits for clients (Clague *et al.* 2011; Waldie *et al.* 2011) but preferentially exploit them by eating mucus (Grutter & Bshary 2003), while non-predatory clients lack the option to cheat in return (they cannot eat a cleaner). Lacking the option to reciprocate, clients face the challenge of inducing cleaners to feed against their preference, in order to obtain a good cleaning service. A frequent observation in the field is that clients terminate cleaning interactions earlier in response to cheating by a cleaner (Bshary & Grutter 2002). We conducted a laboratory experiment to explore whether the clients' threat of early departure might suffice to suppress net exploitation by the partner, as suggested by the model described above (Johnstone & Bshary 2002).

In order to manipulate all parameters of interest, we replaced clients, and their mucus and ectoparasites with Plexiglas plates, and a preferred and a less-preferred alternative food, respectively. The plates were attached to a lever, allowing the experimenter to respond to the foraging behaviour of the cleaners in pre-determined ways. As the simple model predicts that the effectiveness of this partner control mechanism should depend on the potential benefits of cheating and on the ease with which victims may escape from exploitation, we manipulated both parameters and measured their relative impact on the foraging behaviour of cleaners.

Any evidence of the cleaners adjusting their foraging behaviour could be interpreted in two ways. First, adjustments may reflect an evolved capacity to adjust levels of cooperation to circumstances, as our experimental design corresponded to feeding on ectoparasites or mucus on mobile client reef fishes. However, there is the alternative explanation that we tested cleaners with a learning task that involved optimal foraging decisions. Thus, the task may not be linked to any adaptation to interactions with client reef fishes and hence could be equally well solved by species that do not engage in cleaning interactions. Therefore, in order to assess whether the experiment had the ecological relevance intended, we also tested a non-cleaner fish species, the pinstripe wrasse *Halichoeres melanurus*. Both species belong to the Labridae, a large family that originated around 65 million years ago and contains more than 600 species. The genus *Labroides* and *Halichoeres* are part of a more recent clade that originated around 30 million years ago (Cowman *et al.* 2009). The two species are similar with respect to habitat and body size (maximum total length of 11.5 cm for *L. dimidiatus* and 12 cm for *H. melanurus*) (Allen *et al.* 2005).

Material and Methods

Experiments with *L. dimidiatus* were conducted June to July 2004 at Lizard Island Research Station, Great Barrier Reef, Australia (14°40'S 145°28'E). Experiments involving *H. melanurus* were conducted January to February 2012 at the same site. 16 adult cleaner wrasse and 14 adult *H. melanurus* were caught with a barrier net and held alone or in pairs in aquaria of varying sizes for at least 20 days before the experiments. During the acclimatisation phase, cleaners were trained to feed on mashed prawn and fish flakes mixed with prawn (further called 'flake') spread on Plexiglas plates of varying colours. During the same phase, *H. melanurus* individuals were also trained to feed on mashed prawns spread on similar plates.

As cleaners have a strong preference for prawn over flakes (Bshary & Grutter 2005), these two food types could be used in our experiments. Interestingly, *H. melanurus* did not have the same preference. Therefore, during acclimatisation, combinations of various food types were offered to *H. melanurus* to find a suitable combination. The protocol followed Bshary and Grutter (Bshary & Grutter 2005): *H. melanurus* individuals were presented with plates covered with 7 items of each of two types of food tested. We considered an individual to have reached a preference when, in each trial's first seven items, one type of food was chosen more than 80% of the time over three subsequent trials. We eventually found that all individuals showed such a preference for an 80:20 prawn:flake mixture against a 70:30 sand:prawn mixture. In the process of determining a suitable combination of foods, *H. melanurus* experienced in total between 29 and 51 food trials.

The basic experimental design is very similar to the protocol used in previous experimental laboratory studies on *L. dimidiatus* (Bshary & Grutter 2005) and therefore will be described here only briefly. As a first step before the actual experiment, all fish were familiarised with the situation in which a plate with both preferred and less-preferred items would remain in their tank as long as they ate the less-preferred items but would be removed immediately if they ate one preferred item. Immediate reaction was possible because the plate was attached to a lever held by the experimenter. Therefore, the fish had to feed against their preference if they wanted to increase their food intake. During six trials, 14 less-preferred items were presented together with only two preferred items. Under this condition, all fish regularly ate less-preferred items and thus experienced both the positive and the negative consequences of their foraging decisions, giving them the opportunity to learn.

During the experiment, we offered the fish two Plexiglas plates (12 x 7 cm) with distinct novel colour patterns and with six black circles (1 cm diameter), each circle containing a food item. Three items were of the preferred food type and three items were of the less-preferred type. Plates were removed with a lever as soon as the fish ate one preferred item.

For each fish, one plate was always removed so rapidly (< 1 s) that the individual could not eat another item off of it (corresponding to strong victim control over the duration of interaction), while the other plate was removed slowly enough that the fish could eat other items (corresponding to weak victim control over the duration of interaction). The slow removal was adjusted to the speed of subjects foraging and would typically take 2-3 seconds. The slow removal functioned as intended, as all fish ate additional food items in these trials (average values for individuals: minimum, median, maximum items per round: 1.1, 1.5, 1.9, respectively for *L. dimidiatus* and 0.9, 1.6, 2 for *H. melanurus*). Variation in the concentration of the less-preferred food type (50% or 10% flakes in the flake-prawn mixture for cleaners; 70% or 20% sand in the sand-prawn mixture for pinstripes) yielded either a high or a low temptation to eat the preferred item. All fish were confronted with each of the four combinations of speed of removal and temptation (Fig. 1) seven times, yielding a total of 28 trials per animal. Half of the subjects first completed the 14 trials involving low temptation on the first day and then the 14 high temptation trials on the second day, while the other half was exposed to the reversed order. Within the 14 trial units, the sequence of slow moving and fast moving plate trials was counterbalanced. All combinations of plate colour with speed of plate removal were equally balanced between all the individuals of each species to avoid any colour effects explaining the results. All fish were released at the site of capture after the experiment.

For each interaction, we scored the number of less-preferred items eaten by an individual prior to eating a preferred item. The number of less-preferred items was then used as response variable in a generalized mixed model (GLMM) with treatment as explanatory variable and individual as grouping factor. Pairwise comparisons between treatments were obtained with Tukey contrasts for each species separately. In order to assess the effect of the species and the initial temptation treatment, models including these additional explanatory variables were compared to the original model by means of an ANOVA.

To calculate the average number of flake items eaten before the first prawn item under the 0-hypothesis that cleaners eat indiscriminately, we assumed that cleaners ate items at any stage according to their availability. Therefore, the probability that the first item eaten was a prawn was 0.5. The probability of eating a flake followed by a prawn was 0.5 x 0.6 = 0.3 The probability of eating flake, flake, and then prawn was 0.5 x 0.4 x 0.75 = 0.15, and the probability of eating all the flakes before a prawn item was 0.5 x 0.4 x 0.25 = 0.05. Therefore, the null hypothesis predicts that cleaners ate on average 0.5 x 0 flakes plus 0.3 x 1 flake plus 0.15 x 2 flakes plus 0.05 x 3 flakes = 0.75 flake items before the first prawn item (dashed line in Fig. 1). We compared this value against the mean number of less-preferred items eaten per round for each fish in every treatment combination with Wilcoxon tests. All statistics were performed with R 2.14.0 (R Core Team 2013), GLMMs were performed with the R package 'lme4' (Bates & Maechler 2009) and Tukey contrasts were obtained with the R package 'multcomp' (Hothorn *et al.* 2015). The dataset is available in the Dryad repository (doi:10.5061/dryad.r70n0).

Results

Cleaner wrasse *L. dimidiatus* foraging varied considerably between treatments (Fig. 1a). Both temptation (high temptation: HT, low temptation: LT) and victim control (high control: HC, low control: LC) had significant effects: all pairwise comparisons were significant (GLMM fit by Laplace: HT-LC vs. HT-HC: z = -2.568, p = 0.049; HT-LC vs. LT-LC: z = 5.041, p < 0.001; HT-LC vs. LT-HC: z = -6.567, p < 0.001; HT-HC vs. LT-LC: z = 2.653, p = 0.039; HT-HC vs. LT-HC: z = 4.357, p < 0.001) except between the low and high control treatments when temptation was low (GLMM fit by Laplace: z = -1.781, p = 0.279). The combination of high temptation and low victim control led to cleaners 'exploiting' their 'victims' by favouring the preferred prawn items (observed foraging behaviour against random expectation indicated by dashed line in Fig. 1a; n = 16, V = 8, p = 0.002). When the temptation to cheat and victim control were both high, foraging behaviour was not significantly different from random (n = 16, V = 67, p = 0.979). Finally, in both treatments in which the temptation to eat prawn was low, cleaners 'cooperated' by eating significantly against their preference (LT-HC: n = 16, V = 113, p = 0.021, n = 16; LT-LC: n = 16, V = 113, p = 0.003).

Foraging was significantly different between the two species (comparison between GLMMs with and without species as explanatory variable: ANOVA, df = 4, p < 0.001, Fig. 1b). Indeed, *H. melanurus* foraging did not differ significantly between treatments (GLMM fit by Laplace: all pairwise comparisons: -1 < z < 1.3, p > 0.5). In all treatments, they ate preferred food items significantly more often than expected by random choice (n = 14, all V = 0, all p ≤ 0.001). Whether individuals began the experiment with the high or the low temptation treatment did not affect the results significantly (comparison between a GLMM with both the species and initial condition as explanatory variables and a GLMM with only the species as explanatory variable: ANOVA, df = 8, p = 0.109).

Discussion

Our results provide experimental evidence that a most basic partner control mechanism, the threat of early termination of an interaction, may be sufficient to maintain cooperative behaviour in cleaning mutualism as long as the temptation to cheat is low. In our experiment, the degree of temptation influenced the foraging behaviour of cleaners more strongly than did the extent of their control over the duration of interaction. Nevertheless, both parameters had a significant influence. With respect to likely natural conditions, we note that the temptation to cheat should be relatively low: while cleaners preferred mucus over gnathiid isopods in a choice experiment they nevertheless regularly ate the latter. Another abundant type of ectoparasites, monogenean flatworms, was eaten with similar probability relative to mucus (Grutter & Bshary 2003). Furthermore, client control over duration of interactions appears to be very high (Bshary & Grutter 2002). Thus, the conditions favouring a mutualistic outcome, even in the absence of additional control mechanisms, seem to be fulfilled.

Terminating an interaction in response to cheating yields immediate benefits for the client in that it allows escaping further exploitation. In addition, it inflicts a cost on the cleaner since it reduces its foraging opportunities. As a result, cooperation might be promoted as a by-product of a self-serving behaviour (escaping exploitation), which is a form of negative pseudo-reciprocity (Connor 1986; Bshary & Bergmüller 2008; Raihani *et al.* 2012), also called 'sanctions' by various researchers (Herre *et al.* 1999; Kiers *et al.* 2003). While some authors refer to such a mechanism as 'self-serving punishment' or 'no cost punishment' (Cant & Johnstone 2006), we prefer to restrict the use of the term punishment to actions that entail a cost to the perpetuator, following Clutton-Brock & Parker (1995) and Rahiani *et al.* (Raihani *et al.* 2012). Sanctions provide a relatively simple mechanism to promote cooperation. Unlike punishment, it does not rely on future benefits arising from the increase in cooperative behaviour of the target to be under positive selection (Raihani *et al.* 2012). Our results add further evidence to the growing perception that while more complex mechanisms like punishment or reputational effects may receive more attention from researchers, rather simple partner control mechanisms like sanctions may often be responsible for stable cooperation in natural examples (Connor 1986; Brosnan *et al.* 2010; Cant 2010; Archetti *et al.* 2011; Bshary & Bronstein 2011): a plant's selective abortion of fruits infested with seed-eating larvae of the pollinator species (Pellmyr & Huth 1994), a plant's selectively reduced investment in root growth in areas in which nitrogen fixation by rhizobia bacteria is low (Kiers *et al.* 2003, 2011), reduced probing duration by a pollinator if nectar quantities are low (Smithson & Gigord 2003; Brandenburg *et al.* 2012), the avoidance of a cleaner wrasse that has been observed cheating another client (Pinto *et al.* 2011) or the premature departure of a client in response to cleaner cheating (Bshary & Grutter 2002).

What is intriguing about our results involving the cleaners is that there is experimental evidence that clients often do not just terminate interactions prematurely in response to cheating but that they may in addition punish cleaners, switch to another cleaner for their next inspection and may observe cleaner-client interactions to avoid cheating cleaners (Bshary & Grutter 2005; Pinto *et al.* 2011). In the future, we should ask how far punishment or partner switching, in addition to the threat of early termination, may push the quality of service provided by cleaners towards higher levels of cooperation (for models addressing this issue, see Hamilton & Taborsky 2005; Cant & Johnstone 2009; Akçay & Simms 2011). More generally, given that there are so many possible mechanisms of partner control, we suggest that it is only by studying the impact of each on the level of cooperation vs. exploitation, that empirical studies can determine which forms of partner control are of real biological significance.

Pinstripe wrasses *H. melanurus* were not capable of feeding against their preference in order to maximize their food intake and none of the treatments appeared to influence their foraging behaviour. This non-cleaner species eats small invertebrates off substrates (Myers 1991) and hence should not face foraging decisions applicable to our experiments in real life. The results involving *H. melanurus* show that the adjustment of foraging behaviour to the experimental treatment is not a trivial optimal foraging task that can be easily solved by any species. Instead, we conclude that the experiment can only be solved by species for which the problem is ecologically relevant. More specifically, the non-cleaners failed to show the ability to feed against their preference. We predict that cleaner wrasse *L. dimidiatus* are indeed specifically adapted to this problem because it is very likely to be rare in nature that an animal faces this problem. Even cleaning gobies can focus on eating preferred food as they prefer fish ectoparasites over mucus (Soares *et al.* 2010), which probably applies to most facultative cleaner fish species as well (Barbu *et al.* 2011). As it stands, the cleaner wrasse *L. dimidiatus* shows strategic abilities adapted to interactions with clients that are absent in primates (Salwiczek *et al.* 2012), despite the general importance of cooperation in primates' social life (de Waal & Suchak 2010; Jaeggi *et al.* 2010). As the experiments involving plates and food represent learning tasks, the cleaner wrasse's superior performances are linked to adaptations in the cognitive machinery, as predicted by the ecological approach to cognition (Kamil 1998; Shettleworth 2010).

In conclusion, in interactions in which the payoffs are a positive function of duration, cooperative outcomes may be achieved in cases in which only one player may cheat, as predicted by game theoretic models (Johnstone & Bshary 2002; Bowles & Hammerstein 2003; Bshary *et al.* 2008). The victim's control over early termination of the interaction and low temptation to cheat combine to affect the potential cheater's level of cooperation. Despite the apparent simplicity of the concept, it appears that stable cooperation still depends on partners being adapted to the specifics of the game structure.

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Figures

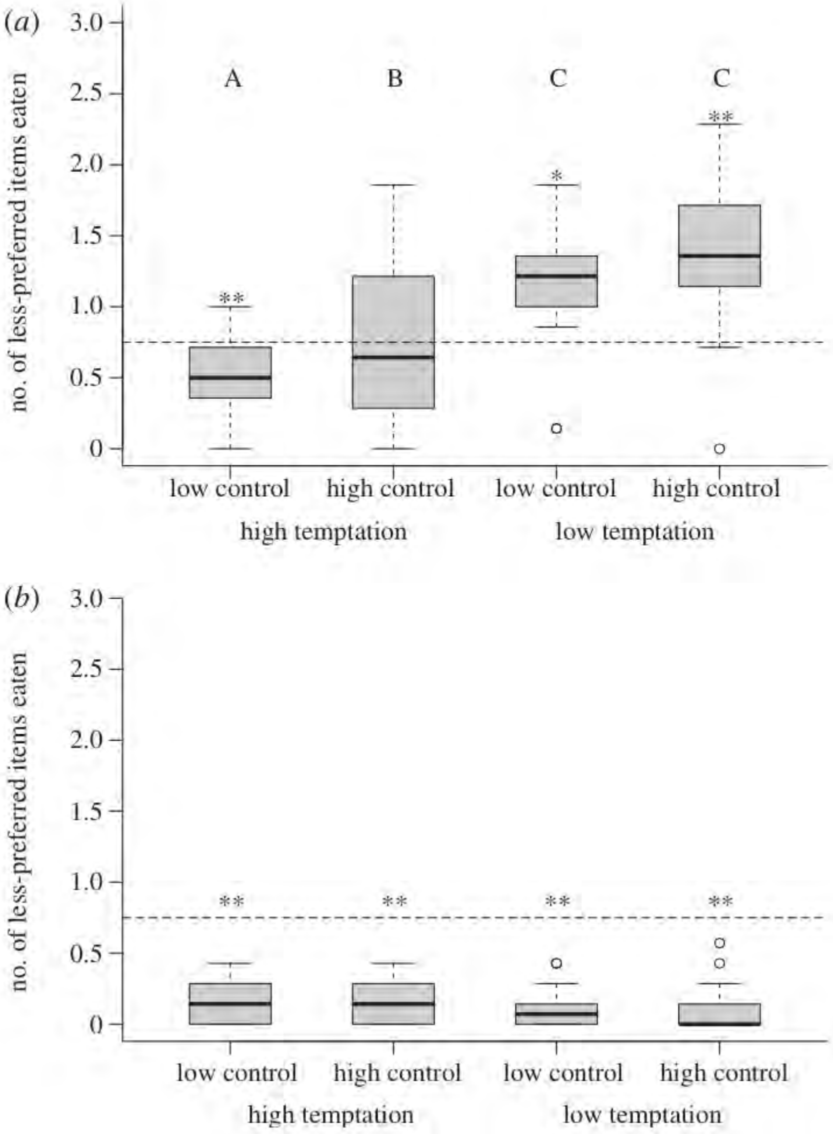

Figure 1: Box-and-whisker plots of mean number of less-preferred items eaten per round by a) 16 cleaner wrasse *Labroides dimidiatus* and b) 14 non-cleaner pinstripe wrasse *Halichoeres melanurus* in four different treatment combinations. Temptation to cheat was either 'low' because of a large proportion of the preferred food type in the less-preferred food or 'high' because of a smaller proportion of the preferred food type in the less-preferred food, and after a fish ate a preferred food item the plate was removed either quickly (no more feeding possible = high victim control over duration) or slowly (some more feeding possible = low victim control over duration). For *L. dimidiatus*, letters indicate significant differences among treatment groups. There were no significant differences between treatments for *H. melanurus*. The asterisks indicate significant differences with the value expected from random foraging (dashed line, calculated by assuming that at each bite, the probability of eating a less-preferred item corresponds to its relative abundance). One asterisk indicates a p-value between 0.05 and 0.01, two asterisks indicate a p-value lower than 0.01. Outliers are represented as circles.

CHAPTER 2 - The cleaner wrasse outperforms other labrids in ecologically relevant contexts, but not in spatial discrimination

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Abstract

The ecological approach to cognition provides a clear prediction regarding cognitive performance: performance should be higher in contexts that are ecologically valid than in invalid contexts. Here, we tested this prediction by comparing juvenile and adult cleaner wrasse, *Labroides dimidiatus*, with juveniles and adults of five related labrid species. Only the former fully depend on interactions with a large variety of so-called 'client reef fish' for their diet, which involves feeding largely on ectoparasites rather than on preferred client mucus. Our results show that cleaners outperform the other species tested in two contexts that are tightly linked to cognitive challenges during cleaning interactions: the willingness to explore novel objects and the ability to feed against preference in order to increase energy intake. In contrast, all species performed similarly in a spatial discrimination task, which was chosen for its limited ecological relevance to any of the species tested. In conclusion, the cognitive abilities of cleaners seem to be tightly linked to the domain-specific challenges they face in nature. We found no support for the alternative hypothesis that selection for social competence in cleaning interactions leads to domain-general cognitive abilities that also enhance performance in unrelated contexts.

Contributions: SG collected all the data. SG & RB jointly wrote the manuscript.

Introduction

Broadly defined, cognition refers to the mechanisms by which animals acquire, process, store and act on information from the environment (Shettleworth 2010). The ecological approach to cognition emphasizes ecological validity as a main predictor of a species' performance in a given task, independent of the underlying cognitive mechanism (Shettleworth 2010). This approach is particularly suitable for explaining why various complex behaviours and cognitive traits such as tool use, self-recognition and many of the features associated with the demands of social living appear to have evolved independently in distant clades. Furthermore, it explains why species with less complex central nervous systems may perform better than species with more complex central nervous systems, including humans, given tasks that are ecologically relevant only to the former. For instance, it has been shown that bees are capable of quicker conceptual learning than primates and infants (Avarguès-Weber *et al.* 2011; Chittka & Jensen 2011), that pigeons, *Columba livia*, outperformed humans in a probability puzzle (Herbranson & Schroeder 2010) and that the bluestreak cleaner wrasse, *Labroides dimidiatus*, outperformed primates in a foraging task relevant only to the cleaners' ecology (Salwiczek *et al.* 2012). Unsurprisingly, an increasing number of studies suggest that phylogenetic relatedness is often a poor predictor of related abilities when it comes to cognition. For example, food hoarding in birds correlates with hippocampal volume better than phylogenetic relationships do (Krebs *et al.* 1989; Sherry *et al.* 1989), although the exact nature of the relationship is still debated (Healy *et al.* 2005). In primates too, social ecology appears to explain the evolution of brain size better than phylogeny (Barton 1996; Dunbar & Shultz 2007). Furthermore, it was recently proposed that high general intelligence has independently evolved at least four times within primates alone (Reader *et al.* 2011). Cognitive skills thus appear to emerge in order to respond to challenges present in nature, and cannot be predicted from phylogenetic relationships alone (Bolhuis & Wynne 2009).

The ecological approach has been highly successful in yielding examples of high performance linked to ecological pressures in various taxa, such as in birds (Kamil & Balda 1990), mammals (Gaulin & Fitzgerald 1989) or fish (White & Brown 2015). However, it has so far contributed little to the long-standing question of the degree to which cognition is domain specific (modular) or driven by a general-purpose machine (Samuels 1998; Magphail & Bolhuis 2001; Heyes 2003; Healy *et al.* 2005; MacLean *et al.* 2012; van Schaik *et al.* 2012; Krause 2015). It has often been argued that forms of general intelligence (i.e. intelligence in which performance correlates across very different cognitive domains) require the presence of a large brain, while domain-specific abilities can be wired in small brains, and may be based on 'simpler' cognitive processes (van Schaik et al., 2012). A key control in this context is to test species not only in a context in which the ecological approach predicts high performance but also in a context where the ecological approach predicts 'low' or 'average' performance (Shettleworth 2010). To assess whether a particular aspect of a species' ecology relates to its cognitive performance, it is crucial to compare species that are similar on as many levels as possible (e.g. habitat, ancestry, size, diet), yet differ for the trait of interest. The best and most studied example supporting the ecological approach is the link between spatial memory abilities and food caching in birds. In this system, the ecology of the species (dependency on food caching) appears to be a very good predictor of performance in spatial memory tasks in the laboratory (Balda & Kamil 1989; Kamil & Balda 1990; Kamil *et al.* 1994), and also appears to correlate with hippocampus size (Sherry *et al.* 1989). To our knowledge, only a few studies have explicitly tested a control condition in the form of a task for which high performance would not be predicted by the ecology of the species, and thus allow us to distinguish between general and specific cognitive abilities (Olson *et al.* 1995; Pravosudov & Clayton 2002). Thus, we see a need to expand proper testing of the ecological approach to other taxa and other contexts. Here, we used a comparative approach to investigate the degree to which the cleaner wrasse, a fish that is renowned for demonstrating complex decision-making skills in social interactions, may excel compared to closely related species in either ecologically relevant or irrelevant tasks.

The bluestreak cleaner wrasse (hereafter 'cleaner') is a small coral reef fish that interacts with dozens of other species (called 'clients'), including predatory fishes, having more than 2000 interactions per day in which it removes ectoparasites from the clients (Grutter 1996). Conflicts of interest between cleaner and client arise because cleaners prefer to eat the protective client mucus over ectoparasites, where consumption of the former constitutes cheating (Grutter & Bshary 2003). As a consequence, clients use various partner control mechanisms such as partner switching, punishment, premature termination and image scoring in order to promote cooperative behaviour in cleaners (i.e. feed against their preference and focus on ectoparasites as opposed to mucus; (Bshary 2011). In response to these client control mechanisms, cleaners have evolved the ability to fine-tune service quality to the specific dynamics of each interaction: the quality of a client as a food patch (i.e. the potential benefits of interacting with it), the client's strategic options, the presence of bystanders, and the co-inspection with partners as well as internal states are all taken into consideration when cleaners determine whether to cheat or not (Bshary 2011; Pinto *et al.* 2011; Raihani *et al.* 2012; Gingins *et al.* 2013; Soares *et al.* 2014). While food acquisition in cleaners leads to a sophisticated interspecific social life, it does not require environmental cognitive abilities such as advanced spatial memory. This is because the cleaners' food patches actively visit them when they are replenished with ectoparasites. We thus expect cleaners to face the same spatial memory challenges as other fishes that share the same habitat (i.e. navigating within their territory on the reef). Three of the five other wrasse species tested here are facultative cleaners. As facultative cleaners do not rely solely on cleaning for their nutritive intake, only some individuals engage in cleaning behaviour, and those that do evidence less sophisticated strategies than obligate cleaners such as *L. dimidiatus* (Côté 2000; Barbu *et al.* 2011). Whether facultative cleaners prefer mucus like *L. dimidiatus* (Grutter & Bshary 2003) or ectoparasites like Caribbean cleaning gobies is unknown (Soares *et al.* 2010). However, field observations from the Red Sea support the idea that they may prefer ectoparasites: client jolt rate, a correlate of cleaner cheating (Bshary & Grutter 2002) is lower in interactions with facultative cleaners than with the obligate cleaner *L. dimidiatus* (Barbu *et al.* 2011). These results, together with the fact that facultative cleaners mainly feed on prey other than ectoparasites, suggest that feeding against preference is of little or no ecological relevance for facultative cleaners. We therefore expect feeding against preference to be of no ecological relevance for noncleaning wrasse species.

Based on the specific aspects of *L. dimidiatus* ecology, we decided to test their performance relative to other wrasses in three different paradigms. First, we investigated whether their high levels of interactions with many client species, including predators, select for increased levels of exploration. While exploration is not as such a cognitive trait, it has been documented repeatedly that exploration or neophobia may have an important impact on cognitive performance especially within species (Dugatkin & Alfieri 2003; Sneddon 2003; Boogert *et al.* 2006; Guillette *et al.* 2009; Carazo *et al.* 2014; Trompf & Brown 2014), and between species (Webster & Lefebvre 2001; Day *et al.* 2003). Second, we investigated whether, due to the conflict of interest with clients, cleaners are more able to feed against their preference than other wrasses that do not face this problem in their foraging behaviour. This experiment expands on a previous study that compared cleaners with one other wrasse species which found that feeding against preference represents a major challenge for noncleaner and facultative cleaner species alike (Gingins *et al.* 2013). On the cognitive level, this task has all the features of a self-control task, in which the subject has to choose between two options and can access the more valuable reward for a certain cost such as a time delay or larger effort (Beran 2015). Finally, we tested all our study species in a spatial discrimination task, which does not appear to be of particular ecological relevance to any of them. Rather than calculating correlations of individual performances across tasks as is often done in within-species comparisons (Isden *et al.* 2013; Shaw *et al.* 2015), we asked whether performance differed between species in some tasks but not in others. More specifically, if the cognitive abilities of cleaner wrasse are domain specific and tightly linked to their ecological needs, we expected that they should not learn to solve the spatial discrimination task faster than the other species. In contrast, if the interactions with clients have led to the evolution of more domain-general cognitive abilities, then we expected that cleaners would learn faster than the other species even in this ecologically nonrelevant task. Domain-general abilities could, for example, indicate a general ability to learn faster based on associations, or a more general understanding of the existence of food patch variation could allow cleaners to readily use location as an alternative cue to client identity. For the comparison between facultative cleaners and noncleaners, we were interested in whether our data would support the notion that cleaning interactions per se may enhance cognitive performance, or whether facultative cleaning is not important enough to drive the acquisition of specific cognitive skills. For the former hypothesis we expected facultative cleaners to show levels of performance that are intermediate between the noncleaners and the obligate cleaner. Alternatively, we predicted that facultative cleaners should not perform better than noncleaners. Finally, most facultative cleaning is performed at the juvenile stage (Côté 2000; Barbu *et al.* 2011), and hence there is a possibility that juveniles of obligate and facultative cleaners express similar cognitive capabilities, which could diverge only in the later stages of life. To investigate such potential ontogenetic effects, both juveniles and adults of each species were tested in this study.

Methods

Study species

Six species of wrasses (Labridae) were involved in this study (Fig. 1): *Labroides dimidiatus*, *Pseudocheilinus hexataenia*, *Halichoeres melanurus*, *Thalassoma lunare*, *Hemigymnus melapterus* and *Labrichthys unilineatus*. Species were selected because they (1) represented the trophic diversity present in the Labridae, (2) were spread across the Labridae phylogenetic tree, (3) represented differences in cleaning activity (obligate cleaners, facultative cleaners and noncleaners) and (4) shared the same habitat. Classification as juvenile or adult was done according to morphological criteria for each species (see Fig. 1). However, *P. hexataenia* does not show any notable difference between juveniles and adults, and therefore a size rule was applied for this species (juveniles: < 4.5 cm; adults: >5.0 cm), which corresponds to adults being at least two-thirds of their maximum size according to Allen (Allen *et al.* 2005).

Capture and housing

All fish were caught on the reefs surrounding Lizard Island, Australia (14°40'S 145°28'E), either by herding them towards a barrier net with small hand nets or by using clove oil to sedate them when hidden inside the reef matrix. The animals were transported immediately after capture to the Lizard Island Research Station and held in tanks with constant flow-through sea water. Because of space restrictions, some individuals were first held in small groups in holding tanks before being kept solitarily in the experimental tanks, which were plastic or glass tanks of different sizes according to the fish body length (minimum 29x37 cm and 30 cm deep, maximum 38x90 cm and 38 cm deep). In the experimental tank, they were all given at least 3 days of acclimation before any experiment was undertaken. All fish were fed daily on a diet of mashed prawns smeared on a grey Plexiglas plate (ca. 8x8 cm) with a blue portion at the top (Fig. 2a), and a PVC tube served as shelter. The plate was left in their holding tank all day and refilled when found empty. A total of 188 wrasses (97 adults, 91 juveniles) were involved in this study. Experiments were conducted over four field trips of 2 months each, between August 2012 and October 2014. Three trips took place during the Australian winter (July–September) and one during summer (January–February). All fish were first tested for their reaction towards novel objects. Ninety-one of them (48 adults, 43 juveniles) were subsequently tested in the feeding against preference experiment, and 88 (45 adults, 43 juveniles) in the spatial discrimination task (see details below). After the novel object tests, nine individuals could not be used in further experiments for various reasons (not participative in the experiments, infection or death). Fish were returned to the reef after completion of the experiments. All experiments were carried out in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes, and under the approval of a Queensland Government (Australia) Animal Ethics Committee (Proposal Reference Number CA 2012/05/611).

Exploration: Reaction towards novel objects

Novel object tests were performed as soon as the fish was well acclimated to the experimental environment, i.e. on the day after being witnessed approaching the food plate within 5 cm in less than 10 s and feeding in less than 20 s. Novel object 1 was a 1 cm diameter black circle which was added to the centre of their feeding plate (Fig. 2b). The usual food (mashed prawns) was placed inside the circle. The plate was then gently placed inside the experimental tank and the fish's reaction was observed for a maximum of 5 min. Time to approach within 5 cm and time to feed off the plate were recorded with a stopwatch. No fish had previously been fed on the day of the experiment. All fish were given a full day of rest and then tested with a second novel object. Novel object 2 was a much larger white plate (16 x 8 cm), and hence looked very different from the feeding plate (Fig. 2c). Reaction towards this object was measured in the exact same way as for the first novel object.

Original food preference

In the feeding against preference task described in the next section, fish were tested for their ability to feed selectively on one type of food in order to maximize their intake: eating the less preferred food type allowed them to continue foraging while eating the preferred one led to the removal of the feeding plate. Every fish was thus first tested for its food preference. While *L. dimidiatus* was known to consistently prefer pure prawn over fish flakes mixed with prawn (Bshary & Grutter 2005), not all other species had the same preference and there was often variation between individuals of the same species. As a consequence, we had to resort to using several combinations of food types. Food types all consisted of different mixtures and concentrations of mashed prawns, flakes (red or green) and/or sand. During the acclimation period, all fish were tested for their food preference using the following protocol. (1) Fourteen items of food (seven of one type and seven of a second type) were randomly placed on a plate (Fig. 2f). (2) The plate was presented to the subject and the first seven items it ate were recorded (the plate was left in the tank until it ate all the items or no longer showed interest in the feeding plate). (3) The subject was considered to have reached a preference for one type of food when, in six subsequent trials, the fish consistently ate more than 50% (i.e. at least four items out of the first seven items) of this type. (4) If no preference was reached after 20 trials using the same combination of food types, the individual was considered to have no preference (*N* = 9) and the type of food it ate the most was used as the 'preferred' type in the subsequent experiments. When the food preference was determined for all fishes, the experiment began.

Feeding against preference

A maximum of 12 fish were tested in parallel over 3-day periods using the protocol described below. All the other fish waiting to be tested were left largely undisturbed and fed on mashed prawns. Since not all subjects could be tested simultaneously, there was variation in the time between the original food preference tests (described in the previous section) and the start of the experiment (mean = 13 days; maximum = 27 days). Therefore, we measured fishes' food preference again on day 1 of the experiment, and used these data as the baseline preference for the statistical analyses. To calculate initial percentage of preferred items consumed, a first rehabituation trial was followed by three trials for which we recorded the first seven items eaten. Seventy individuals, including all the individuals of *L. dimidiatus*, consumed more than 50% of the originally preferred food type (as determined with the procedure described in the previous section) during these three trials. Twenty-one individuals did not show consistency regarding their original preference, i.e. they ate more of their originally less preferred food type. These 21 individuals were given an additional three trials. Fifteen of these fish eventually ate overall more than 50% of initially preferred items when exposed to an additional three trials. In contrast, six individuals had apparently changed their food preference between the original tests (see previous section) and the beginning of the experiment (this section). Therefore, 85 individuals were tested with the same preferred and less preferred food types as determined earlier, while for the remaining six fishes, we exchanged the two types (i.e. the preferred type became the less preferred one, and vice versa). For these six fishes, the time between the original food tests and the experiment ranged from 12 to 27 days. The inconsistencies we encountered in some individuals might reflect a weak food preference (or no preference at all). For these fishes, we argue that the task would be easier because they only have to learn to feed preferentially on one food type to access more items, and do not actually need to go against their preference. None of the inconsistent individuals belonged to *L. dimidiatus*, and the results indicate that weak or inconsistent preferences in the other species did not facilitate learning in our experiment.

On day 2, all fish were subjected to six learning trials. Learning trials were designed to give the fish the opportunity to learn the negative consequences of feeding according to their preference. The plate (Fig. 2g) was covered with 13 items of the less preferred food type and two items of the preferred one, randomly placed. Each fish was allowed to eat less preferred items, but the plate was immediately removed after the fish ate one of the two preferred items. The plate was kept out of the water for 60 s, and then placed back until the second of the preferred items was eaten. For the experiment it was crucial that each fish experienced the consequences of eating both types of food. Therefore, if an individual ate none or only one of the less preferred items during all six trials (three adults and six juveniles, all belonging to species other than *L. dimidiatus*), the plate was placed back in the tank at the end of the last learning trial, i.e. without any preferred items, and the fish was left some time to experience the consequence that eating the less preferred food type did not lead to the removal of the plate. When presented again with the food plate, all nine individuals ate at least eight of the 13 less preferred food items.

On day 3, subjects experienced 10 experimental trials: three items of each food type were placed on the plate in a random order, inside a grid on the top right corner of the plate (Fig. 2g). As in the learning trials, the plate was removed as soon as the fish ate a preferred item; only it was not presented again in this case. The number of less preferred items eaten prior to a preferred one was recorded. The interval between trials was always at least 15 min during this experiment.

Spatial discrimination

In this task, fish were tested for their ability to associate a food source with a location. Subjects were presented simultaneously with two identical Plexiglas plates (Fig. 2d, e), placed next to each other approximately 10 cm apart. Between the two plates, a vertical Plexiglas partition was placed to prevent the fish from easily moving from one plate to the other. The fish was kept behind an opaque partition (placed at about a third of the tank length on the opposite side of the experimental set-up) between the trials and was given full access to the tank only during the trials. One of the two plates had accessible food (mashed prawns) at the back while the other had the same amount of food in an inaccessible place (Fig. 2e). Therefore, both plates smelled of prawn, preventing subjects using smell as a cue. For every fish, the location (left or right) of the plate with food was constant throughout all the trials. Before the trials started, the fish was first presented with the set-up and food on both sides. It was observed for a maximum of 15 min and the first plate it chose was recorded. The fish was considered to have chosen a plate when its nose went past said plate (i.e. as soon as it tried to go behind it where the food reward was located). If after 15 min the fish still had not approached the plates, its PVC shelter was removed and the fish gently pushed towards the set-up until it went to one of the plates, and then given additional time to habituate to the set-up. This first trial was useful both as habituation to the experimental set-up and to allow us to determine the side (left or right) that would offer food in all subsequent trials. That is, food was placed on the initially neglected side. The logic behind this rule was that the initial choice might potentially be indicative of a lateralization or side bias. Thus, by baiting the other plate we reduced the risk that a significant preference for the food-offering plate reflected a bias rather than evidence for learning. The fish was considered to have solved the task when it chose the correct plate (the one with food on it) at least nine times in 10 subsequent trials, or twice eight times out of 10 subsequent trials in a row. The maximum number of trials was set to 60, and the task was considered not solved if the fish had not reached the criterion within the 60 trials.

On the days fish were to be tested, they were all first placed in the holding compartment and allowed at least 30 min to acclimate before the first trial of the day. They experienced up to 60 trials (depending on their ability to solve the task) over a maximum of 6 subsequent days. Every trial was conducted as follows: (1) the transparent partition and the two plates were placed on the side of the tank opposite to the holding compartment, (2) the opaque partition was lifted to allow the subject full access to the tank and the plates, (3) as soon as the fish chose a plate, the other one was removed to prevent the fish from being able to access both plates in one trial and (4) the fish was placed back in the holding compartment until the next trial (minimum 15 min later). Some fish required more trials than others to fully habituate to the set-up. The trial was considered null (not taken into account) if the fish did not choose a plate within 5 min. An individual was removed from the subject pool if it never approached the plates within the first 20 trials, which was the case for 11 individuals. In total, 77 wrasses (41 adults and 36 juveniles) from six different species participated in the experiment.

Data analysis

The response variable in the reaction towards novel objects experiment is an ordinal variable (number of seconds), which is right censored (we stopped after 300 s). We analysed this type of data with a survival analysis. The proportional hazard assumption, required to perform parametric tests, was not fulfilled and thus we used the nonparametric log-rank test. This test does not support multiple response variables (in our case: species and status) and we therefore used a series of log-rank tests to investigate the overall effect of species and pairwise differences across species (juveniles and adults pooled). In a separate analysis, we investigated differences between juveniles and adults within each species. *P* values were adjusted for multiple comparisons.

In the feeding against preference experiment, we wanted to document the change in foraging behaviour after the learning phase. Therefore, for each fish, we calculated the proportion of preferred items eaten over the total during the experimental tests (day 3), as well as the predicted values based of their baseline preference (day 1). The way we calculated the predicted values is described in the supplementary material. The difference between the predicted and the observed values was used in the analysis. Differences across species and between juveniles and adults were investigated through a generalized least squares (GLS) model fitted by restricted maximum likelihood (REML).

Data on spatial discrimination follows the same pattern as the data on novel objects (number of trials, right censored after 60 trials). In this case, however, the proportional hazard assumption was fulfilled, and thus we used a Cox proportional hazard (CoxPH) test. In contrast to the analysis of reaction towards novel objects, we could build a single model in order to detect differences in ability to solve the spatial discrimination task across species and status (juvenile or adult). All statistics were performed with R 3.0.2 (R Core Team 2013). *P* values were adjusted for multiple comparisons using Holm's sequentially rejective multiple test procedure (Holm 1979). The R package 'survival' was used for the two survival analyses and the package 'nlme' (Pinheiro *et al.* 2013) for the GLS analysis.

Results

Exploration: Reaction towards novel objects

Species differed significantly in time to approach novel object 1 (log-rank test: $\chi^2_5 = 71.9$, $P < 0.001$; Fig. 3a). *Labroides dimidiatus* typically approached the first novel object within seconds, and significantly faster than all five related species tested (log-rank tests: all adjusted $P < 0.001$; see Appendix 1 for details on all pairwise comparisons). Within every species, juveniles and adults did not differ significantly in latency to approach the first novel object (log-rank tests: all adjusted $P > 0.15$; see Appendix 1 for details). Species differed significantly regarding the time to feed from novel object 1 (log-rank test: $\chi^2_5 = 11.4$, $P = 0.04$). However, all pairwise comparisons showed nonsignificant differences between species (log-rank tests: all adjusted $P > 0.2$; see Appendix 1 for details). Even though the analysis failed to detect significant differences, it is worth noting that *L. dimidiatus* was the fastest species to feed from novel object 1 (Fig. 3b). No significant differences in latency to feed from novel object 1 appeared between juveniles and adults (log-rank tests: all adjusted $P = 1$; see Appendix 1 for details).

Novel object 2, which looked notably different from the feeding plate (see Fig. 2), appeared to challenge some species more than others (Fig. 3c, d), and we did not observe the same pattern as with novel object 1. Species differed significantly both in time to approach (log-rank test: $\chi^2_5 = 58.6$, $P < 0.001$) and in time to feed (log-rank test: $\chi^2_5 = 61.9$, $P < 0.001$) when confronted with novel object 2. As with the first novel object, *L. dimidiatus* approached this second object significantly faster than all other species (log-rank tests: all adjusted $P < 0.04$; see Appendix 1 for details). It was also the fastest species to feed off the second novel object (log-rank tests: all adjusted $P < 0.025$; see Appendix 1 for details). We also found additional variation between species in time to approach and time to feed off novel object 2 (see Appendix 1). With novel object 2 we generally observed that adults approached and fed faster than juveniles. Juveniles from both *L. dimidiatus* and *T. lunare* were significantly slower than adults to approach or feed from the second novel object (log-rank tests: all adjusted $P \leq 0.002$; see Appendix 1 for details). For *H. melanurus* and *H. melapterus*, this effect is significant only for the time to approach (log-rank tests: *H. melanurus*: $\chi^2_1 = 8.99$, adjusted $P = 0.035$; *H. melapterus*: $\chi^2_1 = 8.09$, adjusted $P = 0.049$).

Feeding against preference

In the feeding against preference experiment, *L. dimidiatus* appeared to be the only species able to adjust its behaviour to the experimental conditions (Fig. 4). Our analysis showed a strong effect of species when *L. dimidiatus* was included in the model (GLS fitted by REML: $F_5 = 4.73$, $P = 0.0008$), which disappeared once we excluded this species from the model (GLS fitted by REML: $F_4 = 1.17$, $P = 0.33$). It thus appears that *L. dimidiatus* was the only species standing out in this task, with all five other species performing similarly. Even though the status of the fish (juvenile or adult) did not appear to have an effect at first (GLS fitted by REML: $F_1 = 2.11$, $P = 0.15$), we detected an interaction between species and status (GLS fitted by REML: $F_5 = 4.58$, $P = 0.001$). Post hoc comparisons showed a significant difference between juvenile and adult *H. melanurus* (test for general linear hypotheses: $z = -4.00$, adjusted $P < 0.001$). All other species showed no significant difference between adults and juveniles (all adjusted $P > 0.54$).

Spatial discrimination

The spatial discrimination task appeared to represent a similar cognitive challenge for all species involved in this study (Fig. 5; CoxPH: likelihood ratio test = 3.8, P = 0.7). Furthermore, juveniles and adults appeared to respond similarly to this challenge (CoxPH: P = 0.97).

Discussion

We investigated whether the bluestreak cleaner wrasse's cognition differed from that of related species in two ecologically relevant contexts (explorative behaviour and feeding against preference) and in one ecologically nonrelevant context (spatial discrimination). As predicted by the ecological approach to cognition, we found that cleaners were generally more explorative than five related species of noncleaner and facultative cleaner labrids, that they outperformed these same fishes in the feeding against preference experiment, and crucially that the cleaners' performance in the spatial discrimination task was very similar to that of the other species. Our study thus aligns with results on food caching in birds (Olson *et al.* 1995; Pravosudov & Clayton 2002), but it also goes further in that it provides a full test of the ecological approach to other species (fish) and contexts (social behaviour).

Our results indicate that the natural tendency of *L. dimidiatus* to seek out and interact with other fishes appears to translate into increased exploration of novel objects compared to species with lower or no dependency on cleaning. Behavioural traits such as exploration or boldness have been linked to performance in cognitive tasks in the past (Webster & Lefebvre 2001; Day *et al.* 2003; Dugatkin & Alfieri 2003; Sneddon 2003; Boogert *et al.* 2006; Guillette *et al.* 2009; Carazo *et al.* 2014; Trompf & Brown 2014). The choice of the cognitive task used to test this hypothesis is very important. In some of the studies cited above, fishes had to learn to wait at a specific location for a reward (Dugatkin & Alfieri 2003; Sneddon 2003), birds and monkeys were tested in a task that was increasingly likely to be solved with perseverance (Webster & Lefebvre 2001; Day *et al.* 2003; Boogert *et al.* 2006) and lizards were taught the location of a safe refuge via simulated predator attacks (Carazo *et al.* 2014). In these experiments, one could argue that the bolder or more explorative individuals are more likely to perform better only because they are more at ease with the experimental set-up. In our spatial discrimination experiment, each trial comes down to a binary choice (left or right). In this context, the more explorative individuals might make their choice faster than the others, but this should not affect the outcome of the decision. In the feeding against preference experiment, eating a preferred item led to the immediate removal of the plate, which tended to scare the fish away (S. Gingins & R. Bshary, personal observation). One could expect that the more explorative individuals might be less affected by plate retrieval and hence more likely to take a bite at these items. Our results indicate that this is not the case, since the more explorative species, *L. dimidiatus*, was also the one feeding the most against its preference after the learning trials. It thus appears that we can rule out explorative behaviour as the major cause for the different levels of performance in our cognitive tests, and hence that our results are best explained by differences in ecological demands. Indeed, *L. dimidiatus* was the only species able to adjust its behaviour in a feeding against preference paradigm, which was specifically aimed at mimicking cleaner–client interactions in the wild. This ability has previously been shown in cleaners (Bshary & Grutter 2005), and appeared to be absent in *H. melanurus* in a previous study (Gingins *et al.* 2013). Our study confirms that the ecology of cleaners requires specific cognitive skills that are apparently absent in closely related species. Finally, our results on spatial discrimination indicate that the performance of *L. dimidiatus* diminishes to normal outside of their domain of expertise, which further supports the ecological approach to cognition.

Interestingly, our results do not indicate any tendency for facultative cleaners to perform differently from noncleaners. Facultative cleaning thus does not appear to require particular skills in the cognitive domains tested in this study. Many of the strategic behaviours of *L. dimidiatus* revolve around the fact that they prefer to eat mucus over ectoparasites (Grutter & Bshary 2003), and hence accessing their preferred food has negative consequences (e.g. termination of interaction, punishment). In contrast, Caribbean cleaning gobies have the opposite preference (Soares *et al.* 2010) and apparently lack sophisticated strategies (Soares *et al.* 2008). Data and field observations on facultative cleaner wrasse (Barbu *et al.* 2011) suggest that at least the majority of facultative cleaners prefer ectoparasites to mucus and hence lack cognitive adaptations linked to the opposite preference, like the ability to refrain from eating a preferred food. Clearly, our tested facultative cleaners are not notably different from noncleaners in this respect. It thus seems that the cognitive skills of the obligate cleaner *L. dimidiatus* are linked to the specialization in cleaning, rather than a prerequisite for engaging in this behaviour.

We argue that previously recorded high cognitive performance in *L. dimidiatus* is tightly linked to the ecological demands on cleaners, such as biological markets (Bshary & Grutter 2002), image scoring (Bshary & Grutter 2006) or fine-tuning of cooperative behaviour (Gingins *et al.* 2013). Cleaners performed very well in these experiments, and we would argue that these tasks are more cognitively challenging than that used in this study (feeding against preference). For instance, in a biological markets task in which subjects have to learn to give priority to an ephemeral food source over a permanent one in order to double food intake, cleaners even outperform capuchins (*Cebus apella*), orang-utans (*Pongo spp.*) and chimpanzees (*Pan troglodytes*) (Salwiczek *et al.* 2012). In contrast, with respect to control experiments that allow distinguishing between domain-specific and domain-general cognitive abilities, we tested cleaners in an explicitly ecologically nonrelevant task for the first time. Thus, more such experiments are needed to determine whether these cognitive abilities are domain specific and hence tightly linked to their ecology, as suggested by our results.

The genus *Labroides*, which contains five species of obligate cleaners, diverged in the late Miocene (Cowman *et al.* 2009), less than 10 million years ago, roughly at the same time that the ancestors of humans and chimpanzees diverged (Pozzi *et al.* 2014). Our results indicate that other labrids do not share the same capabilities as cleaners. It thus seems that within this time frame, *L. dimidiatus* has acquired the cognitive toolbox necessary for cleaner–client interactions. Current theories on the evolution of cognition argue that the demands of sociality might select for increased cognitive abilities (Byrne & Whiten 1988; Whiten & Byrne 1997; Bshary 2011), and correspondingly for larger brains (Dunbar 1992; Dunbar & Shultz 2007; van Schaik *et al.* 2012). However, despite its sophisticated social decision-making rules, *L. dimidiatus* has a brain size relative to body size as predicted by the regression line for wrasses (Chojnacka *et al.* 2015). In the absence of more detailed information on brain structure, we note that at least a part of the cleaners' ability might be due to changes in their psychological attributes, without the need to increase brain complexity. For example, being more explorative may simply offer more opportunities to learn with operant conditioning. Similarly, the cleaners' superior ability to feed against their preference compared to other wrasse species might be based on selection acting on a psychological parameter, self-control, rather than and/or in addition to learning capacity (Wismer *et al.* 2014). For the vast majority of species, optimal foraging theory would predict that one should always eat preferred food (first) and focus on it if it is abundant enough (Charnov 1976). Recent comparative studies on self-control in animals indicate that absolute rather than relative brain size is a major predictor of performance in self-control tasks (MacLean *et al.* 2014; Stevens 2014). These studies argue that self-control could be linked to physiological demands (Stevens 2014) larger individuals have more energetic reserves and can thus afford to wait) or higher cognitive abilities linked to absolute brain size (MacLean *et al.* 2014). Even though it was not explicitly tested in our analysis, the performance of the six species tested in the feeding against preference task does not appear to follow the same pattern. The species that performed best, *L. dimidiatus*, is among the smallest species tested, and its absolute brain size is as expected for a wrasse of its body weight, and hence smaller than the brains of *T. lunare* and *H. melapterus* (Chojnacka *et al.* 2015). Therefore, in our study, differences in self-control abilities appear to match ecological demands rather than being scaled according to body mass or absolute brain volume.

While advanced partner choice abilities (where cleaners even outperform primates) appear to exist only in adult *L. dimidiatus* (Salwiczek *et al.* 2012), we found only marginal differences between juveniles and adults in the current study, suggesting that ontogenetic effects are minimal (see also Wismer *et al.* 2014). Except for juveniles' tendency to approach and feed from novel objects later than adults, our results do not indicate major shifts through ontogeny for the cognitive domains tested here. A recent phylogenetic analysis of cleaning within wrasses suggests that evolutionary transitions to obligate cleaning occur from a juvenile cleaning state (Baliga & Law 2015), and the authors hypothesized that it evolved via a heterochronic process, i.e. adults maintain features of juveniles to continue cleaning. In our study, juvenile facultative cleaners performed similarly to juvenile noncleaners, while juvenile obligate cleaners stood out in both ecologically relevant tasks (novel objects and feeding against preference). It thus seems that obligate cleaners already differ from facultative cleaners at the juvenile stage. Therefore, the heterochrony hypothesis for the evolution of cleaning does not seem applicable to the cognitive domains tested here. For *L. dimidiatus*, the minor differences between juveniles and adults suggest that both their ability for self-control and their propensity to explore novel situations are species characteristics. The mechanisms behind these psychological attributes are most likely to be neuronal. For instance, arginine vasotocin (AVT) was shown to decrease most interspecific cleaning activities (Soares *et al.* 2012), and cleaners have smaller and/or less numerous AVT-immunoreactive neurons than the closely related species *L. unilineatus* (Mendonça *et al.* 2013). With fewer or smaller neurons sensitive to AVT, cleaners might be less restricted to approach and interact with client fishes, and also approach novel objects faster in the laboratory, as shown in our tests. Differences in neuronal phenotype could thus give cleaners predispositions to perform better in cleaning-like situations than related species.

The modular view of cognition is often criticized as oversimplified (Fuster 2000; Bressler & Menon 2010; Lefebvre 2010), based on the arguments that the cognitive processes involved in domains such as innovation, tool use or tactical deception require multiple cognitive domains to work in concert (Lefebvre 2010) and that cognitive processes in general often result from interactions of distributed brain areas (Bressler & Menon 2010). According to this view, selection is expected to work on cognition as a whole rather than on specific modules. However, this approach fails to explain why various complex behaviours and cognitive traits appear to have evolved independently in distant clades and why species with less complex central nervous systems may perform better than species with more complex central nervous systems in specific tasks (see above). In contrast, the strength of the ecological approach lies in predicting in which domains cognitive skills should emerge and why, and it helps explain many of the observed patterns in the evolution of cognition that a strictly generalist approach fails to account for. Van Schaik et al. (2012) suggested that predominantly using domain-general abilities in a given context could lead to automatization and hence produce many of the features associated with modularity, a process called 'secondary modularization'. Lotem and Halpern (2012) suggested that learning and data acquisition mechanisms might be tuned together in order to facilitate effective learning using little memory and computation. This could translate into facilitated associative learning in ecologically relevant contexts, and also produce features associated with modularity. For instance, cleaners could associate a negative experience such as the departure of a client fish with their foraging behaviour more quickly than noncleaning species, which could explain the results we observed in the feeding experiment. The ideas of both secondary modularization and fine-tuned data acquisition mechanisms allow for the reconciliation of the generalist and the modular approaches, in that cognitive skills can emerge from a general-purpose brain to face specific challenges. The ecological approach focuses on the outcome and does not make predictions about the underlying mechanisms. Therefore, these recent conceptual developments can readily be integrated within the framework of the ecological approach, and still facilitate the generation of instructive and testable predictions about the distribution of cognitive skills in animals based on their ecology.

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Figures

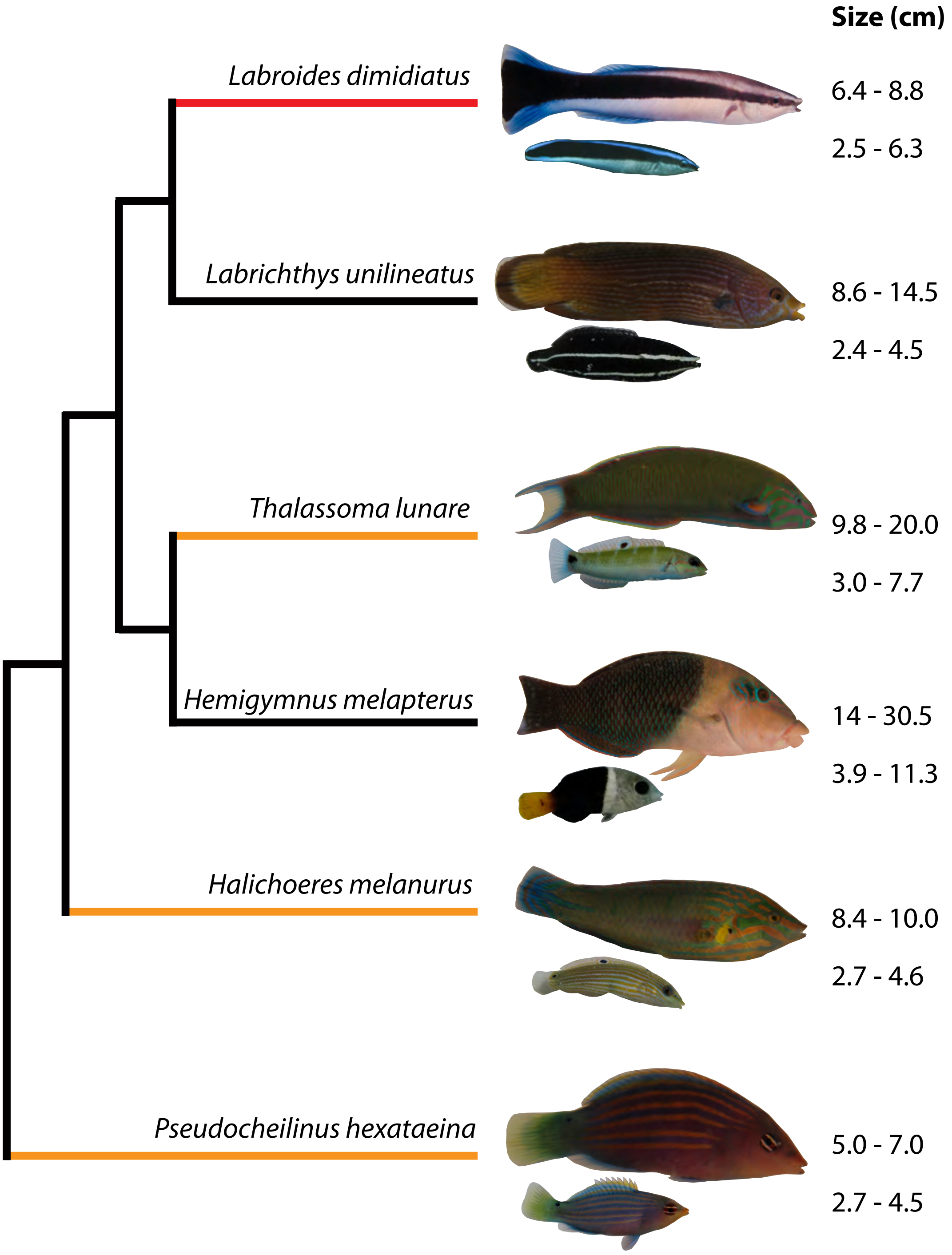


Figure 1. Schematic representation (i.e. branch lengths are not scaled) of the phylogenetic relationships between study species, based on the phylogeny of Cowmann and Bellwood (2011). The colour of the branch indicates dependency on cleaning: red = obligate cleaner; orange = facultative cleaner; black = noncleaner. The total length range for the fishes used is given for both juveniles and adults. Pictures are not reproduced to scale.

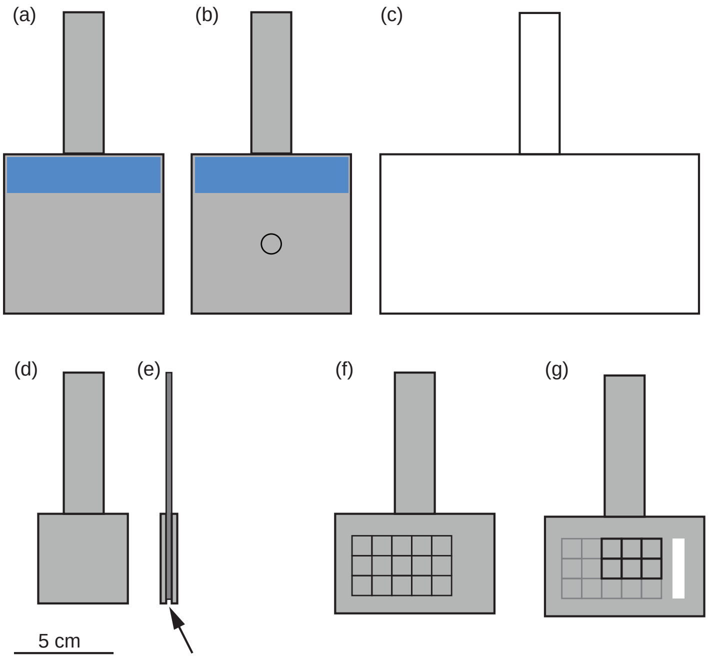


Figure 2. Plexiglas plates used for the different experiments. (a) Normal feeding plate. (b) Novel object 1. (c) Novel object 2. (d) Front view of the plates used for the spatial discrimination experiment. (e) Side view of the plates used for the spatial discrimination experiment. To control for odour cues, a piece of food was placed on each plate in a small interstice (arrow) that prevented the fish accessing it. (f) Plate used for determining initial food preference. (g) Plate used for the feeding against preference experiment: the highlighted part at the top right-hand corner of the plate shows the portion used in the experimental tests when only six items were presented, whereas in the learning phase items were laid in all 15 squares. The white bar on the right was added in order to help the fish understand that this second plate goes away as soon as a preferred item is eaten. All plates are drawn to scale. In the feeding against preference experiment, a larger version of the plates was used for fishes larger than 15 cm (all adult *H. melapterus* and some adult *T. lunare*), with 3x3 cm grid squares instead of 1x1 cm.

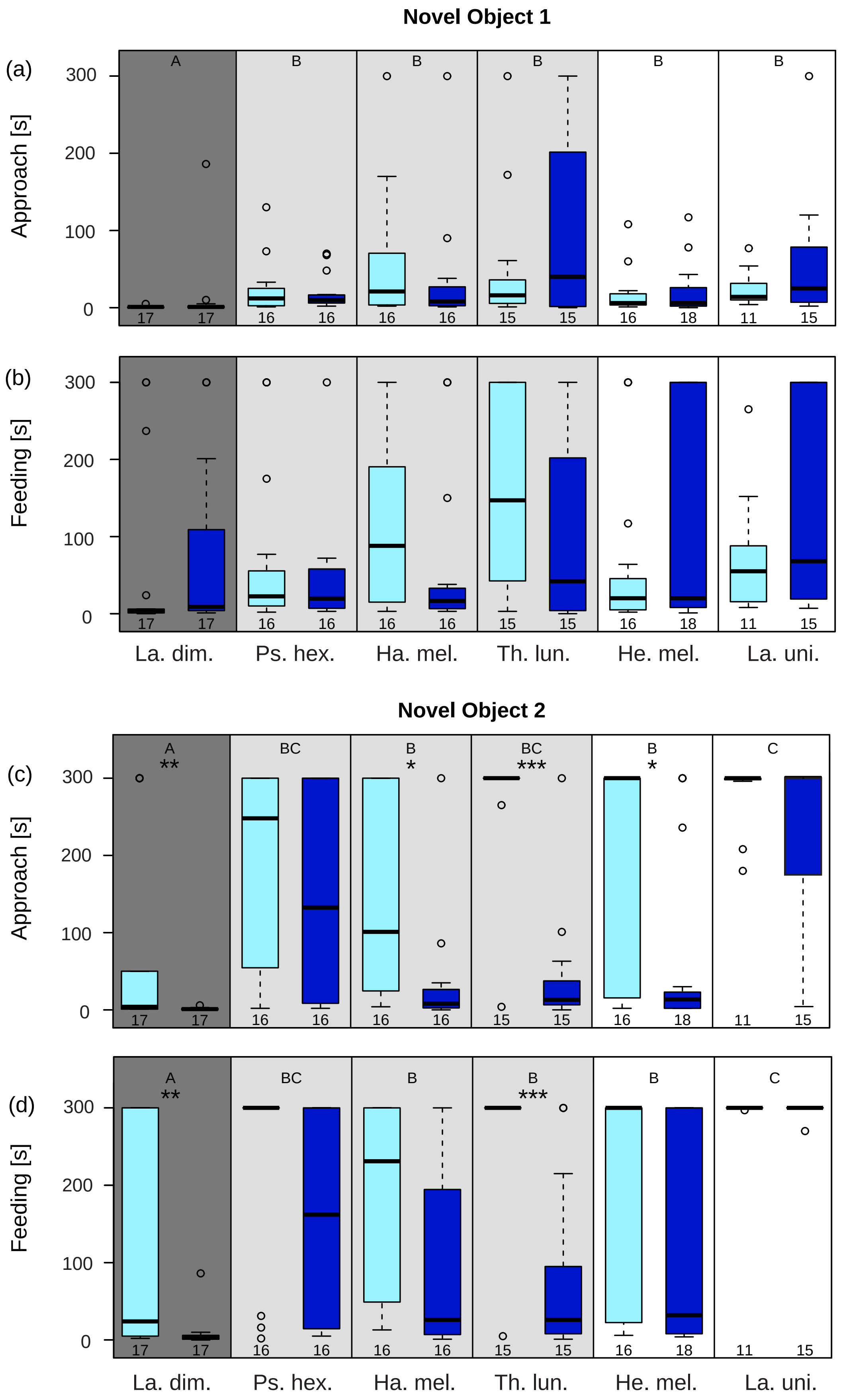


Figure 3. Box plots of (a, c) time to approach and (b, d) feed from novel objects for the six study species. Juveniles are represented in light blue, adults in dark blue. The dark grey background stands for obligate cleaner, the light grey background for facultative cleaners and the white one for noncleaners. Different

letters at the top indicate significant differences between species, and asterisks indicate significant differences between juveniles and adults of the same species: *$P<0.05$; **$P<0.01$; ***$P<0.001$. Sample size is given below every box. Each box covers the second and third quartiles, the black bar represents the median and the whiskers delimit 95% of the distribution. Outliers are represented as circles. Species names are abbreviated (see Fig. 1 for full names).

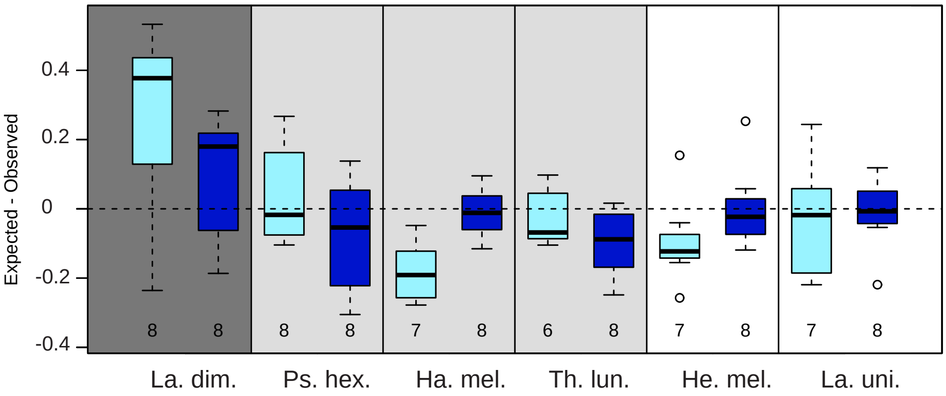


Figure 4. Box plots of the difference between the predicted and the observed ratio of preferred items in the feeding against preference experiment. Values above the dotted line indicate that the fish ate fewer preferred items after the learning sessions than predicted by their original preference. Juveniles are represented in light blue, adults in dark blue. The dark grey background stands for obligate cleaner, the light grey background for facultative cleaners and the white one for noncleaners. Sample size is given below every box. Each box covers the second and third quartiles, the black bar represents the median and the whiskers delimit 95% of the distribution. Outliers are represented as circles. Species names are abbreviated (see Fig. 1 for full names).

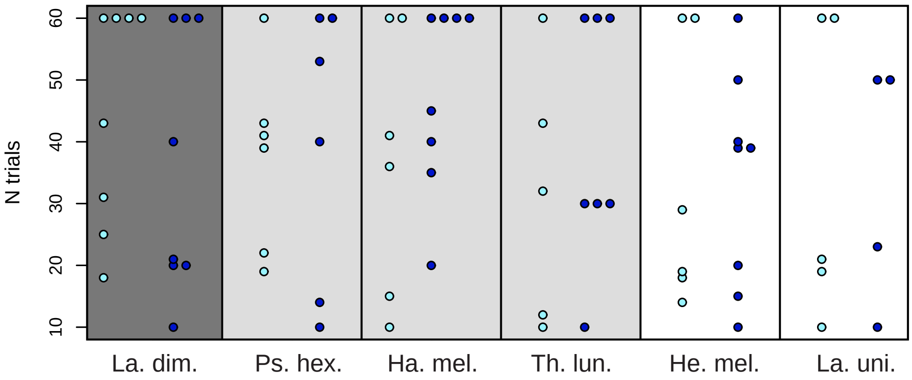


Figure 5. Number of trials required to complete the spatial discrimination task for the six study species. The maximum number of trials was set to 60; hence all individuals at the 60 mark did not solve the task. Juveniles are represented in light blue, adults in dark blue. The dark grey background stands for obligate cleaner, the light grey background for facultative cleaners and the white one for noncleaners. Species names are abbreviated (see Fig. 1 for full names).

Appendix

Comparison	NO 1: approach			NO 1: feeding			NO 2: approach			NO 2: feeding		
	χ^2	*P*	*P* adj.	χ^2	*P*	*P* adj.	χ^2	*P*	*P* adj.	χ^2	*P*	*P* adj.
La. dim–Ps. hex.	24.4	<0.001	<0.001	0.89	0.345	1	20.6	<0.001	<0.001	21.4	<0.001	<0.001
La. dim–Ha. mel.	26.0	<0.001	<0.001	2.55	0.111	1	8.62	0.003	0.040	9.55	0.002	0.024
La. dim–Th. lun.	28.1	<0.001	<0.001	5.82	0.016	0.318	18.26	<0.001	<0.001	14.6	<0.001	0.002
La. dim–He. mel.	17.8	<0.001	<0.001	2.43	0.119	1	13.0	<0.001	<0.001	14.4	<0.001	0.002
La. dim–La. uni.	28.7	<0.001	<0.001	4.66	0.031	0.588	32.5	<0.001	<0.001	39.9	<0.001	<0.001
Ps. hex–Ha. mel.	1.54	0.214	1	1.33	0.249	1	4.53	0.033	0.300	4.00	0.045	0.454
Ps. hex–Th. lun.	4.64	0.031	0.436	6.52	0.011	0.223	0.01	0.906	1	0.62	0.432	1
Ps. hex–He. mel.	0.51	0.475	1	0.29	0.588	1	1.63	0.202	1	1.68	0.194	1
Ps. hex–La. uni.	2.90	0.089	1	3.37	0.066	1	5.01	0.025	0.252	6.65	0.010	0.109
Ha. mel.–Th. lun.	1.17	0.279	1	1.62	0.203	1	4.19	0.041	0.325	1.53	0.217	1
Ha. mel.–He. mel.	2.44	0.118	1	0.10	0.753	1	0.46	0.499	1	0.42	0.513	1
Ha. mel.–La. uni.	0.15	0.700	1	0.43	0.513	1	18.77	<0.001	<0.001	21.8	<0.001	<0.001
Th. lun.–He. mel.	6.14	0.013	0.211	2.46	0.117	1	1.56	0.212	1	0.26	0.613	1
Th. lun.–La. uni.	0.65	0.421	1	0.61	0.435	1	4.09	0.043	0.325	11.2	<0.001	0.011
He. mel–La. uni.	5.12	0.024	0.35	0.955	0.328	1	10.7	0.001	0.015	14.4	<0.001	0.002
JUV–AD *La. dim.*	2.00	0.157	1	1.78	0.183	1	14.8	<0.001	0.002	13.8	<0.001	0.002
JUV–AD *Ps. hex.*	0.32	0.574	1	0.23	0.633	1	0.67	0.413	1	2.91	0.088	0.705
JUV–AD *Ha. mel.*	0.98	0.323	1	2.48	0.115	1	8.99	0.003	0.035	3.22	0.073	0.653
JUV–AD *Th. lun.*	1.19	0.275	1	1.18	0.258	1	21.93	<0.001	<0.001	19.1	<0.001	<0.001
JUV–AD *He. mel.*	0.04	0.848	1	0.62	0.431	1	8.09	0.004	0.049	2.58	0.109	0.760
JUV–AD *La. uni.*	1.44	0.230	1	2.14	0.144	1	0.25	0.619	1	0.04	0.845	1

Appendix 1: Post hoc pairwise comparisons for the novel object tests

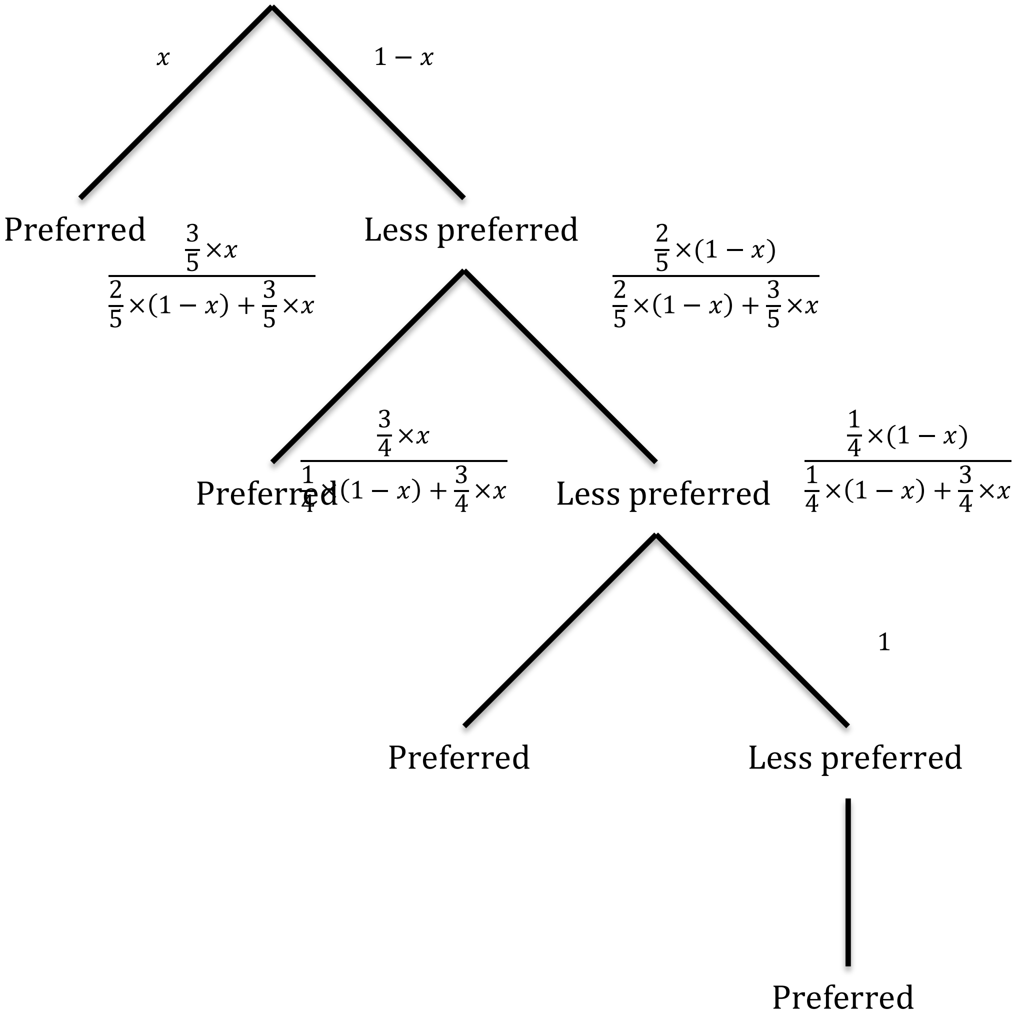
All pairwise comparisons between species, and between juveniles and adults within each species, were made with log-rank tests. *P* values were adjusted for multiple comparisons with Holm's sequentially rejective multiple test procedure. NO = novel object; JUV = juvenile; AD = adult.

Supplementary Material

Calculating predicted values for feeding against preference

In order to calculate the predicted values in the feeding experiments, we used the proportion of preferred food items eaten on day 1 (x) for each individual (see Methods). During the experimental trials on day 3, the fish is presented with three items of each food type, and the trial stops as soon as it eats a preferred item. If this fish keeps eating preferred items with the same probability (x), the probability of eating each type of item during the sequence is described in the tree below. For instance, the probability of eating a less preferred item is 1-x. Once this item has been eaten, there are only two less preferred items left, out of a total of five, and thus the probability of eating a second one is 2/5 × (1-x), divided by the total probabilities at this step (the probability of eating a less preferred item plus the probability of eating a preferred one). The same logic is applied at each step of the tree.

On the plate, there are three preferred and three less preferred items



We thus expect an individual to eat on average z less preferred items before eating a preferred one, calculated as follows:

$$z = (1-x)\times\frac{\frac{3}{5}\times x}{\frac{2}{5}\times(1-x)+\frac{3}{5}\times x}+2\times\left((1-x)\times\frac{\frac{2}{5}\times(1-x)}{\frac{2}{5}\times(1-x)+\frac{3}{5}\times x}\times\frac{\frac{3}{4}\times x}{\frac{1}{4}\times(1-x)+\frac{3}{4}\times x}\right)$$

$$+3\times\left((1-x)\times\frac{\frac{2}{5}\times(1-x)}{\frac{2}{5}\times(1-x)+\frac{3}{5}\times x}\times\frac{\frac{1}{4}\times(1-x)}{\frac{1}{4}\times(1-x)+\frac{3}{4}\times x}\right)$$

Since there are 10 experimental trials on day 3, in which the fish always ate exactly one preferred item, the expected proportion of preferred items is:

$$\frac{10}{z+10}$$

For each individual, we calculated this expected value based on its original preference (day 1, x) and calculated the difference from the actual proportion of preferred items it ate during the experimental trials (day 3). This difference (expected – measured) was used in our analysis as a measure of the amount of change in foraging preference due to the learning sessions on day 2.

CHAPTER 3 - Professionally equipped to clean: obligate cleaners adopt a specific posture in cleaning interaction that is more efficient in comparison with other labrids.

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Status: In preparation

Abstract

The bluestreak cleaner wrasse *Labroides dimidiatus* feeds on the ectoparasites present on other fishes (called 'clients'). During cleaning interactions, this species appears to adopt a very low body angle whit regards to the client fish, while species from the same family typically forage with a more perpendicular position relative to the substrate. We hypothesised that *L. dimidiatus* evolved its peculiar foraging posture for increased efficiency at finding parasites in cleaning interactions. Here, we compared *L. dimidiatus* with four related species of wrasses that differ in terms of dependency on cleaning in laboratory experiments. Our results confirmed that the body angle adopted by *L. dimidiatus* when foraging is lower than the other species tested. While *L. dimidiatus* was generally faster at finding food items than the other species in our experiments, its foraging posture did not appear to allow for better efficiency in contexts that require navigation around tridimensional structures such as clients. However, our results suggest that foraging with a low body angle is more efficient for detecting cryptic preys. Such a posture might thus facilitate detecting the outline of a parasite, even when the latter blends nicely with the background. Finally, we investigated whether the arrangement of photoreceptors in the retina of our study species reflected their foraging technique.

Contributions: SG collected all the data. All authors participated in the redaction of the manuscript.

Introduction

Food acquisition is one key component for survival. According to their niche, species must adapt to the type of food they specialize on, and also to the way they acquire it (O'Brien *et al.* 1990). As a consequence, various aspects of a species bauplan reflect adaptations to foraging. While such adaptations are studied in many clades (e.g. Gilbert 1981; Norberg & Rayner 1987; Patt *et al.* 1997; Svanbäck & Eklöv 2003; Nebel *et al.* 2005), we use fishes as an example. In fishes, a variety of traits have been linked to foraging ecology, such as jaw morphology (Wainwright 1988; Alfaro *et al.* 2001), dentition (Motta 1989), or locomotion (Collar *et al.* 2008). In addition, vision plays a major role in foraging and many aspects of the visual system also appear match foraging ecology (see Archer *et al.* 1999). In response to ecological demands, selective pressures might act upon the location, the size or the shape of the eyes, the type and the distribution of photoreceptors, or even the shape of the pupil (Collin & Shand 2003). Vision relies on recreating an image of the visual environment on the retina and transmitting the information to the brain for further processing. When light hits the retina, it is first transformed by photoreceptors (rods, cones) into a neural signal. The signal then transits through ganglion cells and is finally sent to the brain through axons (Archer *et al.* 1999). For a given species, the distribution (or topography) of retinal cells reflects the importance of obtaining a better resolution in certain areas of the visual field (Collin 1999). On a broad scale, different types of visual environment appear to select for different retinal cells arrangements (Collin 1999). For instance, in species that live in environments where the horizon is a major feature of the visual field, a horizontal streak with high cell densities allows for higher resolving power near the horizon (Hughes 1975, 1977). On a finer scale, retinal topography also appears to respond to the specific needs of a species. The archerfish (*Toxotes chatareus*) is a good illustration of this concept. These fish prey upon terrestrial insects by spitting jets of water at them, and the location of their high-density area is perfectly aligned with the spitting angle (Temple *et al.* 2010). Within species, retinal topography can also shift through ontogeny in order to match the different needs of juveniles and adults (Collin & Shand 2003). Retinal topography has been reported to follow ontogenetic changes in feeding behaviour in several species of fishes (Shand 1997; Shand *et al.* 2000a, b; Miyazaki *et al.* 2011; Gomi & Miyazaki 2015). It is thus apparent that retinal topography can flexibly adapt to ecological demands both at the species level and within species.

Here, we investigated potential behavioural and visual adaptations of the cleaner wrasse *L. dimidiatus* to its peculiar foraging niche. *L. dimidiatus* obtains food almost exclusively from interactions with 'client' reef fishes (Côté 2000). In this mutualism, clients visit cleaners to have ectoparasites removed. Indeed, stomach analyses reveal high numbers of ectoparasites, but also other client material like mucus, skin and scales (Randall 1958; Grutter 1996; Soares *et al.* 2008). Cleaners thus face the problem of finding small prey (ectoparasites) on 3-dimensional structures (clients) that only available for short interspersed periods of time. Since they interact with dozens of different species of fish every day (Grutter 1996), they are also facing the problem of finding parasites over a great variety of backgrounds. In addition, some parasitic monogeneans are translucent and hence rather cryptic against any background colour (Grutter 2002). The feeding on small cryptic benthic preys seems to be a peculiarity of cleaners, while most wrasses eat free-swimming and/or larger benthic preys (Randall 1967; Westneat 1995; Wainwright *et al.*

2004; Berkström *et al.* 2012). Various other species in the Labridae family have also been documented to clean (Côté 2000) but the vast majority of those are classified as facultative cleaners because they have mainly other sources of food and thus do depend very little on cleaning. We hence decided to compare *L. dimidiatus* with other Labrids that could either be facultative cleaners or non-cleaners.

In a first step we asked whether the peculiar foraging niche might have led to the evolution of a more parallel position to the substrate during foraging in *L. dimidiatus* compared to other wrasse species. Such a pattern may be predicted from studies on wrasse jaw anatomy. The lower jaw of *L. dimidiatus* is quite elongated and relatively flat (Wainwright *et al.* 2004), and high speed videos of feeding events (Baliga & Mehta 2015) show that a forward motion is usually associated with a bite (i.e. "scraping" the surface of the client), while in two closely related species no forward movements have been witnessed (Baliga, personal communication). First, a low body angle might help to scrape parasites (or also mucus) off clients. Second, a parallel position during foraging would also help *L. dimidiatus* to provide tactile stimulation to clients. Cleaners perform tactile stimulation with their ventral fins on the body of their clients (Potts 1973). Tactile stimulation is used to manipulate client decisions in favour of the cleaners' interest (Bshary & Würth 2001), a manipulation that works apparently because clients benefit from receiving tactile stimulation through a reduction in cortisol levels (Soares *et al.* 2011). Third, a parallel position with regards to the client might allow for better manoeuvrability and hence increase their efficiency at finding parasites. Finally, a low angle of vision relative to the clients' skin might help discriminating a parasite's outline, even when the parasite blends in perfectly with the background. In line with the various potential benefits linked to a parallel inspection of client bodies during cleaning interactions, preliminary observations indeed suggested that *L. dimidiatus* adopts a lower body angle both during inspection and when picking on the clients' surface, while other labrids appeared to adopt a perpendicular position with respect to the substrate when foraging (personal observation), including facultative cleaners during interactions with client fish. Based on these premises, we thus first measured the angle between body and substrate when fish took a bite for five different labrid species. Assuming that the results would support the conclusions from the preliminary observations, we hypothesised that the swimming position of *L. dimidiatus* might be more efficient for foraging during cleaning interactions. Furthermore, we investigated whether the retinal topography of *L. dimidiatus* might have undergone changes due to selective pressures to adapt to the peculiar foraging technique.

In order to investigate these questions, we first compared the feeding angle of our five study species when picking small food items off a Plexiglas plate in the lab. We then conducted two experiments to compare the relative foraging efficiency when challenged to find and pick small food items off a substrate. The first experiment aimed at assessing whether the peculiar swimming position of *L. dimidiatus* allowed for better efficiency when dealing with a 3-dimensional structure. Since the cleaner wrasse is used to searching for parasites all around the body its clients, we predicted that it should be equally efficient at finding food items on a 2-dimensional or a 3-dimensional structure. For the other wrasses tested, however, we expected a reduction in efficiency on the 3-dimensional plate. The second experiment was designed to understand whether a low angle of vision facilitated foraging in conditions when food items are well camouflaged against the background compared to the control situation in which food was easily visible. If a low body angle helps to discriminate outlines, we expected the efficiency of *L. dimidiatus* to be less reduced when the food items are cryptic compared to species that forage in a more perpendicular angle to the substrate. Finally, we compared the retinal topography of the same five species in order to investigate whether the distribution of cones in the retina followed the different foraging techniques adopted by these species. Most studies investigate the distribution of ganglion cells because they represent how the visual information is sent to the brain (e.g. Collin & Pettigrew 1988a, b). However, photoreceptors, which we used to investigate retinal topography in our study, also play an important role because they are responsible for collecting the information. As a result, the topography of both cell populations usually match each other and provide very similar information (Collin & Shand 2003; Litherland *et al.* 2009; Temple *et al.* 2010).

Methods

Study species

For both the behavioural experiments and the retinal topography analysis, we examined 5 species of wrasses (Labridae) that co-occur on the Great Barrier Reef. The bluestreak cleaner wrasse *Labroides dimidiatus* is an obligate cleaner: it gets its entire energy intake from cleaning interactions (see Fig. 1 for examples of cleaning interactions). *Halichoeres melanurus* and *Pseudocheilinus hexataenia* feed on small crustaceans, and occasionally small fishes for *Thalassoma lunare* (Elliott & Bellwood 2003; Berkström *et al.* 2012), but have also been witnessed to occasionally engage in cleaning activity (Barbu *et al.* 2011, personal observation for *H. melanurus*). These 3 species were thus categorized as “facultative cleaners”, while the corallivore *Labrichthys unilineatus* was categorized as “non-cleaner” (Cole 2009). All fish were collected on reefs surrounding Lizard Island, Australia (14°40'S 145°28'E), and captured using a barrier net and small hand nets. We used clove oil to momentarily sedate species that enter the reef matrix when chased (*P. hexataenia*). After capture, we immediately transferred all fishes to the Lizard Island Research Station (LIRS), where they were kept in tanks with constant flow-through seawater and fed mashed prawns.

Behavioural experiments

All behavioural experiments took place between August and September 2014 at the LIRS. Large individuals (12-20cm TL) were held alone in 67 x 38 x 38 cm glass aquaria. For smaller individuals (5-11cm TL), 2 individuals shared a larger glass aquaria (90 x 38 x 38 cm) divided in half with an opaque Plexiglas partition, preventing any interactions between the two fish. We originally tested a total of 40 individuals (8 per species). Since we were interested in efficiency at finding food items, it was crucial for individuals to be well habituated to the experimental setup. For *L. unilineatus,* 3 individuals had to be discarded from the experiments because they never got habituated well enough. Furthermore, 3 *P. hexataenia*, 1 *L. dimidiatus* and 1 *H. melanurus* did not participate in the second experiment (crypsis experiment) for various reasons (reacting badly to one of the treatment plates, not willingly feeding on novel food items, and bacterial infection respectively). Each fish was given at least 5 days (mean = 7.2, SD = 1.7) of acclimation in the experimental tank prior to the experiments, during which they were also habituated to the experimental procedure. For a fish to be considered well habituated, it had to successfully complete 4 practice trials in each treatment.

Measuring feeding angle

Each fish was presented with a feeding plate with 3 separate items and filmed (with a Canon 7D at 30fps) from the side in order to evaluate the angle they adopt relative to the substrate when foraging ('feeding angle'). The angle formed between the plate and a line passing through centre of the eye and the food item was then measured for each item (on the frame preceding the contact with the food item). To obtain measures that are representative of the fishes' natural behaviour, it is crucial for them to be very well habituated to foraging on feeding plates. We collected data on feeding angles only after the completion of the two foraging experiments described below, so that the fish were all well habituated to their tanks and to feeding from plates.

Shape experiment

The first series of tests consisted of two foraging situations in which fishes had to find 6 food items either on a flat surface (2D treatment) or on a circular surface (3D treatment) (Fig 2a-c). The size of the surface over which the items were placed, as well as the relative position of the items was identical for the two types of plates (2D or 3D). Each fish experienced 3 trials of each treatment per day, over two days. Fish were not fed before the experiments started. In our sequence of trials, one treatment always followed the other one (e.g. 3D then 2D) and within each species, half of the individuals began with the 3D plate and half with the 2D plate. For every trial, the plate was gently introduced in the tank and the lever attached above the tank so that the plate hung in the middle of the tank. All trials were filmed with a camera (Canon G11) placed on a tripod in front of the tank. If the subject did not approach the plate within 300s, the trial was considered null (not taken into account). Once the fish ate the first item, it was given a maximum of 120s to forage on the plate.

Crypsis experiment

A minimum of 4 days (mean = 5.4; max = 12) after completing the shape experiment, each fish was tested in the crypsis experiment. Here, 6 food items were either laid on a white plate (conspicuous treatment), or on a plate that matched the colour of the food items (cryptic treatment) (Fig 1 d-f). We mixed mashed prawns with fish flakes in order to give a green colour to the food items. The cryptic treatment plate consisted of a laminated picture of the white plate that we had entirely covered with the green food mixture. In order to document the contrast between the food items and the two types of backgrounds, we measured their colour with an Ocean Optics (Dunedin, FL) USB2000 spectrometer connected to a computer running OOIBASE32 software. We took measurements on 6 food items laid on the conspicuous plate, 6 on the cryptic plate, as well as 6 locations on each of the plates (without the items). For each measurement, 10 replicates were taken and averaged. The light reflected was measured through a 200 μm bifurcated fibre optic UV/VIS cable connected to a PX-2 pulsed xenon light source (Ocean Optics, Dunedin, FL), handheld at a 45° angle. We used a Spectralon 99% white reflectance standard (LabSphere, USA) for calibration. Our measurements confirmed that the green food items created more contrast over the conspicuous than the cryptic background (Fig. 1g), and hence that our treatment had the intended effect. The habituation criterion and the experimental procedure were the same as for the 1st experiment. All fish were brought back to the reef upon completion of the experiment. Experiments were conducted in accordance with the with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes, and under the approval of a Queensland (Australia) Government Animal Ethics Committee (Proposal Reference Number CA 2012/05/611).

Video analysis

We analysed the videos with JWatcher and recorded the time at which each item was eaten, starting the timer when the plate entered the tank. We then calculated the time difference between the 3rd and the 1st (t_{half}) and between the 6th and the 1st item (t_{tot}). These values were then used in the statistical analysis to compare the efficiency of species across treatments.

Statistical analysis

We first compared the feeding angle across species with a Linear mixed-effects model (lme) with fish identity as a random factor. Our two experiments investigated efficiency at finding food items, yet each was designed to answer a different question. We thus analyzed the 3D and 2D treatments separately from the cryptic and conspicuous ones.

First, the number of successful trials (i.e. trials in which the fish ate all 6 items) was computed for each individual. In some groups, 100% of the trials were successful and thus the lack of variation prevented the use of a standard binomial glm for the analysis. Success was thus compared across species and treatments with a Bayesian binomial generalized linear model (bayesglm command in the R package "arm" (Gelman & Su 2015)). Post-hoc comparisons were computed in order to compare both success and treatment effect across species.

We used the time to eat half of the items (t_{half}), and the time to eat all of the items (t_{tot}) to investigate efficiency. For both t_{half} and t_{tot}, incomplete trials were first removed from the analysis. Differences across species and treatments were then analyzed with linear mixed effects models in the form response~species*treatment, with fish ID as a random factor and allowing for different standard deviations across species. We applied either the Box-Cox variance stabilizing transformation or the log transformation on the response variable (t_{half} or t_{tot}) to fit the model's assumptions, and assessed normality and homoscedasticity of the residuals with qqplots and plots of residuals vs. fitted values. Post-hoc comparisons were computed in order to compare both efficiency and treatment effect across species. *P*-values were adjusted using the single-step method, which implements adjusted *P*-values based on the joint normal or t distribution of the linear function. All statistics were performed in R 3.2.2 (R Core Team 2013). Box-Cox power transformations and the linear models were computed using the R packages "MASS" (Venables & Ripley 2002) and "nlme" (Pinheiro *et al.* 2013) respectively. Pairwise comparisons were obtained with the simultaneous tests for general linear hypotheses procedure from the R package "multcomp" (Hothorn *et al.* 2008).

Retinal topography

Mapping the density of photoreceptors across the retina allows inferring the importance of obtaining a better resolution in certain areas of the visual field (Collin 1999) (see Fig. 3). In order to determine the retinal topography for our five study species, fish were euthanized at the LIRS in a solution of 0.2ml clove oil / litre seawater, followed by swift decapitation. Eyes were enucleated immediately after euthanasia, the cornea and lens dissected off and the whole eyecup fixed in 4% paraformaldehyde (in 0.1 M phosphate buffer) for at least 1h. Retinas were later extracted and prepared for microscopy. We followed the Ullmann *et al.* (2012) procedure for retinal dissection and wholemounting. If possible, the retinal pigmented epithelium (RPE), which is a pigmented cell layer between the retina and the choroid, is removed for visualization under the microscope. However, for our samples, it was not possible to remove the RPE mechanically and we thus bleached the RPE for at least 30 minutes (longer if the sample still looked dark) with a 10% H_2O_2 (diluted in 0.1M phosphate buffer) solution, for which we adjusted the pH to 11.95 with potassium hydroxide (Ullmann *et al.* 2012). Each retina was then wholemounted, photoreceptor layer facing up, on a microscopy slide and analysed using the optical fractionator technique (West *et al.* 1991) adapted by Coimbra et al. (2009) for retinal wholemount. The retina was digitised using a x5 objective (numerical aperture 0.16) mounted on a compound microscope (Zeiss Imager.Z2) equipped with a motorised stage (MAC 6000 System, Microbrightfield, USA), a digital colour camera (Microbrightfield, USA) and a computer running StereoInvestigator software (Microbrightfield, USA). Both single and double cones were counted using a x63 oil objective (numerical aperture 1.40). We set the number of sites to be counted across each retina to 250. The size of the counting frame was adjusted according to the size of the cells to be counted, and varied between 31 and 41 μm across samples (Table 1). Photoreceptors counts were very challenging for the species analysed and we were not able to accurately count cells for several counting sites. As a result, the retinal topography was analysed for 2 samples per species for which the number of the sites counted were satisfactory (>50% sites counted). For *L. unilineatus*, we could only obtain one sample of good enough quality and for *L. dimidiatus*, one of the two samples included had a ratio of sites counted slightly below 50%, (113 sites counted out of a total of 246). In the samples used to produce the topography maps, an average of 157 (s.d. = 27.4) sites could be counted (see Table 4 for details). Maps were created in R 3.2.2 with the package “retina” (Cohn *et al.* 2015).

Results

Behavioural experiments

Feeding angle

Species differed significantly in feeding angle (lme, d.f. = 4, $F = 48.12$, $p < 0.0001$; Fig. S1). *L. dimidiatus* adopted a mean angle of 51.14° with respect to the plate, which was significantly lower than all four other species (all pairwise comparisons < 0.0001). *P. hexataenia* fed from the plate with a mean angle of 77°, which was also significantly different from all the other species (all pairwise comparisons < 0.0001). The other 3 species all adopted a mean feeding angle above 90° and did not differ significantly from each other (Fig. S1).

Shape experiment

In terms of success at finding all 6 items within the allocated 120 seconds, species differed significantly in the shape experiment (Fig. 4a, Table 2). *L. dimidiatus* scored almost perfectly in both treatments (Fig. 4a). While the other species performed very well (all >80%) in the 2D treatment, their performance dropped in the 3D treatment, as low as 63% for *L. unilineatus*. Post hoc pairwise comparisons detected minimal effects after adjustments (Table S1), and all species suffered a similar decrease in success when foraging on the 3D structure (Table 1, Fig. S2e). In this experiment, both the time to eat half of the items and the time to eat all the items differed significantly across species (Fig. 5a-b, Table 3). Furthermore, species took significantly more time to eat the items (t_{half} and t_{tot}) in the 3D than in the 2D treatment. While *L. dimidiatus* was significantly faster than the other species to find the food items (Fig. 5a-b, Table S2), the effect of the treatment generally affected all species in a similar way (Fig. S2a-b).

Crypsis experiment

Success was very high in the crypsis experiment (Fig. 4b), and species differed significantly in their reaction to the treatments (Table 2). Interestingly, *L. dimidiatus* scored lower (92%) in the conspicuous than in the cryptic treatment (98%) (Fig. 4b). The other species performed very well in this experiment (> 90% of successful trials in both treatments), except for *L. unilineatus* that suffered a dramatic decrease of success in the cryptic treatment. Species also differed significantly in both t_{half} and t_{tot}, and the effect of the treatment was significantly different across species (Table 3). The fastest species was also *L. dimidiatus* (Fig. 5c-d), yet post hoc pairwise comparisons failed to detect many differences after adjustment of the p-values (Table S2). In this experiment, *L. dimidiatus* suffered a subtle decrease in efficiency due to the treatment, while the other species reacted generally more strongly (Fig. S1c-d).

Retinal topography

The mean total (single and double) cone photoreceptor density varied between 45,000 and 77,000 cells/mm^2 across samples (Table 4). For two of the species analysed (*L. dimidiatus* and *T. lunare*), the topography was not consistent across individuals (Fig. 6a & 6c), thus making it difficult to draw conclusions at the species level in these cases. For *L. dimidiatus*, the sample from the first individual shows a high-density area in the nasal part of the retina (Fig. 6a.2). For this sample, a high proportion of the sampling sites could be properly counted (> 70%) and the quality of the wholemount was very satisfactory (good visibility of the photoreceptors). The topography of the second individual shows a more common pattern for a coral reef fish (Fig. 6a.3), with a high-density area located dorso-temporally, and a second area centrally (on the nasal side of the falciform process). This pattern was also found in most of the species analyzed. However, the quality of this second *L. dimidiatus* sample was generally lower and thus fewer sites could be counted (Table 4). In *T. lunare* we also found rather different topographies across the two individual analysed (Fig. 6c). The first sample indicates a horizontal streak of high density in the lower part of the retina (Fig. 6c.2), while the location of the high-density are in the second sample is located in the nasal part (Fig. 6c.3). In this sample, the photoreceptors have been flattened in certain areas during preparation, which could cause some errors in counting. However, the flattened areas do not appear to match with the sites where we observe unexpectedly high or low densities. The quality of the first sample appears to be much better, yet more samples will be required to get a correct representation of the typical topography in this species. The topography from the two samples of *H. melanurus* is similar, with a high-density area in the temporal part, near the optic nerve (Fig. 6b). Both samples also have similar densities and hence are likely representative of the topography of this species. In *P. hexataenia*, both samples also show a similar topography, with a high-density area in the dorso-temporal part of the retina and a smaller area in the central part, next to the optic nerve (Fig. 6d). These samples are thus again likely representing the actual topography for this species. The densities in the two samples are different though, with maximum counts reaching 139,000 cells/mm^2 in the first sample and only 82,000 cells/mm^2 in the second sample (Table 4; Fig. 6). Size cannot explain these differences since the total length of the two individuals sampled was very similar (Table 1). Finally, we were able to obtain only one sample with sufficient counts in *L. unilineatus* (Fig. 6e). The retinal topography of this sample shows a very similar pattern to the ones found in *H. melanurus* or *P. hexataenia*, with two high-density areas on both sides of the optic nerve, in the dorso-temporal part of the retina.

Discussion

We had asked whether the cleaner wrasse evolved a foraging posture that increases efficiency in cleaning interactions. Cleaning requires finding small and often cryptic preys over short and interspersed periods of time (interactions), on a mobile substrate (the client fish). Wrasses of the genus *Labroides* are all specialized in cleaning and all adopt low body angle with regards to the client fish during interactions. Our results confirm that the body angle of *L. dimidiatus* when foraging is significantly lower in comparison with 3 species of facultative cleaner and one species of non-cleaner. Our first prediction was that foraging at a low body angle allowed manoeuvring with more ease around the 3-dimensional structure that is a client fish. In our shape experiment, which aimed at mimicking foraging on a flat substrate versus a client fish, *L. dimidiatus* was overall more efficient than the other species. Most cleaning interactions only last a few seconds (Bshary 2001; Gingins & Bshary 2015), and thus cleaners might be prompted to search for parasites as fast as possible. The other species tested typically forage on small invertebrates with limited mobility, and hence pressures on foraging speed is not crucial in this context. However, it appeared that manoeuvring around a 3-dimensional structure represented a similar challenge for *L. dimidiatus* and the other species, since they all experienced a comparable reduction in efficiency in comparison with their performance on a flat structure. Therefore, we conclude that the foraging posture of *L. dimidiatus* has not been selected specifically for manoeuvring around client fishes. Our second prediction was that a low body angle allows for better abilities at spotting cryptic ectoparasites. Even when very small, ectoparasites' outline is more likely to be detected from the side than at a perpendicular angle if they are cryptic. The results of our crypsis experiment support this hypothesis, since facultative cleaners all experienced a reduction in efficiency in the cryptic treatment compared to the conspicuous one, while *L. dimidiatus* performed quite similarly in both treatments. Our results thus suggest that the low body angle adopted by *L. dimidiatus* is beneficial for detecting small cryptic preys. Furthermore, such a position also allows cleaners to give tactile stimulation, and is potentially associated with their jaw morphology for scraping parasites or mucus from the body surface of client fishes (see introduction). The peculiar foraging position of *L. dimidiatus* thus appears to offer several advantages in the context of cleaning, and likely evolved with the specialization in cleaning behaviour.

Feeding posture and retinal topography

In the second part of our study, we had asked whether the retinal topography of the different species would mirror their foraging technique. Unfortunately, we found important differences across samples of the same species for both *L. dimidiatus* and *T. lunare*, and only obtained one sample that was exploitable for *L. unilineatus*. We are thus planning to obtain more samples for these two species in order to get a better picture of their retinal topography. As a result, we will discuss adaptations of the visual system in labrids below, and proceed with care in the interpretation of our current data. Coral reef fishes display a variety of retinal topographies, depending on their habitat and their ecological needs (Collin & Pettigrew 1988a, b). While many studies investigated ganglion cells, we used cone photoreceptor densities for producing the topographic maps. However, since ganglion and photoreceptor densities usually align (Collin & Shand 2003; Litherland & Collin 2008; Temple *et al.* 2010), we discuss the arrangement of retinal cells in general in this section. In the five species we investigated here, it seems that high-density areas are mostly located in the temporal and central parts of the retina (see Fig. 3 for a description of the terminology). A temporal area increases the resolving power in the frontal part of the visual field and is found in many species, such as the coral cod (*Cephalopholis miniatus*), the blue tuskfish (*Choerodon albigena*) or the painted flutemouth (*Aulostoma chinensis*) (Collin & Pettigrew 1988a, b). These three species have very different ways of feeding: the coral cod ambushes preys while hiding in dark corners of the reef, the tuskfish moves rubble around to find invertebrates and the flutemouth hides behind larger fishes and strikes at unsuspecting preys. However, as many other reef fishes, they all strike at preys that are in front of their mouth, which explains why they share a high-density temporal area. *P. hexataenia* and *H. melanurus* feed on small invertebrates and their retinal topography suggests that the frontal part of their visual field is also important for their feeding ecology. In both species, high densities of cones are also found in the central part of the retina. While the front of the visual field is often subtended by binocular vision, depending on the position of the eyes, the central area might be used for monocular vision of their surroundings (Collin & Shand 2003). Our only sample from *L. unilineatus* suggests that the topography in this species is also similar, however, more samples are needed to ascertain this claim. For our focal species, *L. dimidiatus*, our two samples indicate a retinal topography very similar to *P. hexataenia* and *H. melanurus*, with high photoreceptor densities in both the dorso-temporal and the central parts of the retina. However, in one sample we also found high densities of photoreceptors in the nasal region. Nasal areas subtend the rear part of the visual field (see Fig. 3), yet it is unclear why *L. dimidiatus* would require a higher resolving power in this part of its field of view. Such specializations have been found in some species that use branching corals for shelter, such as the damselfish *Amblyglyphidodon curacao,* and was suggested to facilitate navigation between coral branches in escape situations (Collin & Pettigrew 1988b), as well as in species that can swim backwards, such as balistids (Ito & Murakami 1984). Furthermore, it could help to detect predators coming from behind. However, the ecology of *L. dimidiatus* does not fit with these explanations because it does not spend an important amount of time within branching coral, it cannot swim backwards and it generally does not appear to face threats coming from behind. Interestingly, we could have predicted a nasal area in *P. hexataenia* and *L. unilineatus* because they spend a significant amount of time within branching corals, yet our retinal samples did not show any sign of increased photoreceptor densities in the nasal part of their retinas. In *L. dimidiatus*, the position it adopts for foraging could be paralleled to benthic feeding. In benthic feeders, a high-density area is found in the dorsal part of the retina to allow for better resolution in the lower part of the visual field (Shand *et al.* 2000a; Gomi & Miyazaki 2015). However, our *L. dimidiatus* samples so far do not suggest that its retinal topography is similar to benthic feeding species, but rather that it matches that of other species that look forward when foraging. This might be explained by the fact that in *L. dimidiatus*, the eye is located straight behind the mouth, and despite its low body angle when foraging, it is very likely looking forward rather than downwards when foraging. In contrast, in the black bream *Acanthopagrus butcheri*, the eye is located higher up on the head relative to the mouth, and thus needs to look downwards to see in front of its mouth, which could explain why a dorsal area is developed for benthic feeding in this species (Shand *et al.* 2000a) but not in *L. dimidiatus*. Nevertheless, we need more samples in order to determine the retinal topography in our focal species unambiguously.

Service quality in cleaning interactions

According to biological market theory (Noë & Hammerstein 1994; Noë *et al.* 2001), if there is variation between potential partners with respect to the benefits they provide, partner choice will evolve. In cleaning mutualism, clients should thus preferentially seek interactions with cleaners that provide the best service. Given that there are many sympatric cleaning species on indo-pacific coral reefs, one has to explain why clients mainly seek the service of *Labroides species*, allowing them to fully dependent cleaners, while the services of other cleaning species are rarely used, making those species facultative cleaners. Previous evidence yielded a rather mixed picture. One the one hand, all *Labroides* species provide clients with tactile stimulation and hence the benefits of reduced cortisol levels (Soares *et al.* 2011). For facultative cleaners, only *Larabicus quatrilineatus*, the sister clade to Labroides, is known to provide tactile stimulation as well and it is also quite popular with clients (Barbu *et al.* 2011). On the other hand, client jolt frequency – a correlate of cheating by cleaners (Bshary & Grutter 2002) – was found to be higher in interactions with *L. dimidiauts* than in interactions with facultatively cleaning wrasses in the Red sea (Barbu *et al.* 2011). The latter result implies that clients should actually prefer facultative cleaner species. However, cheating rate only scores the negative effects of cleaner foraging behaviour. The negative effects should be contrasted with the positive effects of parasite removal. In this context, the efficiency at removing parasites is a major component of service quality. Our data clearly suggest that *L. dimidiatus* provides more efficient removal of cryptic parasites than the facultative cleaners. This new evidence suggests that the net service quality of dependent cleaners exceeds the service quality of facultative cleaners. From an *L. dimidiatus* perspective, benefits from increased efficiency at finding parasites thus materialize not only through higher energetic gains per time unit during cleaning interactions but also through an increase in the number of interactions due to client partner choice.

In conclusion, our study suggests that *L. dimidiatus* evolved a foraging posture that increases efficiency and thus service quality in cleaning interactions. As facultative cleaners do not show similar foraging postures, it appears that more opportunistic interactions with clients do not pose enough selective pressures to drive adaptations. This view is supported by recent results on the comparison of *L. dimidiatus* with related species of facultative cleaners and non-cleaners, which suggest that strategic and cognitive adaptations to cleaning appeared in specialized species only (Gingins & Bshary 2016; Gingins *et al.* 2013).

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Figures

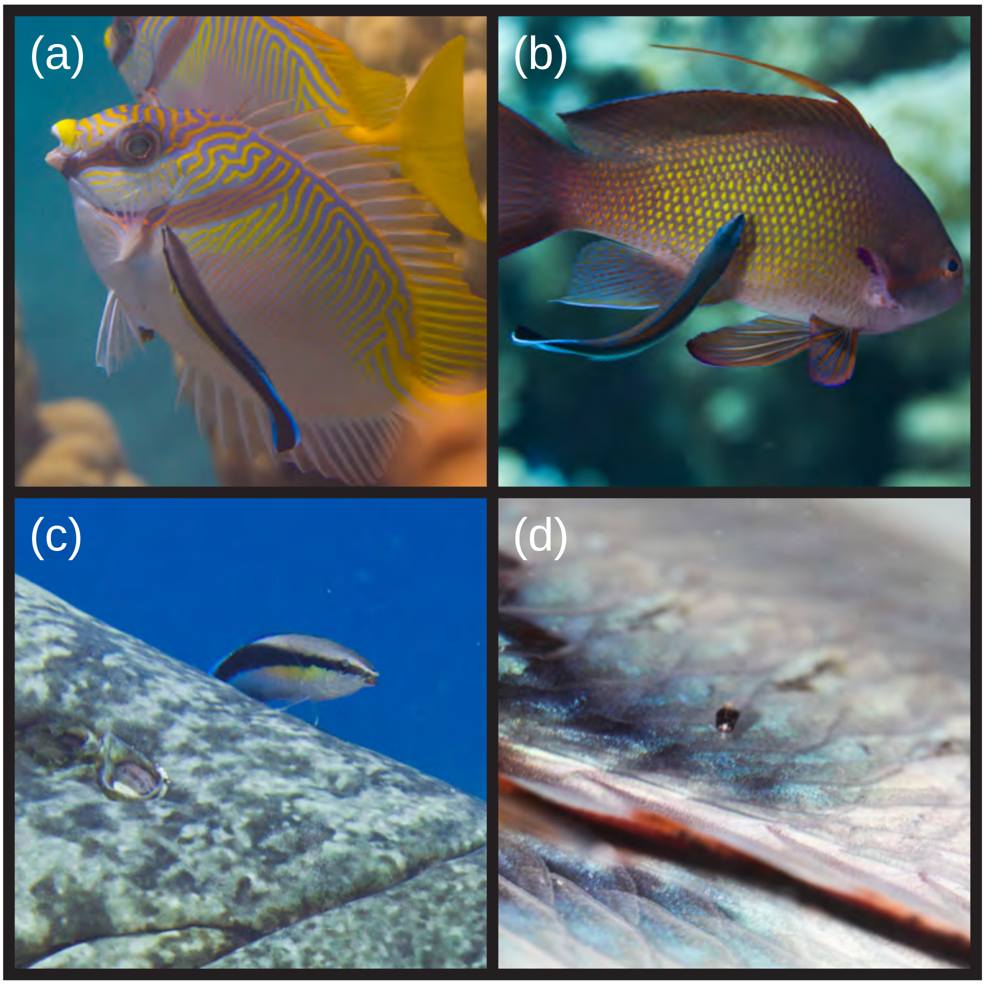

Figure 1. Cleaning interactions in the cleaner wrasse *L. dimidiatus*. This species typically inspects client fishes for parasites with a low body angle relative to the client fish, as illustrated here with *Siganus doliatus* (a) and *Pseudanthias squampinnis* (b). A low body angle also allows *L. dimidiatus* to give tactile stimulation to clients with their ventral fins, as shown in (c) during an interaction with the potato grouper *Epinephelus tukula*. In (d), a gnathiid isopod, one of the most common ectoparasites consumed by *L. dimidiatus*, is attached to the back of a monocle bream *Scolopsis bilineata*. All pictures were taken in the wild, except for (d) that was photographed in the lab at Lizard Island.

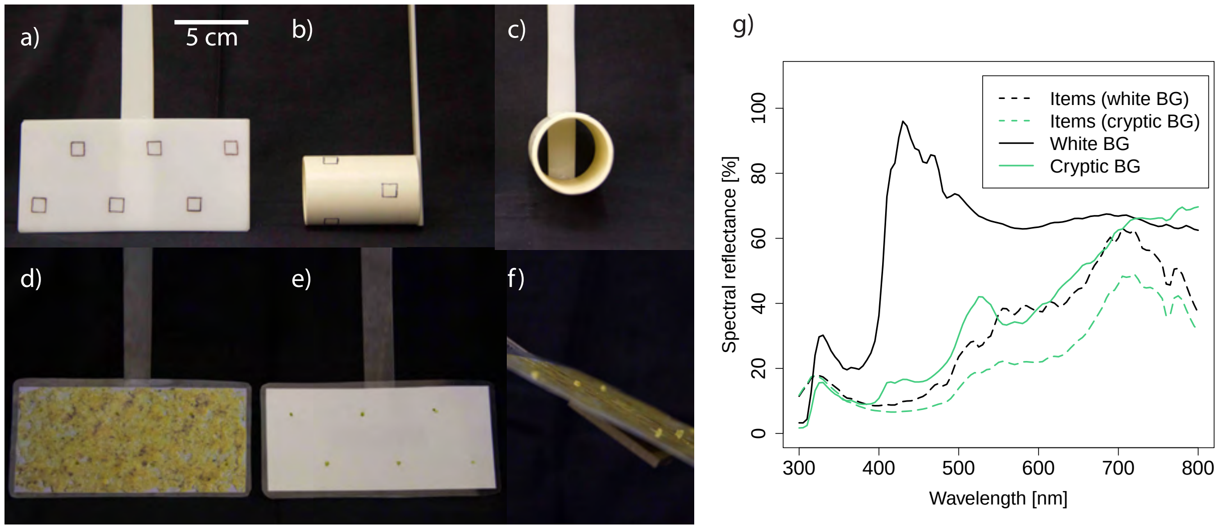

Figure 2. Plates used for the experiments. In the shape experiment, fishes had to find 6 food items either laid on a flat (a) or tridimensional substrate (b: side view; c: front view). Items were always placed inside the black squares. In the crypsis experiment, green food items were either laid over a cryptic background that matched the colour of the items (d), or on a white background. Both plates (d,e) have food items laid on them. When the items blend with the background nicely (d), it is much easier to detect them from an angle (f). The graph in (g) shows the colour profile for the cryptic and conspicuous backgrounds, as well as for the food items laid on either type of background.

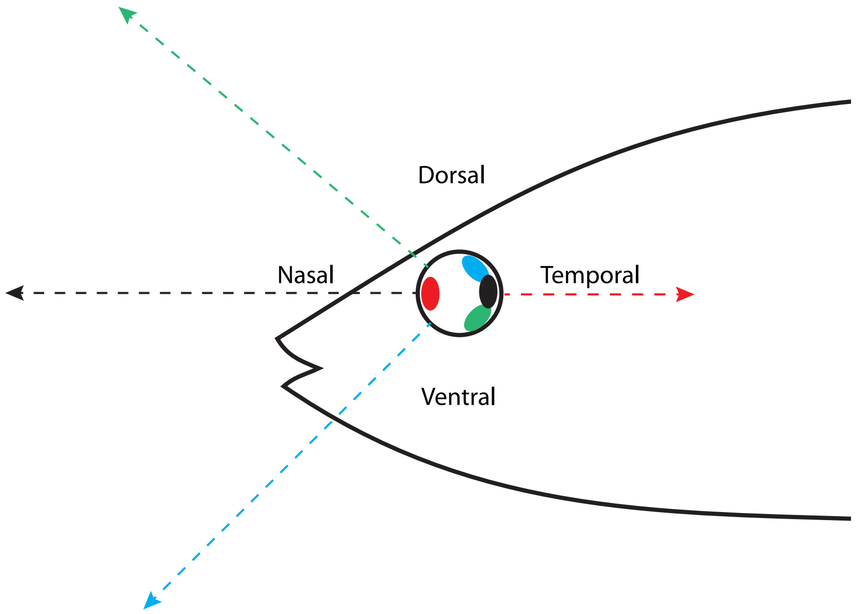

Figure 3. Schematic representation of a fish head and the portions of the visual field subtended by various areas of the retina. The terminology used to describe the location of areas in the retina is indicated on the figure (dorsal, temporal, ventral and nasal). Four areas are highlighted in the retina and the arrows indicate the part of the visual field that is subtended by the area of the same colour. For instance, the area highlighted in blue is located dorso-temporally in the retina and subtends the lower part of the visual field (blue arrow). The outline of the fish head was drawn from a picture of one of the study species (*Halichoeres melanurus*).

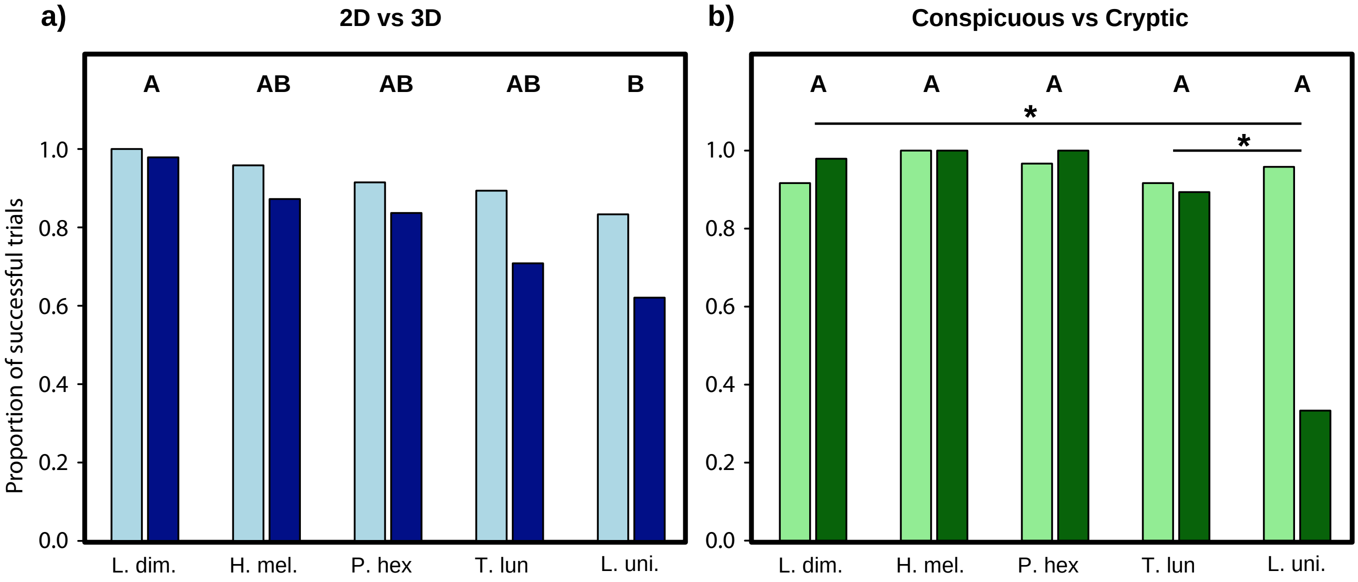

Figure 4. Proportion of trials in which the subject found all six food items for the shape (a) and crypsis (b) experiments. Light blue and dark blue respectively stand for the 2D and 3D treatments. Light green and dark green respectively stand for the conspicuous and cryptic treatments. Different letters at the top indicate significant differences between species. Stars indicate significant differences in treatment effect across species (**P*<0.05). Each box covers the second and third quartiles, the black bar represents the median and the whiskers delimit 95% of the distribution. Outliers are represented as circles. Species names are abbreviated.

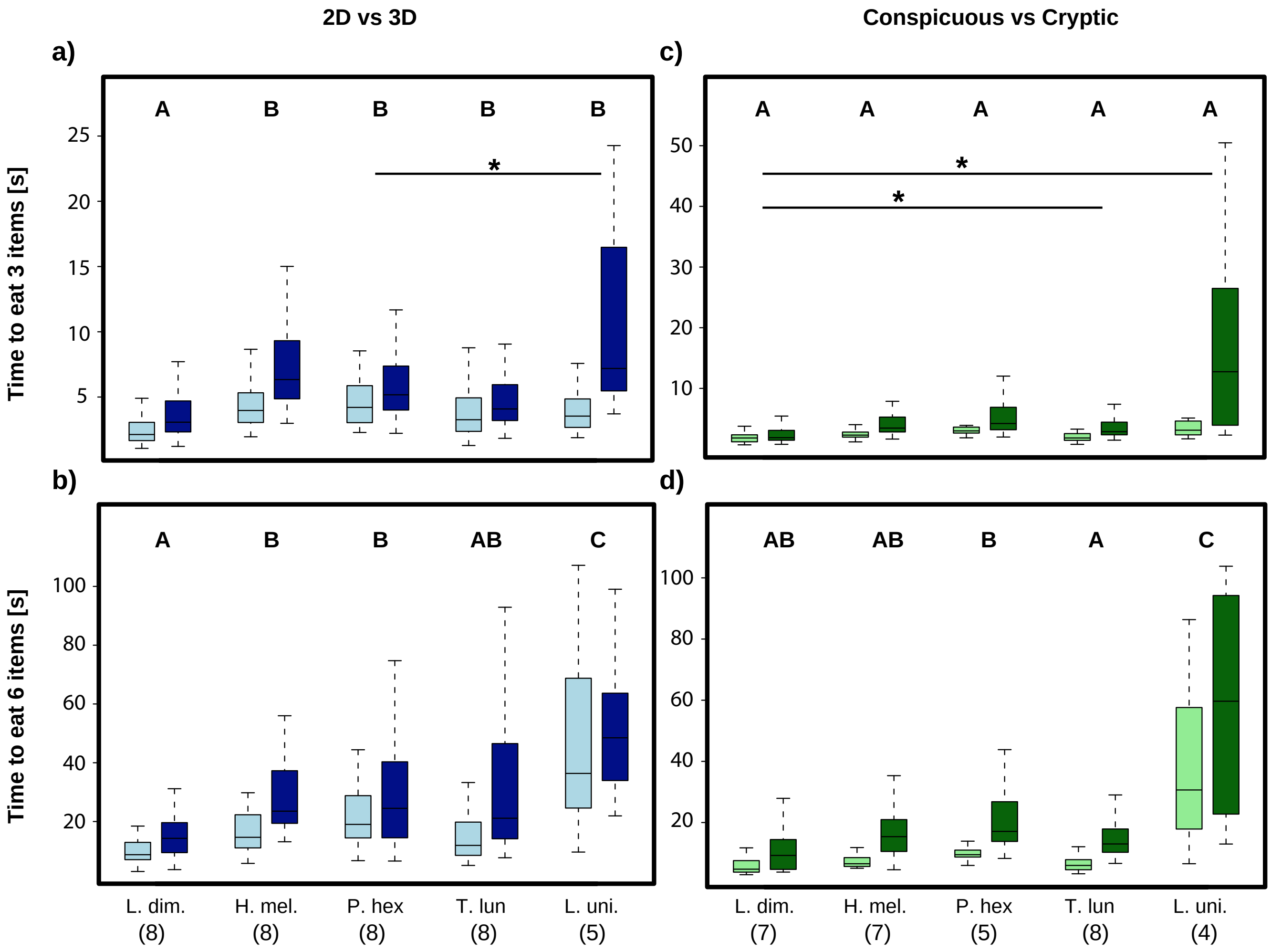

Figure 5. Time to eat 3 (a,c) and 6 (b,d) food items in the shape (a,b) and the crypsis (c,d) experiments. Light blue and dark blue respectively stand for the 2D and 3D treatments. Light green and dark green respectively stand for the conspicuous and cryptic treatments. Different letters at the top indicate significant differences between species. Stars indicate significant differences in treatment effect across species (**P*<0.05). Sample size is given in parentheses for every species. Each box covers the second and third quartiles, the black bar represents the median and the whiskers delimit 95% of the distribution. Outliers are represented as circles. Species names are abbreviated.

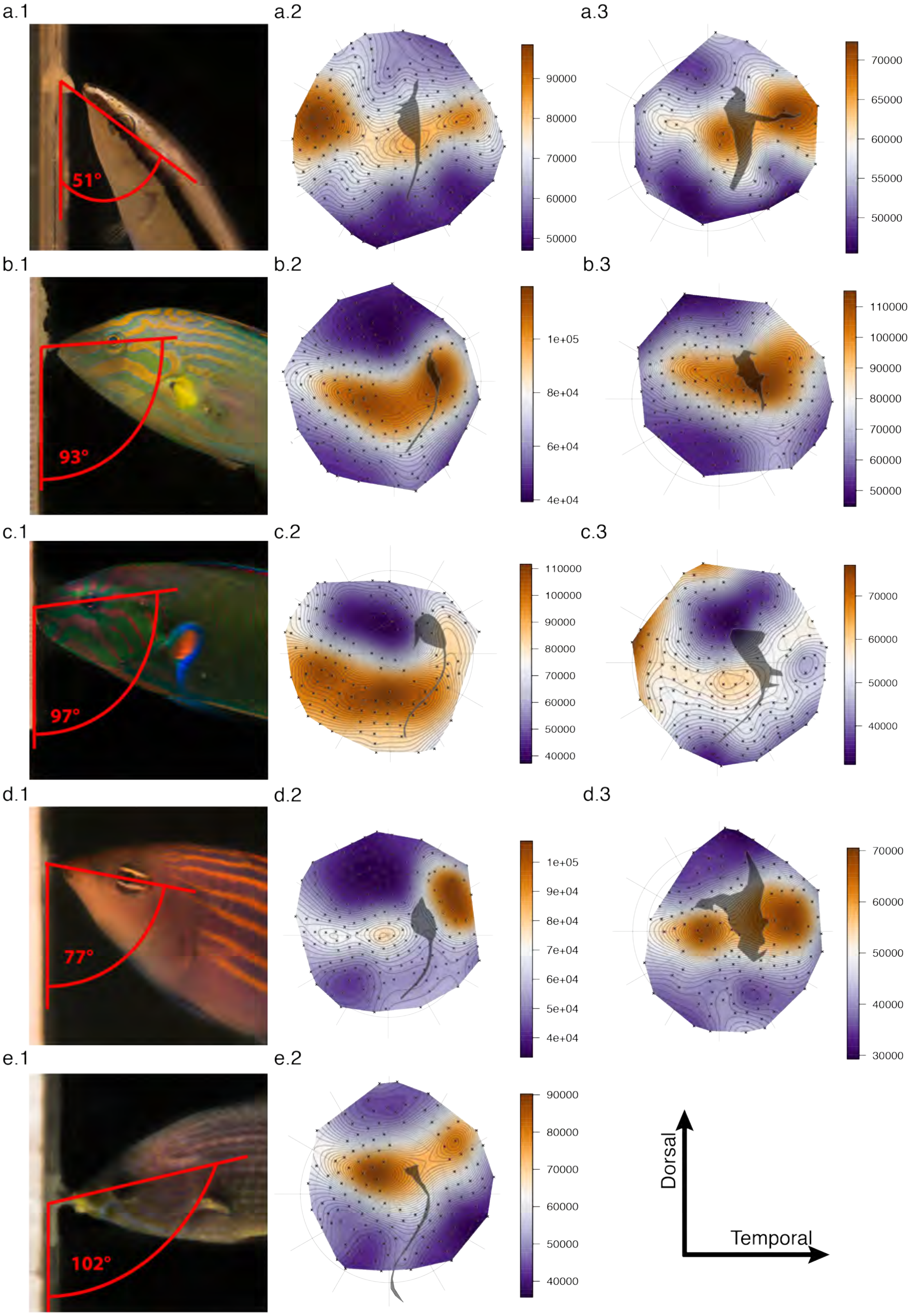

Figure 6. Foraging posture and retinal topography for *L. dimidiatus* (a), *H. melanurus* (b), *T. lunare* (c), *P. hexataenia* (d) and *L. unilineatus* (e). For each species, the body angle adopted when foraging from a plate is illustrated on the left hand side of the figure. In red, the mean body angle is given to the closest degree. Retinal topography maps for two samples, each from different individuals, are shown on the right hand side. Each map is oriented as a left eye for easier comparisons. All maps are oriented with the dorsal part at

the top and the nasal part on the left, as indicated in the bottom right part of the figure. Dark grey shapes represent the location of the falciform process, which is terminated with an enlargement that represents the optic nerve, in each sample. Small crosses indicate the locations of the sites at which photoreceptor counts could be performed. The bar on the left of each map gives the colour coding used to represent photoreceptor densities (cells/mm2). For *L. unilineatus* (e), only one sample could be obtained.

Tables

Table 1. Summary of the fish eyes sampled and the stereological parameters used for topographic analyses.

Species	**Ind.**	**Eye**	**TL**	**Eye ø**	**Sites**	**Counting Frame**	**Grid**
	(cm)	**(mm)**	**Sampled**	**(µm x µm)**		**(µm x µm)**	
L. dimidiatus	a.2	Left	8.6	1.5	259	41.1 x 41.1	291 x 268
	a.3	Left	7.6	1.6	246	31.2 x 31.2	247 x 257
H. melanurus	b.2	Right	9.2	4.9	243	38.9 x 38.9	355 x 328
	b.3	Right	9.3	4.8	251	33.3 x 33.3	349 x 335
T. lunare	c.2	Right	16.4	7.0	251	32.0 x 32.0	444 x 439
	c.3	Left	15.3	7.0	253	55.9 x 55.9	567 x 542
P. hexataenia	d.2	Left	6.1	4.4	243	35.3 x 35.3	285 x 297
	d.3	Right	6.8	4.8	251	41.1 x 41.1	339 x 334
L. unilineatus	e.2	Left	13.4	6.8	253	40.4 x 40.4	456 x 466

Individuals (Ind.) are referred to in the same way as in Table 2 and Figure 5.

Table 2. Differences across species and treatments in number of successful trials for the shape and crypsis experiments.

		D.F.	Deviance	*P*
Shape				
Species	4	33.05	<0.0001***	
Treatment	1	12.89	0.0003 ***	
Species : Treatment	4	0.97	0.915	
Crypsis				
Species	4	48.72	<0.0001***	
Treatment	1	6.02	0.0141 *	
Species : Treatment	4	19.36	0.0007 ***	

D.F., degrees of freedom ; *P, P*-value ; **P*<0.05; ***P*<0.01; ****P*<0.001

Table 3. Differences across species and treatments in time to eat half of the items and time to eat all items for the shape and crypsis experiments.

			t_{half}				**t_{tot}**	
		D.F.	*F*	*P*		D.F.	F	*P*
Shape								
Species	4	16.03	<0.0001***		4	14.93	<0.0001***	
Treatment	1	85.66	<0.0001***		1	48.33	<0.0001***	
Species : Treatment	4	2.51	0.0416 *		4	1.192	0.3141	
Crypsis								
Species	4	5.73	0.0031 **		4	10.47	<0.0001***	
Treatment	1	83.52	<0.0001***		1	184.32	0.0001 ***	
Species : Treatment	4	3.96	0.0037 **		4	3.07	<0.0001***	

D.F., degrees of freedom ; *F, F* ratio, *P, P*-value ; **P*<0.05; ***P*<0.01; ****P*<0.001

Table 4. Summary of the fish eyes sampled and the stereological parameters used for topographic analyses.

Species	**Ind.**	**Sites Counted**	**Peak cell density (cones x 10^3 /mm^{-2})**	**Mean cell density (cones x 10^3 /mm^{-2})**
L. dimidiatus	a.2	187	101	68
	a.3	113	128	91
H. melanurus	b.2	173	143	68
	b.3	152	123	70
T. lunare	c.2	147	123	77
	c.3	179	83	49
P. hexataenia	d.2	192	139	55
	d.3	127	82	45
L. unilineatus	e.2	143	100	55

The number of sites that could be counted during stereology is given for each sample. Individuals (Ind.) are referred to in the same way as in Table 1 and Figure 5.

Supplementary Material

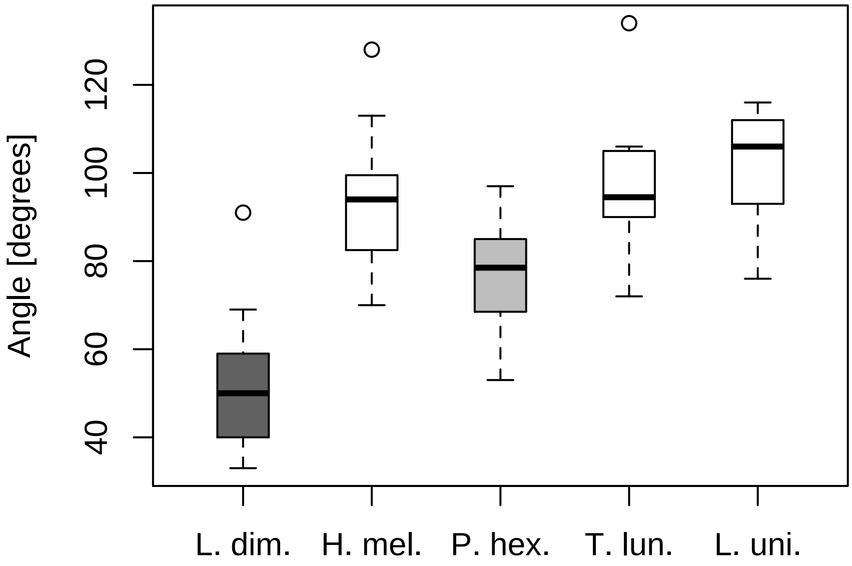

Figure S1. Body angle relative to the feeding plate for each study species. Different colours of boxes indicate significant differences between species. Each box covers the second and third quartiles, the black bar represents the median and the whiskers delimit 95% of the distribution. Outliers are represented as circles. Species names are abbreviated.

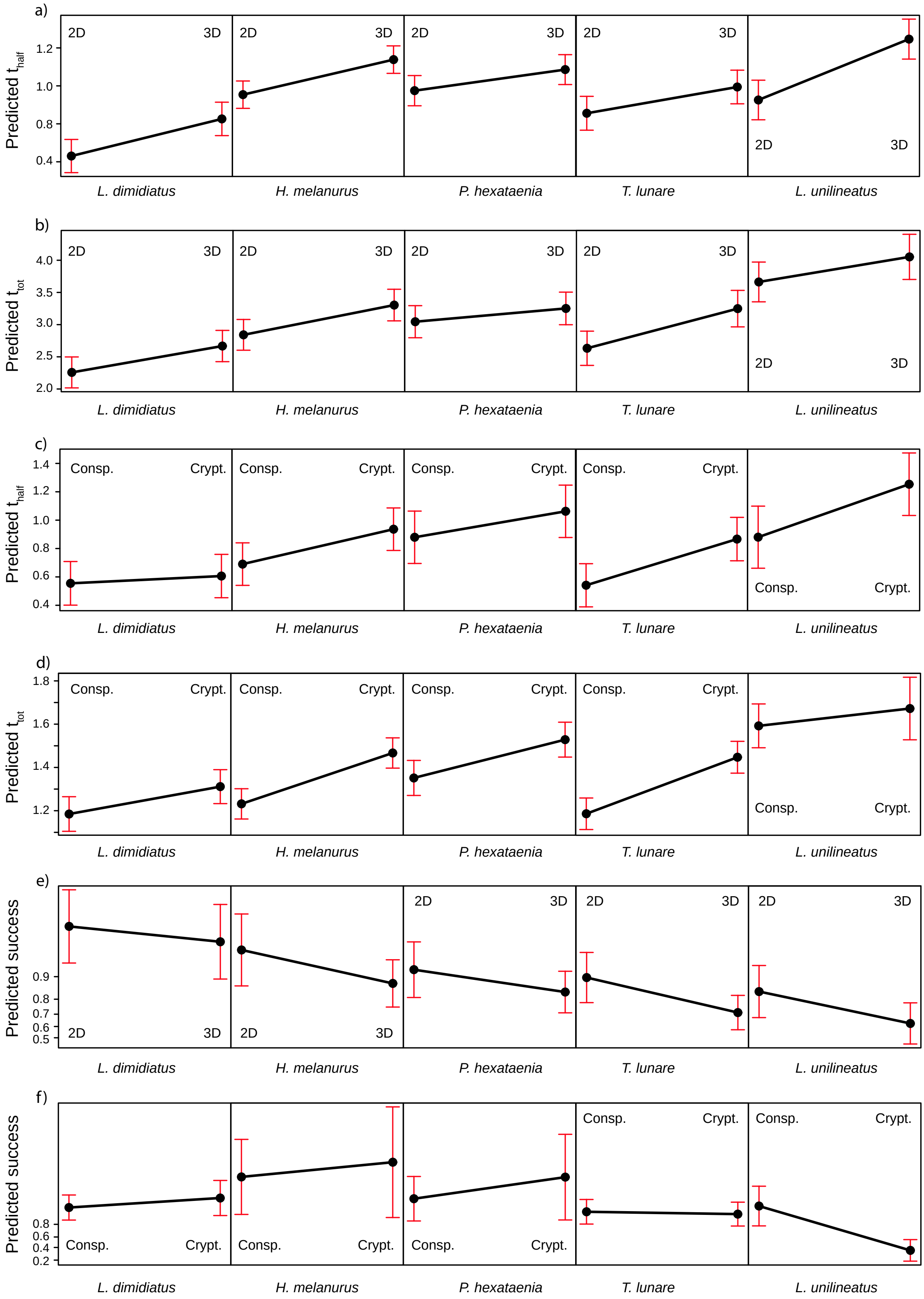

Figure S2.
Model predictions for time to eat 3 items (a,c), time to eat 6 items (b,d) and proportion of successful trials (e,f) for the shape (a,b,e) and cryptic (c,d,f) experiments. Black dots represent the means and red bars the 95% confidence interval.

Table S1. Pairwise comparisons in number of successful trials. Secies were compared in terms of absolute values (top) and effect of the treatment (bottom) for the shape and crypsis experiments.

	Shape					**Crypsis**				
	Est.	**S.E.**	***Z***	***P***	***P* adj.**	**Est.**	**S.E.**	***Z***	***P***	***P* adj.**
Species										
H. mel - L. dim	-0.846	0.858	-0.986	0.324	0.957	2.373	1.501	1.581	0.114	0.695
P. hex - L. dim	-1.557	0.792	-1.965	0.049	0.413	0.682	0.946	0.721	0.471	0.995
T. lun - L. dim	-1.839	0.774	-2.375	0.018	0.194	-0.329	0.655	-0.502	0.616	1.000
L. uni - L. dim	-2.343	0.786	-2.981	0.003	0.042	0.115	0.872	0.132	0.895	1.000
H. mel – P. hex	0.710	0.822	0.864	0.388	0.979	1.691	1.708	0.990	0.322	0.966
H. mel – T. lun	0.992	0.795	1.248	0.212	0.867	2.702	1.552	1.741	0.082	0.582
H. mel – L. uni	1.496	0.806	1.858	0.063	0.484	2.259	1.664	1.357	0.175	0.833
P. hex – T. lun	0.282	0.681	0.414	0.679	1.000	1.011	0.996	1.015	0.310	0.961
P. hex – L. uni	0.786	0.693	1.135	0.256	0.913	0.568	1.166	0.487	0.626	1.000
T. lun – L. uni	0.504	0.658	0.765	0.444	0.990	-0.443	0.915	-0.484	0.628	1.000
Treatment effect										
H. mel - L. dim	-0.652	0.94772	-0.688	0.491	0.995	0.399	1.946	0.205	0.838	1.000
P. hex - L. dim	-0.253	0.87556	-0.288	0.774	1.000	0.933	1.684	0.554	0.580	0.999
T. lun - L. dim	-0.708	0.84557	-0.837	0.403	0.982	-0.920	0.936	-0.986	0.324	0.967
L. uni - L. dim	-0.594	0.86926	-0.683	0.494	0.995	-4.174	1.107	-3.762	<0.001	0.003
H. mel – P. hex	-0.401	0.97284	-0.411	0.681	1.000	-0.534	2.551	-0.209	0.834	1.000
H. mel – T. lun	0.056	0.93165	0.060	0.952	1.000	1.319	2.102	0.628	0.530	0.998
H. mel – L. uni	-0.058	0.95910	-0.060	0.952	1.000	4.573	2.184	2.094	0.036	0.340
T. lun – P. hex	-0.456	0.82205	-0.555	0.579	0.999	-1.853	1.801	-1.024	0.306	0.959
L. uni – P. hex	-0.342	0.85347	-0.401	0.688	1.000	-5.106	1.904	-2.683	0.007	0.092
T. lun – L. uni	-0.114	0.80398	-0.141	0.888	1.000	3.254	1.094	2.975	0.003	0.041

Est., estimate ; S.E., standard error ; *Z*, *Z*-value; *P, P*-value ; *P* adj., adjusted *P*-value.

Table S2. Pairwise comparisons in time to eat half of the items and time to eat all items. Species were compared in terms of absolute values and effect of the treatment for the shape and crypsis experiments.

Shape	t_{half}					t_{tot}				
	Est.	**S.E.**	**Z**	**P**	**P adj.**	**Est.**	**S.E.**	**Z**	**P**	**P adj.**
Species										
H. mel - L. dim	0.323	0.055	5.854	<0.001	<0.001	0.584	0.172	3.386	<0.001	0.012
P. hex - L. dim	0.344	0.058	5.956	<0.001	<0.001	0.788	0.176	4.473	<0.001	<0.001
T. lun - L. dim	0.225	0.061	3.673	<0.001	0.004	0.375	0.182	2.057	0.040	0.395
L. uni - L. dim	0.295	0.067	4.392	<0.001	<0.001	1.405	0.199	7.061	<0.001	<0.001
H. mel – P. hex	-0.021	0.052	-0.405	0.685	0.999	-0.204	0.176	-1.163	0.245	0.934
H. mel – T. lun	0.098	0.056	1.751	0.080	0.596	0.209	0.182	1.148	0.251	0.939
H. mel – L. uni	0.028	0.062	0.449	0.653	0.999	-0.822	0.199	-4.139	<0.001	<0.001
P. hex – T. lun	0.119	0.059	2.035	0.042	0.399	0.413	0.185	2.228	0.026	0.291
P. hex – L. uni	0.049	0.065	0.759	0.448	0.994	-0.617	0.202	-3.060	0.002	0.036
T. lun – L. uni	-0.070	0.068	-1.032	0.302	0.962	-1.031	0.207	-4.973	<0.001	<0.001
Treatment effect										
H. mel - L. dim	-0.011	0.059	-0.185	0.853	1.000	0.053	0.165	0.321	0.748	1.000
P. hex - L. dim	-0.085	0.063	-1.338	0.181	0.856	-0.204	0.171	-1.192	0.233	0.925
T. lun - L. dim	-0.057	0.070	-0.821	0.411	0.991	0.206	0.189	1.089	0.276	0.954
L. uni - L. dim	0.125	0.074	1.685	0.092	0.643	-0.021	0.207	-0.099	0.921	1.000
H. mel – P. hex	0.074	0.052	1.416	0.157	0.816	0.256	0.171	1.499	0.134	0.780
H. mel – T. lun	0.046	0.060	0.776	0.438	0.993	-0.153	0.189	-0.810	0.418	0.993
H. mel – L. uni	-0.136	0.065	-2.091	0.037	0.363	0.073	0.207	0.354	0.723	1.000
T. lun – P. hex	0.027	0.064	0.428	0.668	0.999	0.410	0.195	2.102	0.036	0.365
L. uni – P. hex	0.210	0.069	3.035	0.002	0.038	0.183	0.212	0.863	0.388	0.989
T. lun – L. uni	-0.182	0.075	-2.431	0.015	0.185	0.227	0.227	0.998	0.318	0.972

Crypsis	t_{half}					t_{tot}				
	Est.	**S.E.**	***Z***	***P***	***P* adj.**	**Est.**	**S.E.**	**Z**	**P**	**P adj.**
Species										
H. mel - L. dim	0.135	0.105	1.296	0.195	0.892	0.047	0.054	0.871	0.384	0.989
P. hex - L. dim	0.325	0.118	2.751	0.006	0.087	0.167	0.058	2.890	0.004	0.058
T. lun - L. dim	-0.014	0.105	-0.131	0.896	1.000	0.002	0.055	0.028	0.977	1.000
L. uni - L. dim	0.325	0.132	2.459	0.014	0.180	0.408	0.065	6.229	<0.001	<0.001
H. mel – P. hex	-0.189	0.117	-1.617	0.106	0.712	-0.120	0.054	-2.209	0.027	0.299
H. mel – T. lun	0.149	0.104	1.436	0.151	0.824	0.045	0.051	0.886	0.376	0.987
H. mel – L. uni	-0.190	0.132	-1.443	0.149	0.820	-0.361	0.062	-5.779	<0.001	<0.001
P. hex – T. lun	0.338	0.117	2.881	0.004	0.061	0.165	0.055	2.992	0.003	0.043
P. hex – L. uni	-0.001	0.143	-0.005	0.996	1.000	-0.241	0.066	-3.665	<0.001	0.004
T. lun – L. uni	-0.339	0.132	-2.573	0.010	0.138	-0.406	0.063	-6.418	<0.001	<0.001
Treatment effect										
H. mel - L. dim	0.195	0.070	2.775	0.006	0.081	0.109	0.048	2.284	0.022	0.259
P. hex - L. dim	0.132	0.084	1.572	0.116	0.742	0.050	0.049	1.040	0.298	0.966
T. lun - L. dim	0.274	0.081	3.388	<0.001	0.012	0.135	0.052	2.575	0.010	0.134
L. uni - L. dim	0.322	0.104	3.083	0.002	0.033	-0.046	0.080	-0.584	0.559	0.999
H. mel – P. hex	0.063	0.073	0.869	0.385	0.989	0.058	0.038	1.546	0.122	0.752
H. mel – T. lun	-0.080	0.069	-1.148	0.251	0.943	-0.026	0.042	-0.615	0.539	0.999
H. mel – L. uni	-0.127	0.096	-1.330	0.184	0.877	0.155	0.073	2.113	0.035	0.357
T. lun – P. hex	0.143	0.083	1.718	0.086	0.641	0.084	0.043	1.938	0.053	0.475
L. uni – P. hex	0.190	0.106	1.795	0.073	0.586	-0.097	0.074	-1.309	0.190	0.882
T. lun – L. uni	-0.048	0.104	-0.458	0.647	0.999	0.181	0.077	2.366	0.018	0.217

Est., estimate ; S.E., standard error ; *Z*, *Z*-value; *P, P*-value ; *P* adj., adjusted *P*-value.

CHAPTER 4 - Conflict in mutualistic interactions maintains high escape performance in the cleaner fish *Labroides dimidiatus*

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Status: In preparation

Abstract

In the mutualism between the cleaner wrasse *Labroides dimidiatus* and its coral reef fish clients, predators regularly visit cleaners to get their parasites removed yet show no interest in eating cleaners. According to the concept of compensatory trait loss, characters can be lost if a mutualistic partner performs the same function. Following this logic, one could predict that selective pressures on escape performance have relaxed in *L. dimidiatus* thanks to their mutualistic relationship with predators. However, predation events on cleaners might be rare but the cost of failing to escape is extreme. Furthermore, cleaners regularly escape from non-predatory clients that punish cleaners for cheating (i.e. biting the client). On could thus also predict that successfully fleeing from the close proximity of predators or punishing clients relies on advanced escape capabilities. Here, we compared the fast-start escape performance of *L. dimidiatus* with that of five closely related species that do not clean (or only occasionally). Interestingly, *L. dimidiatus* consistently scored amongst the top performers in our lab experiments. It thus seems that cleaners' mutualistic relationship with predators is not compensated for by a decay in escape performance, but rather that conflicts in cleaning interactions maintained significant selective pressures on this trait.

Contributions: SG & DR collected the data, SG analysed the data. All authors jointly wrote the manuscript.

Introduction

Predation is a major force driving the evolution of species (Darwin 1859; Dawkins & Krebs 1979; Vermeij 1994). Natural selection can act on a wide array of features for prey to avoid being eaten by predators. For example, prey can become more cryptic (Endler 1978), faster at escaping (Watkins 1996; Domenici *et al.* 2008; Langerhans 2009b), or develop protective features such as body armour or spines (Harvell 1984; Reimchen 1995). However, anti-predator traits have costs and divert resources from other fitness-enhancing activities such as growth and reproduction. Therefore, evolutionary theory predicts that anti-predator traits should decay and resources be reallocated elsewhere if predation pressure relaxes on a given species. A classic example involves the loss of anti-predator behaviour in species that have colonised isolated islands devoid of their natural predators (Blumstein & Daniel 2005). Recent studies also suggest that trait loss can be driven by non-predatory interspecific interactions (Ellers *et al.* 2012). For example, characters can be lost if a mutualistic partner performs the same function, a phenomenon termed compensatory trait loss (Ellers *et al.* 2012). Several studies have linked compensatory trait loss to mutualistic partners that provide protection against predators: for example, fungal endophytes providing grass hosts with chemical defences against herbivores (Müller & Krauss 2005) and ants protecting acacia trees from herbivores in exchange for nesting sites (Janzen 1966). Similar to defences against predators, defences against pathogens or competitors can also be reduced due to protection by mutualistic partners. This is exemplified by ants that protect fungus (Currie *et al.* 1999; Currie & Stuart 2001) and damselfish that garden algae (Hata & Kato 2006). Here, we ask whether reduced predation pressure on fish that provide cleaning services to predators can lead to decreased fast-start escape performance via compensatory trait loss.

Labroides dimidiatus is a small coral reef fish (Labridae) that feeds on the ectoparasites of other reef fishes (hereafter 'clients'). Every day, dozens of client species visit the territory of a single cleaner fish to get their ectoparasites removed (Grutter 1996). Piscivorous predators are regular clients at these 'cleaning stations' (Potts 1973) and receive a high quality service relative to non-predatory client species (Bshary 2001). Specifically, cleaners do not cheat predators (i.e. they feed only on parasites and do not bite clients to eat mucus or other live tissues) and provide tactile stimulation (i.e. a 'massage' with the pelvic and pectoral fins) (Bshary 2001; Bshary & Würth 2001; Grutter 2004). In contrast, non-piscivorous clients (e.g. herbivores, corallivores, microbenthic predators) receive tactile stimulation to a lesser degree and are often cheated (Bshary 2001). Cheating opportunities with non-predators are asymmetric because only cleaners can cheat their partner. In contrast, piscivores can cheat cleaners (via predation) and benefit from the threat of reciprocity to enforce cooperation (i.e. trying to eat a cleaner that has cheated) (Bshary & Bronstein 2004). Therefore, interacting with predatory species is risky for cleaner fish. Despite the inherent risks, cleaners readily approach predators and enter their mouth without getting eaten (supplementary video). To date, only anecdotal evidence of predation on cleaners exists and there are no observations of a predation event during a cleaning interaction despite extensive field observations by numerous researchers (Feder 1966; Côté 2000). Predatory clients have also been documented to reduce predatory activities in the vicinity of cleaner fish (Cheney *et al.* 2008). In fact, rather than hiding from predators, cleaners advertise their presence and status to prospective clients via a characteristic oscillating 'dance' (Feder 1966; Youngbluth 1968; Potts 1973) (supplementary video). Finally, *L. dimidiatus* has evolved some of the most conspicuous colours and patterns in the marine environment (Cheney *et al.* 2009), which some species of scale-eating fangblennies mimic to decrease their own predation risk (Cheney 2013).

Several lines of evidence suggest that predators avoid eating cleaners (or fishes that look like them) because the service they provide makes them more valuable alive than dead (Feder 1966; Trivers 1971). Following this logic, one could predict that a reduction in predation pressure has caused selective pressures on escape performance to relax in *L. dimidiatus*. Cleaners might therefore provide a unique example of compensatory trait loss because: 1) there are few examples of compensatory trait loss for vertebrates and none for fish (Ellers *et al.* 2012); 2) the lost (or reduced) trait would be a behavioural response (i.e. the fast-start escape response); and 3) the loss is not compensated for in the cleaners' phenotype but rather by the mutualist predators refraining from predating on cleaners.

The fast-start escape response is the primary behaviour used by fishes to escape from a predatory attack. This behaviour consists of a rapid swimming burst (lasting tens of milliseconds) in which a fish bends its body into a characteristic 'C' shape and then performs a high-energy propulsive stroke (Domenici & Blake 1997; Domenici 2011). In nature, escape performance appears to be tightly linked with predation pressure. Namely, predator induced morphology has been shown to improve fast-start performance in carp (Domenici *et al.* 2008) and mosquitofish (Langerhans 2009a). Conversely, various components of the escape response (e.g. response latency, velocity) tend to decline if other anti-predator strategies are used, such as body armour or schooling (Domenici 2010). In some species that have protective features, such as the spiny eel (Eaton *et al.* 1977) or the lionfish (Webb 1978), researchers have failed to elicit fast-starts. Escape performance can thus respond flexibly to changes in an animal's environment or phenotype.

Here, we use a comparative approach to test the prediction that escape performance has diminished in *L. dimidiatus* due to reduced predation pressure. We compare the fast-start escape performance of *L. dimidiatus* with that of five closely related wrasse species. Wrasses (Labridae) are one of the largest and most diverse family of coral reef fishes (Cowman & Bellwood 2011). Despite their high trophic diversity (Cowman *et al.* 2009), protective features such as body armour, spines and toxins are generally absent in this family (Randall *et al.* 1997), and most labrids employ escape responses to evade predators. Therefore, we expect that performance in this context has been under steady positive selection in this family. In addition to *L. dimidiatus*, we studied two non-cleaner species and three facultative cleaners, which engage in cleaning (mostly as juveniles), but do not exclusively rely on this activity for food acquisition. The cleaning behaviour of facultative cleaners is less specialized than that of *L. dimidiatus* because they do not cheat clients and are not known to interact with predators (Barbu *et al.* 2011).

Based on the concept of compensatory trait loss, we could predict a decay in the fast start performance of cleaner fish in our system. However, there are two arguments for why such an effect might be absent or even reversed. First, while cleaners might experience lower predation risk in general, they might also experience rare, high-risk predation events. Given the high fitness costs of such events, survival relies on advanced fast start performance such as the ability to flee from a predator in close proximity or out of a closing mouth. Second, cleaners have to flee from non-predatory clients on a regular basis because they often feed on the protective mucus of their clients rather than their ectoparasites (i.e. they cheat) (Bshary 2001; Grutter & Bshary 2003). Some client species respond to cheating by terminating the interaction and visiting a different cleaner on their next inspection. Client species without the option to switch partners (because their territory is too small to have access to other cleaners) regularly punish cleaners by chasing them (Bshary & Grutter 2002; 2005). Cleaners must evade these chases to avoid being bitten and injured (see examples of punishment in supplementary video). Here again, successful fleeing might rely on advanced fast-start performance due to the physical proximity of the punisher.

By comparing *L. dimidiatus* with closely related species that differ in their dependency on cleaning for food, we aimed to understand whether selective pressures on escape performance in cleaners have (1) relaxed because of reduced predation by mutualist predators, or (2) been maintained or increased because of the necessity to escape from conflicts. Since individuals from facultative cleaner species typically clean as juveniles but not as adults, we tested both the juvenile and adult life stages of our six study species. Facultative cleaners interact with fewer clients than *L. dimidiatus*, seldom cheat, but are occasionally chased by clients (Barbu et al. 2011). If fast-start escape responses are critical in the context of cleaning interactions, we expect juvenile facultative cleaners and *L. dimidiatus* (obligate cleaner) to exhibit similar performances, higher to that of non-cleaners. Facultative cleaners generally shift away from cleaning at the adult stage, which might cause selective pressures on escape responses to also shift. Therefore, we would expect the performance of obligate and facultative cleaners to diverge more at the adult than the juvenile stage.

Methods

Study species

We examined 122 individuals belonging to 6 species of wrasses (*Labridae*) that co-occur on the Great Barrier Reef (Fig. 1). *L. dimidiatus* is an obligate cleaner, i.e. all of its energy input comes from cleaning interactions; three species (*Pseudocheilinus hexataenia*, *Thalassoma lunare* & *Halichoeres melanurus*) are facultative cleaners, i.e. they might occasionally clean, primarily as juveniles; and two species (*L. unilineatus* & *H. melapterus*) are non-cleaners. We chose these species because they are locally abundant, represent a diversity of trophic niches present in Labridae, and have been used in previous studies aimed at identifying specific selective pressures on *L. dimidiatus* (Gingins & Bshary in press; Gingins *et al.* in prep.). Fish were classified as juveniles or adults based on body colouration (Randall *et al.* 1997; Allen *et al.* 2005) (Fig. 1). Since *P. hexataenia* does not exhibit an ontogenetic colour shift, adults were distinguished from juveniles based on size differences. Adults were greater than 5 cm total length (TL), which corresponds to two thirds of the maximum size for this species (Allen *et al.* 2005). Fish were collected on reefs surrounding Lizard Island, Australia (14°40'S 145°28'E), and captured using a barrier net and small hand nets. We used a 10% clove oil solution to momentarily sedate species that enter the reef matrix when chased (*P. hexataenia* and some juveniles from other species). Fish were transported to the Lizard Island Research Station immediately following capture and housed in individual aquaria with flow-through seawater pumped directly from the reef. We allowed a minimum acclimation time of 24 hours prior to experimentation (mean = 22.5; SD = 14.9 days). Fish were last fed the day before the experiments so they were all tested in a standardized, post-absorptive state.

Fast-start experiments

Juveniles were tested during the Australian winter (August – October 2012) and adults, during summer (January – February 2013). The experimental setup consisted of an acrylic tank (70 x 60 x 35 cm) mounted on a wooden structure, with a mirror placed at a 45° angle beneath the tank (Supplementary figure S1). We filmed the fish through the mirror at 420 frames per second (fps) using a high-speed camera (Casio Exilim EX-FH100, Casio Computer Co., Tokyo, Japan). Escape responses were triggered by releasing a 50ml cylindrical plastic vial filled with lead weights suspended above the tank with an electromagnet. A string prevented the vial from hitting the bottom of the tank as it fell. The vial fell inside an opaque PVC tube (10 cm diameter) suspended 1cm above the water surface, which prevented visual stimulation before contact with the water. The water level was kept between 10 and 20cm (depending on the size of the fish tested) to minimize vertical displacement while allowing full extension of the anal and dorsal fins. Lighting was provided by three 150 W halogen work lights, positioned ~ 75 cm above the sides of the tank, at a 45° angle. A 5cm scale was affixed to the bottom of the tank for distance measurements (see video analysis). Prior to an experiment, a focal fish was transferred from its holding tank to the experimental tank; its TL was measured, and it was allowed a minimum of 30 minutes of acclimation time. To minimize variation in performance due to differences in positioning relative to the stimulus, we stimulated fish when they were at an angle of ~90° relative to the stimulus (mean= 93.9°, s.d.= 34.6°) and ~10 cm from the stimulus (mean= 11.3 cm, s.d.= 4.3 cm). Each fish was tested three times with a minimum time interval of 30 minutes between trials to increase the accuracy of maximum performance estimates (Jornod & Roche 2015). If a test fish did not respond or moved and considerably changed its position immediately prior to the stimulation, additional trials were conducted until a satisfactory response was obtained. Each fish experienced on average 3.85 (s.d.= 1.75) trials. Some individuals and/or species frequently swam to the center of the arena whereas others tended to remain near the edges. In the latter case, we gently moved a PVC pipe along the walls of the arena, encouraging fish to move away from the edges. Following the experiments, one juvenile and one adult of each species were euthanized with an overdose of Aqui-S (100 mg/L, New Zealand Ltd.) to measure their centre of mass (CoM). The position of the CoM relative to the tip of the snout was obtained for each species and age class and used in subsequent video analyses. All other fishes were returned to the reef upon completion of the experiments.

Field observations

We conducted field observations of our six study species in July-September 2014 on SCUBA or snorkel. Eight adults per species were observed for a 15-minute period and we noted, every 30 s, whether an individual was in sight or inside the reef matrix. We also estimated fish TL to the nearest 0.5 cm, and recorded the number of cleaning interactions.

Video analysis

C-starts are escape responses used by fishes, in which they bend their body into a characteristic C shape and use a propulsive stroke to achieve high speed and acceleration. Double-bend C-starts comprise a 1st and 2nd stage separated by the change in direction of the anterior body midline (Domenici & Blake 1997) whereas single-bend C-starts consist of only the first stage. We used the software ImageJ 1.48v (Rasband 1997) and the plugin MTrackJ (Meijering *et al.* 2012) to extract behavioural and kinematic variables from escape response videos. Two experimenters performed the video analysis. For each trial, we measured a fish's escape latency (the time from the onset of the stimulus to the first head movement of the fish), the duration of stages 1 and 2, and the location of the fish's CoM every 2.38 ms during the escape response. We used these data to compute the following variables: (1) stage 1 turning rate (calculated as the angle between the segment joining the CoM and the tip of the snout at the beginning and end of stage 1 divided by the duration of stage 1); and distance-time variables including (2) cumulative escape distance (D_{esc}), (3) maximum velocity ($U_{\max}$), and (4) maximum acceleration ($A_{\max}$) calculated over the mean escape response duration (i.e. stages 1 + 2) across all trials and species (29 ± 14 ms; mean ± SD) (Domenici & Blake 1991). We also measured the distance from the snout of the fish to the stimulus and the angle between the snout, the COM and the stimulus to control for the variation in the position of the fish when startled (Jornod & Roche 2015). $U_{\max}$ and $A_{\max}$ were smoothed using a five-point quadratic polynomial regression (Lanczos 1956).

Statistical Analysis

We analysed escape response trials only when the angle of the fish's body relative to the stimulus was above 25° or below 155° to avoid potential biases due to the stimulus falling frontally or dorsally. The final dataset comprised 271 trials, corresponding to a mean of 2.22 (SD=0.89) trials per individual. We tested for interspecific differences in five measures of escape performance using general linear models: escape latency [ms], maximum velocity [cm s^{-1}], $A_{\max}$ [m s^{-2}] cumulative distance [cm], and turning rate [° ms^{-1}]. In addition to these five standard kinematic variables, we computed the cumulative distance travelled in 34 ms, which corresponded to the time between the onset of the stimulus to the end of stage 2 (approximately). This metric includes a fish's response latency and therefore provides an ecologically relevant measure of how far a fish can escape because it captures the actual distance covered from the onset of a threat. For each variable, we selected an individual's best performance for the analysis (Gibson & Johnston 1995; Langerhans *et al.* 2004; Marras *et al.* 2011). We controlled for any effect of observer, distance to the stimulus and angle relative to stimulus by including the following terms in the models: response.variable ~ sin(angle.stim) + dist.stim + observer + species. Distance and angle to the stimulus were centred prior to the analysis. We assessed normality and homoscedasticity of the residuals with qqplots and plots of residuals vs. fitted values. Latency was log transformed to comply with model assumptions. Juvenile and adult fishes were analysed separately because they were not tested at the same period of the year and water temperature is known to influence escape performance (Domenici 2011). We did not perform pairwise comparisons due to the large number of tests required. Rather, we interpret pairwise differences between species by referring to the overlap of the confidence intervals (C.I.) of the means predicted by the linear models. With sample sizes above 10, *P*-values should be significant (<0.05) if the fraction of the C.I. arm overlapping between two groups is smaller than 0.5 (Cumming *et al.* 2007).

Body length can have an effect on D_{esc} and U_{max} (Webb 1976; Domenici 2011), and researchers often control for size by reporting relative values (i.e. in body lengths and body lengths s^{-1} ; see Domenici & Blake (1997). However, we did not control for body length for three reasons: 1) size and fish species were collinear (see supplementary figure S2), 2) D_{esc} and U_{max} appear to be size-independent when measured in a fixed time frame (Domenici & Blake 1993), and 3) we were interested in absolute rather than relative performance. Whether a species can escape punishment and/or predation depends on its performance in absolute, not relative terms. Since escape latency and absolute cumulative distance correlate with size in opposite ways (Domenici & Blake 1997) , it is hard to predict how size could influence our composite measure of cumulative distance (from the stimulus onset), which integrates both latency and distance covered. Thus, we investigated this relationship separately for adults and juveniles of each species, using Spearman rank correlations.

We used a Kruskal-Wallis test to examine differences in the time spent by the six species inside the reef matrix during our field observations ("kruskal" function in the R package "agricolae") (de Mendiburu 2015). All analyses were done in R 3.2.2 (R Core Team 2013).

Results

Adults

We found significant interspecific differences in escape latency (Table 1; Fig. 2a), turning rate (Table 1; Fig. 2b), U_{max} (supplementary material: Table S1; Fig. S3.a), and D_{esc} both from the onset of the stimulus and from the onset of the response (Table 2; Fig. 3.a-b). The only measure that did not differ across species was A_{max} (supplementary Table S1).

P. hexataenia and *L. dimidiatus* were the two species that had the shortest response latency: their performance was significantly better than that of *H. melanurus*, *T. lunare* and *H. melapterus* (no overlap of C.I.; Fig.2a.2). *P. hexataenia* outperformed all the other species with regards to turning rate (Fig. 2b.2). The turning rate of *L. dimidiatus* was also very high and significantly exceeded that of *T. lunare*, *L. unilineatus* and *H. melapterus* (Fig. 2b.2). *H. melapterus* preformed significantly worse than all other species for both escape latency and turning rate (Fig. 2a-b).

Species clustered into two groups with regards to D_{esc} from the first head movement (Fig. 3a). *L. dimidiatus* and *T. lunare* covered significantly more distance than *H. melanurus*, *L. unilineatus* and *H. melapterus* (Fig. 3a.2). *P. hexataenia* also performed very well and covered a significantly larger distance than both *H. melanurus* and *H. melapterus* (Fig. 3a.2). Results for U_{max} produced a similar clustering of species (supplementary material Fig. S3a.1). When D_{esc} was measured from the onset of the stimulus rather than from the first head movement, *L. dimidiatus* and *P. hexataenia* outperformed all other species (Fig. 3b.2). This pattern was similar to the one observed for escape latency (Fig. 2a). We found no significant correlation between D_{esc} from the onset of the stimulus and size (Spearman rank correlations, all $P > 0.05$). Nevertheless, the relationship between performance and size tended to be positive for *L. dimidiatus* (rho = 0.09), *T. lunare* (rho = 0.18), *L. unilineatus* (rho = 0.16) and *P. hexataenia* (rho = 0.36; *P* = 0.051) and negative for *H. melanurus* (rho = -0.05) and *H. melapterus* (rho = -0.24).

Juveniles

We found significant differences across species in response latency (Table 1; Fig. 2a), turning rate (Table 1; Fig. 2b) and D_{esc} from the onset of the stimulus (Table 2; Fig. 3b). There were no differences in D_{esc} from the first head movement (Table 2; Fig. 3a), $U_{\max}$ and $A_{\max}$ (supplementary material: Table S1; Fig S3.a). *L. dimidiatus* had a significantly shorter escape latency than all other species (Fig. 2a.3). The turning rate of *P. hexataenia* and *H. melanurus* was significantly higher than that of *L. unilineatus* and *H.melapterus*. While for the adults, *P. hexataenia* clearly outperformed other species, the turning rate of juveniles was less variable than that of adults (Fig. 2b.3). There was little variation in D_{esc} from the onset of the stimulus across species (Fig. 3a-b): *L. dimidiatus* perfomed better than *L. unilineatus* and *H. melapterus* but not than other species (Fig. 3b.3). These differences reflected those observe for escape latency (Fig. 2a). We found no significant correlation between size and D_{esc} from the onset of the stimulus (Spearman rank correlation test, all $P > 0.05$). This relationship tended to be positive for *L. dimidiatus* (rho = 0.25), *T. lunare* (rho = 0.005), *L. unilineatus* (rho = 0.37), *P. hexataenia* (rho = 0.34) and *H. melapterus* (rho = 0.47; *P* = 0.0503) and negative for *H. melanurus* (rho = -0.20).

Field observations

Species differed significantly in the percentage of time spent inside the reef matrix (Kruskal-Wallis, df=5, chi^2=18.420, *P*=0.0024). *P. hexataenia* spent over 20% of its time inside the reef, which was significantly more than any other species (Table 3). Other species spent less than 2.5% of their time inside the reef. We recorded 469 cleaning interactions across the eight *L. dimidiatus* observed over two hours (15 min per individual). Facultative cleaner species participated in few cleaning interactions: one *P. hexataenia* (6cm) interacted with a client on two occasions and two *H. melanurus* (6.5 & 7 cm) were involved in 4 and 1 cleaning interactions, respectively.

Discussion

We asked whether the cleaner wrasse *L. dimidiatus* has evolved reduced fast-start escape performance due to lower predation risk resulting from mutualist predatory clients refraining from hunting it. Our results do not support this hypothesis. Instead, they provide evidence to the contrary: compared to five closely related wrasse species that are facultative- or non-cleaners, *L. dimidiatus* consistently figured amongst the top two performers. Below, we discuss the implications of our results for our understanding of marine cleaning mutualisms and links between ecology and fast start performance.

Conflict in a mutualism selects for high fast-start performance

Fast-start escape performance has often been linked to predation pressure in fishes (Andraso 1997; Domenici *et al.* 2008; Langerhans 2009a; Domenici 2010; Beck & Rooker 2011). Accordingly, the cleaner wrasse *L. dimidiatus* could exhibit reduced escape performance because it benefits from reduced predation pressure (Feder 1966; Trivers

1971; Côté 2000; Cheney *et al.* 2008). The high escape performance observed in this species may be explained by two non-mutually exclusive factors.

First, predation events are extremely rare but their fitness costs are high (i.e. death or severe injury). Because of their close physical proximity to predators during cleaning interactions, cleaners are potentially easy preys to capture. Although the distinctive colour pattern of cleaners helps reduce the risk of predators mistakenly attacking them (Cheney *et al.* 2009), rare mistakes might constitute a considerable threat. Currently, we do not know whether mistakes occur and whether predatory clients are sometimes tempted to cheat by eating a cleaner during an interaction. Such events have never been observed (Feder 1966; Côté 2000) but, given associated costs, cleaners might still require excellent fast-start capabilities in the event that they do. Additionally, cryptic sit-and-wait predators like scorpionfish and hawkfish are not clients of cleaners and hence also pose a threat that requires high escape performance to avoid predatory strikes in close proximity.

Second, chases used by non-predatory clients to punish cheating cleaners might also be an important factor selecting for high escape performance (see Bshary 2001). Based on the results from observations in the field (Bshary & Grutter 2002), we can estimate that cleaners experience punishment approximately twice per 100s of interaction, and thus need to escape in this context multiple times every day. On very rare occasions, the chaser might be a predator (observed once by RB over hundreds of hours of observations) and the cleaner risks losing its life ("threat of reciprocity" in Bshary & Bronstein 2004).

Our results do not allow determining the relative importance of predation risk and punishment in influencing the fast-start performance of *L. dimidiatus*. However, cleaning gobies (*Elacatinus* spp.) that occur in the Caribbean also regularly inspect predators and would provide an ideal system to tease apart these effects. Unlike *L. dimidiatus*, *Elacatinus* spp. prefer eating ectoparasites over client mucus (Soares *et al.* 2010). Therefore, clients of *Elacatinus* spp. do not use partner control mechanisms such as punishment or partner switching to prevent cheating (Soares *et al.* 2008). Evidence that the escape performance of cleaning gobies exceeds that of co-occuring, non-cleaning gobies would indicate that the threat of predation is sufficient to maintain selection on fast-start performance despite the typically cooperative nature of cleaning interactions. Conversely, similar performance across cleaning and non-cleaning gobies would suggest that punishment is the main driver of fast-start performance in cleaners.

Three of the wrasse species we examined are facultative cleaners (*H. melanurus*, *P. hexataenia* and *T. lunare*). However, we recorded few cleaning interactions by adults during field observations (5, 2 & 0 respectively). In contrast we recorded over 450 cleaning interactions for the obligate cleaner *L. dimidiatus* in the same time. Escaping from conflicts during cleaning interactions might be particularly important for specialist cleaners such as *L. dimidiatus.* Indeed, in our fast-start experiments, facultative cleaners generally performed worse than *L. dimidiatus*, and quite similarly to non-cleaners. Since facultative cleaners engage in few cleaning interactions, there might be insufficient conflict with clients to create significant positive selection on escape performance. Facultative cleaning is predominantly performed by juveniles (Côté 2000). Accordingly, we found less variation in escape performance across species at the juvenile rather than the adult stage. Differences in selective pressures throughout ontogeny could explain these differences.

Predation pressure on juvenile coral reef fishes is high, and decreases throughout ontogeny (Anderson 1988; Hixon 1991; Almany & Webster 2005). However, the need to escape client chases remains constant throughout *L. dimidiatus'* life history because it cleans both as a juvenile and as an adult (Barbu *et al.* 2011). Greater variation in escape performance among adults than juveniles also supports the idea that cleaning originated independently many times at the juvenile stage (Baliga & Law 2015), and that specific behaviours are selected for at the adult stage in species that specialize in cleaning (Gingins & Bshary n.d.; Barbu *et al.* 2011).

Linking ecology and fast start performance

Two species exhibited very high escape performance in our experiments: *L. dimidiatus* and *P. hexataenia*. Both are relatively small in size compared to the other four species we examined (Fig. 1; Fig. S1). However, *L. dimidiatus* and *P. hexataenia* differ strongly in their habitat use, with *P. hexataenia* being the only study species that spent a considerable amounts of time (> 20%) inside the reef matrix (Table 3). Below, we discuss potential implications of size and habitat use for escape performance.

We did not attempt to control for the effects of body size on fast-start escape performance by choosing similar-sized fishes: species were selected for parallel studies examining inter-specific differences in behaviour, cognition and sensory ecology among coral reef wrasses (Gingins & Bshary in press; Gingins *et al.* in preparation).Phylogenetic relationships and the distinction between obligate cleaner, facultative cleaner and non-cleaner were therefore key factors in our choice of study species.

Size can affect a fish's escape performance, with larger fish achieving a greater absolute distance and velocity than small species during fast-starts (Domenici & Blake 1997; Domenici 2011). This is mainly due to the fact that larger fishes take more time to complete a fast-start (Webb 1976; Domenici & Blake 1993). However, when comparing fishes of different sizes in a fixed time period (as was done here), these variables are size-independent (Domenici & Blake 1993). Therefore, we did not correct for fish size in our analyses. Size also did not correlate with D_{esc} from the onset of the stimulus for any of the species examined. Despite being non-significant, these relationships were positive for most species. The two species that performed best in our experiments were the two smallest, which is contrary to known effects of size on absolute measures of escape performance and the positive but non-significant trends between size and D_{esc}. Thus, we feel confident that most of the interspecific variation in escape performance observed cannot be explained by size, but rather reflects differences in ecological demands.

Trade-offs between various aspects of swimming performance such as steady and burst swimming are often linked to habitat use and predation pressure (Domenici 2003). *P. hexataenia* occupies a small territory inside branching corals and spends most of its time within centimetres of the reef. Navigating in the narrow interstices between coral branches requires high manoeuvrability, which might explain why adults of this species exhibited very rapid turning rates (Fig. 2). The ecology of this species therefore shares some similarities with that of *L. dimidiatus*: living in a highly structured environment means that many encounters with predators do not involve the early stages of a predator-prey interaction (e.g. detection, approach) because predators typically become visible only at close range (Brown *et al.* 2011). Therefore, both *L. dimidiatus* and *P. hexataenia* might have experienced strong selective pressures on short-range escape performance.

Conclusions/outlook

Our study of fast-start escape performance complements other research aiming to identify how interactions with client reef fishes affect the behaviour and cognitive abilities of cleaner fish (Gingins & Bshary in press; Barbu *et al.* 2011; Salwiczek *et al.* 2012; Gingins *et al.* 2013). The goal of these studies is to understand the evolutionary consequences of interspecific interactions on phenotypic traits, both with respect to the evolution of novel traits and the loss of ancestral traits. The absence of evidence for compensatory trait loss in *L. dimidiatus* (i.e. reduced escape performance compensated for by a mutualistic relationship with piscivorous predators) has interesting implications for future research. It suggests that the evolutionary dynamics of traits differ between mutualisms that are characterised by high interdependence and low conflicts of interest on the one hand, and mutualisms characterised by low interdependence and high conflicting interests on the other hand. Only in the former case can interacting partners rely on each other and hence experience relaxed selection on traits that one of the partners compensates for. When conflicts exist and individuals are tempted to cheat and increase their benefits at the expense of others (Axelrod & Hamilton 1981; West *et al.* 2007), compensatory trait loss may occur only in cases of high interdependence, such as in specialised host-parasite systems (Ellers *et al.* 2012). Our results therefore emphasize the importance of conflicts in the evolution and maintenance of species' traits in cleaning mutualisms.

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Figures

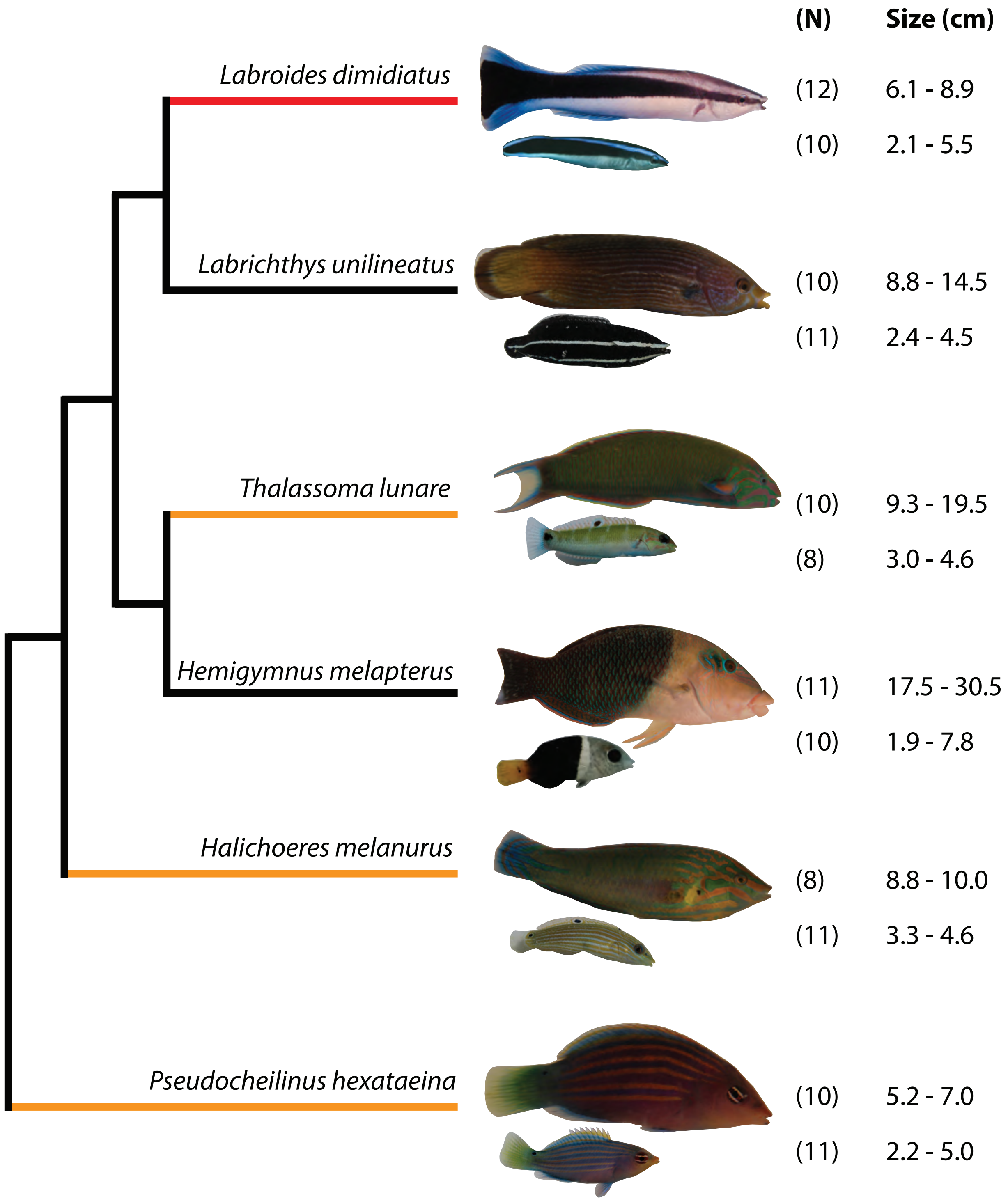


Figure 1. Schematic representation (i.e. branch lengths are not scaled) of the phylogenetic relationships between study species, based on the phylogeny of Cowmann and Bellwood (2011). The colour of the branch indicates dependency on cleaning: red = obligate cleaner; orange = facultative cleaner; black = non-cleaner. The range of total length (TL) for each species is given for juveniles and adults. Pictures are not to scale. The number of individuals tested (*N*) is given in parenthesis.

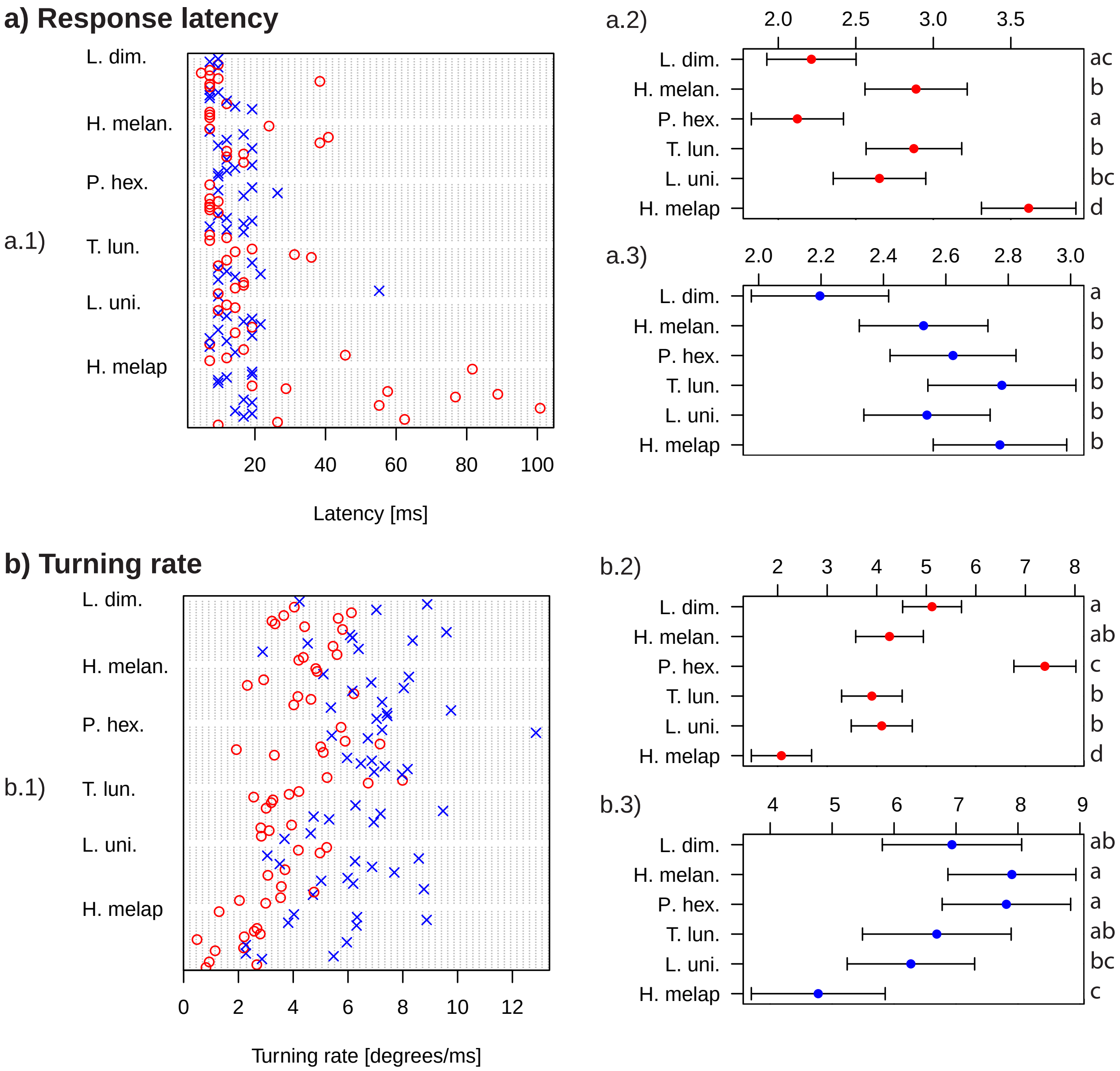
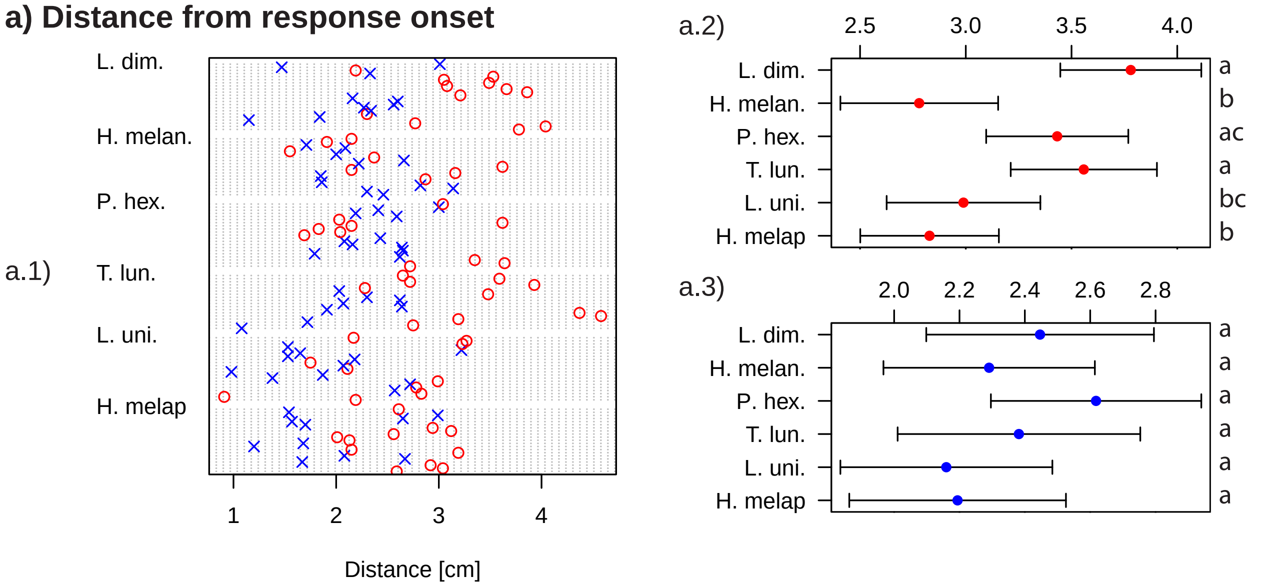


Figure 2. The best performance of fishes for (a) response latency and (b) turning rate. Left panels (a.1; b.1) represent the raw data. Red circles = adults; blue crosses = juveniles. Right panels show the mean and 95% confidence interval (C.I.) predicted by the general linear models for adults (a.2; b.2) and juveniles (a.3; b.3). On the right panels, different letters indicate that the C.I. do not overlap for more than half of the bar length, and thus represent significant differences below the 0.05 threshold (Cumming *et al.* 2007). Plots of model predictions were created with the R package "effects" (Fox 2003). Units for the x-axes of these plots are the same as plots of the raw data except for latency that was log-transformed. Species names are abbreviated (see Fig. 1 for full names).

a) Distance from response onset



b) Distance from stimulus onset

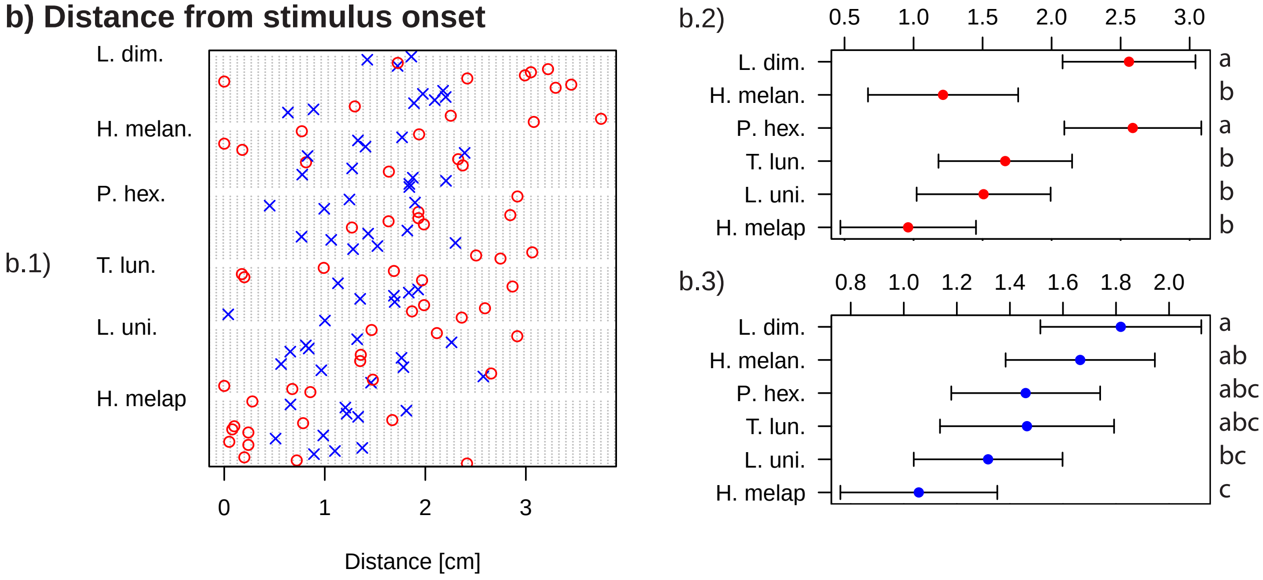


Figure 3. The best performance of fishes for (a) cumulative distance travelled (D_{esc}) from the onset of the response and (b) from the onset of the stimulus. Left panels (a.1; b.1) represent the raw data. Red circles = adults; blue crosses = juveniles. Right panels show the mean and 95% confidence interval (C.I.) predicted by the general linear models for adults (a.2; b.2) and juveniles (a.3; b.3). On the right panels, different letters indicate that the C.I. do not overlap for more than half of the bar length, and thus represent significant differences below the 0.05 threshold (Cumming *et al.* 2007). Plots of model predictions were created with the R package “effects” (Fox 2003). Units for the x-axes of these plots are the same as plots of the raw data. Species names are abbreviated (see Fig. 1 for full names).

Tables

Table 1. Results from the linear models with escape latency (a) and turning rate (b) as response variables, for both adults and juveniles.

			Adults			Juveniles		
Response	**Predictor**	**d.f.**	**Sum Sq.**	**F**	**P-value**	**Sum Sq.**	**F**	**P-value**
a) Latency								
	sin(Angle)	1	0.03	0.12	0.732	0.10	0.90	0.347
	Dist. stim.	1	11.52	53.72	1.47 e^{-9} ***	1.60	14.41	3.85 e^{-4} ***
	Observer	1	0.02	0.11	0.740	0.16	1.41	0.241
	Species	5	14.06	13.11	2.87 e^{-8} ***	2.06	3.72	5.92 e^{-3} **
b) Turning rate								
	sin(Angle)	1	2.44	2.66	0.108	5.20	1.82	0.183
	Dist. stim.	1	18.76	20.42	3.61 e^{-5} ***	20.26	7.11	1.02 e^{-2} *
	Observer	1	4.85	5.28	2.56 e^{-2} *	0.06	0.02	0.886
	Species	5	144.11	31.38	1.41 e^{-14} ***	66.60	4.67	1.35 e^{-3} **

d.f., degrees of freedom ; Sum Sq., Sum of Squares; *P<0.05; **P<0.01; ***P<0.001

Table 2. Results from the linear models with Cumulative distance from response onset (a) and from stimulus onset (b) as response variables, for both adults and juveniles.

			Adults			Juveniles		
Response	**Predictor**	**d.f.**	**Sum Sq.**	**F**	**P-value**	**Sum Sq.**	**F**	**P-value**
a) Cum. dist. (from response onset)	sin(Angle)	1	0.03	0.11	0.737	1.59	5.87	1.89 e^{-2} *
	Dist. stim.	1	0.56	2.06	0.157	0.21	0.79	0.379
	Observer	1	0.45	1.65	0.205	0.18	0.65	0.425
	Species	5	7.43	5.44	4.22 e^{-5} ***	1.44	1.06	0.391
b) Cum. dist. (from stimulus onset)	sin(Angle)	1	0.31	0.56	0.458	1.28	6.05	1.73 e^{-2} *
	Dist. stim.	1	18.55	32.42	5.83 e^{-7} ***	5.99	28.29	2.24 e^{-6} ***
	Observer	1	1.08	1.89	0.176	0.00	0.00	0.988
	Species	5	22.48	7.86	1.44 e^{-5} ***	3.32	3.14	1.51 e^{-2} *

d.f., degrees of freedom ; Sum Sq., Sum of Squares; *P<0.05; **P<0.01; ***P<0.001

Table 3. Proportion of time that each species spent hiding in the reef matrix over 2 hours of observations.

Species	% time in reef	std	Min	Max	Pairwise comparisons
L. dimidiatus	0.004	0.012	0	0.033	b
H. melanurus	0.025	0.050	0	0.133	b
P. hexataenia	0.212	0.215	0	0.633	a
T. lunare	0.000	0.000	0	0.000	b
L. unilineatus	0.008	0.024	0	0.067	b
H. melapterus	0.021	0.059	0	0.167	b

std., standard deviation; Min, Minimum; Max, Maximum; different letters indicate significant differences across species.

Supplementary Material

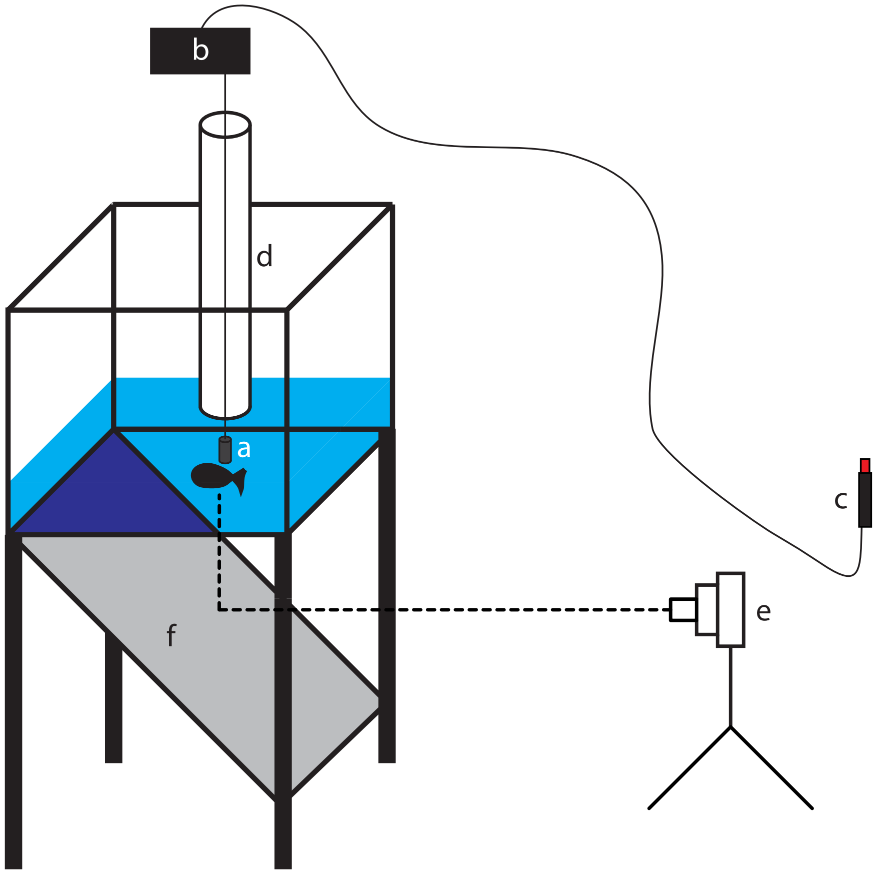


Figure S1. Experimental setup for the fast-start experiments. The stimulus (a) was attached to an electromagnet (b). When the focal fish (in black) was in the right position, the experimenter released the stimulus by switching off the electromagnet (b) with a remote control (c). The stimulus fell inside an opaque PVC tube (d) suspended 1cm above the water surface, which prevented visual stimulation before contact with the water surface. Escape responses were filmed with a high-speed camera (e). A mirror placed at a 45° angle allowed filming through the bottom of the experimental arena (f). See methods for details.

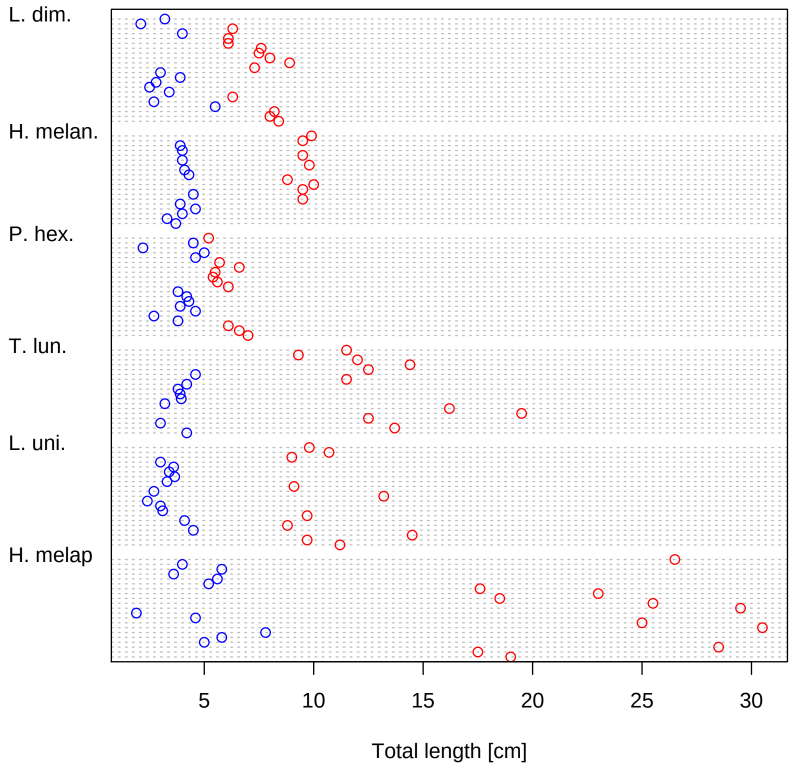
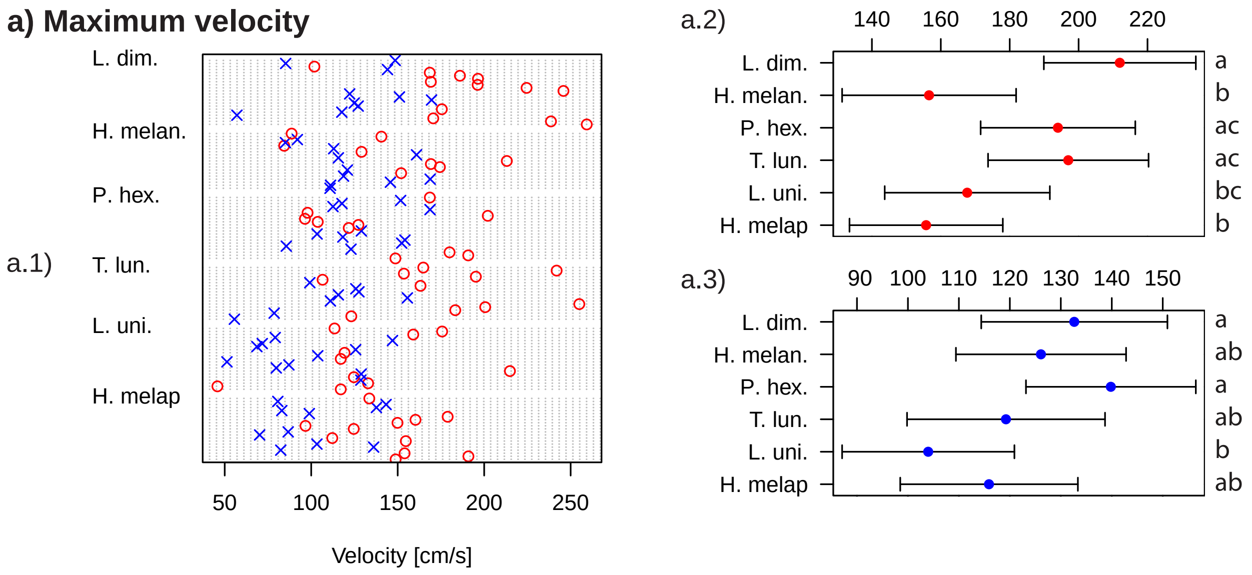


Figure S2. Size (total length) of every individuals tested in the fast-starts experiments. Red = Adults; Blue = Juveniles.

a) Maximum velocity



b) Maximum acceleration

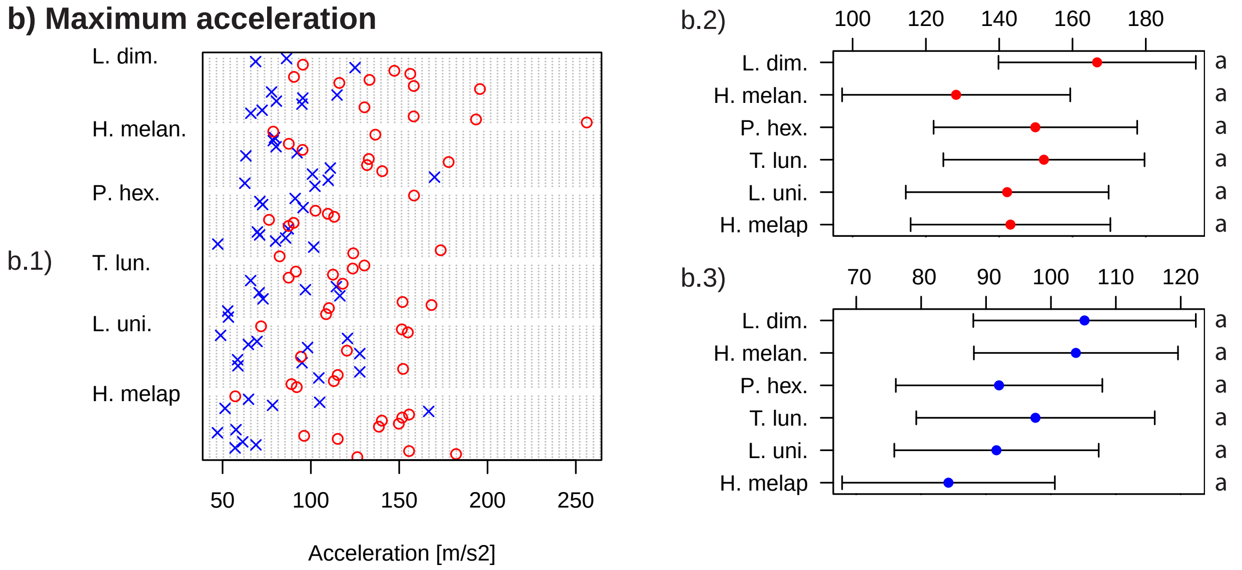


Figure S3. The best performance of fishes for (a) maximum velocity (U_{max}) and (b)maximum acceleration (A_{max}). Left panels (a.1; b.1) represent the raw data. Red circles = adults; blue crosses = juveniles. Right panels show the mean and 95% confidence interval (C.I.) predicted by the general linear models for adults (a.2; b.2) and juveniles (a.3; b.3). On the right panels, different letters indicate that the CIs do not overlap for more than half of the bar length, and thus represent significant differences below the 0.05 threshold (Cumming *et al.* 2007). Plots of predictions were created with the R package "effects" (Fox 2003). Units for the x-axes of these plots are the same as plots of the raw data. Species names are abbreviated (see Fig. 1 for full names).

Table S1. Results from the linear models with maximum velocity (a) and maximum acceleration (b) as response variables, for both adults and juveniles.

			Adults			**Juveniles**		
Response	**Predictor**	**d.f.**	**Sum Sq.**	**F**	**P-value**	**Sum Sq.**	**F**	**P-value**
a) Velocity								
	sin(Angle)	1	2564	2.10	0.153	3957	0.90	2.51 e^{-2} *
	Dist. stim.	1	1552	1.27	0.264	941	14.41	0.266
	Observer	1	2257	1.84	0.180	368	1.41	0.485
	Species	5	24724	4.05	3.54 e^{-3} **	8048	3.72	7.42 e^{-2}
b) Acceleration								
	sin(Angle)	1	187	0.10	0.751	1572	2.37	0.130
	Dist. stim.	1	3	1.8 e^{-3}	0.966	2161	3.26	7.68 e^{-2}
	Observer	1	2444	1.32	0.255	402	0.61	0.440
	Species	5	7386	0.80	0.554	3200	0.97	0.447

d.f., degrees of freedom ; Sum Sq., Sum of Squares; *P<0.05; **P<0.01; ***P<0.001

B - DISCUSSION

In order to understand how cleaners evolved for their highly social ecology, I had asked in which aspects they differed from closely related species that do not face the same ecological challenges. The results from the various experiments in my thesis (see Figure 1) suggest that the behaviour of the obligate cleaner *L. dimidiatus* is highly specialized for cleaning interactions. First, it was able to fine-tune its cooperative behaviour to the specifics of different cleaning-related situations, an ability that was absent in the closely related *H. melanurus*. Second, *L. dimidiatus* outperformed five other wrasses in tasks relevant for cleaning. Conversely, all species performed similarly in a context that had little ecological relevance, indicating that cognition in cleaners is tightly linked to ecological demands.

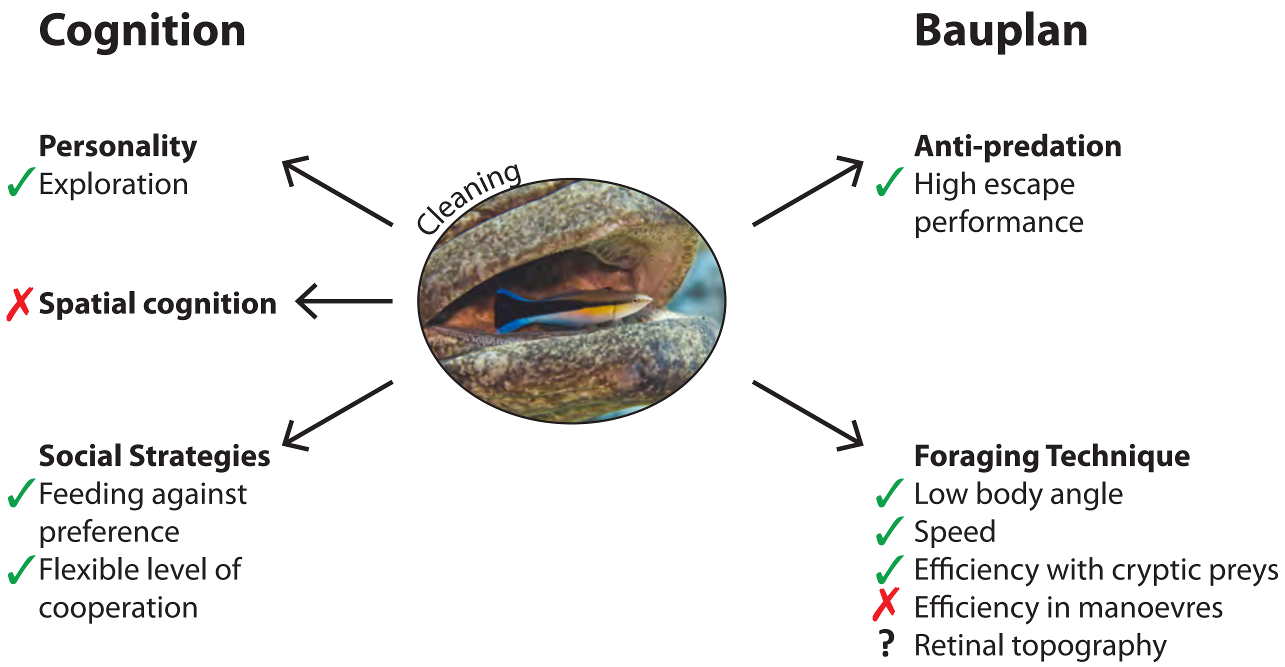


Figure 1. Schematic representation of the main results from my thesis. Green checkmark indicate characteristics that differed between cleaners and the other species tested; a red cross indicates no difference; a question mark indicates that the current data did not allow drawing conclusions. I use the term 'bauplan' to describe behaviours that have a strong morphological underpinning, in contrast with cognitive traits that relate to the way animals acquire, process and act upon information.

Third, foraging experiments suggest that *L. dimidiatus* evolved a feeding posture that increases efficiency at finding cryptic parasites on the body of client fishes. With the current data, however, I could not assess whether the visual system of *L. dimidiatus* also adapted for this foraging ecology. Fourth, it appeared that conflicts in cleaning interactions maintained a high escape performance in cleaners in comparison with related species. The ecological niche of *L. dimidiatus* thus appears to have exerted selective pressures on a wide array of features, ranging from behaviour and cognition, to escape performance and foraging posture. In species that do not clean, or only occasionally, the same traits did not appear to have been under selection.

B.1- The emergence of cleaning in Labridae

In *Labridae*, more than 50 out of the approximately 600 species engage in cleaning at some point in their life (Côté 2000). For most species, cleaning is a minor component of their ecology (facultative cleaners), and only 6 species rely almost exclusively on this activity for food acquisition (obligate cleaners). My comparisons between the obligate cleaner *L. dimidiatus* and facultative cleaners suggest that specific adaptations linked to cleaning only occur in specialist species. A previous study also supports this view, as the strategic behaviours employed by *L. dimidiatus* were absent in facultative cleaners (Barbu *et al.* 2011). Furthermore, I could not identify characteristics that might facilitate the emergence of cleaning since facultative cleaners and non-cleaners performed very similarly in my experiments. So far, the only evidence that facultative cleaners might differ from non-cleaners stems from a recent study, which suggests that facultative cleaners of the genus *Thalassoma* have jaws with low mobility and weaker bite force than non-cleaners (Baliga & Mehta 2014). However, these findings should be taken with caution because three of the six species categorized as non-cleaners in this study have been observed to clean in the past. In labrids, facultative cleaning emerged independently in numerous taxa distributed throughout the phylogeny (Baliga & Law 2015). Moreover, with the increasing amount of field observations, species are constantly added to the list (see introduction section 1.3). Within facultative cleaners, there is important variation in dependency on cleaning both across and within species (Barbu *et al.* 2011). In some species, cleaning can represent a major component of their ecology at some stage of their life (Cole 2009), while it appears to be extremely rare in other species. As a result, binning all of these species in a single category for comparisons with obligate cleaners or non-cleaners is not ideal. However, gathering field observations in order to determine cleaning dependency in each species of labrid is a gigantic task, and using discrete categories is the best we can do with the information currently available. The fact that a great diversity of labrids engage in cleaning, and the apparent lack of specific adaptations in this category of cleaners suggests that the emergence of facultative cleaning is opportunistic. For species feeding on small invertebrates on the reef, occasionally eating a prey located on another fish might thus not represent a difficult transition. It is very likely only in species that specialize for cleaning that selective pressures become important enough to drive adaptation.

For a better understanding of the emergence of cleaning, it would be interesting to determine which of the client or the cleaner drives the relationship in facultative cleaners. I have tried on two occasions to answer this question by translocating individuals that I had observed cleaning with conspecifics that were not cleaning. The rationale is that if the non-cleaner individual starts cleaning in its new location, it would suggest that clients are driving the relationship by visiting and posing in front of the facultative cleaner. Conversely, if the individual that used to clean in its previous location also engages in cleaning interactions at its new location, it would suggest that the cleaner drives the relationship. Unfortunately, the scarcity of facultative cleaning behaviour at the study sites prevented me from obtaining enough replicated to draw any conclusions.

B.2 - Specializing for cleaning

Once a species specializes in cleaning, such as *L. dimidiatus*, various traits appear to be under selection (Fig. 2). Adjusting the level of cooperation to the category of clients (Bshary 2001), the presence of bystanders (Bshary & Grutter 2006; Pinto *et al.* 2011), the clients' control over the end of an interaction (chapter 1, Gingins *et al.* 2013), the presence of a co-inspecting partner (Bshary *et al.* 2008; Gingins & Bshary 2015), or the outcome of previous interactions with a specific client (Bshary & Grutter 2005) are all challenges that are quite unique to cleaning interactions. Moreover, these rather complex behaviours might only emerge in certain species of obligate cleaners. Indeed, adjusting the level of cooperation is only necessary when a conflict of interest between clients and cleaners exists, i.e. when cleaners prefer exploiting their clients by taking bites of mucus rather than focusing on ectoparasites. For instance, some species of gobies (*Elactinus* spp.) are also obligate cleaners, yet they prefer ectoparasites to mucus (Soares *et al.* 2010b) and hence the interests of the two interacting partners align. As a result, the same strategic behaviours are not required in cleaning gobies because clients do not use partner control mechanisms to enforce cooperation in this system (Soares *et al.* 2008).

In order to perform the complex behaviours observed in *L. dimidiatus*, its cognition is expected to have undergone some changes. In the evolution of cognition, the degree to which cognition is modular or the result of an all-purpose machine is a long-standing question (Samuels 1998; Magphail & Bolhuis 2001; Heyes 2003; MacLean *et al.* 2012; van Schaik *et al.* 2012; Krause 2015). The results of chapter 2 suggest that cognitive abilities in obligate cleaners have been acquired specifically for their ecology, and hence support a modular view of cognition.

One of the key aspects in the relationship between *L. dimidiatus* and its clients is the ability of the cleaner to feed against its preference, which appears to represent a significant challenge for species that do not face the same issue in nature. Cleaners' ability to further adjust how much they feed against their preference in various conditions is likely through associative learning. With more than 2000 interactions per day (Grutter 1996), opportunities for operant conditioning are plentiful. For instance, the decision rules of adult cleaners from socially simple and complex environments differ, while the juveniles from both environments performed similarly in these experiments (Wismer *et al.* 2014). It thus appears that cleaners are able to learn from past experiences. Since cognitive performance is often related to brain size (Deaner *et al.* 2007), we could have expected cleaners to have evolved a larger brain, but it appeared that this is not the case (Chojnacka *et al.* 2015). So how did *L. dimidiatus* acquire its exceptional cognitive skills? First, it appears that they might have undergone a restructuration of the brain rather than an overall increase in size (Chojnacka *et al.* 2015). Second, their neuronal phenotype appears to have been modified in order for them to have a higher propensity to engage in interspecific interactions (Mendonça *et al.* 2013). Third, their endocrine system is an important factor in the modulation of their cooperative behaviour (Soares *et al.* 2012, 2014), and differs from non-cleaner species (Kulczykowska *et al.* 2015). Through rather subtle changes in the organization of the brain and the endocrine system, the propensity to interact and the ability to learn of *L. dimidiatus* thus appear to have been primed for the specifics of cleaning interactions.

While many aspects of their behaviour are related to the costs and benefits reducing the quality of service they provide to clients, the overall service quality also appears to have been under positive selection in *L. dimidiatus*. In chapter 3, I suggest that they evolved a foraging posture that allows them to be more efficient at finding parasites on clients. This is beneficial for both interacting partners, because it means that clients will have more parasites removed per time unit, and cleaners more prey to eat in the same period of time. Another way to increase the service quality is by providing tactile stimulation to the clients (Grutter 2004; Soares *et al.* 2011), a behaviour that is exclusively found in obligate cleaners. Natural selection might have acted on service quality for various reasons. First, client fishes are expected to preferentially visit cleaners that give them the best service, which will create competition both between and within species of cleaners for providing the best quality of service. Second, clients might be more likely to tolerate some exploitation from cleaners that otherwise provide a high service. For instance, cleaners use tactile stimulation as a pre-conflict management strategy (Grutter 2004), suggesting that conflicts with clients can be altered by the quality of the service provided.

Finally, the results of chapter 4 suggest that conflicts in cleaning interactions have maintained a high escape performance in *L. dimidiatus.* This is interesting because according to the concept of compensatory trait loss (Ellers *et al.* 2012), we could have predicted that the escape performance of *L. dimidiatus* decayed thanks to the service they provide to predatory fishes. However, engaging in numerous close encounters with client fishes appears to entail enough risks for this species to express a very strong escape response. In summary, a wide range of characteristics are important for cleaning interactions, and species that specialized in this activity appear to have undergone very different selective pressures than fishes with more standard ecologies.

B.3 - Cleaning and the evolution of helping

In cleaners, helping behaviour in itself does not appear to represent a major challenge. Yet once it gains in importance, individuals might start exploiting the interacting partners. In response to exploitation, partner control mechanisms might emerge, such as partner choice, sanctions, or punishment (Connor 1986; Herre *et al.* 1999; Bshary & Bronstein 2011; Raihani *et al.* 2012). While punishment is quite complex because it requires for the actor to pay a cost in order to harm a cheating partner (Clutton-Brock & Parker 1995), the results from the first chapter provide evidence that simple mechanisms such as the threat of terminating an interaction can be sufficient to maintain cooperative behaviour. Because of partner control mechanisms, the cost of exploitation increases and cooperative behaviour becomes more beneficial. Strategic behaviours can subsequently evolve in order to determine under which conditions a switch from cooperation to exploitation is beneficial. This is clearly exemplified in the first chapter, with cleaners flexibly adjusting their level of cooperation according to the partner's abilities for partner control.

Since helping is widespread in nature and can take many forms (Dugatkin 2002; Lehmann & Keller 2006; West *et al.* 2007a; Bshary & Bergmüller 2008), identifying general patterns of evolution requires studying as many taxa as possible. Cleaning behaviour provides an excellent case study. In this system, the emergence of cleaning did not appear to be hindered by specific behavioural and morphological traits, yet the behaviour of species that further specialized for cleaning differs notably from others. This is not surprising, because in its simplest form, cleaning can be categorized as a by-product mutualism (see

Bshary & Bergmüller 2008), in which both clients and cleaners obtain direct benefits as a by-product of the partner's behaviour (i.e. clients visit cleaners to get their parasites removed, cleaners get fed by removing the parasites). Under such conditions, helping behaviour is self-serving and hence its evolution and stability is straightforward (Dugatkin 2002).

Once species specialize in cleaning though, conflicts of interests with clients and competition between cleaners appear to become major selective forces, as discussed earlier. According to biological market theory (Noë & Hammerstein 1994, 1995), clients' selecting the best interacting partners is predicted to increase overall service quality, which appeared to affect foraging technique in cleaners. Another challenge in cooperative interactions is often argued to be the ability to avoid exploitation by cheaters (Stevens & Hauser 2004; Hauser *et al.* 2009). What is interesting in cleaners is that they are the ones exploiting their partners, and not the other way around. The strategic behaviours they express thus emerged in order for them to exploit rather than to avoid exploitation. This fits well with the machiavellian intelligence hypothesis (Byrne & Whiten 1988), which predicts that exploiting and dealing with the complexity of social interactions in order to increase the individuals' fitness will select for higher cognitive abilities. Interestingly, conflicts of interests did not seem to only affect cognition in cleaners, but also other traits such as escape performance. This system thus provides a good example that helping behaviour is not particularly challenging in itself, and that species do not necessarily experience strong selective pressures just because it expresses this type of behaviour. Instead, it is rather the conflicts over the distribution of the benefits in cooperative interactions that appear to represent more significant challenges and drive species to adapt.

B.4 - Current and future research

During my thesis, I exclusively used an experimental approach and thus was limited in the number of taxa that I could compare. While my approach allowed investigating in details some aspects of the biology of cleaners, using a broader comparative approach would be beneficial to further our understanding the evolution of cleaning. There are more than 600 species in Labridae, and in combination with a well resolved phylogeny (Cowman *et al.* 2009), this provides an excellent ground for phylogenetic analyses. Several authors have used phylogenetic tool to study cleaners (Arnal *et al.* 2006; Cheney *et al.* 2009; Sims *et al.* 2013; Baliga & Mehta 2014; Baliga & Law 2015). In most cases, the Labridae phylogeney was used in order to correct for relatedness in comparisons of specific traits across a wide range of taxa (Arnal *et al.* 2006; Cheney *et al.* 2009; Baliga & Mehta 2014). However, recent developments in phylogenetic analyses allow investigating new questions about the evolution of cleaning. For instance, Baliga & Law (2015) recently investigated how and when cleaning behaviour evolved within labrids, and showed that this behaviour emerged independently on numerous occasions. Since 2014, I have been supervising Océane Krattinger in her master thesis that also aimed at investigating the evolution of cleaning using a phylogenetic approach. First, she collected behavioural data on over 40 species of wrasses (obligate, facultative and non-cleaners) in nature, in order to investigate whether species that engage in cleaning are more alike than non-cleaners in terms of social structure, aggressive behaviour and space use. After controlling for phylogenetic relationships, the different categories of wrasses did not differ significantly in any of the traits tested, suggesting that the emergence of cleaning is not restricted by the social structure or some basic aspects of fishes' behaviour. Second, she extracted morphological data from digitized pictures of labrids available in the literature for 255 species in order to infer evolutionary processes on various morphological traits. Here again, after correcting for relatedness, only minor differences between groups were found for the five traits investigated (i.e. maximum body length, relative position of the eye, the mouth, the ventral fins and the pectoral fins). It thus appears that the gross morphology differed little across cleaning categories. Different models of trait evolution were then applied and compared in order to determine which model described the distribution of traits between obligate cleaners and the rest of the family. It appeared that for the five different morphological traits tested, the models with the strongest support were the ones that allowed for different rates of evolution, strength of selection and trait optimums between the two groups. These results suggest that even though morphological differences are subtle, obligate cleaners experienced very different selective pressures in comparison with the rest of the family.

In order to understand whether the evolutionary patterns that we observe in labrids could be extended to cleaning in general, investigating similar questions in other clades would be very informative. The best candidates for such comparisons are the cleaning gobies from the Caribbean. Within Gobiidae, fourteen species are known to clean, distributed within two genus that also encompass non-cleaner species (Côté & Soares 2011). While cleaning gobies and cleaning wrasses share many similarities in their ecology, there are also notable differences between these two systems, particularly in terms of conflicts of interests with client fishes (Soares *et al.* 2008; Côté & Soares 2011). Since conflicts appear to be a major factor influencing the behaviour of cleaner wrasses, comparing wrasses and gobies would allow discerning between the selective pressures that are associated with cleaning in general and the pressures linked to the specific dynamics of cleaning interactions in each system.

Understanding cleaners' behaviour and evolution also requires an understanding of the mechanisms underlying their behaviour. During her PhD thesis, my colleague Sharon Wismer compared the behaviour of *L. dimidiatus* individuals from environments that differ in terms of social complexity. She showed that cleaners from socially complex environments outperform cleaners from socially simpler environments in various laboratory tasks derived from cleaner-client interactions (Wismer *et al.* 2014). Furthermore, juveniles from both environments performed equally poorly in the same experiments. It thus appears that cleaners acquire at least part of their cognitive skills throughout their life, and that the environment they are exposed to plays a major role in the development of their cognitive repertoire. Further research on this topic aims at understanding which decision rules that emerge in each type of habitat and why.

Currently, there is a growing interest in the hormonal regulation of cooperative behaviour (Soares *et al.* 2010a), and the effect of various hormones on the behaviour of *L. dimidiatus* have already been investigated (Soares *et al.* 2012, 2014; Cardoso *et al.* 2015; Paula *et al.* 2015; Messias *et al.* 2016). For instance, arginine vasotocin, dopamine and serotonin have been identified to impact the propensity of cleaners to engage in interspecific interactions (Cardoso *et al.* 2015; Paula *et al.* 2015; Messias *et al.* 2016), and cortisol and serotonin can affect cleaners' level of cooperation (Soares *et al.* 2014; Paula *et al.* 2015). Furthermore, it was recently suggested that the structure of the social decision-making network, a network of brain nuclei regulating social interactions, is highly conserved within vertebrates (O'Connell & Hofmann 2011, 2012). The remarkable similarities in the organisation of fish brains and other vertebrates thus offers unprecedented opportunities for integrating cognitive performance, decision-making, hormonal control and brain structure across a wide range of taxa (Bshary *et al.* 2014). Cleaners do not appear to have acquired their exceptional cognitive skills through an increase in overall brain size (Chojnacka *et al.* 2015), and hence it is more likely that they underwent more subtle changes in the organization of their brain. Now that we know which brain areas are the best candidates for the neuronal regulation of social behaviour in cleaners, understanding the implication of the different nuclei of the social decision-making network in cleaners represents an important step for future research. Zegni Triki recently started her PhD in our lab in order to investigate such questions. Integrating hormonal and neuronal research will undoubtedly allow for a more detailed understanding of the mechanisms controlling the social behaviour of cleaners.

B.5 - Conclusion

Using a comparative approach allowed me to identify some of the key aspects related to the ecology of cleaners, providing a good example of how the evolution of a species can be affected by the demands of a highly social life. In cleaners, competition among service providers and conflicts of interests between cleaners and clients appear to be the major drivers of adaptation. But each system has its specificities, and results on cleaners might not be readily extended to other systems. Accumulating such data across various forms of helping and various taxa is crucial in order to understand how traits evolve in response to various conditions. By doing so, we will eventually be able to identify what are the major forces driving the evolution of helping.

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