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THE COGNITIVE ECOLOGY OF THE CLEANER FISH *Labroides dimidiatus*

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GENERAL SUMMARY

There is substantial variation in either absolute or relative brain size between vertebrates. Comparing vertebrate species is the most commonly used method when exploring the link between brain size variation and ecological conditions. Nevertheless, there is an ongoing debate about whether the main selective factors on the evolution of brain complexity are driven by social or environmental challenges. Furthermore, the measures of brain complexity that correlate best with cognitive performance remain contested. It has thus been proposed that a "bottom-up" approach, by studying individual variation, may yield important complementary insights on the links between ecological conditions, cognitive performance and brain complexity.

This PhD thesis aimed to use the bottom-up approach in a study on the cleaner fish *Labroides dimidiatus*. Cleaner fish engage in mutualistic cleaning interactions, by removing ectoparasites from a variety of "client" coral reef fishes. Previous research has documented a strong behavioural divergence within the same population in this species. Cleaners differed in their strategic sophistication in laboratory experiments that feature key aspects of cleaner-client interactions: 1) reputation management, wherein the adjustment of service quality in the presence of bystanders; and 2) cleaning service priority to clients with partner choice option. From this, the main question was which ecological factors can explain this behavioural variation. In Chapter I, the succession of environmental perturbations at the study site in Lizard Island, Great Barrier Reef, Australia, provided natural conditions for my experiment as the perturbations significantly altered ecological variables on the reef. The study consisted of collecting fish censuses and behavioural recordings at various reef sites around the island, as well as testing cleaners from these sites in the two laboratory-based cognitive tasks. I found that formerly socially complex sites with high fish densities, and cleaners with high strategic sophistication, recorded very low fish densities after the perturbations with cleaners showing low strategic sophistication in the tasks. This study suggests that individuals adjusted their strategic sophistication to the new ecological conditions from before to after the perturbations.

In Chapter II, an analysis of fish censuses, behavioural recordings and cleaners' performance in laboratory tasks over several years revealed that the reduction in cleaner density (i.e., a reduced supply in the cleaning biological market), was the primary driver of low strategic sophistication. Also, cleaner density was strongly correlated with large client density, suggesting that the results cannot be well explained by changes in the supply-to-demand ratio.

Based on the results of Chapters I and II, I employed cleaner density as a proxy of both the intra- and interspecific social complexity in Chapter III and IV. The aim of Chapters III and IV were thus to investigate potential correlations between social complexity, strategic sophistication and brain complexity. In Chapter III, the magnetic resonance imagery (MRI) method was used to estimate with high precision the volumes of the five main brain major areas (i.e., telencephalon, diencephalon, mesencephalon, cerebellum, and brain stem). I found that cleaner density correlated positively with relative forebrain size (i.e., telencephalon and diencephalon together form the forebrain). Indeed, the forebrain harbours the "social decision-making network"; a network of brain nuclei involved in decision-making within a social context. These findings were mirrored in the outcomes of Chapter IV where I found a positive correlation between social complexity and the number of brain cells and neurons. Interestingly, strategic sophistication did not predict brain complexity. Instead, cleaners demonstrated social competence by displaying strategies that were optimal at their reef site of capture (i.e., low sophistication at low cleaner density, and high sophistication at high cleaner density). These cleaners also had relatively larger forebrains with more cells/neurons. The effect of size was strong, where there was a ~ 40 % difference in relative forebrain neuron count between low and high social complexity.

In conclusion, this thesis provides unique insights on the links between ecology, cognition and brain features within a species. The results support the idea that the bottom-up approach may provide important insights into the selective pressures on brain complexity. Importantly, most of the documented variation is likely due to ontogenetic effects, as the egg and larval stages are pelagic in the cleaner fish species. This implies that laboratory experiments that manipulate key ecological factors during development can be used to test for potential effects on brain structure. According to the results, social complexity is a key factor driving forebrain size and cell/neuron number adjustments. Finally, the social competence analysis suggests that, in the case of cleaner fish, part of the selection on increased forebrain complexity is due to intraspecific social complexity.

Keywords: cleaner fish; marine cleaning mutualism; brain complexity; behavioural ecology; biological market; decision-making; strategic sophistication

GENERAL INTRODUCTION

I.1. Social cognition and the comparative approach

Social cognition is the role played by cognition in processing, storing, and deciding to act on information in a social context [Frith, and Blakemore, 2006; Seyfarth, and Cheney, 2015]. Complex cognitive performance is suggested to be the out-turn of a complex social life, allowing individuals to achieve higher fitness through sophisticated "Machiavellian" strategies [Humphrey, 1979; Byrne, and Whiten, 1989; Byrne, 1996; Bshary et al., 2002]. The comparative cognitive approach views this cognitive performance as a proxy for advanced cognitive abilities [Pearce, 2013; Shettleworth, 2010], whereas the behavioural ecological approach tries to find the ecological conditions (i.e., selection pressure) underlying such performances [Kamil, 1998; Shettleworth, 2010]. Many interdisciplinary research fields emerged as a means by which to bridge the gap between these two approaches by studying animal cognition with respect to its ecology, fields such as "cognitive ethology" and "cognitive ecology", for instance [Shettleworth, 2001; Kamil, 1998].

Since the brain is the centre of the nervous system, its size has been extensively studied as a proxy for cognitive abilities [Logan et al., 2018; Chittka, and Niven, 2009]. Indeed, many studies suggest that larger brains possess "better" cognitive abilities based on the correlations between brain size and performance (see Table 1, and Table 1 in the review by Healy and Rowe [2007]). In birds, for instance, food-storing species have relatively larger hippocampi than species that do not store food, suggesting that this part of the brain plays a role in spatial memory and is linked to the abilities to retrieve stored food [Krebs et al., 1989; Sherry et al., 1989].

Although the evolutionary approach is fundamental in understanding brain size evolution [MacLean et al., 2012; Dunbar, 1992; Schaik, and Burkart, 2011], it still has its limitations: For instance, it has become evident that mixing clades, especially if they live in different environments, may introduce a lot of unexplained variances [van Schaik et al., 2012]. Also, exploring how differences in brain size may translate into cognitive performance are still failing to produce consistent results [Logan et al., 2018]. One potential explanation is that the ecological relevance of the cognitive tasks employed can also play a substantial role in the outcomes. Low cognitive performance in a task does not necessarily reflect low cognitive abilities, especially if the task is not ecologically relevant for the studied species [Gingins, and Bshary, 2016; Salwiczek et al., 2012; Prétôt et al., 2016a; Prétôt et al., 2016b]. Furthermore, the data set on brain features currently have several limitations including; small sample size, potentially imprecise skull scan rather than brain scan measurements, and lack of information on brain part sizes, neuron numbers and connectivity [Logan et al., 2018; Chittka, and Niven, 2009]. Therefore, intraspecific studies are needed to complement the comparative approach (so-called the "Bottom-Up" approach in the review by Logan et al. [2018]). This approach will help to identify variation within the same species, where cognitive tasks will be adapted to the ecology of the studied species. Currently, there are only a few intraspecific studies targeting the link between brain and performance or ecological conditions (see Table 1 and Table 2). However, since more and more reviews are now emphasising the importance of individual variation within the same population and its implications in understanding ontogenetic effects on brain development [Logan et al., 2018; Gonda et al., 2013; Wascher et al., 2018].

I.2. The "intelligence" hypotheses

The correlations between brain traits and multiple ecological conditions witnessed the emergence of several "intelligence" hypotheses that took their names from the studied correlates. These hypotheses make different inferences from such correlations and try to discern which challenges explain most of the variation in brain expansion. This includes: (1) the "social brain hypothesis" with proxies for social complexity as predictors for brain size (i.e., group size, coalition size, social system, sociality, gregariousness, mating system, flock size, etc.) [Dunbar, 1992; Humphrey, 1979; Byrne, and Whiten, 1989]; (2) the "ecological intelligence hypothesis" with non-social ecological conditions as the correlates of brain size (i.e., diet type, food finding, etc.) [Clutton-Brock, and Harvey, 1977; Hutcheon et al., 2002; Iwaniuk, and Nelson, 2001; DeCasien et al., 2017; Rosati, 2017]; and (3) the "cultural intelligence hypothesis" with factors link to cultural knowledge as the brain size predictors (i.e., social learning, teaching, general intelligence, etc.) [Schaik, and Burkart, 2011; van Schaik et al., 2012; Reader et al., 2011; Burkart et al., 2016].

There is an ongoing debate to weigh the relative importance of each of these hypotheses [González-Forero, and Gardner, 2018]. If we take the social brain hypothesis, for example, it appears that it is not necessarily applicable to all primate species: Some large-brained species live in simple social systems and exhibit more complex cognitive abilities, like the orangutans [van Schaik et al., 2012]. This also applies to other taxa, especially birds, wherein the relationship between flock size and brain size is unclear [Boucherie et al., 2019; Beauchamp, and Fernández-Juricic, 2004]. It suggests that group size does not capture the degree of social complexity in all species [Borgeaud, 2016; Kappeler, 2019]: If group size is a good indicator of cognitive abilities for "species A and B" it is not necessarily true for "species C" [see review by van Schaik et al., 2012; Emery et al., 2007]. Thus, identifying variables as a proxy for the "intelligence" hypothesis to be tested is not straightforward. The lack of empirical support to validate a proxy (i.e., group size, diet, home range, etc.) for cognitive performance in the studied species might be the reason behind the contentious and controversial outcomes in the "intelligence" hypotheses between species and taxa.

Again, the current comparative approach of differences in "intelligence" will remain questionable, as comparing different species will suggest that differences might be due to species-specific rather than real differences in cognitive abilities [Kamil, 1987]. Therefore, applying the bottom-up approach to a wild species in its natural habitat will help build a robust framework to understand variation between individuals and highlight the mechanisms underlying such variation in natural conditions. Furthermore, a potential addition to the bottom-up approach is to create selection lines either on behaviour or on brain size. A very successful method developed by Niclas Kolm and his collaborators showed that artificial selection on relative brain size in a guppy (*Poecilia reticulata*) revealed links between brain size and cognitive performance [Buechel et al., 2018; Kotrschal et al., 2013], predator avoidance [Kotrschal et al., 2015a; van der Bijl et al., 2015], and mate choice [Kotrschal et al., 2015b; Corral-López et al., 2017]. As it has been suggested that cognitive performance can shape brain complexity [Wascher et al., 2018], the guppy system can be ideal to test whether it is possible to create selection lines from performance and test whether such selection would impact brain development.

I.3. Study system

An ideal model system to study the mechanisms underlying intraspecific variation of social decision-making is the marine cleaning mutualism involving the cleaner fish *Labroides dimidiatus* (hereafter simply "cleaner"). Cleaners inhabit small territories (i.e., cleaning stations), where they obtain most of their food by engaging in interspecific social interactions with a variety of client reef fish (hereafter "client") that visit the cleaners to have their ectoparasites removed [Randall, 1958; Losey Jr, 1979]. Although the outcome of these interactions is mutually beneficial [Ros et al., 2011; Grutter et al., 2003; Grutter, 1999], conflicts of interest can occur. This is because cleaners prefer to bite the clients' protective mucus rather than ectoparasites, an act that is considered as cheating [Grutter, and Bshary, 2003]. Furthermore, when two or more clients seek a cleaning service simultaneously, competition can then occur between cleaners over access to highly profitable client species (i.e., visitor client). "Visitors" are typically large-bodied client fish with access to multiple cleaning stations, whereas "resident" clients, only have access to the local cleaner. Cleaners thus compete by outbidding, without aggression, by either exhibiting superior service quality or by giving service priority to visitor clients. Together, these conflicts form the basis of the biological market of the marine cleaning mutualism [Bshary, 2001].

Cleaners also have a relatively complex intraspecific social life: they are protogynous hermaphrodites, living in harems composed of one larger male and several smaller females [Robertson, 1972]. Cleaners start reproducing as a female but can change sex by either growing relatively larger bodies and becoming the largest cleaner in the harem and/or by avoiding the male partner's aggression [Robertson, 1972; Nakashima et al., 2000; Sakai, and Kohda, 2001].

All these inter- and intraspecific conflicts are apparently driving a complex behavioural repertoire (see Table 3), wherein the sophistication of cleaners' social strategies has become a textbook example of both the application of evolutionary game theory to cooperation and the ecological approach to cognition [Davies et al., 2012; Shettleworth, 2009]. Of particular importance for this PhD thesis are two cognitive aspects of cleaner fish within the interspecific context:

(i) Field observations and laboratory experiments on cleaners provide strong evidence of cooperative reputation management outside humans [Bshary, and Grutter, 2006; Pinto et al., 2011]. Cleaners increase levels of cooperation in the presence of "image-scoring" bystanders that are more likely to invite for cleaning if they observe an interaction without apparent conflict (i.e., cheating). This ability of cleaners to manage their reputation is a form of inhibitory-control, wherein they refrain from biting highly preferable mucus and eat ectoparasites (i.e., less preferable food) instead [Bshary, and Grutter, 2006].

(ii) Cleaners give priority to visitor clients over resident clients [Bshary, 2001] as the former would switch to another cleaner if it is made to wait [Bshary, and Schäffer, 2002]. Giving priority to an ephemeral food source (i.e., visitors are considered as ephemeral food source) is not a trivial task, as evidenced by the fact that cleaners outperform chimpanzees, orangutans, capuchins, rats and pigeons in a more abstract version of the general problem in laboratory experiments [Bshary, and Grutter, 2002; Salwiczek et al., 2012; Zentall et al., 2016; Zentall et al., 2017].

Cleaner's ability to flexibly adjust their decisions to a given situation (i.e., strategic sophistication) in (i) and (ii) have been recently challenged by Wismer et al. [2014]. Previous research on these particular strategies suggested homogenous levels of strategic sophistication in cleaners [Bshary, 2002; Bshary, and Grutter, 2002; Bshary, and Grutter, 2005; Bshary, and Grutter, 2006; Bshary, and Noë, 2003; Salwiczek et al., 2012]. In 2009, however, Wismer et al. [2014] found that in laboratory-based cognitive tasks cleaners did not all perform similarly. While some cleaners exhibited high strategic sophistication during the tests, others performed quite poorly. By further investigating the differences between these cleaners based on their performance, they found that social conditions at the sites of capture differed significantly. Cleaners with high strategic sophistication were from a site with high cleaner and client densities, and high cleaner-client interactions (i.e., up to 2000 interactions per day), while the low performing ones were from a site with low densities of cleaners and clients, and low interaction frequencies (i.e., 800 per day) [Wismer et al., 2014]. It is important to point out that all tested cleaners belonged to the same population at Lizard Island, on the Great Barrier Reef. The fact that cleaners have a pelagic larval stage and at larger scales have little choice of where they finally settle [Victor, 1986], supports the theory that ontogenetic development is behind this strategic sophistication rather than genetic factors.

I.4. The PhD thesis topic

After the study by Wismer et al. [2014] which documented an intraspecific variation in cleaner fish strategic sophistication for the first time, this PhD thesis was designed to unravel the underlying mechanisms of such variation. Phylogenetically speaking, all vertebrates share a common ancestor, suggesting that fish share the basic genetic features with higher vertebrates [Bshary, and Brown, 2014; O'Connell, and Hofmann, 2012]. Therefore, investigating the mechanisms of strategic behavioural decision-making in a fish species, is an essential contribution to improving our understanding of how individuals of vertebrate species may adapt their decision rules to a given situation. These mechanisms can range from cues from the surrounding environment to the computational power of the brain, and their potential interaction. As mentioned above, there are many "intelligence" hypotheses that tend to classify these ecological cues into either social (group size), non-social (diet), or cultural (social learning) factors [Dunbar, 1992; DeCasien et al., 2017; González-Forero, and Gardner, 2018; Schaik, and Burkart, 2011].

Interestingly, many studies have explored the direct link between the ecological conditions and either "intelligence" correlates or brain neuroanatomical traits, as well as the link between brain features and ecological conditions. However, to our knowledge, no study so far has explored the direct link between ecological conditions, brain traits and "intelligence" in a single analysis (see Table 1 and Table 2). For this reason, the aim of this thesis was to: (1) determine whether the strategic sophistication levels are sensitive to a changing environment; (2) find out which ecological conditions are linked to strategic sophistication and can better explain the variation; (3) assess whether the best explanatory ecological condition(s) from (2) correlate with brain complexity; and finally (4) study the link between brain complexity, best explanatory ecological condition(s) and strategic sophistication.

I.5. Thesis chapters

Throughout the chapters of this PhD thesis, I focused on two complex interspecific social behaviours in cleaner fish: reputation management and service priority (i.e., described above in (i) and (ii)). The choice for these two particular behaviours is due to several reasons: First, because the previously documented variation in cleaner strategic sophistication also explored these two behaviours [Wismer et al., 2014]. Since I am trying to unravel the mechanisms underlying such variation, it made sense to study the same strategies for further comparisons. Second, the importance of these behavioural strategies in the daily social life of cleaner wrasse is crucial, as they are the basis of the repeated interactions with clients. Third, as explained above in (i) and (ii), these capacities of cleaners have been replicated successfully in the last two decades. Therefore, I chose these strategies as the best proxy for cleaner's strategic sophistication. The laboratory-based cognitive tasks to assess these two strategies were namely the "bystander effect task" to test cleaner's reputation management and the "biological market task" to test cleaner's service priority abilities. In the bystander effect task, cleaners are tested for their abilities to adjust their cooperative behaviour in the presence of an image-scoring audience [Bshary, and Grutter, 2006; Wismer et al., 2014]. Cleaners are known to prefer clients' mucus over ectoparasites, which constitute cheating [Bshary, and Grutter, 2005; Bshary, and Grutter, 2006]. Nevertheless, in the presence of an image-scoring bystander client, cleaners refrain from biting mucus from the current client and cooperate instead [Bshary, and Grutter, 2006]. Therefore, in the bystander effect task, cleaners were presented with Plexiglas plates (i.e., as surrogates for clients) with two types of food: a highly preferred food (i.e., prawn as a substitute for mucus) and a less preferred food (i.e., fish flakes as a substitute for ectoparasites). The task consists of testing cleaners' willingness to feed more against their preferences in the presence of an image-scoring bystander plate.

The "biological market task" is a complex foraging choice task based on ephemeral vs permanent food options, where access to the ephemeral food source is granted if it has been chosen first [Bshary, and Grutter, 2005; Salwiczek et al., 2012; Wismer et al., 2014; Prétôt et al., 2016a; Prétôt et al., 2016b]. Cleaners in natural habitats prioritise clients with partner-choice to provide them with a cleaning service [Bshary, and Noë, 2003]. These clients with partner-choice are mainly visitor clients that might seek a cleaning service simultaneously with a resident client. Cleaners' optimal choice in such case, is to always service visitors first, then residents. The reason behind this strategy is that visitors usually leave if not serviced while residents are willing to wait [Bshary, and Noë, 2003; Triki et al., 2018]. Overall, I tested cleaners for their abilities to learn to prefer a visitor plate over a resident plate in a biological market task. The plates were of equal size and while they offered an equal amount of food, they differed in colour/pattern. The resident plate was always willing to remain in the aquarium until the cleaner would feed on it. The visitor plate, however, was an ephemeral food source that can be accessed only if it is the first plate to be inspected by the cleaner. The optimal solution is to always prioritise the visitor plate and then feed off the resident plate to double the food gain.

Given the ecology of cleaner mutualistic behaviour, finding food is an interspecific social task rather than just an environmental foraging task, as in most other species. This makes the differentiation between the non-social ecological and social drivers unlikely in cleaners. Therefore, ecological conditions were divided into socio-ecological and behavioural: The former was assessed from the fish survey data, which provided information on the population density of cleaners, as well as information about client species densities; whereas the latter was measured from behavioural cleaner-client interaction patterns. Cleaner strategic sophistication levels and their socio-ecological and behavioural characteristics were assessed throughout all four chapters of this PhD thesis in a consistent manner.

In **Chapter I**, environmental perturbations hit our usual study field site, the Lizard Island reef, Great Barrier Reef, Australia. Such events created natural conditions to investigate the extent to which the recent natural perturbations affecting the reef were associated with changes in fish composition, cleaner-client interspecific interactions, and as a consequence, cleaner strategic sophistication. **Chapter II** aimed to find the best predictors of cleaner strategic sophistication among the many factors assessed from the fish survey and cleaner-client interactions patterns. The aim was to investigate 12 factors, and determine which factor(s) explain the largest proportion of the variation in cleaner strategic sophistication. The selected factor(s) represent the best ecological condition(s) underlying individual behavioural strategies, and are employed in the following two chapters. In **Chapter III**, the aim was to test whether the best predictor from Chapter II is a good correlate for brain complexity. As the many "intelligence" hypotheses suggest that the brain tissue enlargement is a coping mechanism with complex ecological conditions [Dunbar, 1992; Schaik, and Burkart, 2011; DeCasien et al., 2017; González-Forero, and Gardner, 2018], the aim here was to test the correlation between relative brain size and/or brain regions sizes and the selected ecological condition(s) from Chapter II. The magnetic resonance imagery MRI technic was employed to assess brain morphometrics. The highly advanced MRI technic offered an accurate fine-scale brain tissue measurement [Simões et al., 2012]. Finally, in **Chapter IV** the aim was to go beyond brain morphometrics and explore the neuronal densities in the main brain parts. Of course, brain morphometrics were also assessed but this time with a highly valuable addition in the form of neurons count. Together, brain morphometrics and cells densities will expand our knowledge of brain functionality [Herculano-Houzel, 2017; Herculano-Houzel, 2011; Herculano-Houzel et al., 2014; Kverková et al., 2018]. In this chapter, it was possible to test the direct link between brain features (i.e., size or cells densities), ecological conditions (best predictor from Chapter II) and strategic sophistication. Several questions were asked here: (1) Do brain size or brain part size correlate with ecological conditions and strategic sophistication? (2) Also, whether the outcomes here are in line with the outcomes of Chapter II, given that the measurement methods are quite different: MRI vs stereoscopic dissection; (3) Do cells densities (i.e., neuronal or nonneuronal cells) correlate with ecological conditions and strategic sophistication? (4) Does more brain tissue mean more cell numbers? How will the findings in Chapter IV contribute to the "intelligence" hypotheses?

Taken together, these four chapters should allow us to pinpoint the significant ecological condition(s) driving the development and stability of strategic sophistication in cleaner fish. Both Chapter III and IV should then provide a more complete picture by finding whether brain structural differences under variable ecological conditions drive the variation in strategic sophistication or not. The key working hypothesis is that even at the individual level, ecological complexity drives the adjustment of brain complexity as a means of adaptation to the current conditions, much like a species would adjust to their average social, non-socioecological and/or cultural conditions.

Table 1. Studies exploring the link between brain measurement with either cognitive performance or ecological conditions. In between species comparison, I include here studies published after 2006 as a continuation to Table 1 from the review by Healy and Rowe [2007].

Correl ati-on link	Compari-son level	Brain characteristics	Factors that correlate with brain measurements	Species/taxon	Study
Brain measures and cognition	Between species	Absolute brain size	General intelligence	Primates	[Deaner et al., 2007; Deaner et al., 2006]
		Neocortex ratio	General intelligence	Primates	[Reader et al., 2011]
		Relative brain size	Social learning	Primates	[Street et al., 2017]
		Absolute neurons count in parts of the brain (i.e., mammalian cortex, bird pallium)	General intelligence	Mammals and birds	[Herculano-Houzel, 2017]
		Absolute and relative brain size	Inhibitory control tests	Birds and mammals	[MacLean et al., 2014]
		Relative brain size and brain region sizes	Problem solving by (i.e., puzzle box)	Carnivores	[Benson-Amram et al., 2016]
	Within species	Hippocampus size	Spatial navigation in taxi drivers	*Homo sapiens*	[Maguire et al., 2000]
		Leftward cortex size	Musical skills	*Homo sapiens*	[Schlaug et al., 1995]
		Brain size and brain region sizes	Learning and memory	Honey bee (*Apis melifera*)	[Gronenberg, and Couvillon, 2010]
Brain measures and ecological conditions	Between species	Relative brain size	Group size	Primates	[Street et al., 2017]
		Relative brain size	Diet	Primates	[DeCasien et al., 2017]
		Telencephalon size	Social complexity	Ancient birds	[Burish et al., 2004]
		Brain region sizes	Habitat complexity	Fish (cichlids)	[Gonzalez-Voyer et al., 2009]
		Telencephalon size	Social organisation, number of individuals	Fish (cichlids)	[Pollen et al., 2007]
		Telencephalon size	Habitat complexity	Fish (cichlids)	[Shumway, 2010]
		Brain size	Diet, and parental care	Fish (cichlids)	[Gonzalez-Voyer Alejandro et al., 2009]
		Brain region sizes	Habitat, and mating competition	Fish (cichlids)	[Gonzalez-Voyer, and Kolm, 2010]
	Within species	Brain size	Ecological challenges	*Homo sapiens*	[González-Forero, and Gardner, 2018]
		Hippocampus volume and neurons count	Harsh environment (i.e., higher latitudes)	Black-capped chickadee (*Poecile atricapillus*)	[Roth, and Pravosudov, 2009]
		Neurogenesis	Harsh environment (i.e., higher latitudes)	Black-capped chickadee (*Poecile atricapillus*)	[Chancellor et al., 2011]
		Hippocampus size	Seasonality	Black-capped chickadee (*Parus atricapillus*)	[Smulders et al., 1995]
		Hippocampal neuron counts	Harsh environment (i.e., higher latitudes)	Mountain chickadee (*Poecile gambeli*)	[Freas et al., 2012]
		Neurogenesis	Breeding season	Gambel's white-crowned sparrow (*Zonotrichia leucoph- rys gambelii*)	[Larson et al., 2013]
		Naturally occurring neurons degradation	Breeding season	Gambel's white-crowned sparrow (*Zonotrichia leucoph- rys gambelii*)	[Larson et al., 2014]

Table 2. Studies exploring the link between cognitive performance and ecological conditions

Comparison level	Cognitive performance	Ecological conditions	Species/taxon	Study
Between species	General intelligence	Group size	Primates	[Reader et al., 2011]
	Social learning	Group size	Primates	[Street et al., 2017]
	Problem-solving	Sociality	Primates	[Forss et al., 2016]
	General intelligence	Group size	Birds	[Bond et al., 2003]
	Discrimination task	Sociality	Fish (cichlids)	[Hick et al., 2014]
Within species	Social tasks	Social experience	Cichlid fish (*Neolamprologus pulcher*)	[Arnold, and Taborsky, 2010]
	Reproductive success	Social complexity	Cowbirds (*Molothrus ater*)	[White et al., 2010]
	General intelligence	Group size	Australian magpies (*Cracticus tibicen dorsalis*)	[Ashton et al., 2018]
	Spatial memory and associative learning	Harsh environment (i.e., higher latitudes)	Black-capped chickadee (*Poecile atricapillus*)	[Pravosudov, and Clayton, 2002]
	Social competence	Group size	breeding cichlid fish (*Neolamprologus pulcher*)	[Fischer et al., 2015]
	Spatial memory	Social enrichment conditions	Wolf spider (*Hogna carolinensis*)	[Punzo, and Ludwig, 2002]
	Cooperative behaviour	Intra- and interspecific social enrichment conditions	Cleaner fish (*Labroides dimidiatus*)	[Wismer et al., 2014; Triki et al., 2018; Binning et al., 2017]

Table 3. Cleaner fish behavioural repertoire.

Context	Behaviour	Details
Interspecific	Individual recognition (bookkeeping)	Memorising individual client fish [Tebbich et al., 2002]
	Discrimination	Discrimination between two classes of clients: residents vs visitors [Bshary, 2001]
	Learning and reversal learning	Complex foraging task involving ephemeral food source vs permanent food source [Salwiczek et al., 2012; Prétôt et al., 2016a; Prétôt et al., 2016b; Wismer et al., 2014; Triki et al., 2018; Bshary, and Grutter, 2005]
	Reputation management (inhibitory control-like behaviour)	Adjusting feeding preferences in the presence of an image-scoring bystander [Bshary, and Grutter, 2006; Bshary, 2002; Wismer et al., 2014; Bshary, and Grutter, 2005]
	Tactical deception-like behaviour	Strategic adjustment of reputation management [Binning et al., 2017]
	Feeding against the preferences (inhibitory control-like behaviour)	Eating less preferred food before eating highly preferred food [Grutter, and Bshary, 2003; Gingins, and Bshary, 2016; Wismer et al., 2014]
	Generalisation	Generalising decision rules to pre-existing learned rules in a choice test between client species [Wismer et al., 2016]
	Partner manipulation	Manipulation of client partner via tactile stimulations [Bshary, and Würth, 2001]
	Numerical quantities discrimination	Learning to discriminate 2 vs 5 and 5 vs 8 black squares on white panels [Triki, and Bshary, 2018]
Intraspecific	Third-party punishment	Male cleaner punish female partner upon cheating a current client [Raihani et al., 2010] Male partner adjust punishment level to the cheating female according to the client value [Raihani et al., 2012b]
	Service quality adjustment	Female partner increase cleaning service quality in joined cleaning interactions with the male partner [Bshary et al., 2008] Pairs of cleaner fish increase service quality when cleaning together [Gingins, and Bshary, 2014]
	Territoriality	Aggression towards competitors [Kuwamura, 1984]
	Group membership	Female recognise in-group vs out-group male partner and adjust its cooperative behaviour accordingly [Raihani et al., 2012a]

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CHAPTER I | A decrease in the abundance and strategic sophistication of cleaner fish after environmental perturbations

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Abstract

Coral reef ecosystems are declining worldwide and under foreseeable threat due to climate change, resulting in significant changes in reef communities. It is unknown, however, how such community changes impact interspecific interactions. Recent extreme weather events affecting the Great Barrier Reef, i.e., consecutive cyclones and the 2016 El Niño event, allowed us to explore potential consequences in the mutualistic interactions involving cleaner fish *Labroides dimidiatus* (hereafter "cleaner"). After the perturbations, cleaner densities were reduced by 80%, disproportionally compared to the variety of reef fish clients from which cleaners remove ectoparasites. Consequently, shifts in supply and demand yielded an increase in the clients' demand for cleaning. Therefore, clients became less selective towards cleaners, while cleaners were able to choose from a multitude of partners. In parallel, we found a significant decline in the ability of cleaners to manage their reputation and to learn to prioritize ephemeral food sources to maximize food intake in laboratory experiments. In other words, cleaners failed to display the previously documented strategic sophistication that made this species a prime example for fish intelligence. In conclusion, low population densities may cause various effects on individual behavior, and as a consequence, interspecific interactions. At the same time, our data suggest that a recovery of population densities would cause a recovery of previously described interaction patterns and cleaner strategic sophistication within the lifetime of individuals.

Keywords: Cyclones, El Niño, coral bleaching, marine cleaning mutualism, biological market, learning

1.1 Introduction

Rapid changes in climate conditions pose a severe threat to biodiversity, with coral reef ecosystems providing a prime example [Hoegh-Guldberg et al., 2009; Hughes et al., 2003; Walther et al., 2002]. Global warming is predicted to negatively affect coral reef fish communities, either directly through the rise in seawater temperature or ocean acidification [Ferrari et al., 2011; Pankhurst, and Munday, 2011; Browman, 2016; Messmer et al., 2017; Munday et al., 2017], or indirectly via habitat loss [Munday et al., 2008]. Conditions occurring during or after extreme weather events offer ideal scenarios to evaluate the effects of climate change on ecosystems. Such perturbations may also allow for the documentation of rapid microevolutionary processes, such as changes in beak morphology in Galapagos finches [Grant, and Grant, 2002], to major ontogenetic effects, including shrinking in marine iguanas [Wikelski, and Thom, 2000]. However, we do not know how climate change affects interspecific interactions, either directly through affecting individual behavior or indirectly through changes in community composition. Here, we investigated to what extent environmental damage, caused predominantly by extreme weather events, may affect mutualistic interactions on coral reefs.

The marine cleaning mutualism involving the cleaner fish *Labroides dimidiatus* (hereafter "cleaner") is a classic model system in the study of cooperation, in which cleaners remove ectoparasites from a variety of coral reef fish "clients". Conflict, however, arises as cleaners prefer to feed directly on client mucus, which is energetically costly for the client fish to produce and constitutes cheating (Grutter & Bshary, 2003). Several partner control mechanisms employed by clients reduce the frequency of such cheating events, which includes image scoring. For example, cleaners increase their image score by being more cooperative when in the presence of potential future clients ("bystanders"), which only interact with cooperative cleaners [Bshary, and Grutter, 2006]. Consequently, cleaners are more cooperative in the presence of bystanders [Pinto et al., 2011]. Moreover, in line with biological market theory [Noë et al., 1991], cleaners respond to the partner choice options of clients. Indeed, cleaners can distinguish between "visitor" species with access to several cleaning stations and "resident" species with access only to the local cleaner. Employing such information, cleaners give priority of the cleaning service to visitors, followed by residents, which usually wait for inspection [Bshary, and Noë, 2003]. Cleaners can hence adjust their behavior in the presence of bystanders [Bshary, and Grutter, 2006; Wismer et al., 2014] and prioritize clients with partner choice [Bshary, 2001; Wismer et al., 2014]. Therefore, the sophistication of the cleaners' social strategies has become a textbook example of both the application of evolutionary game theory to cooperation and the ecological approach to cognition [Davies et al., 2012; Shettleworth, 2009].

The coral reefs of Lizard Island, Great Barrier Reef (GBR), have been strongly affected by recent extreme weather events [Hughes et al., 2017; Pizarro et al., 2017]. For example, Cyclone Ita in 2014 and Cyclone Nathan in 2015 caused severe damage to shallow reefs in the northern region of the island [Ceccarelli et al., 2016; Pizarro et al., 2017], where on some reefs, coral cover was reduced from 25% to 9%, and branching corals were destroyed on several reefs (J. Stella at GBRMPA, personal communication). Furthermore, while reefs located in the lagoon were mostly protected from the cyclones [Pizarro et al., 2017], they were negatively affected by the 2016 coral bleaching event. El Niño, a phenomenon that brings warm water to the Indo-Pacific [Collins et al., 2010; Wellington, and Victor, 1985], caused a continued increase in ocean surface temperatures in 2016 (Fig. S1). The event resulted in ubiquitous coral bleaching [Lough, 2016; Hoegh-Guldberg, and Ridgway, 2016; Ainsworth, and Gates, 2016]. We, therefore, investigated to what extent the recent natural perturbations affecting reefs around Lizard Island were associated with changes in fish composition and as a consequence, interspecific interactions between cleaner fish and reef fish clients.

1.2 Materials and Methods

1.2.1 Study site, data comparison, and timeline of perturbations

Field data were collected on reefs adjacent to Lizard Island, Great Barrier Reef, Australia, and laboratory experiments were conducted at Lizard Island Research Station (LIRS) (14.6682° S, 145.4604° E). We compared data collected in June and August of 2014 and 2016 to data collected in 2003 and 2010/2011. Hence, data collected prior to 2014 were published in 2006 and 2014, respectively [laboratory cognitive data, n=12; Bshary, and Grutter, 2006] [field data, n=08; laboratory cognitive data, n=10; fish census data, n=10; Wismer et al., 2014]. All data collection, including published data, was conducted in the Australian winter season, which excludes variation in seasons as a confounding variable. The precise locations of the sites from which we obtained cleaners in the various studies are shown in Fig. S2.

The timeline regarding both data collection and perturbations was as follows. In 2003: testing of cleaners from sites 1, 2 and 3 in experiment I (see below). In 2010: testing of cleaners from site 1 in experiment II (see below). In 2011: field observations and fish censuses at site 1. In May 2014: Cyclone Ita damages site 1 only. In June 2014: field observations, censuses, and experiment I on cleaners from site 2. In May 2015: Cyclone Nathan again damages site 1 only. In February/March 2016: El Niño event, with water temperatures reaching up to 30°C (Fig. S1), induced major coral bleaching on all reefs surrounding Lizard Island. In June 2016, water temperatures were back to normal (25 to 26°C, Fig. S1): here we conducted field observations, fish censuses, experiments I and II on cleaners from site 1 and 2. Thus, for the laboratory data collected in 2003, we do not have precise corresponding field data. The cleaners used in those experiments had been collected from sites 1, 2 and 3 (Fig. S2), without keeping track of site for data analyses. As individuals performed consistently, we decided to include the data in the analysis despite the presence of cleaners from site 3, for which no field observations and censuses before perturbations are available. All data collection followed the same protocols.

1.2.2 Field data

Natural cleaning interactions were filmed for 30 minutes, between 08:30 and 16:00 hours (Canon G15 and G16), for (*n*=8) randomly selected adult female cleaners per site per field trip. Divers kept an approximate distance of 2 m during filming, as a compromise between keeping disturbance low and obtaining good footage. From the videos, we extracted for each cleaner-client interaction the client species and the duration. For the comparison between sites/years, we analyzed the number of interactions per time unit, the number of interactions with large clients per time unit, the duration of interactions and the percentage of time cleaners spent interacting. Furthermore, we quantified the number of occasions in which a client swam off after being ignored by a cleaner in favor of another client. As swimming off is almost exclusively a response to visitor clients, we quantified how often cleaners had simultaneous choices between two clients, of which at least one was a visitor. Simultaneous choices included two situations: two clients arriving and seeking service at the same moment or a client seeking service while the cleaner was interacting with another client. We also calculated the percentage of non-serviced visitors leaving from the total number of visitors seeking cleaning service simultaneously with another client.

The abundance and diversity of cleaner fish and client reef fishes were estimated with fish censuses collected by scuba divers. We used (*n*=10) replicate 30 m transects at each site/year. The transects were placed haphazardly either parallel to the shoreline or parallel to the reef crest. First, we recorded all visible large fish with a body size of TL > 10 cm and cleaners on a 5 m wide area, followed by small fish with a body size of ≤ 10 cm TL on a 1 m wide area along the 30 m transect. Fish were then classified to species levels for the diversity data and to body size for the abundance data. For the comparison between sites/periods, we kept the number of species and fish abundance (per unit surface) per transect separately for large and small species for the data analyses. The classification of clients as small/resident or large/visitor species mostly followed Bshary [2001] and published information on their home range size [Froese, and Pauly, 2016]. All visitor species have a large body size (TL > 10 cm), whereas 70 % of the resident species are small-bodied fish (TL ≤ 10 cm) (Table S1). Species that are not regular clients for the cleaners (*n*=5 excluded from a total of *n*= 159 species) were excluded from the analyses, as they do not have a role in the biological market of the cleaning mutualism. Few client species were by error counted in the non-corresponding transect width (small species in large width transect and large species in small transect width), but further correction did follow up based on the client species' home range (for further details, please refer to Table S2). We calculated cleaner abundance by scaling densities to 100 m^2.

1.2.3 Laboratory cognition experiments

Twenty-five adult female cleaner fish (site 1 in 2016, *n*=10; site 2 in 2014, *n*=5; and site 2 in 2016, *n*=10), were captured using hand and barrier nets (2 m x 1 m, 5 mm mesh). Cleaners were individually housed in aquaria (62 cm x 27 cm x 37 cm) for a minimum of seven days before starting the experiments. Each cleaner was provided with a PVC pipe for shelter. Food was offered smeared on Plexiglas plates (8 x 15 cm), consisting predominantly of mashed prawn, and occasionally a mixture of fish flake (70%) and prawn (30%) (hereafter 'flake'). All individuals learned to feed from the Plexiglas plates within two days in captivity.

Experiment I: the "Bystander effect" task

The "Bystander effect" experiment tested if the cleaners can adjust their feeding behavior when in the presence of an image scoring bystander. Experimental Plexiglas plates containing food items were used as surrogates for reef fish clients [Bshary, and Grutter, 2006]. To begin with, all cleaners were exposed to a total of six learning trials, one every 2h, with a maximum of four trials per day. Cleaners significantly prefer feeding on prawn over flake [Bshary, and Grutter, 2005]; hence, we used prawn and flake items as preferred and less-preferred food items to mimic client mucus and ectoparasites, respectively. During the learning phase, cleaners were offered a novel Plexiglas plate containing 13 flake items and 2 prawn items. Feeding on prawn was scored as cheating, whereas feeding on less-preferred flake was scored as cooperating. Consequently, fish were allowed to feed on flake items, but eating a prawn item lead to the immediate removal of the plate for 60 seconds. If a second prawn item was eaten, the plate was removed until the next learning trial, two hours later. During the learning phase, all cleaners ate flake items and hence, learned that there was no consequence for eating the less preferred flake, while eating prawn led to the immediate removal of a plate.

The following bystander effect task consisted of several rounds, where each round consisted of two trials 30 min apart, one trial as a control and the second trial as a treatment. The following round started 60 min later. The order of presentation within each round was determined for each cleaner individually by flipping a coin. For each trial, cleaners were first confined to one side of the aquarium with a partition, then the plate(s) were placed on the other end of the aquarium. The partition was then lifted, and the cleaner started foraging until the consumption of a prawn item led to the removal of the plate(s). In earlier studies, cleaners had been subjected to 20 and 30 rounds. However, as the performance of cleaners reflects their spontaneous decision rules, which are not affected by learning [Wismer et al., 2014], we opted, therefore, for five rounds for the experiment conducted in 2016, three rounds on the first day and two rounds on the second day.

During the bystander task trials, cleaners were offered novel Plexiglas plates of a similar size (12 cm x 7 cm) either colored with white or yellow stripes. Both plates contained prawn and flake items, where each plate offered two items of each food type. Cleaners faced two situations: a control "non-image scoring situation" with a single plate, and treatment "image-scoring situation" when exposed simultaneously to two plates. In both cases, the plate(s) was immediately removed from the aquarium if the fish fed on a prawn item, which constitutes cheating. In the image-scoring treatment, fish would have access to a bystander plate in case they increased their image score and demonstrated cooperation by feeding only on flake items on the first plate and refraining from eating prawn. This experiment mimics a natural scenario, where cleaners only have access to an image scoring bystander client if they cooperate with a current client [Bshary, 2002; Pinto et al., 2011]. Despite their high preference for prawn, cleaners can maximize their food intake by feeding first on the flake items, then one prawn item. The presence of a bystander plate would thus make cleaners eat more against their preference (i.e., behave more cooperatively) in the image scoring situation than when only a single plate is presented in the non-image scoring situation. The optimal solution in the non-image scoring situation was to feed on the two flake items and one prawn item, whereas, in the image scoring situation, it was to feed on the four flake items on the two plates (the first plate and the bystander plate) before feeding on one prawn item. Subsequently, we calculated the ratio of the flake to prawn items consumed by cleaners from each plate. To establish a bystander effect score, we subtracted the ratio of the single plate (i.e., the non-image scoring situation) from the first plate (i.e., the image scoring situation). Cooperation induced by an audience effect would hence yield positive values, as it refers to that cleaners ate more against their preference (more flake items) in the presence of a bystander, than in the absence of the latter.

Experiment II: the "Biological market" task

In the "Biological market" task, cleaners were tested for their ability to learn to prefer an "ephemeral" plate over a "permanent" plate. The plates represented two different client categories based on their accessibility to cleaners, which is a simple consequence of home range/territory size. Visitors have access to multiple cleaning stations, while residents have access to a single cleaning station. As a result, the latter have to wait for inspection (they are a "permanent" food source for cleaners), while the former may swim off and seek another cleaning station if they are made to wait (they are an "ephemeral" food source for the cleaners). Therefore, when two clients seek cleaning service simultaneously, cleaners have been observed to give cleaning priority to a visitor client over a resident client [Bshary, and Noë, 2003].

Plexiglas plates were equal in size (10 cm x 7 cm), yet differed in color and orientation patterns of decorative stripes (i.e., horizontal green stripes and vertical pink stripes) to allow for individual identification of the plates. Both plates were presented simultaneously and offered an equal value of food of one prawn item. One plate had a role of a resident client, which was willing to wait to be inspected (i.e., permanent plate). The other plate, however, represented a visitor client, which was removed from the aquarium if the cleaner fed on the resident plate first. The optimal solution, to maximize food intake, was hence to always feed first on the visitor plate, then on the resident plate. Otherwise, cleaners will only have access to one prawn item if they choose the resident plate first, versus eating two prawn items if they prioritize the visitor plate. The status of each plate was predetermined, counterbalanced between individuals, and plate positions (i.e., left or right) were counterbalanced over ten successive trials. Trials continued until a cleaner would reach the learning criterion. Success consisted of showing a significant preference for the visitor plate, i.e., at least 9 successful choices out of 10 trials, two consecutive 8/10 or three consecutive 7/10. Upon learning the initial task, cleaners were subjected to a reversal learning task, where the visitor plate became the resident plate and vice versa. The reversal learning task allowed us to be confident that cleaners solved the biological market task based on the status and the behavior of the plate, and not due to a bias of color preference. Following established protocols [Salwiczek et al., 2012; Wismer et al., 2014], we scored the number of trials each cleaner needed to solve both initial and reversal learning. Individuals that couldn't solve the task within a total of 200 trials were categorized as "failed". The trials were conducted over ten days per cleaner. For each trial, cleaners were first confined to one side of the aquarium with a partition, and then the plates were placed on the opposite side. The partition was then lifted, and the cleaners had to make a choice. Choosing a resident plate led to the removal of the visitor plate, while the resident plate was only removed once the cleaner was finished foraging on it. In the optimal choice scenario, both plates (i.e., visitor and resident) would remain in the aquarium until the cleaner would eat the two prawn items. All experiments were conducted between 8:00 and 17:00.

1.2.4 Statistical analyses

Data were analyzed using the software Rstudio® (version darwin.10.08.0). Non-parametric statistics were used for analyses in the present study due to data violating assumptions regarding normality and homogeneity of variances. We used Wilcoxon test (also called Mann-Whitney-U-Test) when groups $n = 2$; and from the R packages (*agricolae*) we used the Kruskal-Wallis test when groups $n > 2$. For the analyses, due to the time lapse between the two data collections before and after perturbations, data from the same site before and after were considered as independent samples. The binomial data from experiment II (success/failure in solving the task within 200 trials) were analyzed with a Generalized Linear Model (GLM), as the data did fit the binomial models' assumptions. Data analyses were conducted on the period level (i.e., before environmental perturbations vs. after environmental perturbations) only if the within level predictors (i.e., site identity) were not significantly different from each other. Therefore, we first conducted analyses between the period levels, then we compared before vs. after (see the Supplementary materials for details).

1.3 Results

Cleaner fish densities were reduced by 80% after environmental perturbations (W = 353.5, $p<0.001$, Fig. 1a). Similarly, densities of large clients were reduced at both sites after perturbations (mean ± SD; before: 78.3 ± 37.8, after: 33.9 ± 12.9 large clients per 150 m^2, Fig. S3b). This decrease in density of cleaners and clients resulted in lower cleaner-client ratios after perturbations (ratio of cleaner to 100 clients (i.e., large and small clients): W= 304, p =0.004; ratio of cleaner to 100 large clients: W= 305.5, p = 0.003) (Fig. 1 b and c). The diversity of large clients and the diversity and abundance of small clients did not yield consistent patterns between sites prior/after perturbations (mean ± SD; before: 18.5 ± 4.9, and after: 16.3 ± 5.4 large client species per 150 m^2, Fig. S3a) (mean ± SD; before: 6.5 ± 2.2, and after: 7.9 ± 3.4 small client species per 30 m^2, Fig. S3c; before: 41.3 ± 25 and after: 21.6 ± 17.8 small clients per 100 m^2, Fig. S3d).

Changes in densities were accompanied by several changes in cleaning interaction patterns. While average duration and number of interactions varied non-systematically between sites/time periods (Fig. S4a, b), overall time spent cleaning systematically increased after perturbations (W = 71, p = 0.031, Fig. S4c), largely driven by significantly more frequent interactions with large clients (W = 11, p < 0.001, Fig. S4d). Importantly, after the perturbations, the frequency of visitors seeking a cleaning service simultaneously with another client, or from a cleaner already interacting with a current client, increased significantly (W = 61, p = 0.010, Fig. 2 a). Nevertheless, the frequency of visitors swimming off when not receiving the cleaning service was significantly lower after the perturbations (W = 186, p = 0.024, Fig. 2 b). Hence, leading to a highly significant decline in the percentage of visitors swimming off if the cleaner gave priority to another client (W = 214, p < 0.001, Fig. 2 c).

The substantial loss in cleaner densities and fluctuations in cleaner cleaning behavior in nature coincided with a significant decline in their cognitive performance. Cleaners responded less to the presence of a bystander, i.e., appeared to care less about their cleaning reputation after perturbations compared to performances of cleaners from the same sites before perturbations (W = 372.5, p = 0.028, Fig. 3 a). Similarly, cleaners tested after the perturbations failed to learn to give priority to a visitor plate over a resident plate than cleaners tested before perturbations (One-Way ANOVA: before vs. after: $X^2(1) = 7.19$, $p = 0.007$, Fig. 3 b).

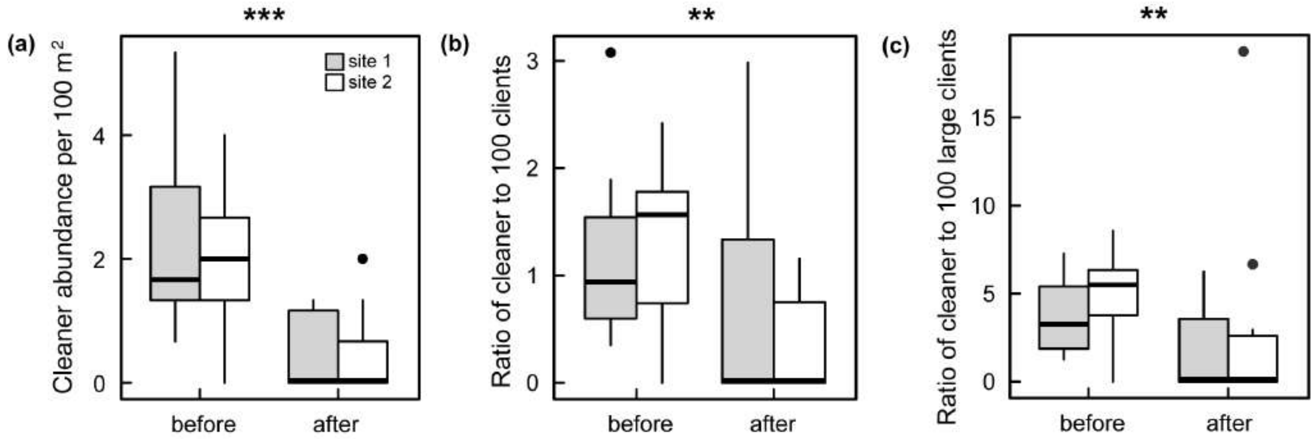

Fig. 1. Cleaner abundance and cleaner-client ratios before and after the environmental perturbations. Boxplots showing median and interquartile range of (a) cleaner fish abundance, (b) ratio of cleaners to 100 clients, and (c) ratio of cleaner to 100 large clients. Wilcoxon Tests: **: $p \leq 0.01$; ***: $p \leq 0.001$.

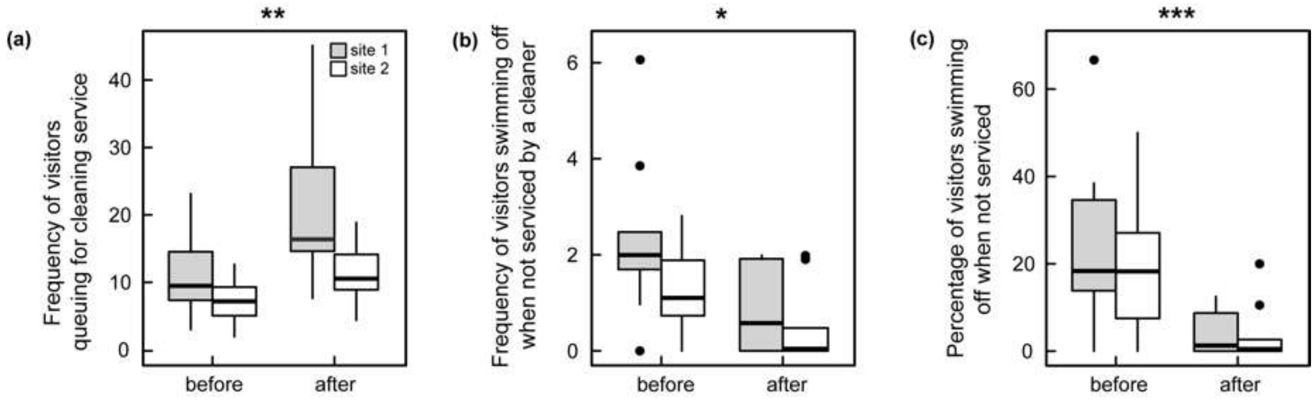

Fig. 2. Learning opportunities in the natural habitat before and after the environmental perturbations. Boxplot median and interquartile range of (a) number of visitors seeking cleaning service with another client, (b) number of visitors swimming off if not serviced, and (c) percentage of visitors were swimming off if not serviced. Wilcoxon Tests: *: $p \leq 0.05$; ** $p \leq 0.01$; ***: $p \leq 0.001$.

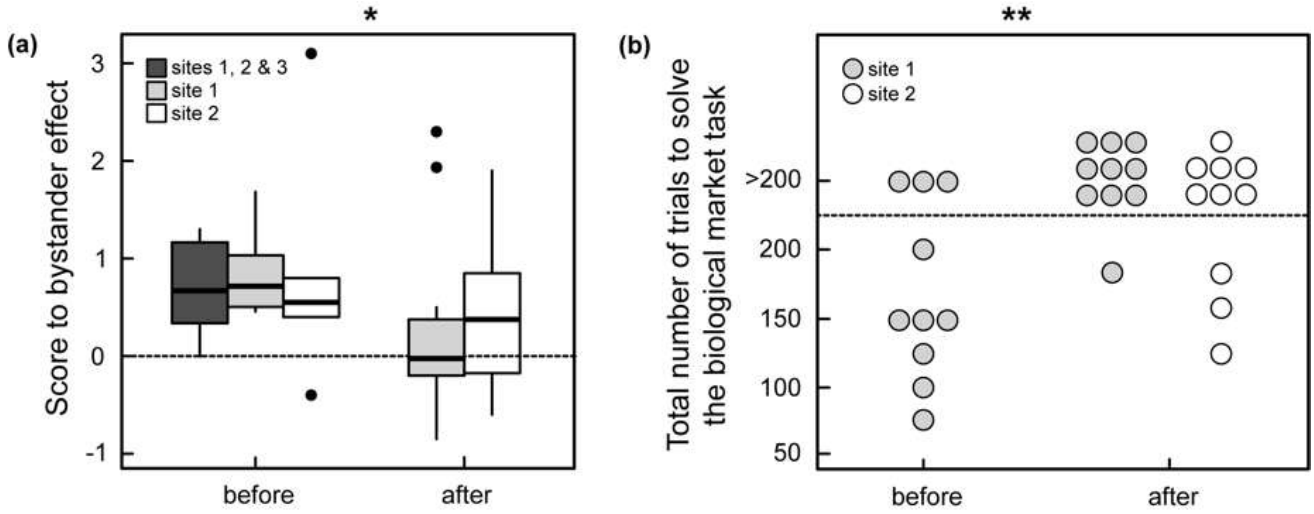

Fig. 3. Cognitive performance in the laboratory before and after the environmental perturbations. (a) Boxplot median and interquartile range of the change in the flake to prawn ratio eaten by cleaners as a performance score in the presence of a bystander plate. (b) Scatterplot of individual performance in the biological market task denoting the number of trials needed to complete the task. The dashed line represents the maximal number of trials during the task. Dots above the line denote individuals that failed to perform the task. Wilcoxon Test: *: p ≤ 0.05. One-Way ANOVA: **: p ≤ 0.01.

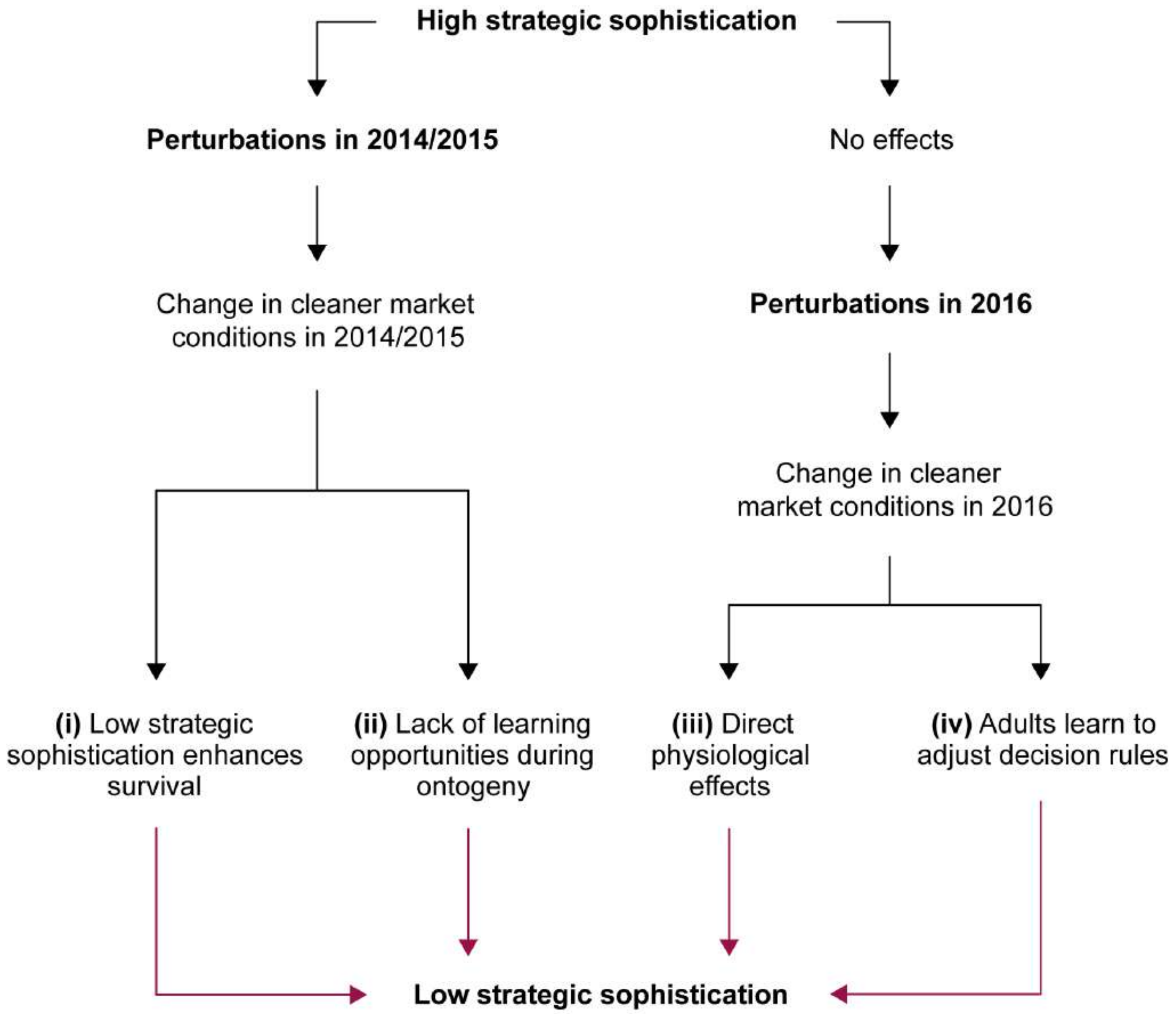

Fig. 4. A detailed outline illustrating four possible mechanisms underlying changes in cleaner strategic sophistication. The scheme shows a timeline in changes of the environmental conditions and consequently the change in the biological market of the cleaning mutualism.

1.4 Discussion

We aimed to determine whether the composition and densities of fish species involved in the *L. dimidiatus* cleaning mutualism system affect cleaning interaction patterns, as well as, the cognitive performance of cleaner fish. Consecutive cyclones and a major coral bleaching event offered natural conditions to test our hypotheses. We documented a loss of fish abundance and biodiversity, with an apparent decline in cleaner fish strategic sophistication that strongly correlates with changes in the biological market conditions.

Fish abundance and cleaning patterns after the perturbations

While reef fish abundance declined, cleaner abundance decreased disproportionally after the perturbations. Indeed, cleaners are a key species in coral reef ecosystems [Bshary, 2003; Grutter et al., 2003; Wagner et al., 2015]. The loss in cleaner abundance may be linked directly to an increased metabolism, due to an elevated water temperature beyond the physiological plasticity of the organism [Neuheimer et al., 2011]. Alternatively, the loss may be indirect, due to a decrease in food availability, as client abundance declined as well. On the other hand, cleaner presence on coral reefs promote local fish diversity, most likely because cleaning service improves the health status of client fish (Bshary *et al.* 2007; Ros *et al.* 2011; Waldie et al. 2011; Soares *et al.* 2011). It is hence possible the documented loss in client abundance may be partially due to a loss in cleaner abundance, in addition to potential direct effects of the environmental perturbations.

The decrease in cleaner-to-client ratio translated into cleaners spending more time cleaning and having disproportionally more interactions with visitor clients. Furthermore, visitor clients were less likely to swim off if the cleaner made them wait in favor of another client, both regarding absolute frequency and relative to the number of occasions in which a visitor had to wait. These results are in line with biological market theory [Noë et al., 1991]. This theory views mutualism between species as an exchange of goods and services between two classes of traders, where supply and demand affect individual decisions [Noë, and Hammerstein, 1994; Noë et al., 1991]. Lower supply and increased demand favor the acceptance of a lower service quality [Johnstone, and Bshary, 2008]. In our case, the low absolute cleaner density and the increased client to cleaner ratio made visitor switching in response to being made to wait costlier as the distance to the next cleaner and the likelihood that this cleaner is again already engaged with another client became larger.

Cleaner strategic sophistication after the perturbations

In the laboratory experiments conducted after the perturbations, cleaners largely failed to show the strategic sophistication necessary to maximize their food intake, in stark contrast to the cleaners caught at the same locations before the environmental perturbations [Bshary, and Grutter, 2006; Wismer et al., 2014]. Low performance in the two laboratory experiments has been documented once before [Wismer et al., 2014], albeit under different circumstances, excluding environmental perturbations. The low-performing cleaners in the Wismer et *al.* [2014] study have in common with the low-performing cleaners in the present study a low cleaner density and low cleaner to client ratios. In other words, the only similarities consisted of a restricted biological market, with low supply and relatively high demand.

Here we suggest four different potential explanations for the low performance of cleaners after the perturbations, three of them linked to the market conditions while the fourth suggestion is directly related to physiological mechanisms (Fig. 4). The change in the cleaning market conditions may have caused low strategic sophistication in different ways. First, low cleaner densities and high client to cleaner ratio may favor cleaners with low strategic sophistication. Either cleaners with low strategic sophistication may preferentially settle in areas with low competition, or they may experience an increased survival if sophisticated decision-making was costly. Alternatively, the other two market explanations assume that cleaners may be able to learn how to adapt their decision-rules based on changes in a visitor's partner choice. At site 1, variations in the biological market most likely occurred 1-2 years ago, because of the cyclones destroying the coral habitat. Therefore, low strategic sophistication could be due to ontogenetic effects: juvenile cleaners turning into adults would not have experienced visitor clients leaving at high frequencies due to being ignored by cleaners, or by observing cleaners cheating current clients. At site 2, on the other hand, the observed changes in the market conditions occurred only within four months after the environmental perturbations. As the lifespan expectancy of cleaners is up to 5 years [Eckert, 1987], the subjects should have been adult cleaners with high strategic sophistication before the perturbations, and hence, learned to adjust their behavior to the new market conditions. While we only documented an increase in visitors' willingness to wait for inspection, the changed market conditions should also make visitor bystanders indifferent to observing how cleaners treat their current client. The fact that visitors rarely swam off made it less risky for cleaners to cheat in the presence of bystanders. Cleaners should hence not behave more cooperatively when observed. Indeed, Bshary & Grutter [2006] showed with the Plexiglas plate paradigm that cleaners readily learn to ignore the presence of bystanders if those do not attribute a reputation to the cleaners. Our current findings showed that cleaners, after the perturbations, neither spontaneously increased their cooperation level in the presence of a bystander nor did they learn to adjust over the course of the experiment.

We rely here on learning as a plausible explanation of the strategic sophistication variation because *L. dimidiatus* has a pelagic larval duration before settlement [Victor, 1986]. Therefore, the hypothesis of a strong genetic basis for variation in strategic sophistication appears unlikely, given that the selection gradient, as a function of the environment, would have to be very strong to explain our results. In contrast, we know that fish behavioral plasticity is subject to change from early stage to adult stage depending on the environmental social cues [Kotrschal, and Taborsky, 2010]. Cleaners, similarly to the vast majority of animal species, can learn through conditioning. The feedback that cleaners receive in response to their behavior during interactions provides ideal conditions for operant conditioning, where both food reward and visitors swimming off, correspond to the positive and negative reinforcement, respectively [Wynne, 2001]. Indeed, several previous studies support the notion that variation in cleaner strategic sophistication is based on learning. For instance, juvenile cleaners were shown to solve the bystander effect task consistently but to fail the biological market task [Salwiczek et al., 2012; Wismer et al., 2014]. Thus, levels of performance change over the lifetime of individuals and can either improve or decrease. The more positive note is that if cleaner cognitive sophistication is indeed based on learning according to local cleaning market conditions, high levels of cognitive sophistication may re-emerge whenever cleaner densities increase to pre-perturbation levels.

Finally, the fourth potential explanation is that direct effects of the perturbations lead to the declined strategic sophistication via an impact on the physiological mechanisms of cleaners. Scientists have focused on direct effects of climate change on fish behavior. For instance, rises in water temperature, CO2 concentration, and ocean acidification can alter behavior [Pörtner, 2001; Jonsson, and Jonsson, 2009; Sih et al., 2011; Browman, 2016]. During the 2016 El Niño, water temperature indeed peaked, which may have induced direct physiological effects on all cleaners in the population, including effects on decision-making through brain functioning. However, such effects would have to be long-lasting as the water temperature was clearly below average summer maxima when we caught the subjects in June (Fig. S2). However, the possibility that extremely high water temperatures cause a permanent loss of cognitive performance certainly needs further investigation. Independently of the precise underlying mechanism, our study suggests that environmental perturbations, largely caused by cyclones and El Niño, affect the functioning of marine cleaning mutualism. Such environmental perturbations are predicted to increase as a consequence of climate change [Cheal et al., 2017]. We, therefore, hypothesize that climate change will affect many interspecific interactions via reduced population densities, with somewhat unpredictable consequences for species communities.

1.5 Ethics

The Animal Ethics Committee of the Queensland government (DAFF) approved the project (CA 2016/05/97).

1.6 Data availability

The data are available in the repository figshare (Data DOI: 10.6084/m9.figshare.4234886).

1.7 Acknowledgements

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1.8 Author's contributions

ZT and RB designed the study; ZT, SW, EL, and RB collected the data; ZT analyzed the data; ZT and RB wrote and finalized the manuscript. SW commented on the manuscript. All authors declare no competing interests.

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1.10 Supplementary Information for Chapter I

Methods S1:

Statistical analyses:

Data analyses were carried out on the period level (i.e. before environmental perturbations vs. after environmental perturbations) only if within level predictors (i.e. site identity) were not significantly different form each other by using Wilcoxon Tests. Therefore, we carried out analyses first within each period, then we compared before vs. after. For data regarding cleaner densities and cleaner-client ratios, we grouped data due to non-significant differences within each period group (cleaner densities: before: W = 53.5, $p = 0.817$; after: W = 50.5, $p = 1$, cleaner to 100 clients ratio: before: W = 41, $p = 0.528$; after: W = 55, $p = 0.700$, cleaner to 100 large clients ratio: before: W = 36, $p = 0.315$; after: W = 49, $p = 0.966$). Whereas, for the rest of the fish censuses factors, the grouping was not possible due to non-systematic differences (richness in large clients: before: W = 92.5, $p = 0.001$; after: W = 48.5, $p = 0.939$, abundance in large clients: before: W = 82.5, $p = 0.0155$; after: W = 64, $p = 0.307$, richness in small clients: before: W = 97, $p < 0.001$; after: W = 0.5, $p < 0.001$, abundance in small clients: before: W = 74, $p = 0.075$; after: W = 2, p <0.001) (Fig. 1B).

From the natural interactions data, we were not able to group within periods of time, the duration of interactions (before: W = 7, $p = 0.006$; after: W = 44, $p = 0.234$) and the total number of interactions (before: W = 56, $p = 0.010$; after: W = 8, $p = 0.013$). However, for total time spent cleaning (before: W = 41, $p = 0.382$; after: W = 29, $p = 0.798$), the total number of interactions with large clients (before: W = 39, $p = 0.494$; after: W = 18, $p = 0.160$), the frequency of visitors seeking cleaning service simultaneously with another client or from a cleaner already interacting with another client increased after the perturbations (before: W = 45, $p = 0.194$; after: W = 61, $p = 0.065$). The frequency of visitors swimming off when not receiving the cleaning service was significantly lower after the perturbations (before: W = 48, $p = 0.102$; after: W = 39, $p = 0.432$), leading to highly significant decline in the percentage of visitors swimming off if not serviced from the total number of visitors seeking the cleaning service (before: W = 37, $p = 0.635$; after: W = 38, $p = 0.507$), hence, grouping was allowed.

Finally, for the cognitive performance analyses from experiment I, we were able to group data within each period time due to non-significant differences between the level within each time period (before: Kruskal-Wallis tests: x^2 (2) = 0.72, $p = 0.694$; after: Wilcoxon Tests: $W= 44$, $p = 0.676$). To test whether the binary response in the biological market task from sites after perturbations can be grouped in one data set, we used Fisher's Exact test, the cleaners performance post-perturbations was similar between sites (fisher test, $p =0.58$) and hence, grouped.

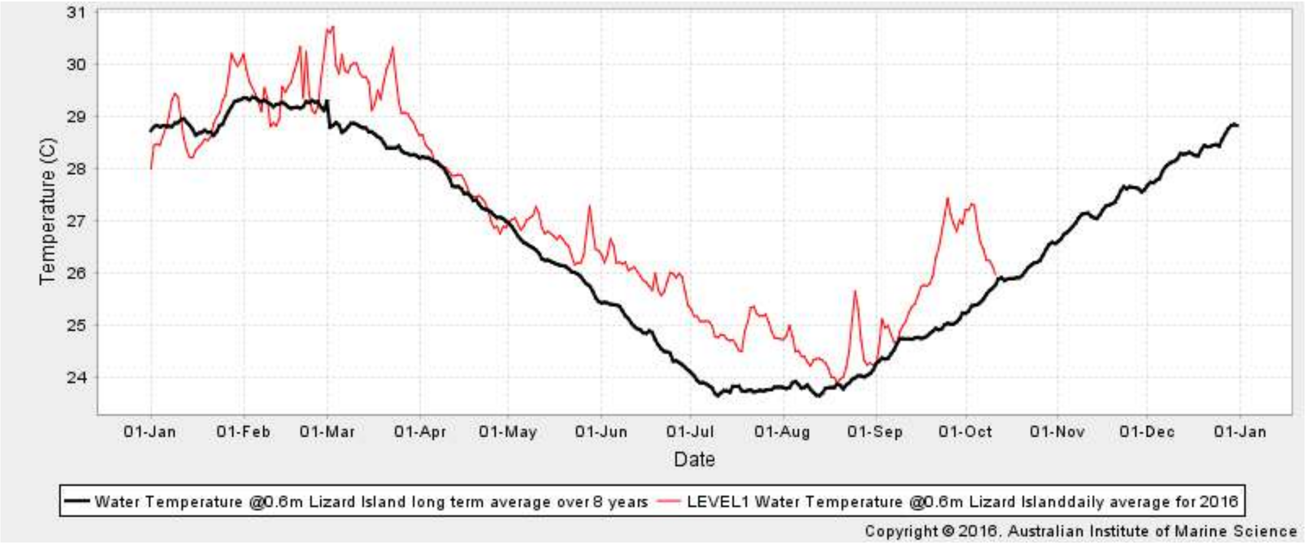

Fig. S1. Water temperature in C° at 0.6 m water depth at Lizard Island.
Red line shows the average daily temperature, starting from January 2016 until mid-October 2016. Black line reports the long term daily temperature average. Graph reproduced with authorization from Australian Institute of Marine Science AIMS®.

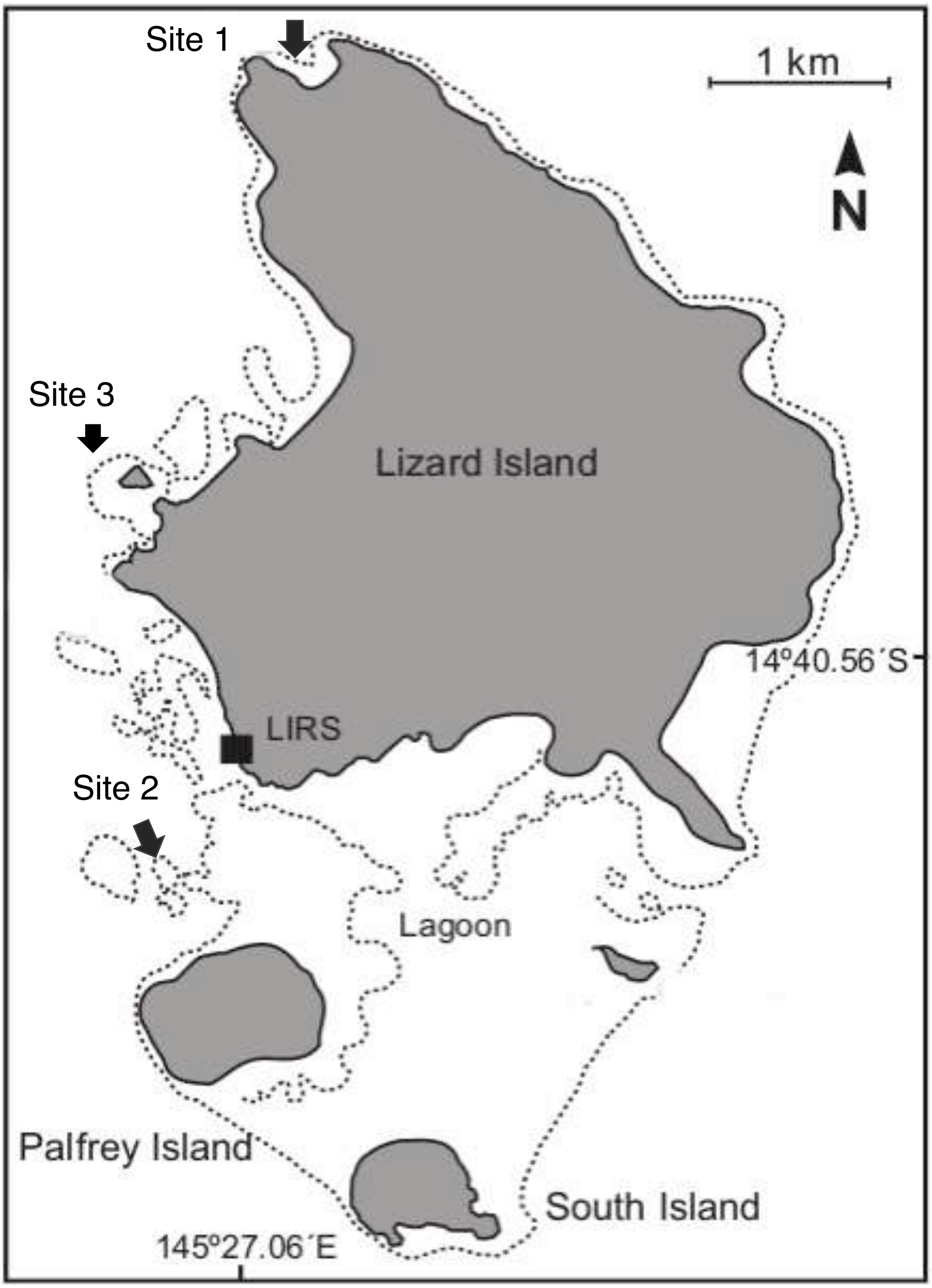

Fig. S2. Map of the Lizard Island Group. Data were collected on the sites indicated by filled arrows: (1) MC, Mermaid Cove; (2) NH, North Horseshoe; (3) Osprey. Prior to climatic perturbations, data were collected in 2003 at MC, NH and Osprey, in 2011 at MC and in 2014 at NH; post-perturbations data were collected in 2016 at MC and NH. MC is a continuous fringing reef of an approximate size of 35,000 m^2 (depth 1 to 7 m) (estimation from maps: https://www.freemaptools.com/area-calculator.htm), located in a small bay on the northern side of the island. NHS is a shallow coral garden (depth 1 to 4 m) of approximately 17,000 m^2 close to the island's main lagoon, and Osprey is a continuous reef an approximate size of 94857.289 m^2.

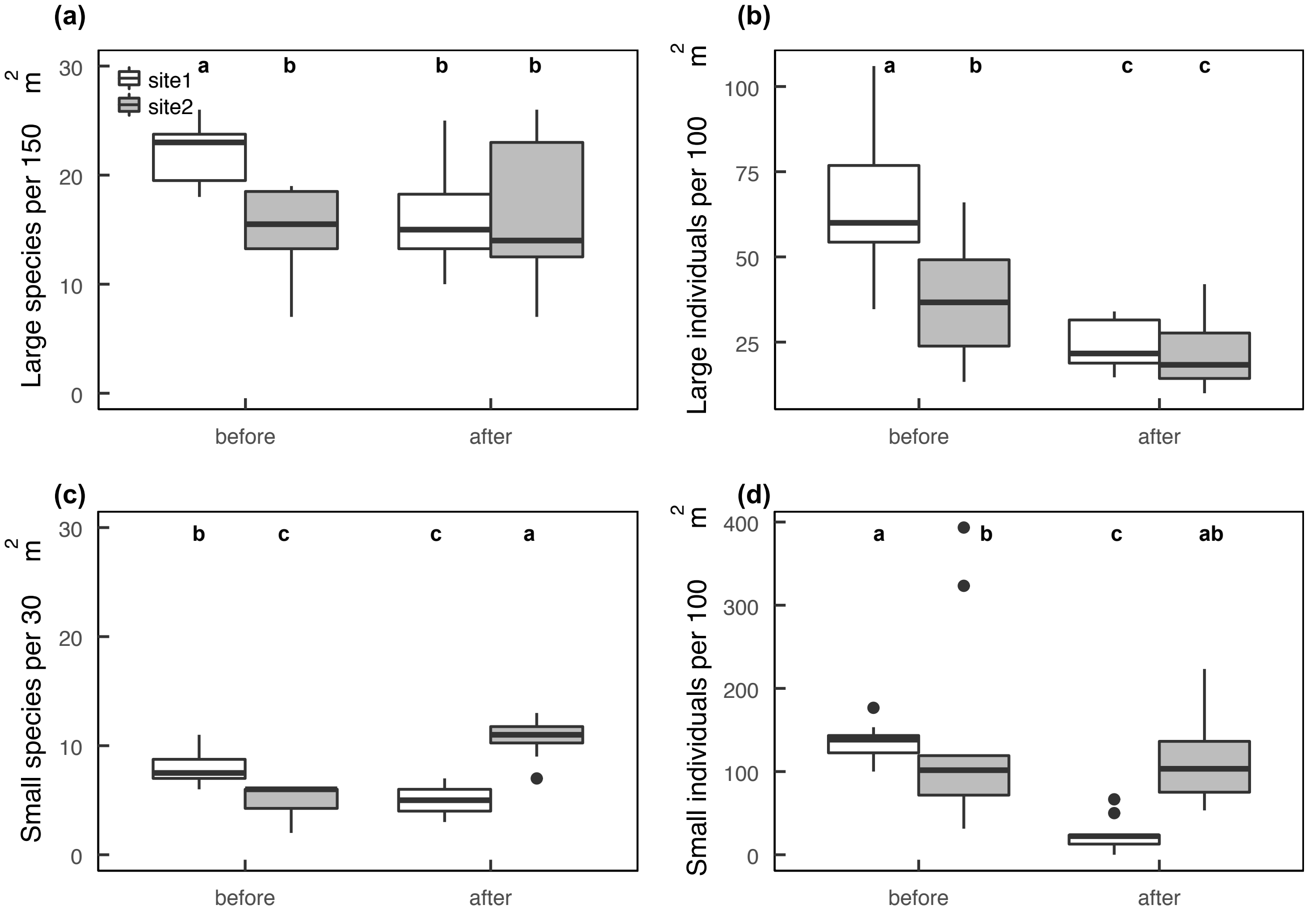

Figure S3. Client fish richness and density before and after environmental perturbations. Boxplots showing median and interquartile range of large client species **(a)** richness and **(b)** abundance in 5 m transect width, and small client species **(c)** richness, and **(d)** abundance in 1 m transect width. Kruskal-Wallis tests: different letter codes indicate significant differences.

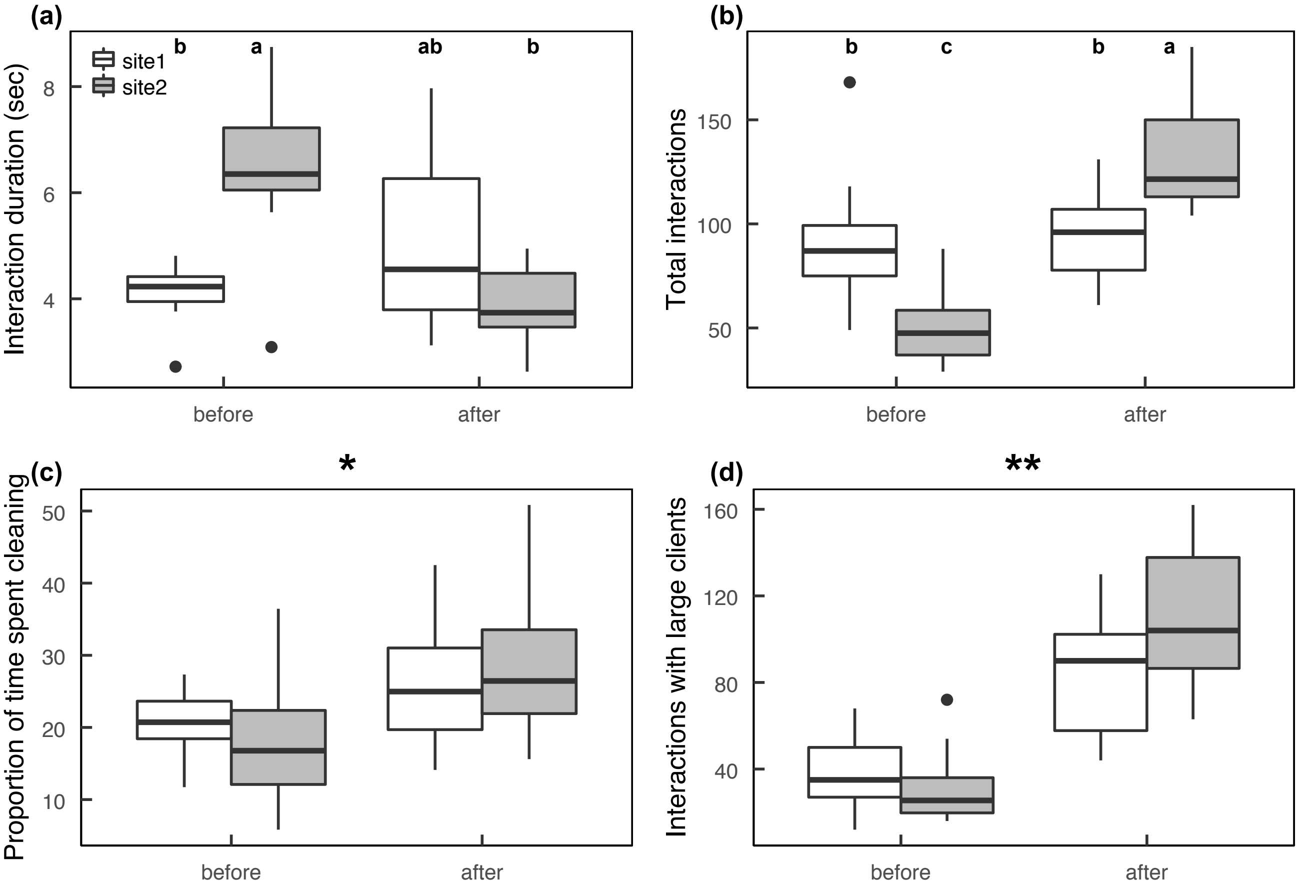

Figure S4. Cleaner-client interaction patterns in natural habitats before and after environmental perturbations. Boxplots showing the median and interquartile range of **(a)** average duration of a cleaning interaction, **(b)** total number of cleaning interactions during 30 min, **(c)** overall proportion of time spent in cleaning, and **(d)** total number of cleaning interactions with large clients during 30 min. Kruskal-Wallis tests: different letter codes indicate significant differences. Wilcoxon Tests: *: $p \leq 0.05$; ***: $p \leq 0.001$.

Table. S1. Species counts per client category and size. Evidence that the classification of species into “sampled on the large (5m) transect width” and ‘sampled on the small (1m) transect width’ provides a strong correlation with the classification of species as “visitor client” with access to several cleaning stations and “resident client” with access to the local cleaning station only. The matrix shows that the visitor class is composed entirely of species sampled on the large transects, whereas the majority resident species were sampled on the small transects.

Species counts		Client Category	
		Visitor	Resident
Transect width	Large	105	13
	Small	0	32

Table. S2. Supplementary information regarding species classification. List of species that by error were not sampled consistently in one transect width, i.e. either in 1m or in 5m width. The 'final classification' column states how the species was classified. For the species richness counts, data collected using the wrong transect width could not be adjusted in any way. In contrast the abundance data were scaled according m^2 sampled, i.e. $30m^2$ or $150m^2$.

family	genus	species	final classification
Apogonidae	*Cheilodipterus*	*quinquelineatus*	small
Labridae	*Halichoeres*	*chloropterus*	large
Labridae	*Halichoeres*	*melanurus*	small
Pomacentridae	*Acanthochromis*	*polyacanthus*	small
Pomacentridae	*Amblyglyphidodon*	*curacao*	small
Pomacentridae	*Dischistodus*	*melanotus*	large
Pomacentridae	*Dischistodus*	*prosopotaenia*	large
Pomacentridae	*Dischistodus*	*pseudochrysopoecilus*	large
Pomacentridae	*Neoglyphidodon*	*melas*	large
Pomacentridae	*Stegastes*	*apicalis*	large
Pomacentridae	*Stegastes*	*nigricans*	large

CHAPTER II | Shifts in the biological market predict cleaner fish strategic sophistication

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Abstract

Variation in partner choice strongly affects payoff distributions in a biological market. Here, we provide first evidence that market conditions may also affect the complexity of social life and its cognitive challenges: the strategic sophistication of "cleaner" fish *Labroides dimidiatus* in laboratory experiments, i.e. their ability to adjust flexibly behavior to the specifics of a situation, correlates with the degree of competition over access to "client" reef fishes. Cleaner fish trade parasite removal in exchange for food with a variety of client species. Crucially, cleaners compete among each other through outbidding over access to visitor clients with access to multiple cleaners. Only cleaners from sites with high outbidding competition, characterised by high cleaner and visitor densities, and visitor client behavior enforcing such competition, consistently increased cooperation to gain access to an image-scoring audience, and/or learned to priorities an ephemeral food source over a simultaneously offered permanent one. Thus, two hallmarks of cleaners' strategic sophistication are apparently linked to strong market competition.

Keywords: reputation management, partner choice, supply and demand, competition, market structure

2.1 Introduction

Biological market theory (Noë and Hammerstein 1994; Noë and Hammerstein 1995) emphasises partner choice as a key feature of cooperative interactions: commodities, services or goods can change value based on shifts in supply and demand only if traders exert partner choice (McNamara et al. 2008; Barclay 2016). A large body of empirical evidence, including elegant field experiments, supports this general concept (Axén et al. 1996; Herre and West 1997; Kutsukake and Clutton-Brock 2008; Fruteau et al. 2009; Kiers et al. 2011). What remains an open question is in how far the possibility to choose between potential partners contributes to selection on cognitive social skills (Barrett et al. 2003; Ashton et al. 2018). Indeed, the fact that biological market theory can be applied to organisms that lack any central nervous system, like bacteria, fungi and plants (Hammerstein and Noë 2016), contrasts with the complexity of decisions in human markets, where detailed knowledge of market conditions and rational decision-making play a major role (Werner et al. 2014). It has even been proposed that regular choosing and being chosen in human social interactions was crucial for the evolution of our sense of fairness and morality (Baumard et al. 2013). One way to investigate potential links between partner choice and social cognition is to test in how far variation in market conditions may predict intra- and interspecific variation in strategic sophistication, i.e. the ability to adjust flexibly own behavior to the specifics of a situation.

An ideal model to study the links between market conditions and intraspecific variation in strategic sophistication is the marine cleaning mutualism involving the cleaner fish *Labroides dimidiatus* (hereafter simply “cleaner”). Cleaners inhabit small territories (i.e., cleaning stations), where “client” reef fishes visit them to have their ectoparasites removed. Despite the mutualistic outcome of interactions (Ros et al. 2011; Waldie et al. 2011), conflicts of interest occur. Most importantly, cleaners prefer to eat the clients’ protective mucus rather than ectoparasites (Grutter and Bshary 2003). Furthermore, clients sometimes compete with each other when two or more simultaneously seek the service of the same cleaner, and cleaners compete with each other over access to visitor client species with access to several cleaning stations. Both cleaners and clients compete by outbidding without aggressive interactions (Bshary 2001). These conflicts are apparently driving the reported high levels of strategic sophistication described for cleaners (Bshary 2007). Of importance for the current study are two aspects: First, field observations and laboratory experiments on cleaners provide a clear-cut example of cooperative reputation management outside humans (Bshary and Grutter 2006; Pinto et al. 2011). Cleaners increase levels of cooperation in the presence of image-scoring bystanders that are more likely to invite for cleaning if they observe an interaction without apparent conflict. Second, cleaners give priority to visitor clients over resident clients (i.e., resident have access to the local cleaner only) (Bshary 2001) as the former would switch to another cleaner if being made to wait (Bshary and Schäffer 2002). Giving priority to an ephemeral food source is not a trivial task, as evidenced by cleaners outperforming chimpanzees, orangutans, capuchins, rats and pigeons in a more abstract version of the general problem in laboratory experiments (Bshary and Grutter 2002; Salwiczek et al. 2012; Zentall et al. 2016; Zentall et al. 2017).

Initially, the high strategic sophistication of cleaners was thought to be homogeneous without variation (Bshary 2002; Bshary and Grutter 2002; Bshary and Noë 2003; Bshary and Grutter 2005; Bshary and Grutter 2006; Salwiczek et al. 2012). Later on, by including cleaners from socially poor habitats due to either poor environmental structure (Wismer et al. 2014) or natural disturbances (Triki et al. 2018), this picture of homogeneity turned out to be wrong. Recent studies document important intraspecific variation in the cleaners’ reputation management and service priority rules (Wismer et al. 2014; Triki et al. 2018). Much of the observed behavioral variation can be attributed to the precise site and year of capture, because all tested cleaners belonged to the same population at Lizard Island, Great Barrier Reef. As cleaners go through a pelagic larvae phase prior to settling on a coral reef (Victor 1986) and can live up to five years (Eckert 1987), a genetic basis for this observed variation is unlikely. Here, we repeatedly collected field and laboratory data across numerous sites and years to investigate whether local market conditions at the time of cleaner capture, i.e. cleaner and client abundances as well as interaction patterns, correlate with cleaners' performance in laboratory tasks. Based on the logic of biological market theory, we predicted that changes in the supply to demand ratio would trigger changes both in client partner choice rules, and in cleaner strategic sophistication.

2.2 Materials and Methods

2.2.1 Study site

Field observations and laboratory experiments were conducted at Lizard Island (14.6682° S, 145.4604° E), Great Barrier Reef, Australia. Data were collected at several sites from adjacent reefs over different time periods. Overall, there were nine sites in total: Bird Islet (BI), Big Vickies (BV), Corner Beach (CB), Horseshoe (HS), Mermaid Cove (MC), Northern Horseshoe (NHS), Osprey (OS), Trawler Beach (TB), and Watson's Bay (WB). Data were collected between 2010 and 2017, but always between June and August of each year of data collection. Further details on the location of the precise sites, and the type of data collected at each site (i.e., either field or laboratory data) are shown in Supplementary Fig. S1 and Table S1, respectively.

2.2.2 Field data

Behavioral observations

Natural cleaning interactions data were encoded from videos of 30 min duration. Videos were recorded between 08:30 and 16:00 hours, using Canon G15® and G16® cameras. For each site, per period, (n = 8) adult female cleaners were selected randomly for video recording (Supplementary Table S1). Scuba divers recorded cleaning behavior from a distance of ~ 2 m, to minimize disturbance, while still obtaining good video footage.

Video encoding consisted of extracting for each cleaner-client interaction the client species and the duration. From this information, we calculated: the total number of interactions per time unit (i.e., 30 min), the total number of interactions with large clients per time unit, the average duration of cleaner-client interaction and the percentage of time cleaners spent cleaning. We also encoded for situations in which the cleaner had simultaneous choices between two clients, at least one of them being a visitor client (i.e., client species with cleaner-partner choice). There were two distinguishable situations of simultaneous choices, one with two clients (i.e., at least one of the two being a visitor client) seeking the cleaning service at the same moment, while the other with a visitor client seeking the cleaning service while the cleaner is already interacting with another client. Then, we counted the number of occasions in which a visitor client swam away after being ignored by a cleaner. Finally, we transformed these counts to percentages of visitors swimming off when not serviced from the total of simultaneous choices counts.

Fish survey

The abundance of adult cleaner fish and its variety of adult client reef fishes were estimated with fish censuses collected by scuba divers. A replicate of (n =10) transects of 30 m each was used at the different sites/periods (Supplementary Table S1). Transects were placed haphazardly either parallel to the shoreline or the reef crest. All large visible fish with a body size of a total length TL > 10 cm and cleaners were first recorded on a 5 m width, followed by small fish with a body size of TL ≤ 10 cm on a 1 m wide area along the 30 m transect. Fish were then classified to body size for the abundance data. This classification mostly followed Bshary (2001) and Froese and Pauly (2016). All visitor species (i.e., client species with cleaner-partner choice) have a large body size (TL > 10 cm), whereas resident species (i.e., client species with no cleaner-partner choice) are small-bodied fish (TL ≤ 10 cm) (Triki et al. 2018). All fish counts were scaled to densities per 100 m^2. From these counts, we estimated the ratio of cleaner to 100 clients, and the ratio of cleaner to 100 large clients.

2.2.3 Laboratory experiments

Animal capture and housing

In total, (*n* =120) adult female cleaner fish were caught at the different study sites over years of data collection. A total of (*n* = 118) were tested in the laboratory experiment "Bystander effect". The remaining (*n* = 2) did not perform in the laboratory experiments, and they were hence excluded from data analyses. In the "Biological market" task, we tested (*n* = 80) from the total of the (n =120) captured fish. Cleaners were captured were captured using barrier nets (2 m x 1 m, 5 mm mesh) and hand nets. Captured fish were individually housed in aquaria of a size 62 cm x 27 cm x 37 cm, and provided with PVC pipes (10 cm x 1 cm) for shelter. Cleaners were acclimated for an average period of ten days before subjecting them to laboratory experiments. Cleaners were fed with smeared paste on Plexiglas plates (8 x 15 cm), consisting mainly of mashed prawn. Gradually, cleaners were offered a mixture of fish flake and prawn, hereafter "flake". All cleaners fed successfully off the Plexiglas plates within the first two days of the acclimation period. All laboratory experiments were conducted between 8:00 and 17:00.

Experiment 1: "Bystander effect" task

A training phase preceded the bystander effect task, in which cleaners were trained to eat less preferred food (i.e., flake) instead of highly preferred food (i.e., prawn). Since cleaners prefer eating prawn over flake in a choice test (Bshary and Grutter 2005), we hence used prawn and flake food items to mimic client mucus and ectoparasites, respectively. Therefore, Plexiglas plates offering food items were used as a substitute for clients reef fish (Bshary and Grutter 2006).

A total of six learning trials were run over two days, with a break of a maximum of 2h between every two trials. In each training trial, cleaners were confined to one side of the aquarium by using an opaque partition. Then, a novel Plexiglas plate offering 13 flake items and two prawn items was positioned in the aquarium on the inaccessible side by the cleaner. At the removal of the partition, cleaners were allowed to forage on flake items (i.e., a homology of feeding on ectoparasites or cooperation), whereas the consumption of a prawn item (i.e., a homology of biting client mucus or cheating) led to the immediate removal of the plate from the aquarium. After 60 seconds, the plate with the remaining food items would return to the aquarium until a second prawn item is eaten. A trial took end when no prawn items are left on the Plexiglas plate. During this learning phase, all cleaners (*n* = 118) were successfully able to feed first on less preferred food (i.e., flake) before eating a preferred food (i.e., prawn).

In the bystander effect task, cleaners were presented with novel Plexiglas plates of identical size (12 cm x 7 cm) exhibiting different decoration with either yellow or white stripes. Here, each plate offered two flake items and two prawn items. Again, feeding on a prawn item, which constitutes cheating, also led to the immediate withdrawal of the plate(s) from the aquarium in this task. During the experiment, cleaners were tested in control and treatment trials. Treatment or "image-scoring situation" consisted in reproducing a natural condition, where cleaners can have access to an image-scoring bystander client only if they cooperate with a current client (Bshary 2002; Pinto et al. 2011). Therefore, cleaners are allowed to feed on a bystander plate only if they show cooperation by eating flake item(s) and refrain from eating a prawn item on a current plate. The control is hence a "non-image scoring situation" where cleaners are offered a single plate without a potential bystander plate. In the treatment trials, the optimal solution to maximize food intake is to feed first on all available flake items (i.e., four items on either plates) and then eat one prawn item, whereas in the control trials the optimal solution is to eat the two available flake items and then eat one prawn item.

The experiment consisted of several rounds composed of two trials each, one as control and one as treatment. With 30 min time interval between the two trials, and 60 min time interval between two rounds. The order of the two trials within each round was randomized by flipping a coin for each cleaner individually. Overall, cleaners were subjected to either five (n = 60), 20 (n = 39) or 30 rounds (n = 19), over a period of two to ten days. From an earlier study by Wismer et *al.* (2014), cleaners were tested in 30 rounds, but their performance was not affected by the number of rounds. It suggests that a cleaner's decision-rule in the bystander effect task is rather spontaneous and not affected by learning. Subsequently, for each cleaner, we established a single bystander effect score from total rounds. To estimate this score, we first calculated the ratio of the total flake items to total prawn items consumed by cleaners on the single plate in control trials, and on the current plate in treatment trials. Then, we subtracted the ratio of the single plate (i.e., control or the non-image scoring situation) from the current plate (i.e., treatment or the image scoring situation). Positive values > 0 refer to occasioned cooperation by bystander effect, in which cleaners fed more against their preference (i.e., eating more flake items) in the presence of an image-scoring bystander plate than in its absence. On the other hand, values ≤ 0 were considered as a failure in exhibiting cooperative feeding in the presence of a bystander. All scores were transformed to binary response: positive values > 0 referred to success, while values ≤ 0 referred to a failure.

Experiment 2: "Biological market" task

The biological market task was designed to reproduce a natural condition in the laboratory (Bshary and Grutter 2002). It has been observed that cleaners would give cleaning service priority to clients with partner-choice (i.e., visitor client) over clients without partner-choice (i.e., resident client) (Bshary and Noë 2003). Therefore, when visitor and resident clients seek cleaning service simultaneously, cleaners' optimal choice is to give cleaning priority to the visitor client that would leave if not serviced, and then service resident client that is willing to wait for cleaner's inspection. Therefore, in the biological market task, cleaners were tested for their ability to learn to prefer visitor plate (i.e., ephemeral food source) over a resident plate (i.e., permanent food source). Here again, two Plexiglas plates of equal size (10 cm x 7 cm), offering an equal amount of food (i.e., one prawn item each), were used as surrogates for visitor and resident clients. The plates were decorated with either vertical pink stripes or horizontal green stripes to facilitate the distinction between the plates by cleaners. The plate that played the role of a resident client was always accessible to cleaners, whereas the other plate that played the visitor client role was withdrawn from the aquarium if the cleaner gives priority to the resident plate and feed on it. In each trial, the two plates were placed on one side of the aquarium while the cleaner is confined to the other side by an opaque partition. Once the setup is ready, the partition would leave the aquarium allowing the cleaner to choose between the two plates. Prioritizing a resident plate led to the withdrawal of the visitor plate from the aquarium, while the resident plate was removed once the cleaner ate the prawn item on it. The optimal solution in the market task is hence to give priority to visitor plate (i.e., ephemeral food resource) then feed on the resident plate (i.e., permanent food resource). Cleaners will hence gain two prawn items instead of only one if they feed on the resident plate first.

A total of 200 trials were run over a period of 10 days. Solving the task consisted of scoring a significant preference for the visitor plate: either ≥ 9 successful choices out of a session of 10 trials, two consecutive sessions with a score of 8/10, or three consecutive sessions with a score of 7/10. After solving the initial task, a reversal task took place with a reversed role of the plates. For instance, if a plate with green stripes had a role of a visitor and pink plate for a resident in the initial task, upon success the green plate will become a resident and pink will become visitor for the remaining trials. The reversal learning task deal with the potential bias due to cleaners' possible color preferences. Also, the decoration (i.e., green or pink stripes), status (i.e., visitor or resident), and spatial location (i.e., left or right) of the plates in the aquarium were predetermined and counterbalanced between individuals. Therefore, cleaners solving both initial and reversal learning task of the biological market were successful to choose based on the status of the plate only. Cleaners that were unable to solve either the initial or reversal task were categorized as "failed".

2.2.4 Statistical analyses

All statistical analyses and figures were generating with the statistical open source software R version 3.3.2 (2016-10-31). First of all, we tested the potential correlation in cleaners' performance from the two laboratory tasks by performing a Jaccard similarity test. Due to the low similarity in cleaners' performance in the bystander effect and biological market tasks (n=80, 20% similarity percentage calculated with *Jaccard* index), each task's outcome was analyzed separately. For the market conditions, we had overall 12 relevant conditions (i.e., predictors) from the fish censuses and the video observations combined. Cleaners' performance, on the other hand, were set as responses variables. By clustering market conditions into ecological and behavioral factors, we had five ecological predictors: **1**- client abundance, **2-**abundance of large client, **3**- abundance of cleaner, **4**- ratio of cleaner to client, **5**- ratio of cleaner to large client; while we had seven behavioral predictors: **1**- percentage of time spent in interactions, **2**- interaction duration, **3**- frequency of interactions, **4**- frequency of interactions with large client, **5**- frequency of visitors queuing for a cleaning service, **6**- frequency of visitor clients swimming away if not serviced by a cleaner, and **7**- the percentage of visitor clients swimming away if not serviced by a cleaner (see Supplementary Fig. S2 to S14). The two response variables were the binary responses (i.e., success or failure) of the tested cleaners in either the bystander effect task or the biological market task (Table 1). For every cleaner tested in the laboratory, we assigned average values in the 12 recorded predictors from the corresponding data of the site and year from which the cleaner was caught. For instance, we encoded the interaction frequency from eight video observations collected at the same site and period. We then calculated the average of these eight values, and attributed the mean value to all the cleaners caught from the same site and period, uniformly.

All continuous predictors were standardized and centered around the mean. Also, we detected the presence of correlations between the 12 predictors (see Supplementary Fig. S15 to S18), which might lead to multicollinearity problem and cause overfitting of the statistical models. After all, we aim to find the important predictors that explain the outcomes in term of statistical significance. We chose the least absolute shrinkage and selection operator (lasso) logistic regression as variable selection method. The lasso approach solves overfitting problems by imposing a constraint on the parameters. It allows for variable selection by shrinking ordinary least squares estimates towards zero and forcing some redundant estimates to be exactly zero. However, a tuning factor lambda should be set for each dataset to control the size of coefficients' shrinkage. A standard method to set the optimal lambda is by cross-validation with *k*-folds, where *k* should be $\leq$ (n-1), where n is the number of variables (Tibshirani et al. 2012; Morozova et al. 2015). The selected variables were first fitted as fixed factors in a Generalized Linear Mixed Model GLMM with a binary distribution, wherein site and year of data collection were fitted as nested random effects. As the estimates of the random factors turned out to be zeros, indicating no effect of site and year, the random factors were then removed from the models and GLMs were run instead (personal communication with the statistician R. Slobodeanu).

2.3 Results

We tested for potential links between field market conditions and cleaners being more cooperative in the presence of a bystander. We fitted four non-collinear variables (i.e., selected from the 12 variables with the least absolute shrinkage and selection operator "*lasso*", see Methods) in a Generalized Linear Model (GLM) with a binomial distribution (Fig. 1a). The four variables included three ecological variables, namely abundance of all clients, abundance of large clients, ratio of cleaner to 100 clients; and one behavioral variable, namely the percentage of time cleaners spent cleaning. In the fitted model, interaction terms were run between the selected ecological and behavioral variables. We found a significant interaction between the abundance of large clients (i.e., visitors) and percentage of time spent interacting (n=118, two-way ANOVA: $X^2 = 4.659$, $P = 0.030$, pseudo $R^2 = 0.18$, Fig. 1 b & c) in explaining success in the bystander effect task, while the other variables were non-significant (Table 2). The probability of success correlated positively with the abundance of large clients but negatively with the percentage of time spent interacting. Accordingly, the statistical model predicted high success only when large clients are in higher densities, which is a strong correlate of cleaner densities ($r = 0.69$), combined with a low percentage of time spent interacting, which is largely equivalent to low interaction frequency with all clients and with large clients ($r = 0.68$, and $r = 0.70$, respectively, Supplementary Fig. S15 & S16). In other words, successful reputation management by cleaners apparently occurs in a market with a high abundance of both traders, but only if there is limited demand by visitor clients, as indicated by the low interaction frequency and subsequently low percentage of time spent interacting.

We tested the links between field market conditions and the cleaners' success in prioritizing visitors over residents in the biological market task. After variable selection, we obtained a model with two best variables: cleaner abundance from the set of ecological variables; and the percentage of visitor clients swimming away if not serviced by a cleaner as a behavioral variable (i.e., visitors using partner choice options) (Fig. 2a, Table 2). Here, we found that cleaner performance was significantly predicted by the interaction of cleaners' abundance and percentage of visitors swimming away if not serviced ($n = 80$, two-way ANOVA: $X^2 = 11.325$, $P < 0.001$, pseudo $R^2 = 0.37$, Fig. 2 b & c). A high probability to solve the biological market task was likely if a high abundance of cleaners co-occurred with a high percentage of visitors swimming away. As cleaner abundance correlated positively with the abundance of large clients ($r = 0.83$, see Supplementary Figures S17 & S18), giving service priority to visitor clients can thus occur in a market with relatively high densities of both traders, in which visitors frequently exert partner choice.

Table 1. Cleaner performance in the laboratory tasks.

Bystander effect task	Bird islet 2014	Big vickies 2014	Corner beach 2010	Corner beach 2017	Horseshoe 2017	Mermaid cove 2010	Mermaid cove 2016	Mermaid cove 2017	Northern horseshoe 2014	Northern horseshoe 2016	Northern horseshoe 2017	Osprey 2014	Trawler beach 2014	Watson’s bay 2014
failure	3	4	3	6	9	0	7	5	1	5	4	1	3	3
success	2	6	6	4	1	10	3	5	4	5	6	4	2	6
Biological market task			Corner beach 2010	Corner beach 2017	Horseshoe 2017	Mermaid cove 2010	Mermaid cove 2016	Mermaid cove 2017		Northern horseshoe 2016	Northern horseshoe 2017			
failure	-	-	9	10	8	3	9	9	-	7	2	-	-	-
success	-	-	1	0	2	7	1	1	-	3	8	-	-	-

Table 2. Effect of selected predictors on cleaner performance in the bystander effect and biological market tasks from the fitted GLMs with a binomial distribution.

Bystander effect task						
Predictor	Mean ± SD	df	LR X^2	*P*-value	95 % CI	vif score
Client abundance per 100 m^2	106.22 ± 48.72	1	3.1463	0.076	-0.05 1.18	1.18
Large client abundance per 100 m^2	24.76 ± 14.67	1	1.715	0.190	-0.44 1.20	1.27
Ratio of cleaner to 100 clients	3.07 ± 3.38	1	0.810	0.368	-0.26 0.82	1.11
Percentage of time spent in interactions per 30 min	21.97 ± 4.76	**1**	**4.456**	**0.034**	**-1.18 -0.04**	1.13
(Client abundance per 100 m^2) x (Percentage of time spent in interactions per 30 min)	-	1	0.125	0.724	-0.60 0.89	.
(Large client abundance per 100 m^2) x (Percentage of time spent in interactions per 30 min)	**-**	**1**	**4.659**	**0.030**	**-2.32 -0.10**	.
(Ratio of cleaner to 100 clients) x (Percentage of time spent in interactions per 30 min)	-	1	1.449	0.229	-0.25 1.08	.
Null deviance: 162.73 on 117 degrees of freedom						
Residual deviance: 146.08 on 113 degrees of freedom						
AIC: 156.08						
Pseudo R^2 = 0.18						
SD: standard deviation; df: degrees of freedom; LR: Likelihood ratio test; 95% CI: lower and upper bound of the 0.95 confidence interval; vif: variance influence factor, 2 was selected as a threshold; AIC: Akaike Information Criterion; R^2: the proportion of explained variance. Statistically significant outcomes ($p \leq 0.05$) are indicated in bold font.						

Biological market task

Predictor	Mean ± SD	df	LR X^2	*P*-value	95 % CI	vif score
Cleaner abundance per 100 m^2	1.03 ± 0.75	1	3.702	0.054	-0.75 0.84	1.21
Percentage of visitor clients swimming away if not serviced by a cleaner per 30 min	14.54 ± 10.43	**1**	**5.673**	**0.017**	**-0.02 1.56**	1.21
(Cleaner abundance per 100 m^2) x (Percentage of visitor clients swimming away if not serviced by a cleaner per 30 min)	**-**	**1**	**11.324**	**< 0.001**	**1.07 5.46**	.

Null deviance: 95.984 on 79 degrees of freedom

Residual deviance: 66.041 on 76 degrees of freedom

AIC: 74.041

Pseudo $R^2 = 0.37$

SD: standard deviation; df: degrees of freedom; LR: Likelihood ratio test; 95% CI: lower and upper bound of the 0.95 confidence interval;
vif: variance influence factor, 2 was selected as a threshold; AIC: Akaike Information Criterion; R^2: the proportion of explained variance. Statistically significant outcomes ($p \leq 0.05$) are indicated in bold font.

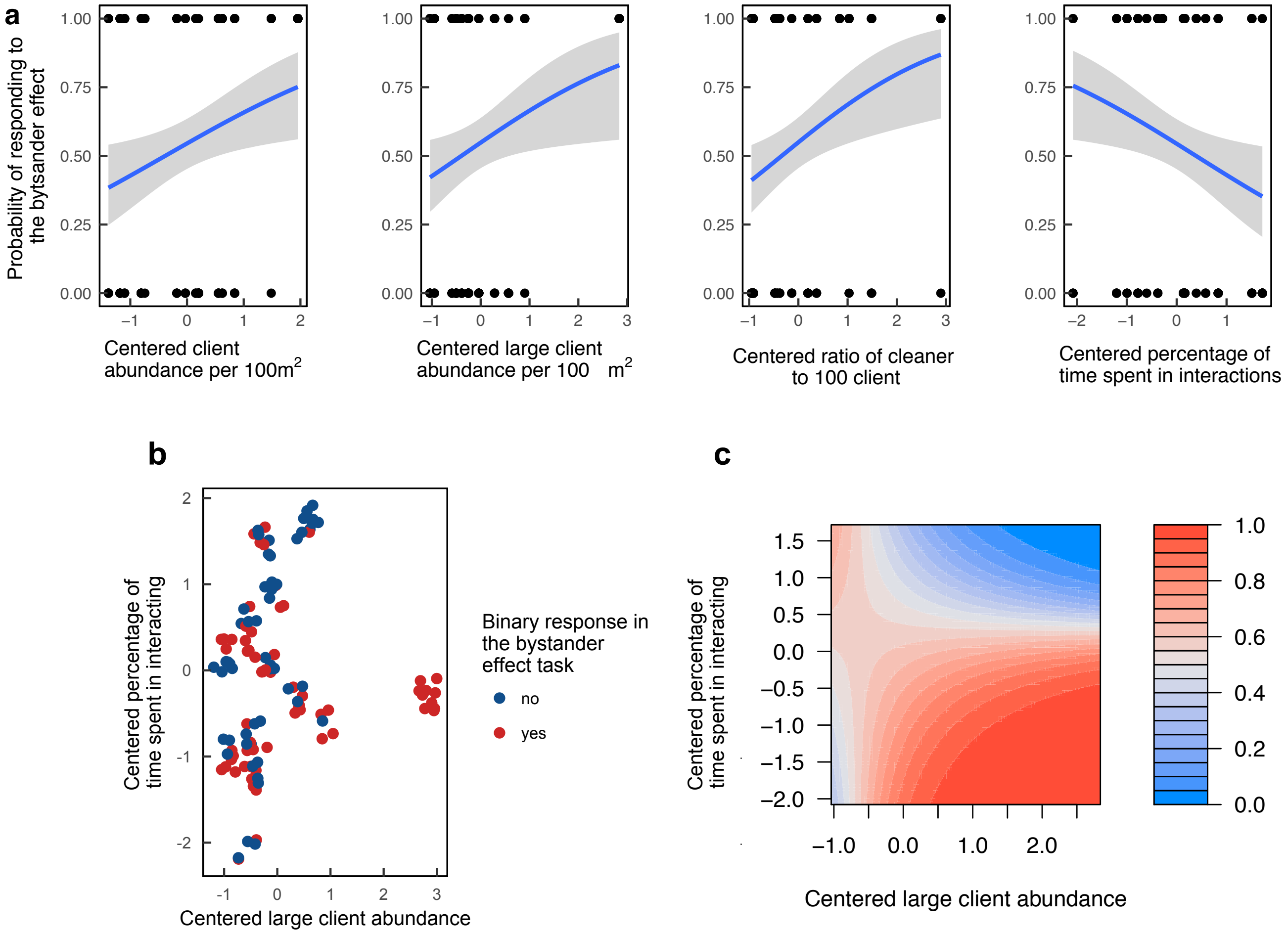

Figure 1. Relationship of performance in the bystander effect task and the selected market characteristics. a, Predicted probabilities for cleaners eating on average more against their preference if a bystander plate was present in the bystander effect task, with 95% confidence interval, along with the four selected predictors. The predictors' values were centered around the mean. **b,** a scatterplot and **c,** filled contour plot of the significant interaction effect of the abundance of large clients and percentage of time spent in interactions (GLM, $p < 0.05$). The color map indicates the probability of success in the task (associated values are shown on the right bar), while the vertical and horizontal axes are centered values of the explanatory factors. The data points in **b** are jittered to avoid overlapping.

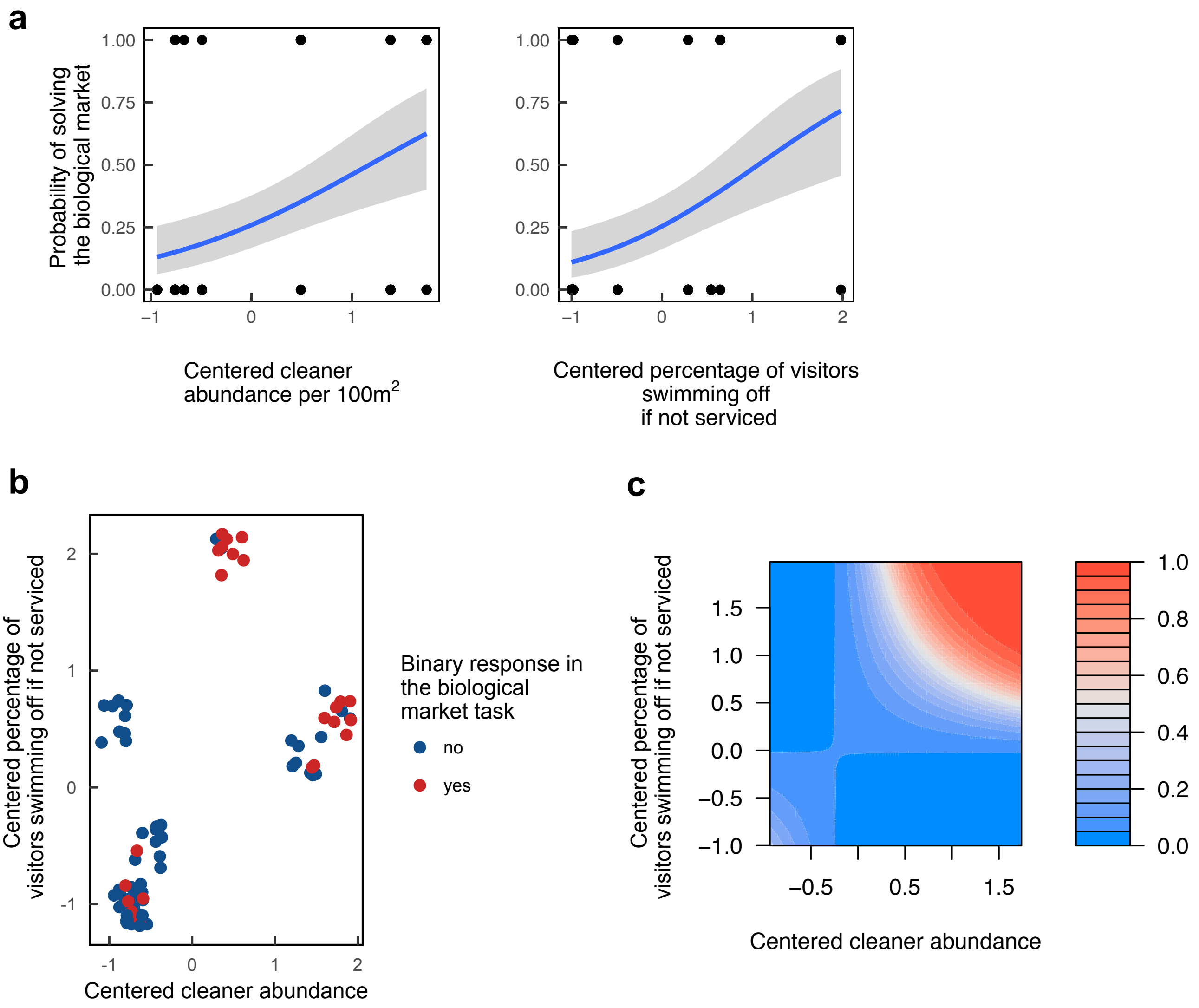

Figure 2. Predicted performance in the biological market task by the selected market characteristics. a, Predicted probabilities for learning to solve the biological market task, with 95% confidence interval, along with the two selected predictors. **b,** a scatterplot and **c,** filled contour plot of the significant interaction effect of the abundance of cleaners and percentage of visitors swimming away (GLM, $p < 0.001$). The color map indicates the probability of success in the task (associated values are shown on the right bar), while the vertical and horizontal axes are centered values of the explanatory factors. The data points in **b** are jittered to avoid overlapping.

2.4 Discussion

Biological market theory has been developed to make functional predictions about payoff distributions between traders. Our results provide first evidence that biological markets may play a major role for the evolution of a social brain and resulting sophisticated social strategies. Indeed, as reputation management and selective prioritizing of clients independently of their value appear to be expressed in cleaners only if visitor clients exert partner choice in nature. Partner choice emerges as a prime selective force to develop these sophisticated strategic abilities.

With respect to market composition, only cleaner abundance and large client (i.e., visitor) abundance highly correlated with strategic sophistication. Hence, the recorded sophistication in our experiments is partly linked to high abundance of clients with partner choice (i.e., visitor clients). Most importantly, the effect of cleaner abundance indicates that biological market theory needs to incorporate additional features of human market theory. Indeed, high numbers of cleaners and visitor clients lead to a "perfect competition" market structure. In this particular structure the service providers (i.e., cleaners) offer a homogenous high service to their partners (i.e., visitor clients) (Stigler 1957). However, cleaners turn into monopolists when in low abundance as switching between them becomes costlier in terms of time and energy budget. These costs of switching are a factor that is independent of supply and demand ratios. For example, given a fixed reef area, the presence of two cleaners and 100 visitor clients may lead to low strategic sophistication, whereas ten cleaners and 500 visitor clients may lead to cleaners showing high strategic sophistication. As variation in densities may occur on small spatial scales and between subsequent years, the differences in strategic sophistication are most likely based on cleaners learning how to respond to local conditions.

While no cleaner-to-client ratio variables correlated with cleaner strategic sophistication, changes in supply and demand nevertheless appear to play a role for predicting reputation management. This is because the percentage of time spent by cleaners in cleaning interactions (i.e., a correlate of frequency of interactions with large clients) is a direct measure of supply and demand: low values indicate low demand, while high values indicate high demand, especially by those who could, in principle, exert choice. According to the analyses, only low demand leads to a strong audience effect. In contrast, the cleaners' ability to priorities visitors appeared to be rather independent of any changes in the supply-to-demand ratio but linked to visitors applying partner choice.

The variation in both client behavior and cleaner strategic sophistication provides additional links with human market theory, where Cook and Emerson (1978) argued that a perfect competition market of non-committed traders is rare and should be seen as an uncommon condition. As a consequence, monopolistic competition may emerge as a market structure where variation in the offered commodities creates imperfect competition (Stigler 1957). Indeed, cleaners with high strategic sophistication apparently compete against cleaners with low sophistication from the same habitats, (see Fig. 1b and Fig. 2b), which is imperfect competition due to the non-homogeneity in the strategic sophistication between these cleaners.

Two variables of likely importance, in particular with respect to cleaner reputation management, were not measured in this study. First, one has to ask why the percentage of time spent in cleaning varied independently of fish densities. Perhaps the parasitic infection load of the visitor clients would have provided more reliable information on their needs to be inspected by a cleaner (Grutter 2001). Second, it would have been important to have quantitative data on the likelihood that visitors arriving at a cleaning station decide to avoid the cleaner because it witnessed the cleaner cheating a client (Bshary 2002). Such image scoring by clients exerts selective pressure on cleaners to behave more cooperatively when observed (Bshary and Grutter 2006). Unfortunately, with video recordings lasting only 30 min per cleaner, these situations were too rare to yield enough observations in the present study. Lacking information to what extent client image scoring may vary between sites could be the reason why the variance explained in the bystander effect task is considerably lower than the variance explained in the biological market experiment.

Conclusions

Partner choice, a core element of biological market theory (Noë and Hammerstein 1994; Noë and Hammerstein 1995), may also affect strategic sophistication. Our data emphasize that shifts in the visitor clients' costs of exerting partner choice, rather than shifts in the supply-to-demand ratio, drive changes in strategic sophistication. Moreover, the current analyses show that the ability of cleaners to manage their reputation develops independently of the ability to preferentially clean visiting clients rather than residents. Apparently, the variation in strategic sophistication of cleaners is based on behavioral flexibility, where individual learning allows adapting to the current market conditions (Komers 1997; McNamara and Leimar 2010). The learned decision rules, shaped at least in part by local social choices of traders (Dugatkin and Wilson 1993), are then applied in our experimental settings too, which can explain the observed differences in performance. Such insights about the mechanisms underlying individual variation in decision-rules and resulting social behavior are crucial for our understanding of how natural selection may promote the evolution and maintenance of cooperation (McNamara et al. 2008; Barta 2016).

2.5 Ethics

The Animal Ethics Committee of the Queensland government (DAFF) approved the project (CA 2016/05/970 and CA 2017/05/1063).

2.6 Data availability

Analyses reported in this article can be reproduced using the data provided by Triki et al. (2019).

2.7 Acknowledgements

We kindly thank the staff of Lizard Island Research Station, Dominique Roche and William McNeely for their field support, and Radu Slobodeanu for statistical consulting. We thank Ronald Noë for his comments on an earlier version. This work was supported by the Swiss National Science Foundation (grants number: 31003A_153067/1 and 310030B_173334/1 to R.B.).

2.8 Author's contributions

ZT and RB designed the study, all authors collected the field data. ZT, SW, OR and EL collected the laboratory data. ZT encoded the video observations with the contribution of SW and OL. ZT conducted the statistical analyses and produced the figures. ZT and RB wrote and finalized the manuscript with input from all authors. All authors declare no competing interests.

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2.10 Supplementary Information for Chapter II

Supplementary figures:

Figure S1. Map of Lizard Island group. Google maps satellite image showing the detailed location of the nine study reef sites at Lizard Island: Bird Islet (BI), Big Vickies (BV), Corner Beach (CB), Horseshoe (HS), Mermaid Cove (MC), Northern Horseshoe (NHS), Osprey (OS), Trawler Beach (TB), Watson’s Bay (WB). Depth at the study sites ranges from 1 to 16 m.

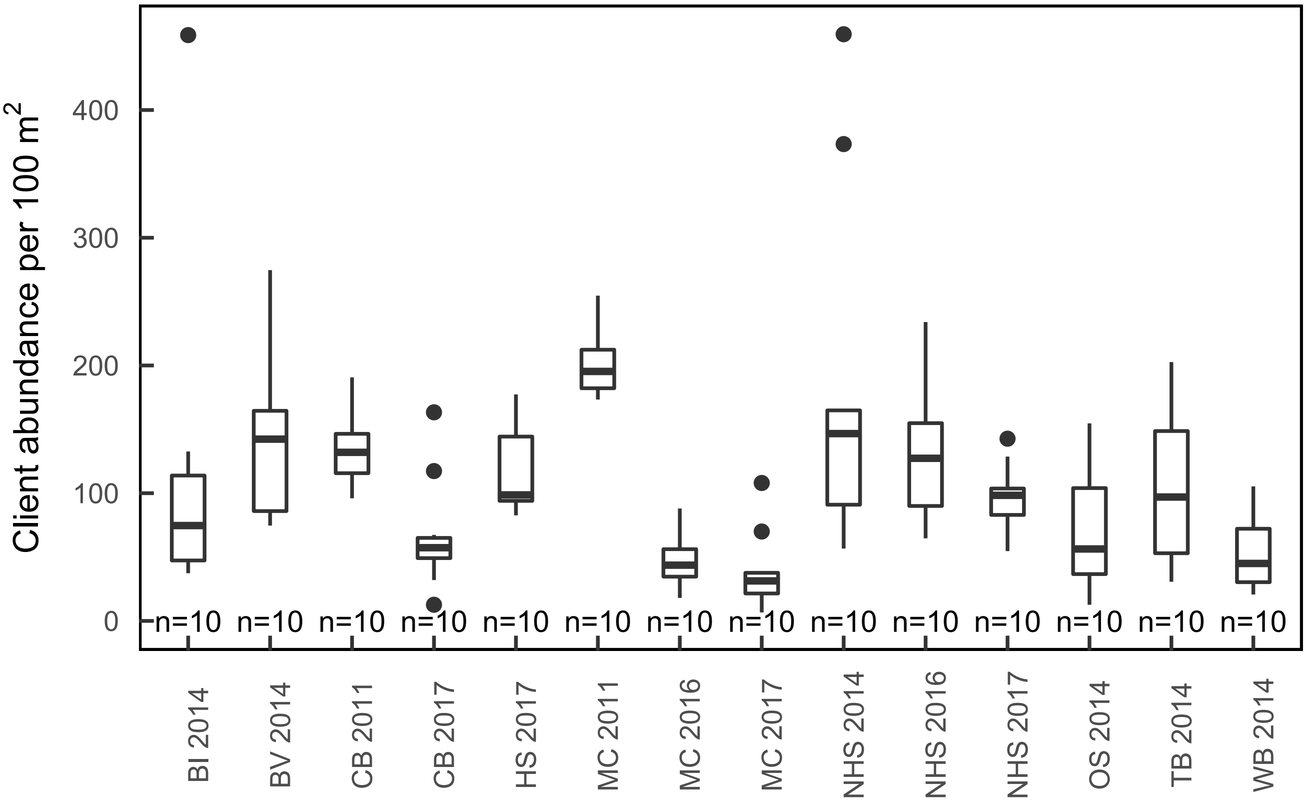


Figure S2. Boxplots and whiskers of the abundance of clients per site/year. The number of transects as sample size per site/year is indicated at the bottom of the plot.

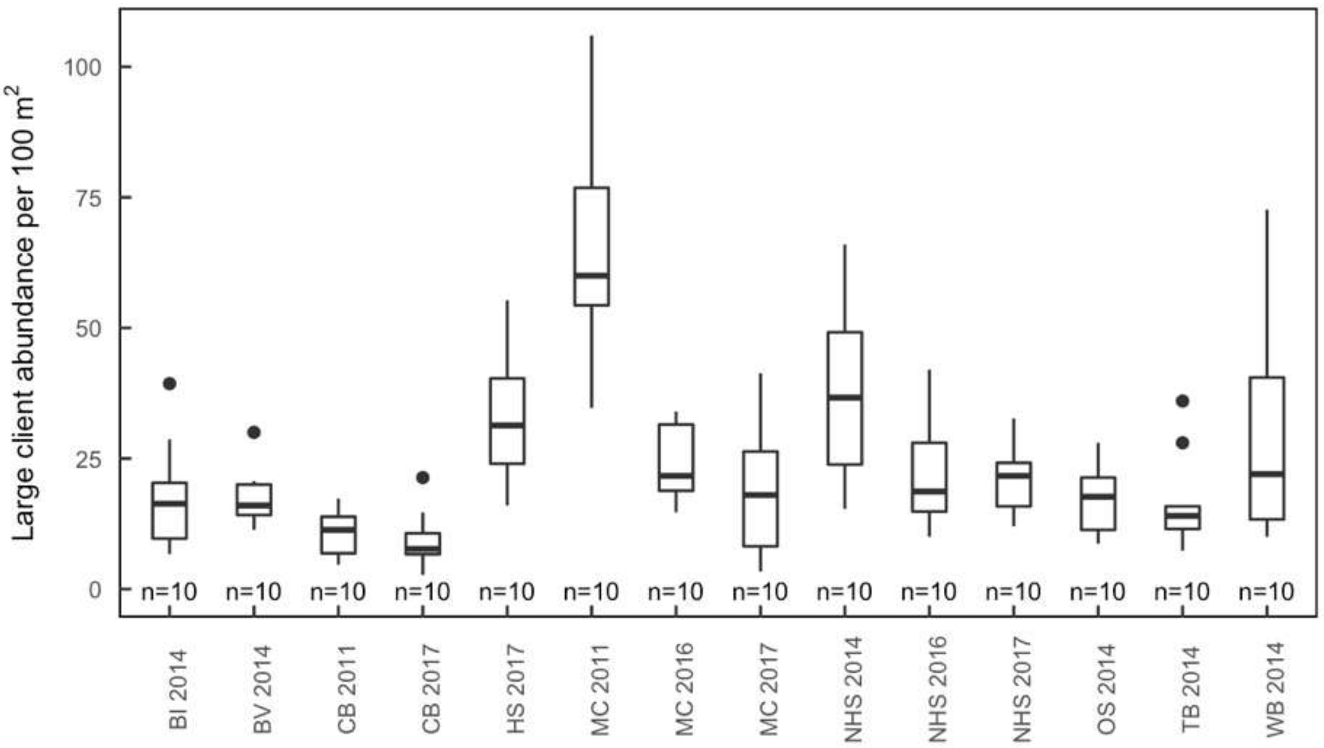


Figure S3. Boxplots and whiskers of the abundance of large clients per site/year. The number of transects as sample size per site/year is indicated at the bottom of the plot.

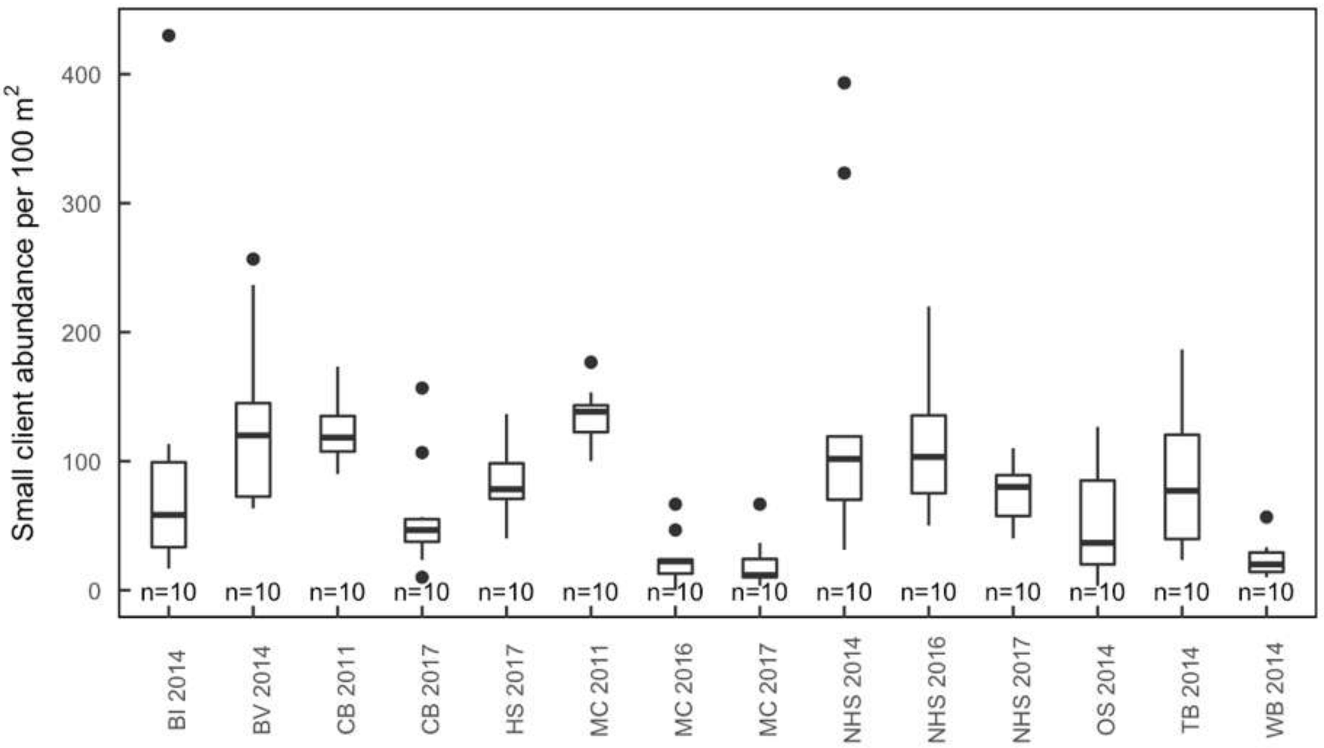


Figure S4. Boxplots and whiskers of the abundance of small clients per site/year. The number of transects as sample size per site/year is indicated at the bottom of the plot.

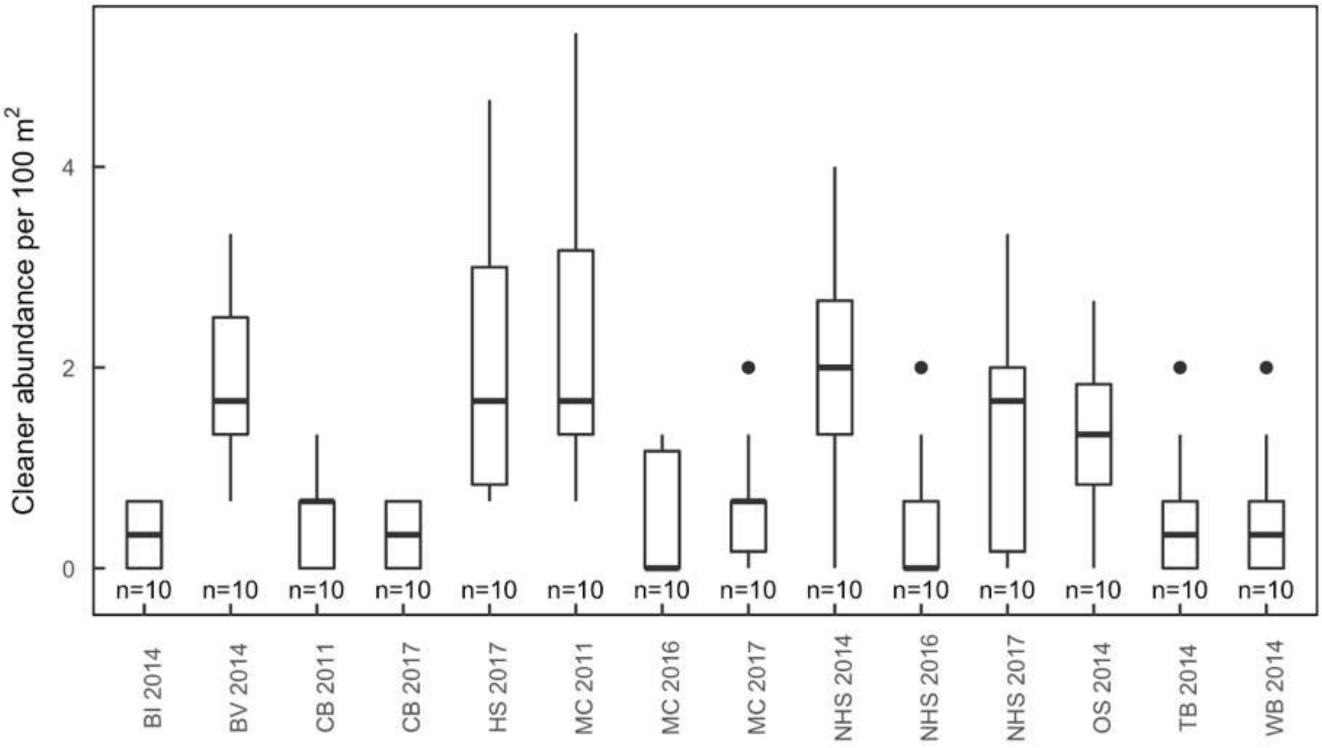


Figure S5. Boxplots and whiskers of the abundance of cleaners per site/year. The number of transects as sample size per site/year is indicated at the bottom of the plot.

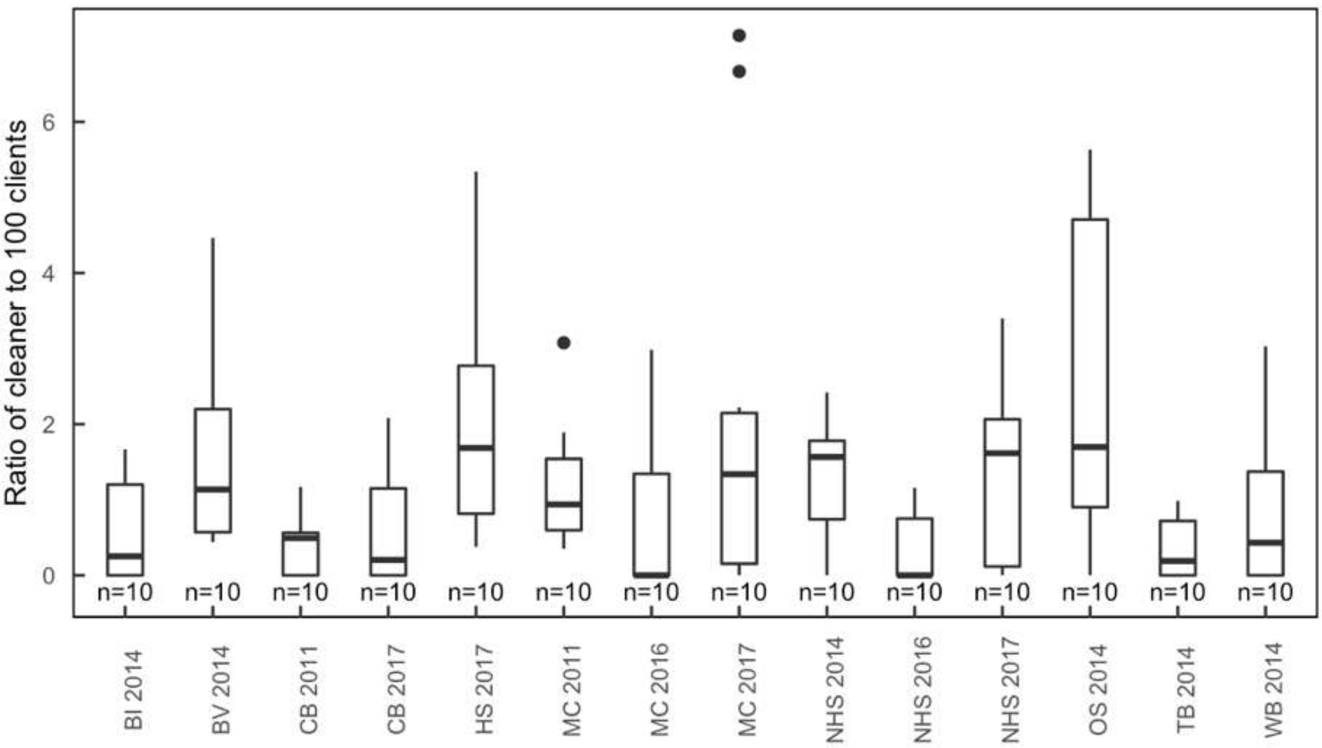


Figure S6. Boxplots and whiskers of the ratio of cleaner to 100 clients. The number of transects as sample size per site/year is indicated at the bottom of the plot.

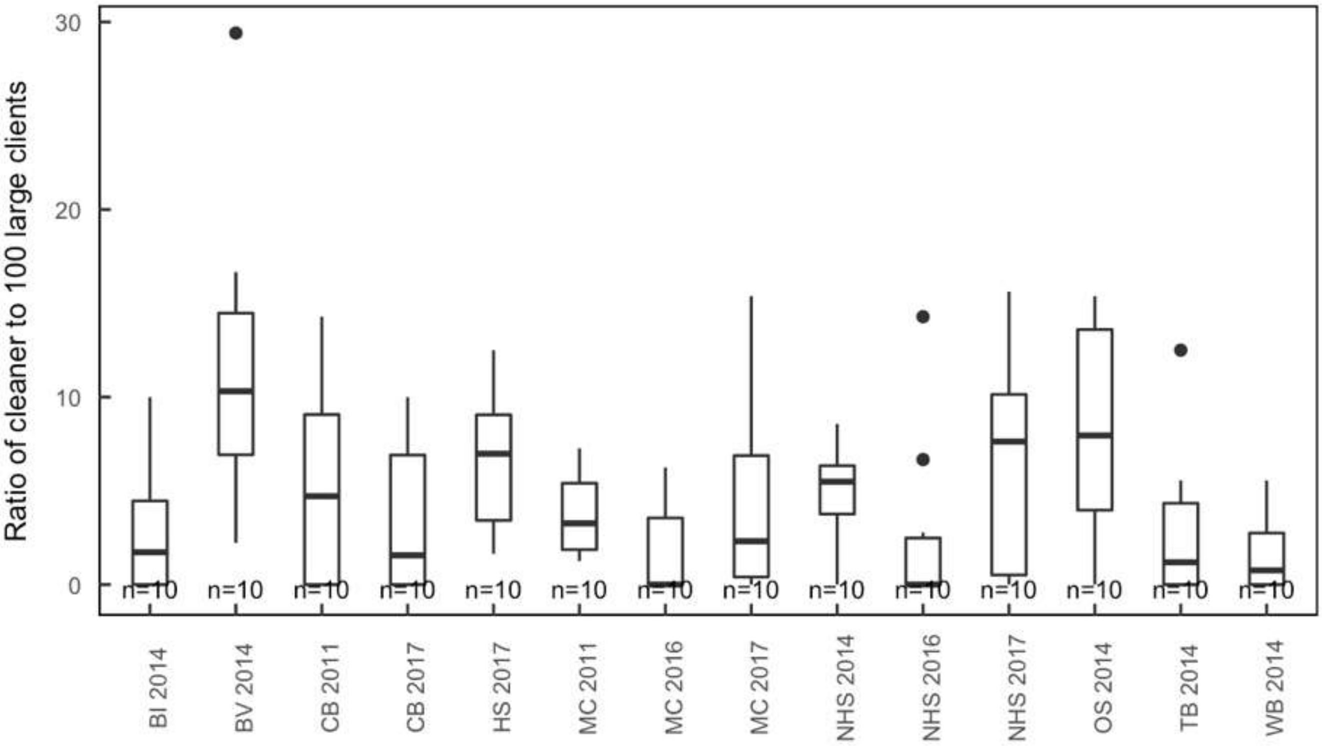


Figure S7. Boxplots and whiskers of the ratio of cleaner to 100 large clients. The number of transects as sample size per site/year is indicated at the bottom of the plot.

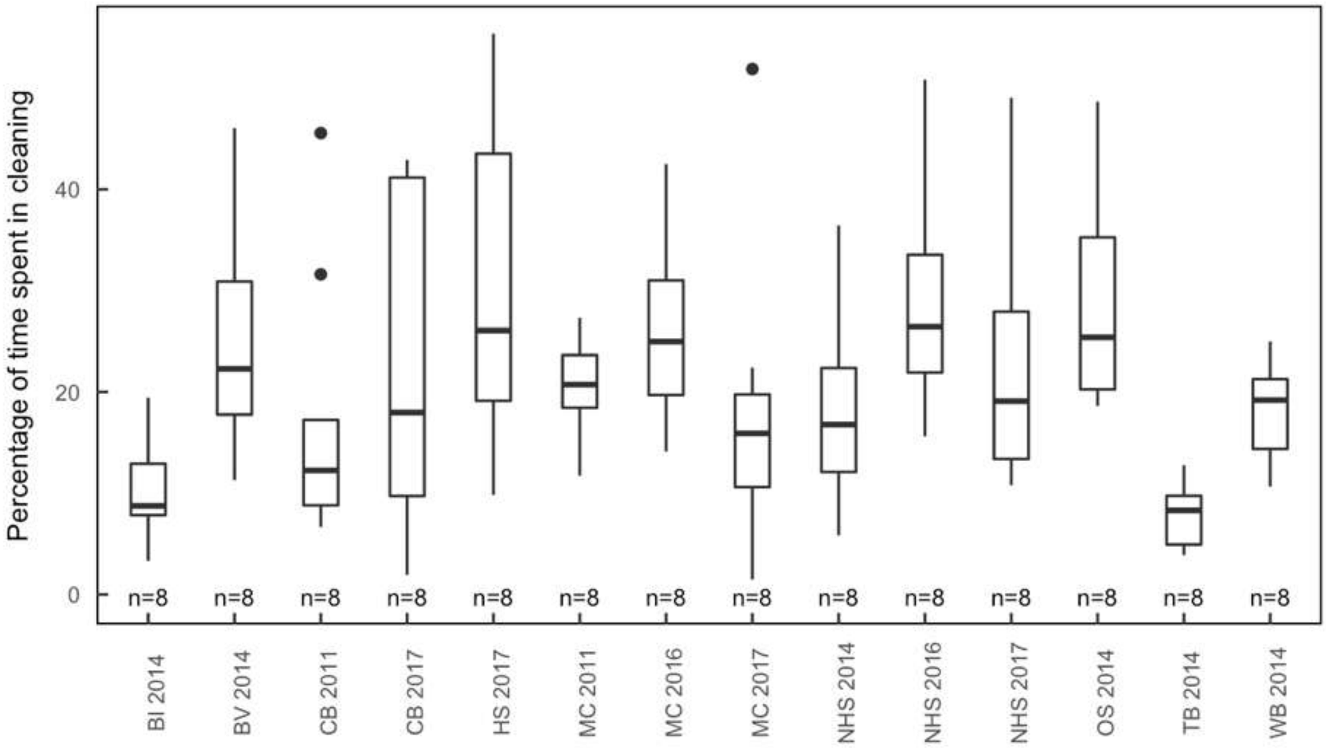


Figure S8. Boxplots and whiskers of the percentage of time spent in cleaning interactions per 30 min of video observation. The number of adult female cleaners observed as sample size per site/year is indicated at the bottom of the plot.

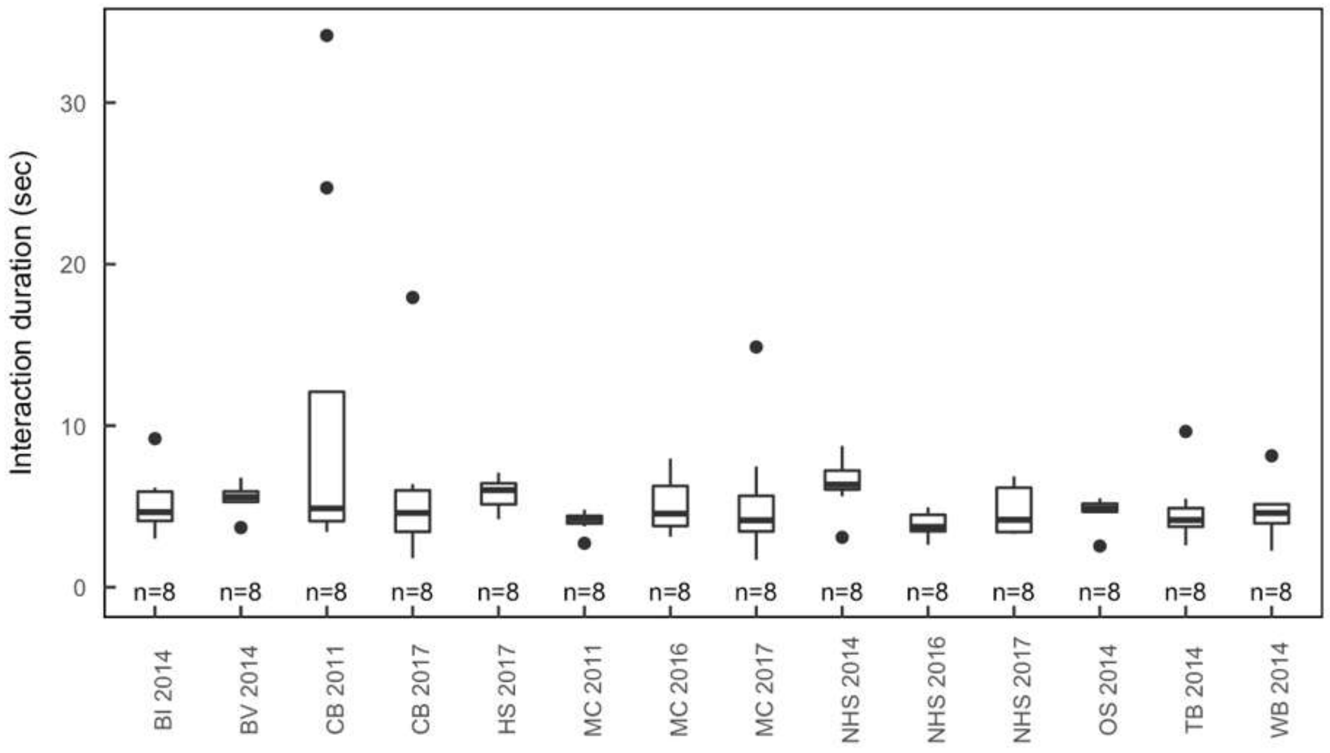


Figure S9. Boxplots and whiskers of the interaction average duration. The number of adult female cleaners observed as sample size per site/year is indicated at the bottom of the plot.

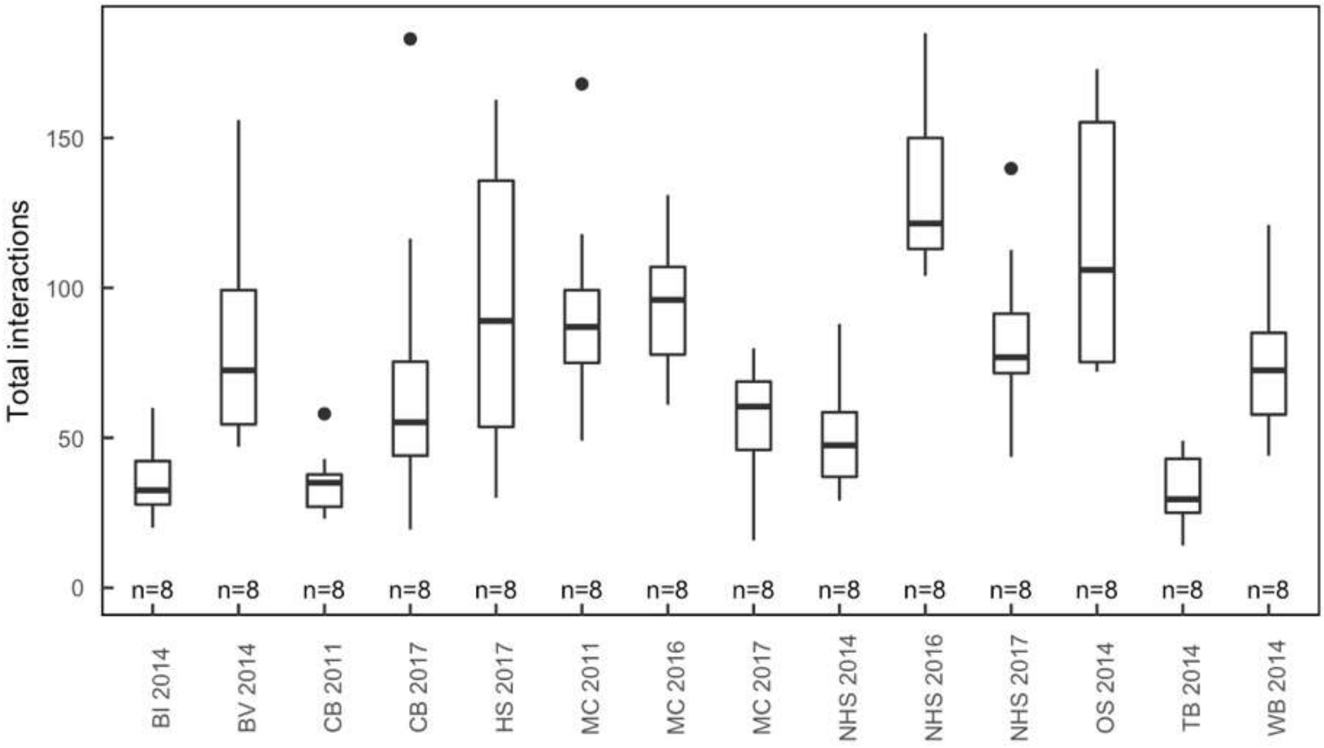


Figure S10. Boxplots and whiskers of the total number of cleaning interactions per 30 min of video observation. The number of adult female cleaners observed as sample size per site/year is indicated at the bottom of the plot.

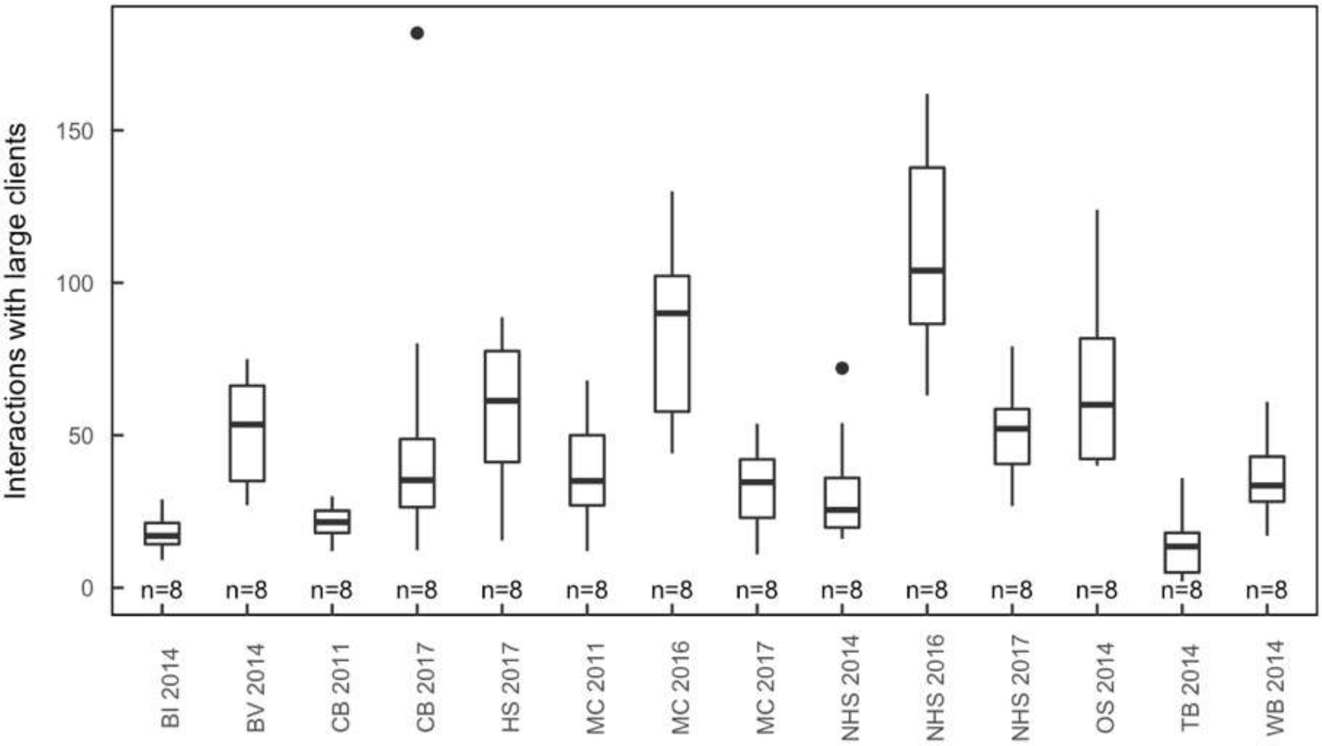


Figure S11. Boxplots and whiskers of the total number of cleaning interactions with large clients per 30 min of video observation. The number of adult female cleaners observed as sample size per site/year is indicated at the bottom of the plot.

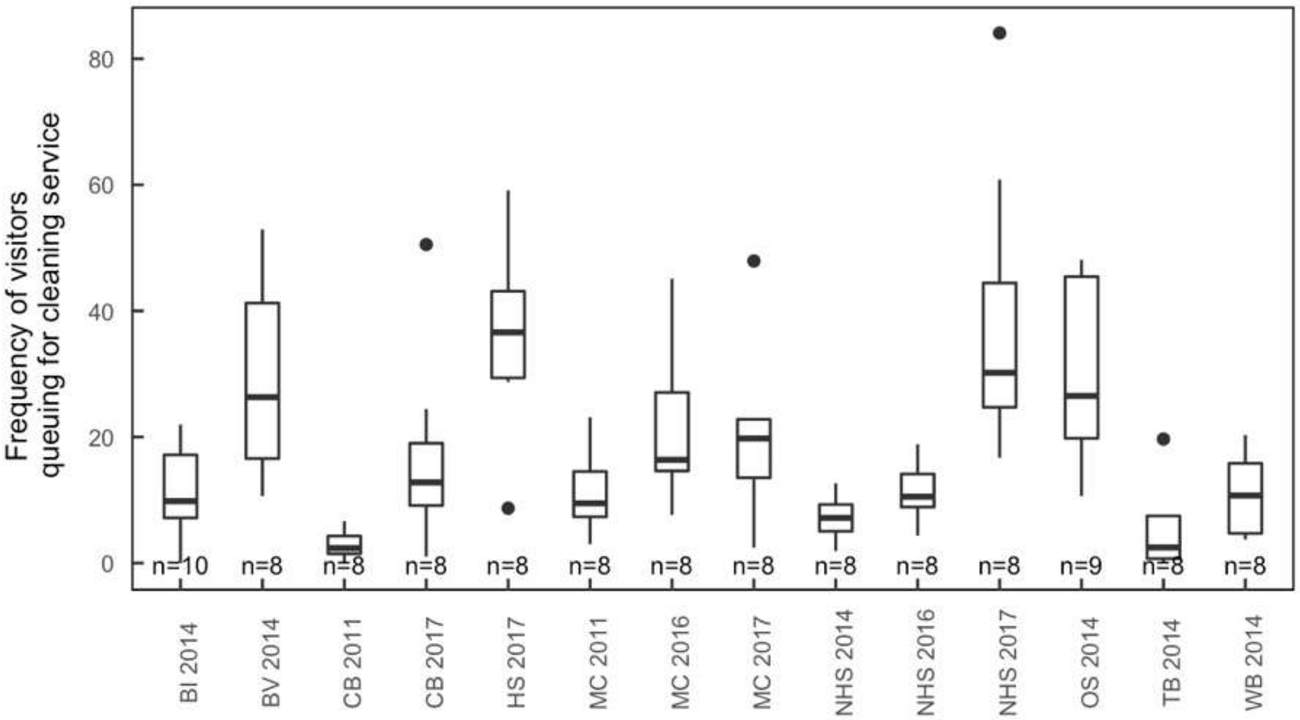


Figure S12. Boxplots and whiskers of the frequency of visitor clients queuing for a cleaning service per 30 min of video observation. The number of adult female cleaners observed as sample size per site/year is indicated at the bottom of the plot.

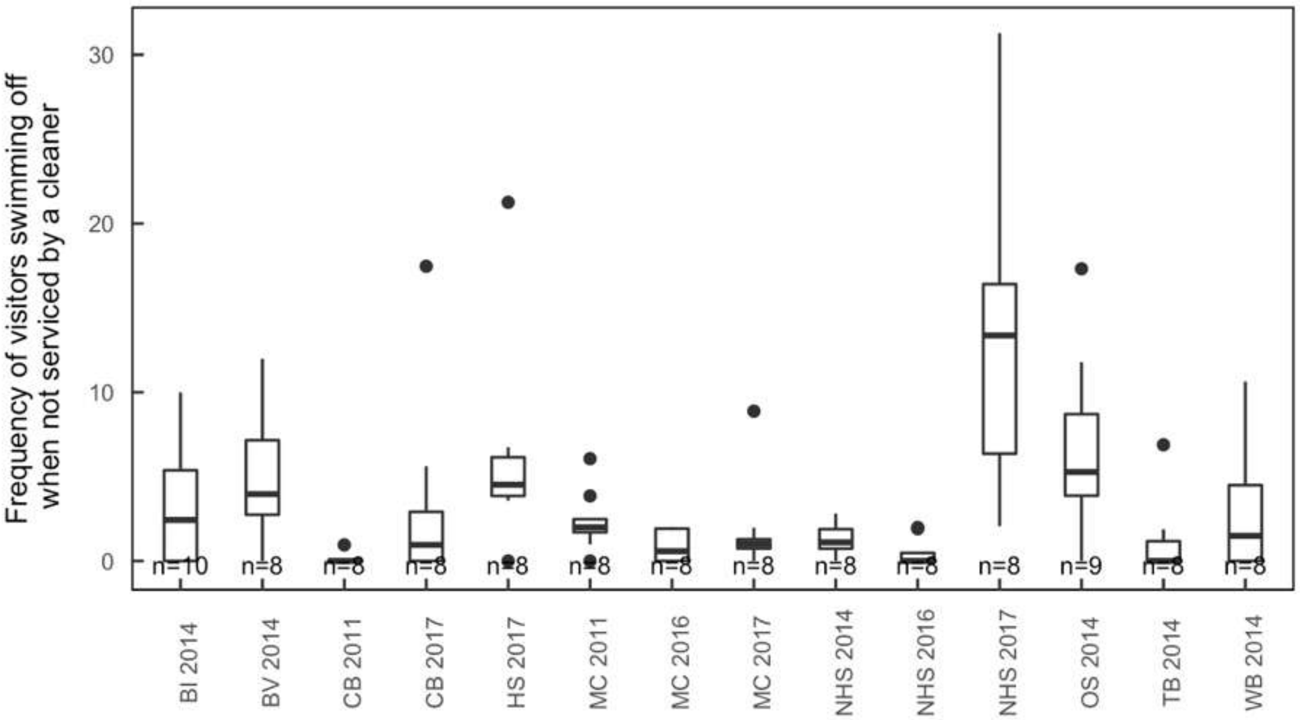


Figure S13. Boxplots and whiskers of the frequency of visitor clients swimming away if not serviced by a cleaner per 30 min of video observation. The number of adult female cleaners observed as sample size per site/year is indicated at the bottom of the plot.

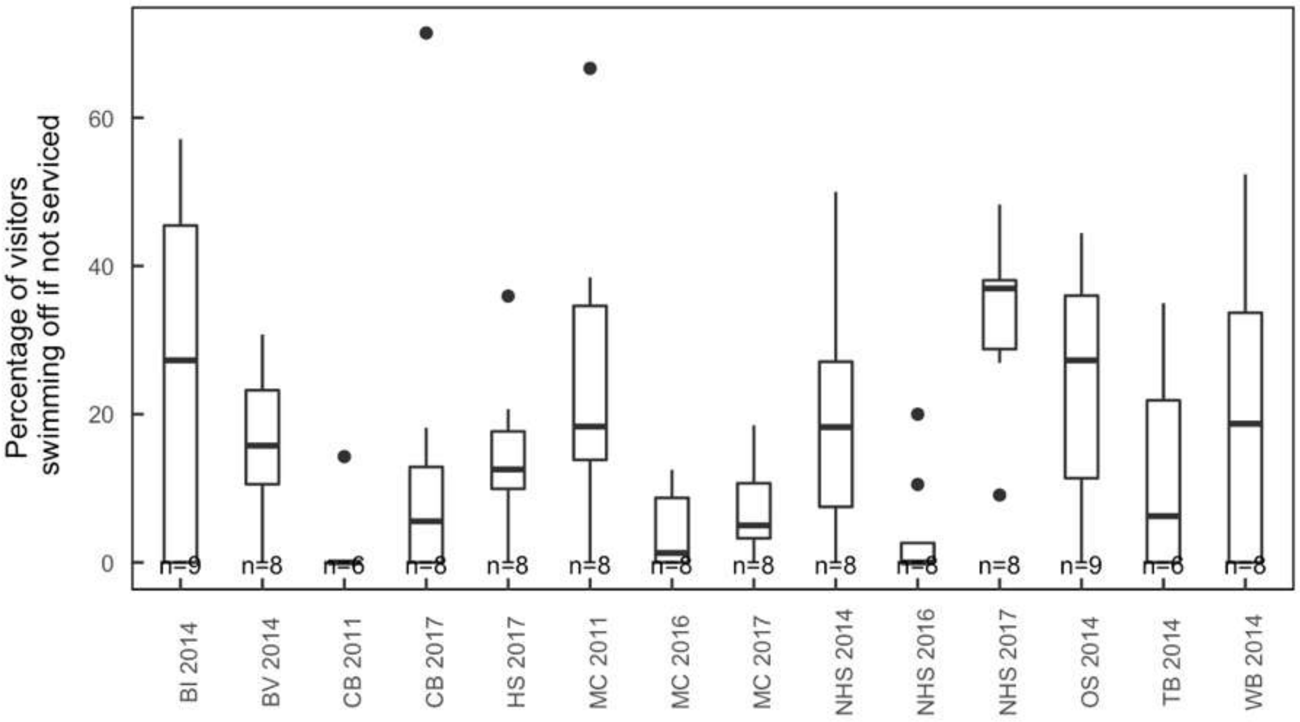
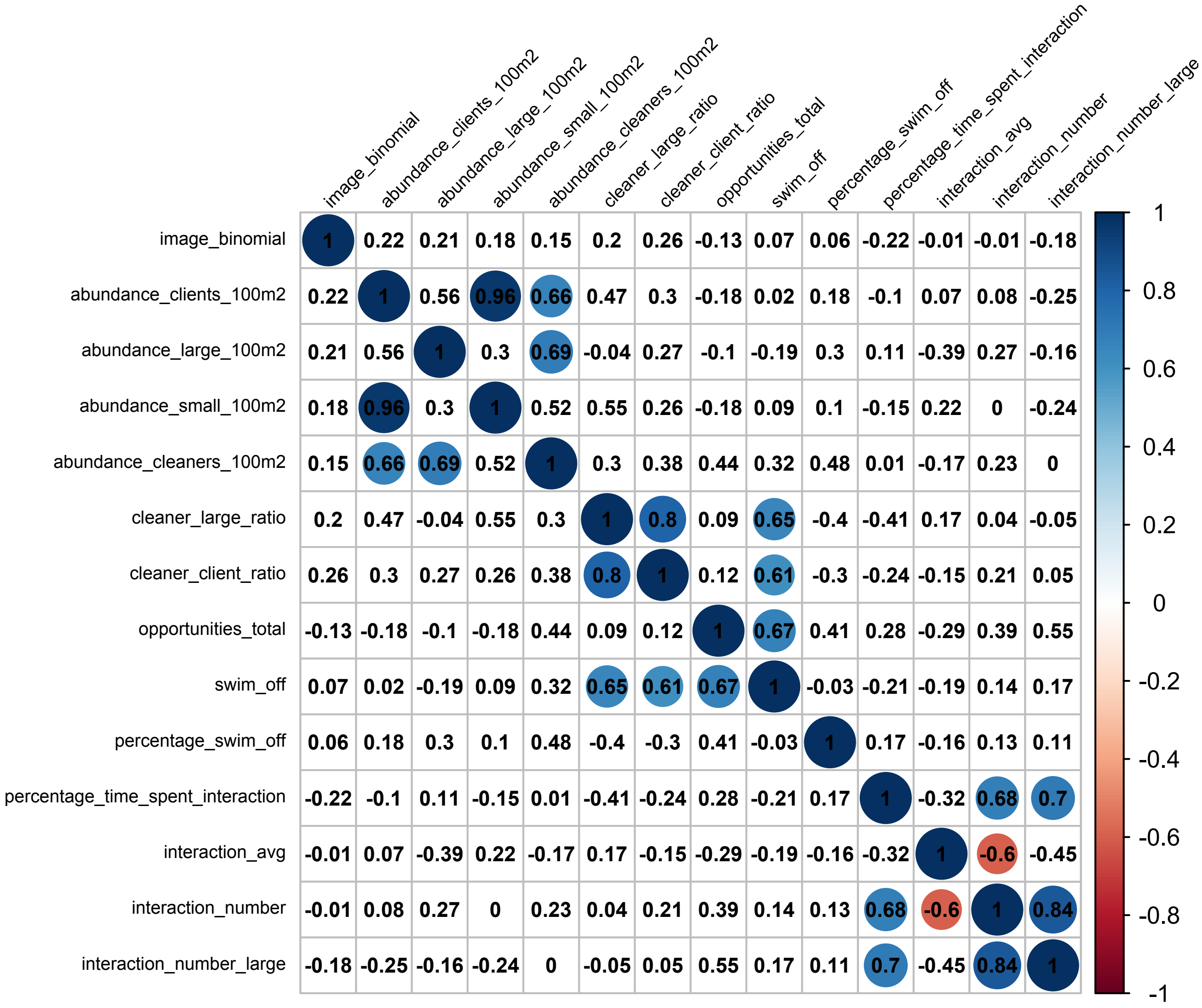


Figure S14. Boxplots and whiskers of the percentage of visitor clients swimming away if not serviced by a cleaner per 30 min of video observation. The number of adult female cleaners observed as sample size per site/year is indicated at the bottom of the plot.



	image_binomial	abundance_clients_100m2	abundance_large_100m2	abundance_small_100m2	abundance_cleaners_100m2	cleaner_large_ratio	cleaner_client_ratio	opportunities_total	swim_off	percentage_swim_off	percentage_time_spent_interaction	interaction_avg	interaction_number	interaction_number_large
image_binomial	1	0.22	0.21	0.18	0.15	0.2	0.26	-0.13	0.07	0.06	-0.22	-0.01	-0.01	-0.18
abundance_clients_100m2	0.22	1	0.56	0.96	0.66	0.47	0.3	-0.18	0.02	0.18	-0.1	0.07	0.08	-0.25
abundance_large_100m2	0.21	0.56	1	0.3	0.69	-0.04	0.27	-0.1	-0.19	0.3	0.11	-0.39	0.27	-0.16
abundance_small_100m2	0.18	0.96	0.3	1	0.52	0.55	0.26	-0.18	0.09	0.1	-0.15	0.22	0	-0.24
abundance_cleaners_100m2	0.15	0.66	0.69	0.52	1	0.3	0.38	0.44	0.32	0.48	0.01	-0.17	0.23	0
cleaner_large_ratio	0.2	0.47	-0.04	0.55	0.3	1	0.8	0.09	0.65	-0.4	-0.41	0.17	0.04	-0.05
cleaner_client_ratio	0.26	0.3	0.27	0.26	0.38	0.8	1	0.12	0.61	-0.3	-0.24	-0.15	0.21	0.05
opportunities_total	-0.13	-0.18	-0.1	-0.18	0.44	0.09	0.12	1	0.67	0.41	0.28	-0.29	0.39	0.55
swim_off	0.07	0.02	-0.19	0.09	0.32	0.65	0.61	0.67	1	-0.03	-0.21	-0.19	0.14	0.17
percentage_swim_off	0.06	0.18	0.3	0.1	0.48	-0.4	-0.3	0.41	-0.03	1	0.17	-0.16	0.13	0.11
percentage_time_spent_interaction	-0.22	-0.1	0.11	-0.15	0.01	-0.41	-0.24	0.28	-0.21	0.17	1	-0.32	0.68	0.7
interaction_avg	-0.01	0.07	-0.39	0.22	-0.17	0.17	-0.15	-0.29	-0.19	-0.16	-0.32	1	-0.6	-0.45
interaction_number	-0.01	0.08	0.27	0	0.23	0.04	0.21	0.39	0.14	0.13	0.68	-0.6	1	0.84
interaction_number_large	-0.18	-0.25	-0.16	-0.24	0	-0.05	0.05	0.55	0.17	0.11	0.7	-0.45	0.84	1

Figure S15. Correlation matrix of the bystander effect's predictors (n = 14) site/year. image_binomial: binomial response in the bystander effect task. "Yes" cleaners scoring > 0 in the test, while "No" is for cleaners failed the task (score ≤ 0). **abundance_client_100m2:** estimated all client species abundance in 100 m^2 from the transect data. **abundance_large_100m2:** estimated large species abundance in 100 m^2 from the transect data. **abundance_small_100m2:** estimated small species abundance in 100 m^2 from the transect data. **abundance_cleaner_100m2:** estimated number of cleaner fish, Labroides dimidiatus, in 100 m^2 from the transect data. **cleaner_large_ratio:** the ratio of 1 cleaner to 100 large clients estimated from count of cleaners divided by count of large clients and multiplied by 100. **cleaner_client_ratio:** the ratio of 1 cleaner to 100 clients estimated from count of cleaners divided by count of clients and multiplied by 100. **opportunities_total:** number of visitors seeking a cleaning service simultaneously with another client (s) or/and with a client already interacting with the cleaner wrasse. **swim_off:** number of visitors from the "all.opportunities" events (see description above) swimming off when not receiving a cleaning service. **percentage_swim_off:** percentage of visitors swimming off when not serviced by the cleaner calculated from number of visitors swimming off (swim_off) from the total number of all opportunities (opportunities_total). **percentage_timpe_spent_interaction:** during 30 min of observation, we have here the proportion of time spent in cleaning interactions. **interaction_avg:** average of time (in seconds) spent in one cleaner-client interaction. **interaction_number:** total number of interactions during the 30 min observations. **interaction_number_large:** number of interactions with large species (a strong correlate with visitor species. Significant correlation coefficient was set at |0.65|.

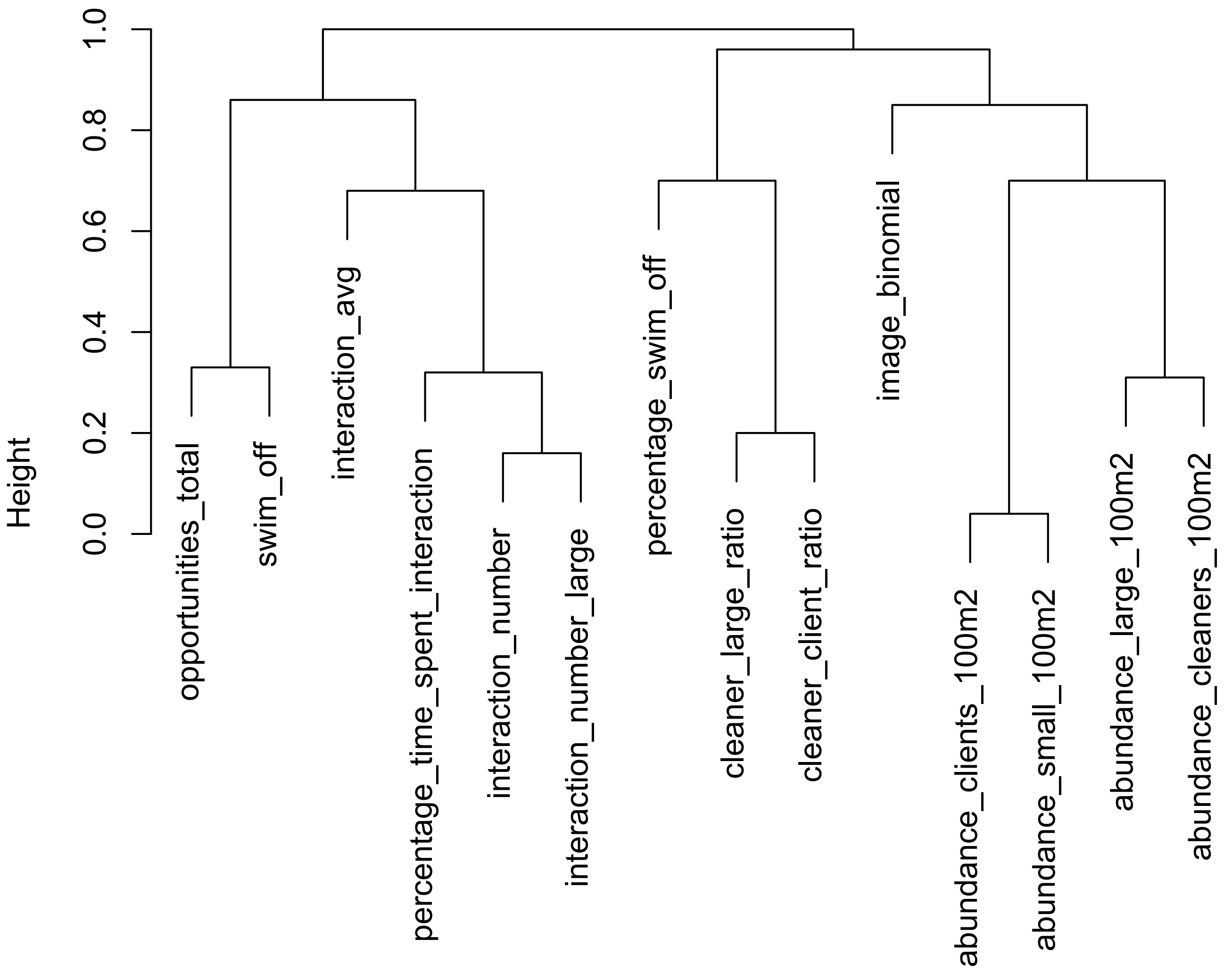
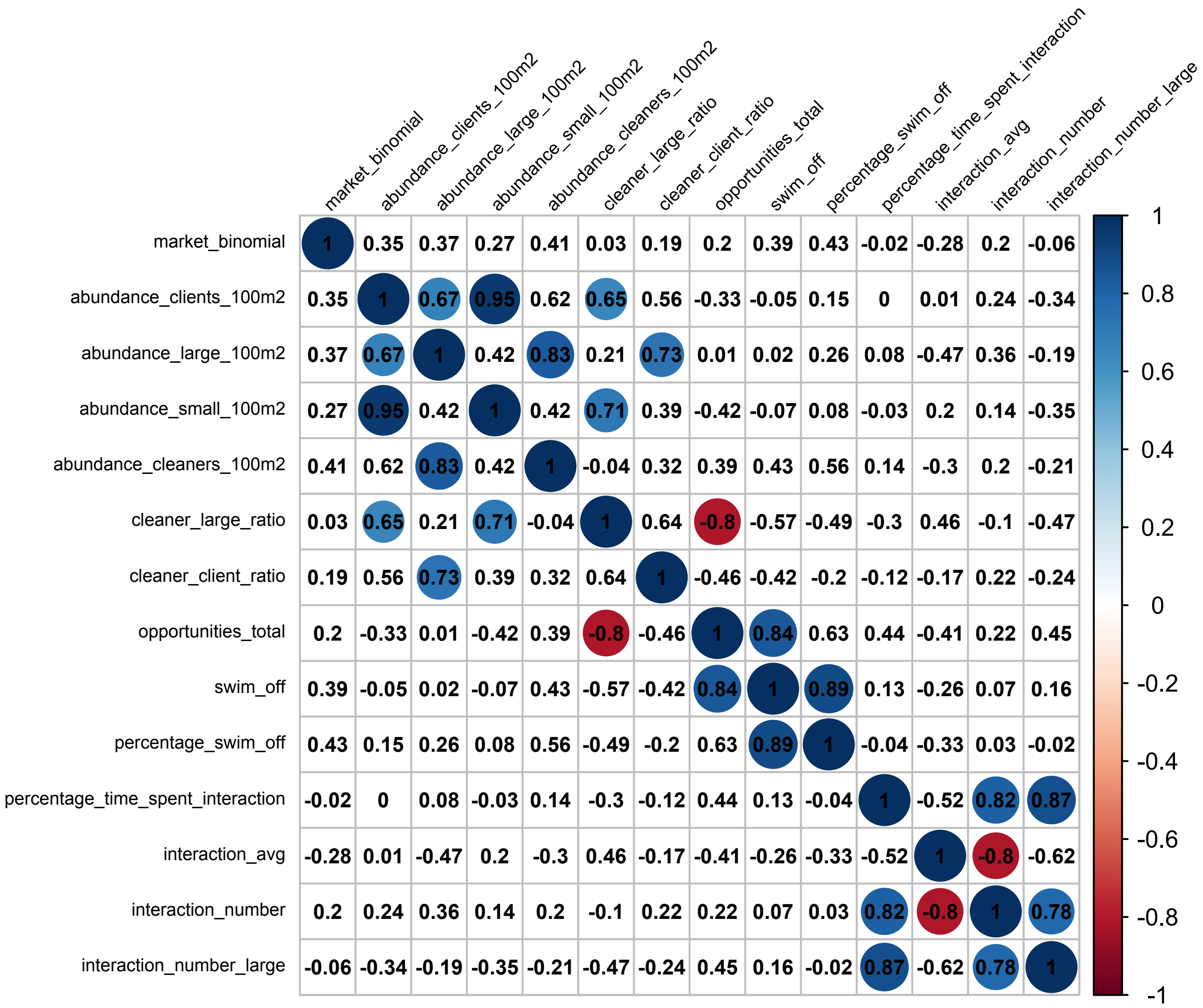


Figure S16. Hierarchical clustering of the bystander effect's predictors (n = 14) site/year. Please refer to Fig. S15's caption for more information about the variables. Values close to zero indicate low dissimilarity (i.e., high similarity or correlation), while high value, close to 1, refer to high dissimilarity among the variables.



	market_binomial	abundance_clients_100m2	abundance_large_100m2	abundance_small_100m2	abundance_cleaners_100m2	cleaner_large_ratio	cleaner_client_ratio	opportunities_total	swim_off	percentage_swim_off	percentage_time_spent_interaction	interaction_avg	interaction_number	interaction_number_large
market_binomial	1	0.35	0.37	0.27	0.41	0.03	0.19	0.2	0.39	0.43	-0.02	-0.28	0.2	-0.06
abundance_clients_100m2	0.35	1	0.67	0.95	0.62	0.65	0.56	-0.33	-0.05	0.15	0	0.01	0.24	-0.34
abundance_large_100m2	0.37	0.67	1	0.42	0.83	0.21	0.73	0.01	0.02	0.26	0.08	-0.47	0.36	-0.19
abundance_small_100m2	0.27	0.95	0.42	1	0.42	0.71	0.39	-0.42	-0.07	0.08	-0.03	0.2	0.14	-0.35
abundance_cleaners_100m2	0.41	0.62	0.83	0.42	1	-0.04	0.32	0.39	0.43	0.56	0.14	-0.3	0.2	-0.21
cleaner_large_ratio	0.03	0.65	0.21	0.71	-0.04	1	0.64	-0.8	-0.57	-0.49	-0.3	0.46	-0.1	-0.47
cleaner_client_ratio	0.19	0.56	0.73	0.39	0.32	0.64	1	-0.46	-0.42	-0.2	-0.12	-0.17	0.22	-0.24
opportunities_total	0.2	-0.33	0.01	-0.42	0.39	-0.8	-0.46	1	0.84	0.63	0.44	-0.41	0.22	0.45
swim_off	0.39	-0.05	0.02	-0.07	0.43	-0.57	-0.42	0.84	1	0.89	0.13	-0.26	0.07	0.16
percentage_swim_off	0.43	0.15	0.26	0.08	0.56	-0.49	-0.2	0.63	0.89	1	-0.04	-0.33	0.03	-0.02
percentage_time_spent_interaction	-0.02	0	0.08	-0.03	0.14	-0.3	-0.12	0.44	0.13	-0.04	1	-0.52	0.82	0.87
interaction_avg	-0.28	0.01	-0.47	0.2	-0.3	0.46	-0.17	-0.41	-0.26	-0.33	-0.52	1	-0.8	-0.62
interaction_number	0.2	0.24	0.36	0.14	0.2	-0.1	0.22	0.22	0.07	0.03	0.82	-0.8	1	0.78
interaction_number_large	-0.06	-0.34	-0.19	-0.35	-0.21	-0.47	-0.24	0.45	0.16	-0.02	0.87	-0.62	0.78	1

Figure S17. Correlation matrix of the biological market's predictors (n = 08) site/year. market_binomial: binomial response in the biological market task. "Yes" cleaners solving both the initial and reversal test, while "No" is for cleaners failed to solve initial and reversal in the biological market task. For the rest of the variables please refer to Fig. S15 caption for more information. Significant correlation coefficient was set at |0.65|.

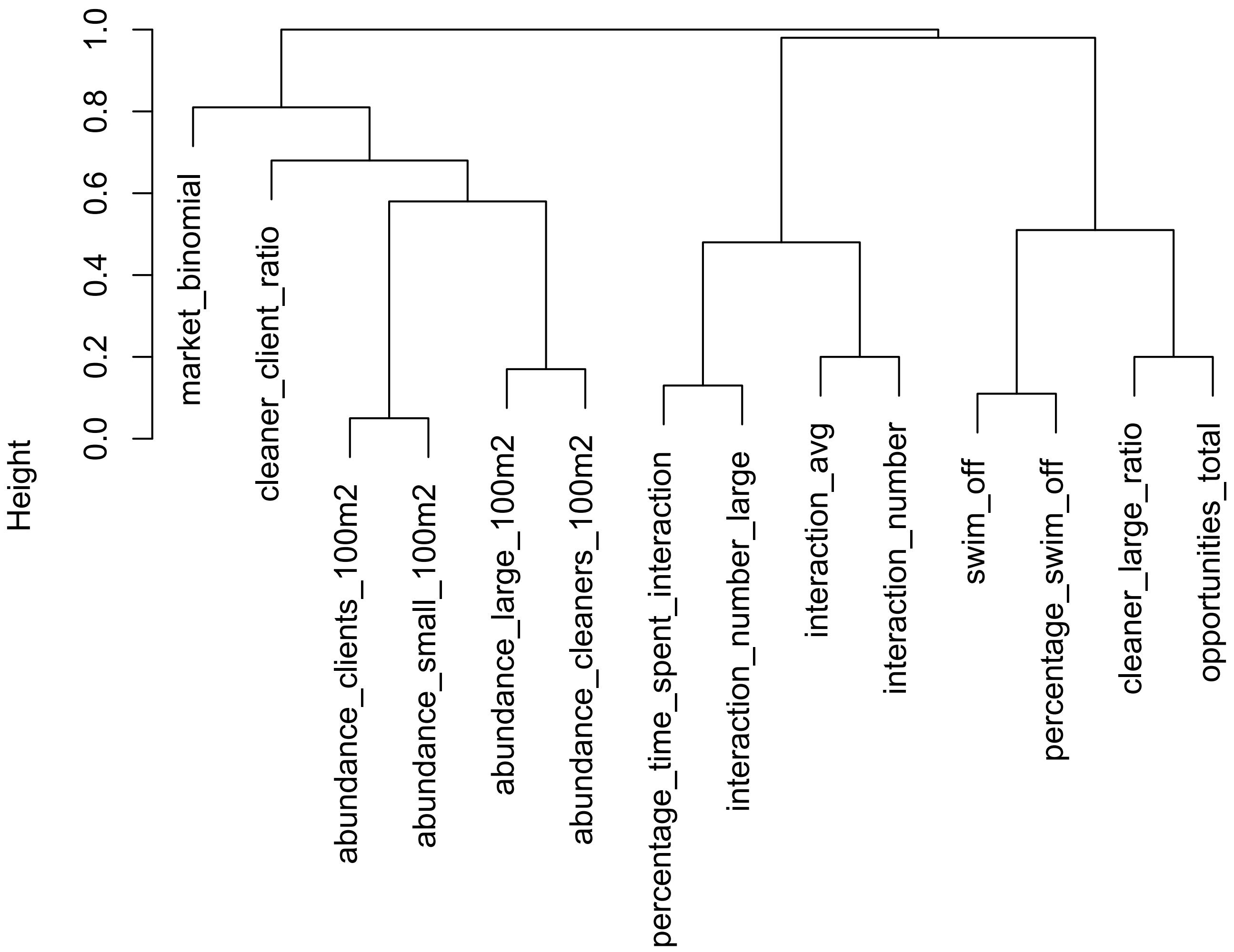


Figure S18. Hierarchical clustering of the biological market's predictors (n = 08) site/year. Please refer to Fig. S15 & S17's captions for more information about the variables. Values close to zero indicate low dissimilarity (i.e., high similarity or correlation), while high value, close to 1, refer to high dissimilarity among the variables.

Supplementary tables:

Table. S1.

Field and laboratory data were always collected between June and August of each year of data collection. The (N) refers to the sample size employed in each condition. *NA*: refers to non-available data.

Site ID	**year**	**Field data collection**		**Experiment 1 “Bystander effect”**	**Experiment 2 “Biological market”**
		Behavioral observations	Fish censuses		
Bird Islet (BI)	2014	N = 08	N = 10	N = 05	*NA*
Big Vickies (BV)	2014	N = 08	N = 10	N = 10	*NA*
Corner Beach (CB)	2010	*NA*	*NA*	N = 10	N = 10
	2011	N = 08	N = 10	*NA*	*NA*
	2017	N = 08	N = 10	N = 10	N = 10
Horseshoe (HS)	2017	N = 08	N = 10	N = 10	N = 10
Mermaid Cove (MC)	2010	*NA*	*NA*	N = 10	N = 10
	2011	N = 08	N = 10	*NA*	*NA*
	2016	N = 08	N = 10	N = 10	N = 10
	2017	N = 08	N = 10	N = 10	N = 10
Northern Horseshoe (NHS)	2014	N = 08	N = 10	N = 05	*NA*
	2016	N = 08	N = 10	N = 10	N = 10
	2017	N = 08	N = 10	N = 10	N = 10
Osprey (OS)	2014	N = 08	N = 10	N = 05	*NA*
Trawler Beach (TB)	2014	N = 08	N = 10	N = 05	*NA*
Watson’s Bay (WB)	2014	N = 08	N = 10	N = 10	*NA*
Total (N)	**-**	**112**	**140**	**120**	**80**

Table. S2.
Selected variables with the lasso regression method. The table shows the best variables remained after applying a penalization factor on the absolute values of the variables' coefficients. The tuning factor lambda was estimated form a Cross Validation method in the package glmnet. Shrunk coefficients to be equal to zero are indicated with a dot "." Alpha set at 1 for the lasso regression formula from glmnet package in R language. Mean and SD are given to provide more information. However, in the lasso regression model all the variables were standardized and centered around the mean.

Bystander effect task		**Lambda = 0.05573, alpha= 1**
Predictor	Mean ± SD	Penalized coefficient
(Intercept)	-	0.17936066
Client abundance per 100 m^2	106.22 ± 48.72	**0.08523518**
Large client abundance per 100 m^2	24.76 ± 14.67	**0.13487581**
Cleaner abundance per 100 m^2	1.07 ± 0.72	.
Ratio of cleaner to 100 clients	3.07 ± 3.38	**0.19972301**
Ratio of cleaner to 100 large clients	0.65 ± 0.66	.
Percentage of time spent in interactions per 30 min	21.97 ± 4.76	**-0.17825824**
Average duration of a single interaction (in seconds)	5.52 ± 1.72	.
Frequency of interactions per 30 min	78.39 ± 27.66	.
Frequency of interactions with large clients per 30 min	44 ± 19.73	.
Frequency of visitors queuing for a cleaning service per 30 min	19.95 ± 12.04	.
Frequency of visitor clients swimming away if not serviced by a cleaner per 30 min	7.72 ± 9.17	.
The percentage of visitor clients swimming away if not serviced by a cleaner per 30 min	11.67 ± 9.9	.
Biological market task		**Lambda = 0.1345, alpha= 1**
Predictor	Mean ± SD	Penalized coefficient
(Intercept)	-	-0.93636199
Client abundance per 100 m^2	104.88 ± 50.67	**0.03928872**
Large client abundance per 100 m^2	25.67 ± 17	**0.07141726**
Cleaner abundance per 100 m^2	1.03 ± 0.75	**0.05003962**
Ratio of cleaner to 100 clients	1.76 ± 1.95	.
Ratio of cleaner to 100 large clients	0.35 ± 0.41	.
percentage of time spent in interactions per 30 min	23.34 ± 4.47	.
Average duration of a single interaction (in seconds)	5.74 ± 2.13	.
Frequency of interactions per 30 min	82.76 ± 27.44	.
Frequency of interactions with large clients per 30 min	46.71 ± 20.22	.
Frequency of visitors queuing for a cleaning service per 30 min	19.71 ± 11.61	.
Frequency of visitor clients swimming away if not serviced by a cleaner per 30 min	3.62 ± 4.23	.
The percentage of visitor clients swimming away if not serviced by a cleaner per 30 min	14.54 ± 10.43	**0.29993958**

CHAPTER III | Social complexity predicts a mosaic brain development in a fish species the cleaner wrasse *Labroides dimidiatus*

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Status: *in preparation for submission*

Abstract

The "social brain hypothesis" proposes a causal link between social complexity and either brain size or the size of key brain regions known to be involved in cognitive processing and decision-making. As testing the hypothesis in an evolutionary comparative approach cannot control for many potentially confounding variables, intraspecific studies of species with variable sociality and flexible brain development may be a useful complementary approach. A suitable study model is the mutualist "cleaner" fish *Labroides dimidiatus* that removes ectoparasites from a variety of "client" coral reef fish in iterative social interactions. Here, we report a positive relationship between the local density of cleaners, as a proxy of social complexity, and the size of the cleaner's diencephalon, part of the forebrain in fishes. Three scaling methods used here provided similar results, wherein the relative diencephalon size correlated with local cleaner density. While telencephalon size estimates were noted as less reliable due to potential artefacts introduced during dissection and fixation, results are at least indicative of the same trend (see methods for further explanation). In contrast, the other three brain region sizes (i.e., mesencephalon, rhombencephalon, and brain stem) were independent of the social complexity level of cleaners' habitats. Importantly, as the forebrain harbour various nuclei of the social decision-making network, it appears that social complexity can be a driver of a selective enlargement of relevant brain regions, supporting the mosaic brain development hypothesis.

Keywords: Diencephalon, forebrain, brain morphology, mosaic brain, social brain hypothesis, fish densities, cleaning mutualism, MRI.

3.1 Introduction

Brain size has been repeatedly suggested as a proxy for cognitive capacities [Jerison, 1973; Byrne, 1996; Roth, and Dicke, 2005; Deaner et al., 2007; Finlay et al., 2011; van Schaik et al., 2012]. However, there is a long-standing discussion around the underlying factors of brain size evolution [see review by Healy, and Rowe, 2007]. Both environmental and social complexity play a crucial role in driving brain changes, and subsequently behavioural flexibility [Dunbar, 1992; Barton, 1996; Reader, and Laland, 2002; DeCasien et al., 2017; Street et al., 2017; González-Forero, and Gardner, 2018]. For instance, and in line with the "social brain hypothesis", primates and ungulates have disproportionate enlargement of the neocortex reflecting the potential increase in social cues and social interactions in larger groups [Dunbar, 1992; Dunbar, 1993; Reader, and Laland, 2002]. Birds also show enlarged forebrain at higher social complexity levels [Burish et al., 2004]. Although, there are efforts to disentangle the environmental complexity from the social complexity effect on brain size evolution [Dunbar, 1992; Gonda et al., 2009; DeCasien et al., 2017; González-Forero, and Gardner, 2018]. It has been suggested that the two factors are not mutually exclusive [Shultz, and Dunbar, 2006]. Indeed, Pollen et al. [2007] show that the physical complexity of the habitat correlates with the social complexity of cichlids, wherein key brain parts such as the telencephalon were larger.

While most brain size research focuses on comparisons between species or across taxa, little is known about intraspecific brain size development [Gonda et al., 2013; Healy, and Rowe, 2007]. Comparative studies between species or across taxa entail caveats such as the phylogenetic distance between species, individual variation within a species, and the various sampling methods/conditions [Garamszegi, and Møller, 2010]. While the species-specific traits may be corrected to some extent with modern statistical analyses, taking in account updated phylogeny trees for example [DeCasien et al., 2017], intraspecific variation is still often ignored when averaging the data at the species level [Gonda et al., 2013]. Also, the heterogeneous methods of data collection may compromise the outcomes [Thornton, and Lukas, 2012]. Intraspecific comparisons in species with flexible brain development offer the opportunity to control for the caveats described above and still investigate the relationship of social complexity and brain morphometrics. The key working hypothesis is that even at the individual level, social complexity drives the adjustment of brain complexity as a means of adaptation to the current conditions in similar ways as species would adjust to their average social conditions.

Comparative analyses tend to standardise the brain or brain region size to body size measures like length or weight [Gould, 1975; Schmidt-Nielsen, 1984; Smith, 1984]. This approach aims to correct for the brain portion responsible for body maintenance, facilitating thus the comparison of the brain component supposedly linked to the various cognitive processes [Jerison, 1973; Martin, and Harvey, 1985; Byrne, 1996]. An alternative practice is to scale brain region sizes to either the whole brain or the other brain regions instead of body size [Kolb et al., 2013]. This scaling method, contrary to body size scaling, allows exploration of potential selective enlargement in key brain zones depending on their functionality. This method is used as a measurement for the mosaic brain development hypothesis, wherein some brain parts might selectively grow bigger while the rest of the brain remains unchanged [Barton, and Harvey, 2000]. As scaling to either body size or other brain regions address different questions regarding brain morphometrics [Deaner et al., 2000], using both scaling methods on the same data set can provide a more complete picture of brain development.

Fish are a promising clade for intraspecific comparative brain studies [Butler, and Hodos, 2005]. Fish brains have the advantage of possessing a relatively simple and plastic neuroanatomical structure in comparison to a mammalian brain [Kotrschal et al., 1998]. Indeed, various studies showed potential links between ecological variables and intraspecific variation in brain size. There is evidence for covariation in fish brain structure development and habitat features [Gonda et al., 2009], migration and growth rules [Kolm et al., 2009], and sensory system shifts during ontogenetic development [Lisney et al., 2007; Wagner, 2003]. Nevertheless, to our knowledge, no study on fish brain development has so far explored the link between social complexity and brain region sizes.

Cleaner fish *Labroides dimidiatus* (hereafter “cleaner”) are an ideal study model to explore potential links between social complexity and brain complexity. They have a well-studied intra- and interspecific social life: The population is female biased, and they typically live in harems comprised of one male and several smaller females [Robertson, 1972]. While male and female harem members cooperate in terms of joint reproduction, female harem members are also potential future competitors to the current male. This is because cleaners are protogynous hermaphrodites. They start reproducing as a female but might change sex by either reaching a larger body size than the current male or/and a lack of being the target of a partner’s aggression [Robertson, 1972; Nakashima et al., 2000; Sakai, and Kohda, 2001]. While not studied in cleaners, research on other sex-change species revealed that conflicts over dominance in a size-based hierarchy lead to strategic growth decisions to avoid aggression by dominants [Wong et al., 2007]. Overall, the complexity of a cleaner’s intraspecific social life will likely be a function of the species densities.

Adding to the complexity, cleaners obtain almost 100% of their diet from interspecific social interactions with a variety of client reef fish (hereafter “client”) that visit the cleaners to have their ectoparasites removed [Randall, 1958; Losey Jr, 1979]. Finding food is hence an interspecific social task rather than just an environmental foraging task as in most other species. This makes the differentiation between the ecological and social drivers of brain development unlikely in cleaners. Furthermore, the interspecific social life of cleaner is not straightforward. For instance, conflicts of interest arise between cleaners and their clients because cleaners in fact prefer to eat client’s mucus instead of ectoparasites, an act that constitutes cheating [Grutter, and Bshary, 2003]. Clients employ various partner control mechanisms to reduce the cheating rates including: switching to another cleaner for future inspections, punishment through aggressive chasing, and the attribution of a negative reputation (i.e., image-score) and hence avoidance upon witnessing a cheating event with a current client [Bshary, and Grutter, 2005; Bshary, and Grutter, 2006]. In turn, cleaners adjust their service quality to the client’s control mechanisms [Bshary, 2001; Bshary et al., 2008; Gingins, and Bshary, 2014].

Cleaners may also give priority to clients with access to several cleaning stations (i.e., able to switch to another cleaner if made to wait) over clients with access to the local cleaner only [Bshary, 2001]. In summary, cleaners show highly advanced levels of fine-tuning in terms of service quality depending on the situation. This has been termed high strategic sophistication. Both the extent and variability in all these interactions provides the potential for the need to be cognitively able and flexible [Bshary, 2006; Triki et al., 2018].

Interestingly, a variation has been documented in strategic behaviour among cleaners ranging from low to high strategic sophistication among different individuals from different habitats [Wismer et al., 2014]. Intra- and interspecific social complexity, in the form of cleaner and client densities has been suggested as the underlying factors for such variation [Binning et al., 2017; Triki et al., 2018; Wismer et al., 2014]. Cleaners from socially complex locations usually outperform cleaners from socially simple sites in laboratory learning tasks [Wismer et al., 2014]. The fact that cleaners have a pelagic larval stage and at larger scales have little choice of where they finally settle [Victor, 1986] supports an ontogenetic development of this strategic sophistication over genetic factors.

Our initial aim was to collect cleaners from sites differing in their cleaner and client density and to evaluate their strategic sophistication in two laboratory-based cognitive tasks before then quantifying their brain morphometrics. This would have allowed us to explore the link between social complexity, strategic sophistication and brain anatomical complexity. Although the brains were sampled according to the performance of cleaners in the laboratory tasks, this variable was later considered as an unreliable proxy for cleaners' cognitive abilities and was discarded from the current study (see methods). The reason behind this change is that the El Niño 2016 event took place three months before our study, altering cleaner cognitive performance [Triki et al., 2018]. We hence focused on exploring potential links between measures of social complexity and brain region sizes.

Based on the social brain hypothesis [Dunbar, 1998], we developed the prediction that increased social complexity and strategic sophistication in cleaner fish would be reflected in ontogenetic adjustments of brain characteristics. We considered the following two scenarios: first, the entire brain might become enlarged in response to high social complexity. Second, a mosaic brain development might increase brain size through a selective enlargement of brain regions that are relevant for social decision-making, i.e., the telencephalon and diencephalon [Goodson, 2005; O'Connell, and Hofmann, 2011], while other brain regions remain unchanged. Our sampling methods may have caused unintentional damage or artefactual extrusion to the frontal part of several telencephalons (see methods). We therefore restrict the statistical evaluation of the selective enlargement hypothesis to the diencephalon and present telencephalon data only in descriptive ways. Nevertheless, we consider the diencephalon of a particular relevance as it is relatively larger in cleaners compared to other perciform fish species [Chojnacka et al., 2015]. With mosaic brain development, key brain regions like the diencephalon and telencephalon would be selectively enlarged or at least proportionally enlarged more than other regions.

3.2 Materials and Methods

3.2.1 Field site and fish survey

We conducted the study on Lizard Island (14.6682° S, 145.4604° E), Great Barrier Reef, Australia, between July and August 2016. Scuba divers conducted an underwater fish survey at four different reef sites (see Figure S1 in the Supplemental Information). Observers counted the abundance of adult cleaner fish *Labroides dimidiatus* and their client coral fishes on transect lines. Up to 10 replicates of transects of 30 m each were conducted at every study site. Large-bodied fish (total length $TL > 10$ cm) and cleaners were recorded on a 5 m width, whereas small-bodied fish ($TL \leq 10$ cm) were recorded on a 1 m wide area along the 30 m transect. All counts were scaled to densities per 100 m^2.

Social complexity, considered to be a correlate of cleaner density was estimated per study site. This choice was based on our previous findings, wherein cleaner density correlates significantly with the abundance of large clients. Also, cleaner and large client densities were the best predictors of cleaner's strategic sophistication (Triki et al. Unpublished data).

3.2.2 Study animals

Cleaners were captured upon accomplishing fish surveys at both Mermaid Cove and Northern Horseshoe. At Horseshoe and Bird Islet locations, however, fish were captured before the surveys due to time constraints. In total, 40 adult female cleaner wrasse were collected from these study sites. Cleaners were captured with barrier nets (2 m x 1 m, 5 mm mesh) and hand nets. Fish were then individually housed in aquaria of a size (62 cm x 27 cm x 37 cm) and provided with PVC pipes (10 cm x 1 cm) as shelter. Fish were fed daily with a paste of mashed prawn smeared on Plexiglas plates (8 x 15 cm). All cleaners fed successfully off the Plexiglas plates upon the first two days of acclimation. Upon an acclimation period of 14 days, fish were tested in two laboratory tasks: bystander effect task and biological market task. Both tasks consisted of testing cleaners' behaviour towards Plexiglas plates as surrogates for client fish [Bshary, and Grutter, 2006].

The two tasks are described in detail in Wismer et al. [2014] and not described here as they are not relevant for the analyses. Our initial hypothesis had been that social complexity predicts performance, which in turn predicts brain features. Therefore, we selected brains based on individual performance (Table S1 in Supplementary information). After analysing the fish survey data, we found that this selection converged to an unbalanced design with asymmetric numbers of high and low performing individuals according to the social complexity of the sites. Also, the predicted correlation social complexity-strategic sophistication became potentially masked by the recent environmental disturbances [Triki et al., 2018], which made the relationship brain features-performance irrelevant. For these reasons, we opted for a one-way correlation test between brain features and social complexity.

3.2.3 **Magnetic resonance imaging scans (MRI)**

Brain samples were from the 20 selected adult female cleaner wrasse (total length TL: mean ± SD, 7.38 ± 0.71 cm). Cleaners were sacrificed by a rapid cervical transection. Immediately after, the whole heads were immersed in a fix solution of 4% paraformaldehyde (PFA). Then, we proceeded to brain dissection under a stereomicroscope Zeiss steREO Discovery.V8 with a zoom set at 7:1. The brains, however, were kept in the braincase, which is the upper and the lower part of the skull containing the brain tissue. The rest of the surrounding tissue and bones were removed. Also, we made a small breach on the frontal side of the brain. This aimed to facilitate the filtration of the fix solution to the brain tissue. After a period of 24h in the PFA at room temperature, the specimens were transferred to a phosphate buffered solution (PBS). The samples were, then, transported to Queensland Brain Institute (QBI) in Brisbane for magnetic resonance imaging (MRI). Overall, the samples stayed in PBS for 48h before being transferred into a gadolinium-based contrast agent of 0.2% Magnevist®. The samples were incubated at 4°C in a Magnevist® solution of 10x the volume of the sample for ten days. Also, the Magnevist® solution was renewed at day 5 of the incubation.

Fish brains were scanned with a Bruker Avance 16.4T MRI spectrometer. They were scanned using a 15 mm surface acoustic wave (SAW) coil, and micro 2.5 gradient. The parameters employed for the scanning were the following: 3D gradient echo FLASH sequence, with TR/TE = 50/8 ms, NEX= 8, flip angle of 30 degrees, field-of-view = 31 x 12 x 10 mm, bandwidth of 100 KHz, and matrix size = 1028 x 400 x 330 providing an image resolution of 30 μm^3 isotropic voxels. Total image acquisition was 8 h 16 min. The 20 brains were run in four rounds of scan, wherein five brains were scanned per round. Every five brains were glued on a small plastic sheet (6 x 1,5 x 0.3 cm): three brains on one side, and two on the other side of the plastic sheet. This spatial distribution helped in identifying the brains' identities from the scans (see Fig. S3 in the Supplemental Information).

3.2.4 **Image processing**

Brain images were analysed and rendered using the software Amira® version 6.0. The delineation was done manually, image-by-image, first on frontal sections, then corrected and confirmed on the sagittal and transversal sections. Two cichlid fishes brain atlases were used as a reference in the brain parts delineation process: the *Oreochromis mossambicus* [Simões et al., 2012], and *Astatotilapia burtoni* (H. A. Hofmann, personal communication). Experimenters were blind to the identity of the samples during image analyses. In total, the brains were segmented into five brain parts: telencephalon, diencephalon, mesencephalon, rhombencephalon, and brain stem (see Fig. 1 and Fig. S4 in the Supplemental Information). The image processing indicated that in several of the samples (n=18) the frontal part of the telencephalon was slightly damaged during the brain dissection (see Fig. S5 in Supplementary material). We still obtained an estimate of telencephalon size for (n=16) brains from the MRI scans. We used the global brain scan images to estimate the relative size of the damaged area, which varied between 0 and 70% (median 30%). The estimate was made blind to the identity of the sample. Two statistical tests indicated that the data might still provide useful information to colleagues. First, there were no systematic differences between telencephalons collected at different sites with respect to estimated damage (one-way ANOVA, df = 1, F = 0.86, p = 0.37). Second, we did not find any correlation between estimated damage and telencephalon volume corrected for body weight (one-way ANOVA, df = 1, F = 0.46, p = 0.51). Therefore, as we were interested in brain part sizes relative to body weight and other brain parts rather than in precise absolute volumes, we decided to present the telencephalon data in a descriptive way.

3.2.5 **Statistical analyses**

All statistical analyses and figures were performed with the software R version 3.3.2 (2016-10-31). In the first scaling method to body size, brain region sizes (in mm^3) were log transformed and corrected by log transformed total body length (in cm), where log body weight (in g) was included in the model as a covariate. In the second scaling method to either the rest of the brain size or to another brain part, no log transformation was needed at this stage. Multivariate analysis of variance (MANOVA) allowed for multiple hypotheses test. Upon a significant MANOVA outcome (significance threshold set at $\alpha \leq 0.05$), we ran separate univariate analyses of variance (ANOVA) for each dependent variable. Models assumptions, such as normal distribution and homogeneity of variance of the residuals, were met and verified with statistical tests and visual plots.

3.3 Results

Here, we tested whether the total size of the four studied brain regions (i.e., diencephalon, mesencephalon, rhombencephalon, and brain stem) correlates with the cleaner density at the study sites. We found no significant relationship for cleaner density and overall size of the four brain parts (two-way ANOVA: n = 20, $F = 0.356$, df=(1,17) , $p = 0.558$). Nevertheless, by fitting the four brain structures in a multivariate analysis of variance (MANOVA), we found a significant effect of cleaner density (MANOVA: n = 20, df = (1,4), F = 6.596, $p = 0.003$). With univariate analyses as *post hoc* tests, we found that the difference was mainly driven by the diencephalon size, wherein a positive correlation relationship between cleaner density and diencephalon relative size to body length existed (two-way ANOVA: n = 20, df = (1,17), F = 6.848, $p = 0.018$, adjusted $R^2 = 0.58$, Fig. 2). The other brain parts, however, did not show a significant relationship with cleaner density (Table 1a). Correspondingly, the size ratio of the diencephalon to the other three brain parts combined (i.e., mesencephalon, rhombencephalon, and brain stem) was also significantly positively correlated with cleaner density (two-way ANOVA: n = 20, $F = 22.721$, df = (1,18), $p < 0.001$, adjusted $R^2 = 0.53$, Fig. 3).

Furthermore, we used another scaling method by estimating the size ratio of every brain part to another brain part. Here, as well, we found a significant effect of cleaner density (MANOVA: n = 20, df = (1,4), F = 6.467, $p = 0.002$, Fig. 4). By running univariate *post hoc* tests, the diencephalon was relatively larger than any of the other three brain parts ($p \leq 0.01$, Table 1b). Among the size ratios of the remaining three brain parts to each other (i.e., mesencephalon, rhombencephalon and brain stem), however, there were no significant correlation with cleaner density (Table 1b).

Despite the potential inaccuracy of the telencephalon data from the present study, Fig. 5 A&B shows a trend of positive correlation between cleaner density and the relative size of telencephalon to either body size or to the combined size of mesencephalon, rhombencephalon and brain stem. In contrast, telencephalon to diencephalon ratio apparently did not change as a function of cleaner density (i.e. both were similarly increased in absolute and relative size). As a consequence, the forebrain size ratio, estimated from the telencephalon and diencephalon size ratio to the rest of the brain (Fig. 5C), showed a positive correlation with cleaner density.

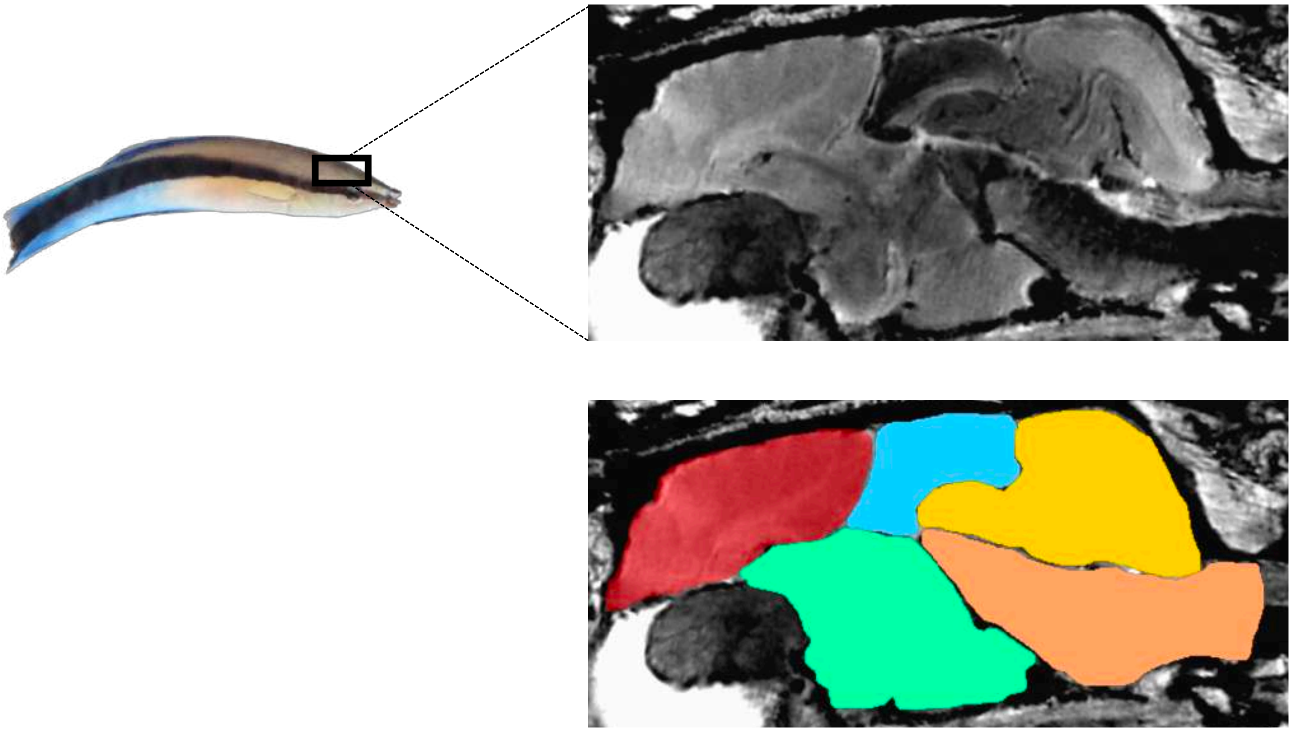

Figure 1. The *Labroides dimidiatus* brain. A representative MRI scan image of a sagittal section of *L. dimidiatus* brain inside its braincase, the lower panel shows the delineation of the five brain structures from: telencephalon (in red), diencephalon (in green), mesencephalon (in blue), rhombencephalon (in yellow), brain stem (in orange). Images generated with OsiriX Lite® V. 9.5.2. Cleaner fish photo credit by *Z. Triki*.

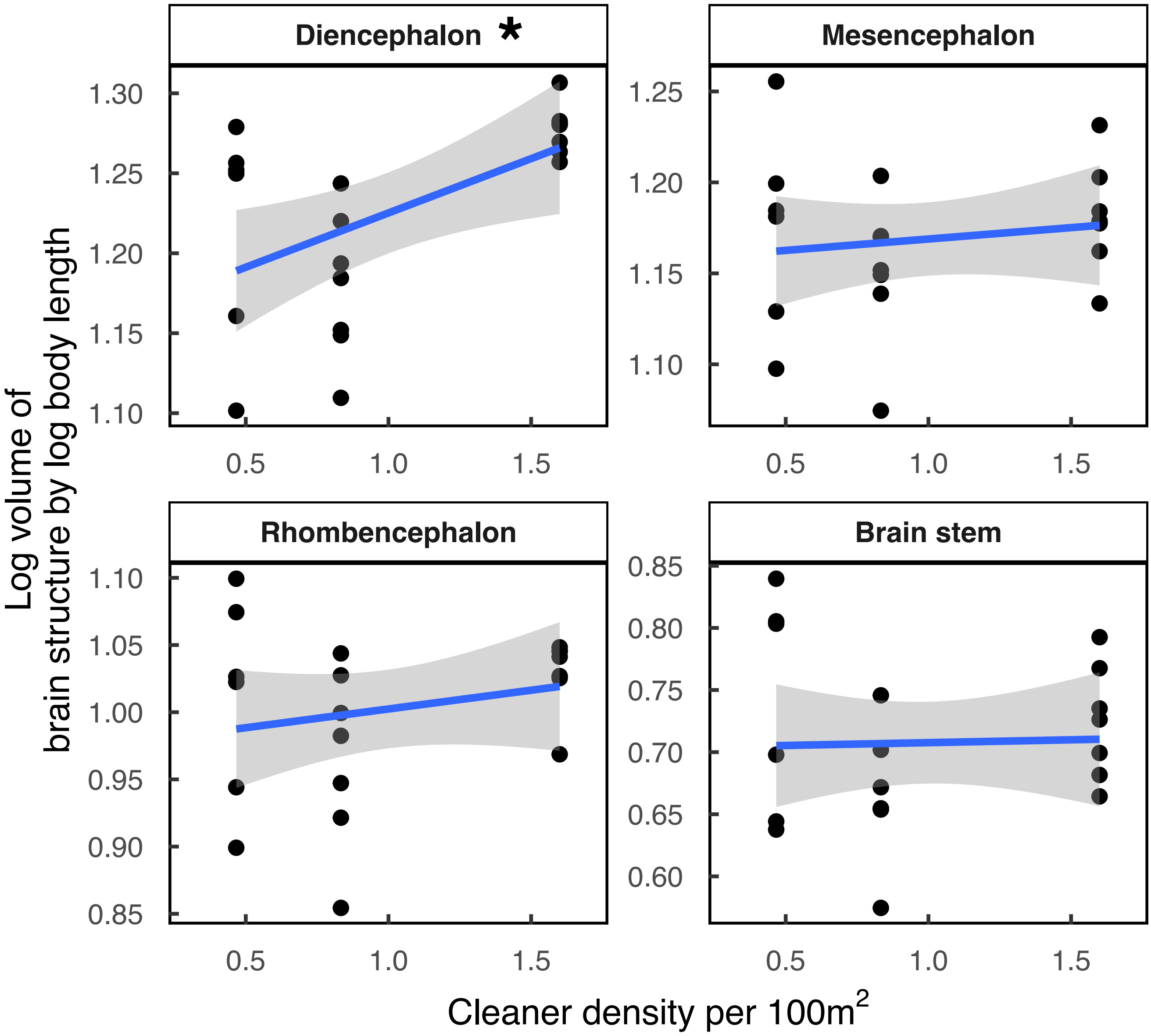

Figure 2. Relationship of relative brain part sizes to body length and cleaner density. Scatterplot and regression line with 95% confidence interval CI represented by the shaded area. Two-way ANOVA: *, $p < 0.05$

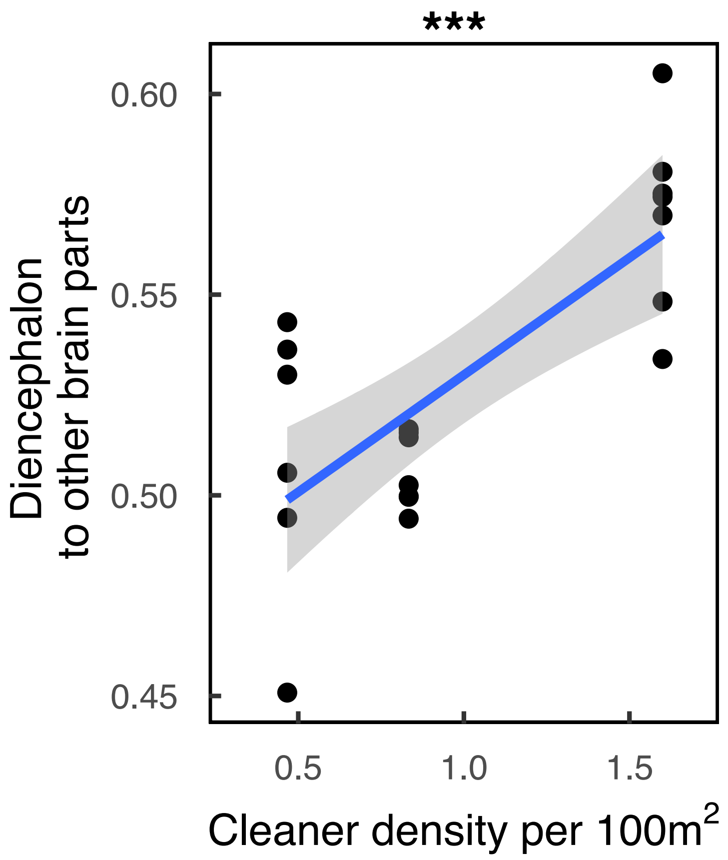

Figure 3. Relationship of the diencephalon ratio to the rest of the brain and cleaner density. Regression line and 95% CI. One-way ANOVA: ***, $p < 0.001$

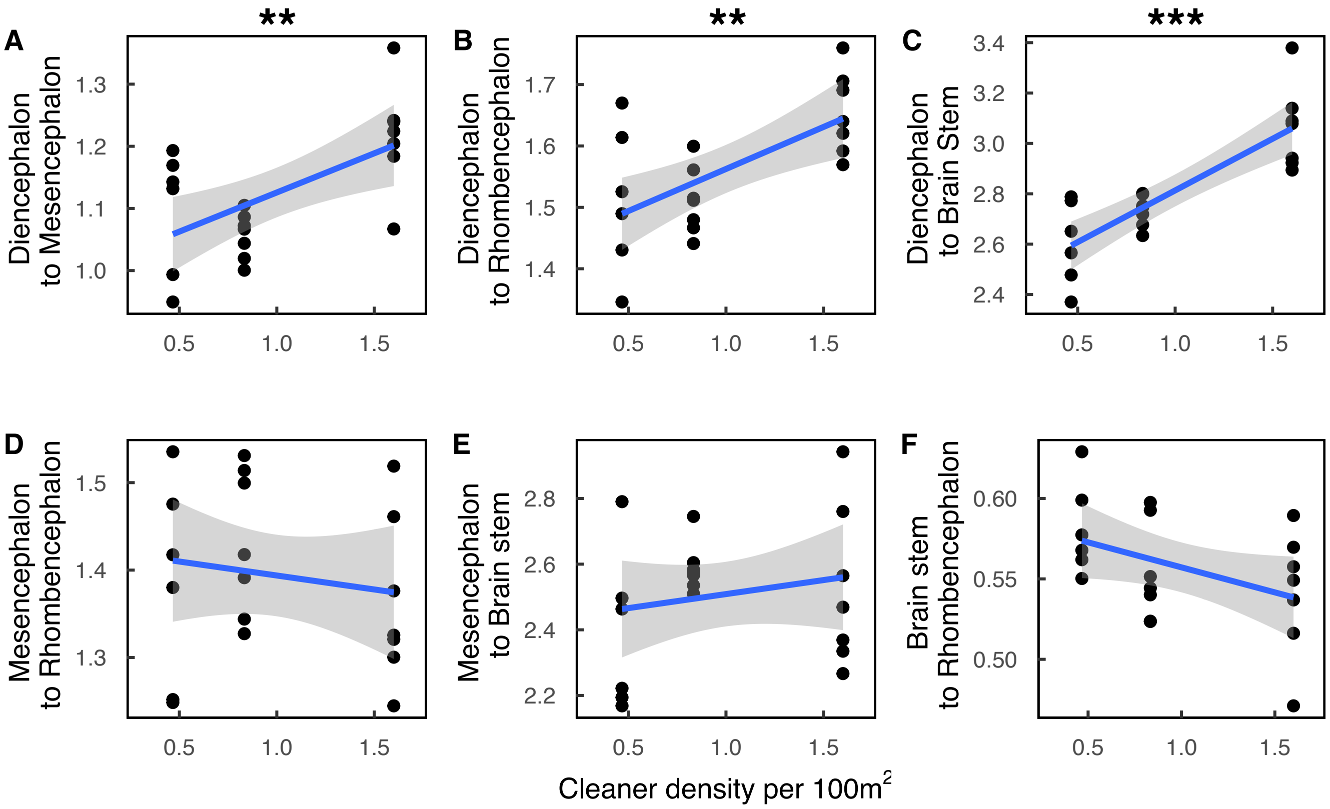

Figure 4. Size ratio of every two brain part sizes and its relationship with cleaner density. Regression line and 95% CI. One-way ANOVA: **: $p < 0.01$; ***: $p < 0.001$

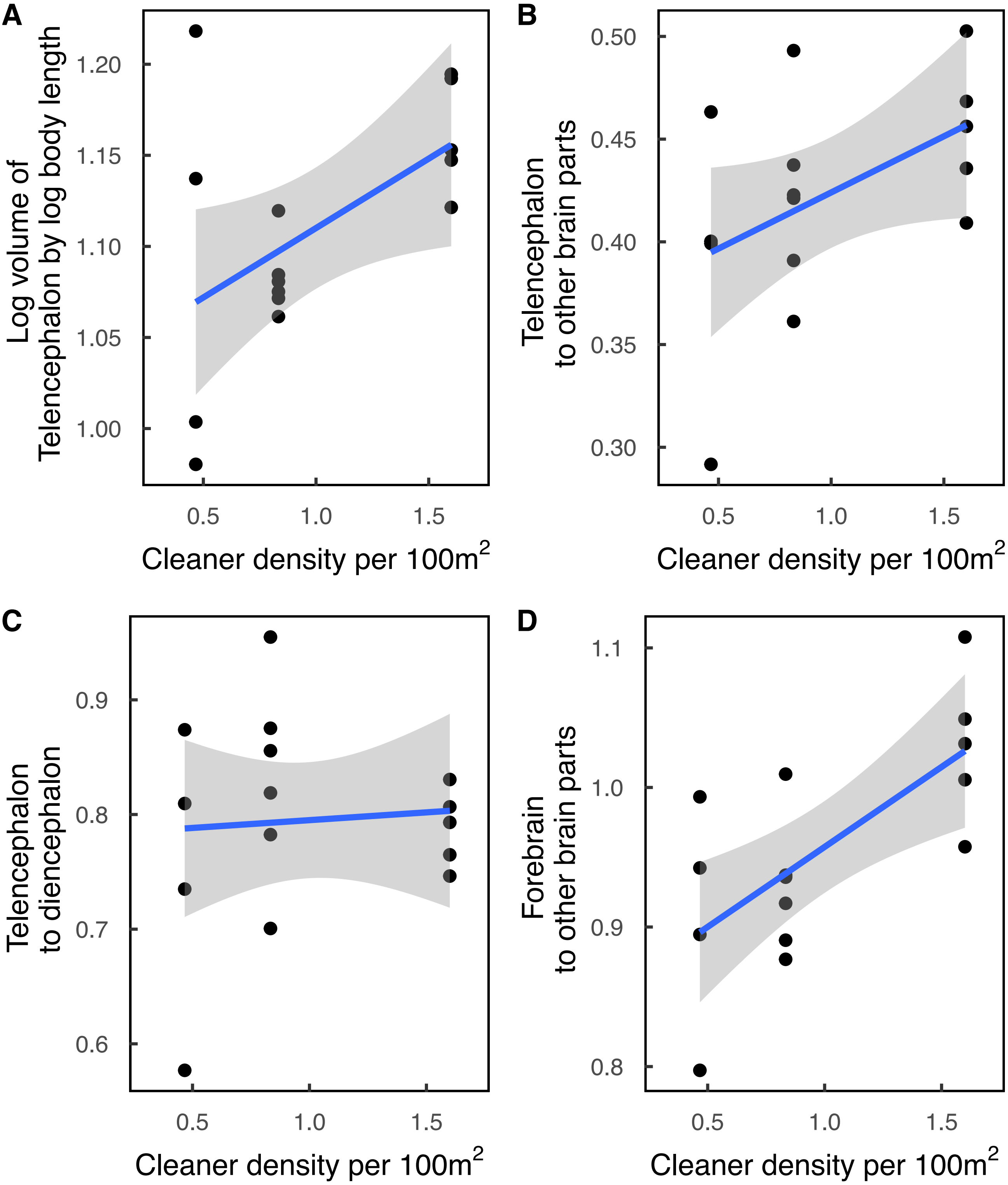

Figure 5. Relationship of brain morphometrics and cleaner density. Scatterplot of (**A**) & (**B**) telencephalon size ratio and (**C**) forebrain size ratio as an estimate of telencephalon + diencephalon / mesencephalon + rhombencephalon + brain stem, regression line and 95% CI.

Table 1. Summary statistics of univariate analyses following the MANOVA tests. The table reports the univariate ANOVA outcomes performed after a significant MANOVA.

a) Brain part sizes

Response variable	**Predictor**	**Degree of freedom (df)**	***F*-value**	***P*-value**	**adjusted *R*-square**
Log Diencephalon / log body length	Cleaner density	**1, 17**	**6.848**	**0.018**	**0.58**
	Log Body weight	**1, 17**	**15.700**	**0.001**	
Log Mesencephalon / log body length	Cleaner density	1, 17	0.025	0.876	**0.17**
	Log Body weight	**1, 17**	**5.457**	**0.032**	
Log Rhombencephalon / log body length	Cleaner density	1, 17	0.167	0.6878	**0.38**
	Log Body weight	**1, 17**	**12.115**	**0.0023**	
Log Brain stem / log body length	Cleaner density	1, 17	0.807	0.381	**0.55**
	Log Body weight	**1, 17**	**25.6034**	**< 0.001**	

b) Size trade-offs between brain parts

Response variable	**Predictor**	**df**	***F*-value**	***P*-value**	**adjusted *R*-square**
Diencephalon / Mesencephalon	Cleaner density	**1, 18**	**9.707**	**0.006**	**0.31**
Diencephalon / Rhombencephalon	Cleaner density	**1, 18**	**11.85**	**0.003**	**0.36**
Diencephalon / Brain stem	Cleaner density	**1, 18**	**40.545**	**< 0.001**	**0.68**
Mesencephalon / Rhombencephalon	Cleaner density	1, 18	0.457	0.507	- 0.03
Mesencephalon / Brain Stem	Cleaner density	1, 18	0.732	0.403	-0.01
Brain stem / Rhombencephalon	Cleaner density	1, 18	3.923	0.0631	0.13

3.4 Discussion

This study shows a robust intraspecific correlation between a specific brain region size and social complexity. Regardless of which scaling method we used, either scaling to body size or to another brain measure, the diencephalon emerged as enlarged in fish from higher cleaner density habitats relative to those from lower density. Our findings suggest that high social complexity has a significant influence on a mosaic brain development in cleaners by enlarging brain parts that are involved in social decision-making.

Previous studies on fish key brain regions have mainly focused on the telencephalon [Gonda et al., 2009; Pollen et al., 2007]. While it would have been ideal to have high quality data of telencephalon size in the present study, the current measurements of this brain region suggest that telencephalon also exhibits intraspecific selective growth together with the diencephalon at high social complexity (Fig. 5). Nevertheless, our results are in line with a previous analysis by Chojnaka et al. [2015], wherein the authors found that the diencephalon is relatively enlarged in cleaner fish in comparison to 24 other perciform fish species. Our study, together with the study by Chojnaka et al. [2015], suggest that the diencephalon size of cleaners is both evolutionarily and ecologically (i.e., within individual lifetime) enlarged to allow the species, or respectively the individual, to cope with a complex social environment. Indeed, the diencephalon plays a crucial role in the connection between the central nervous system and the endocrine system [Bernier, and Peter, 2001], where a large body of literature has documented the effect of neurohormones on social behaviour [Adkins-Regan, 2005; Oliveira et al., 2008; Soares et al., 2010; Reddon Adam R. et al., 2015]. As such, two neuromodulators, vasotocin and isotocin (i.e., homologous of the mammalian vasopressin and oxytocin, respectively) are found to regulate either the inter- or intraspecific social interactions in cleaner fish [Soares et al., 2012]. Therefore, the same brain areas, i.e., those with the relevant receptors, are expected to be affected by changes in both intra- and interspecific complexity.

Socially complex habitats brings richness in both intra- and interspecific social interactions. It simply suggests that the increase in the number of individuals would increase the likelihood of social encounters. For instance, with an increased number of cleaners, the intraspecific interactions can be more frequent. They might take several forms either as aggressive encounters when the cleaner is defending its territory against intruding conspecifics [Kuwamura, 1984], or female cleaner adjusting its cooperation level in the presence of the male partner [Bshary et al., 2008; Raihani et al., 2010; Gingins, and Bshary, 2014]. With few cleaners, these intraspecific interactions would be relatively scarce compared to socially complex habitats. On the other hand, the interspecific interactions show a dynamic variation at high and low social complexity. For instance, studies carried out at Lizard Island from before the environmental disturbances showed that the number of cleaner-client interactions is higher at socially complex sites [Wismer et al., 2014], with a higher frequency of clients with outside option to apply partner choice [Triki et al., 2018]. This might create a competition among cleaners that become strategically sophisticated to outbid neighbouring cleaners [Bshary, and Noë, 2003]. Thus, cleaners living in socially complex habitats deal with much complex and more frequent social interactions. It is then possible that the enrichment of such intra- and interspecific social interactions stimulated neuronal plasticity [van Praag et al., 2000] of the diencephalon in these fish. It is, however, difficult to predict whether it is the intra- or the interspecific social enrichment that drive the diencephalon enlargement in cleaners. In this case, a more suitable study system, where the intra- and interspecific social complexity can be controlled, is needed to address such questions.

We know from previous research that strategically sophisticated cleaners occur in habitats with high social complexity [Binning et al., 2017; Triki et al., 2018; Wismer et al., 2014] (Wismer et al.; Triki et al. unpublished data). In the present study, however, there was somehow a mismatch between social complexity and cleaner performance in the laboratory tasks. It is important to point out that the current study was carried out on Lizard Island, few months after major environmental disturbances hit the island (i.e., the coral bleaching event in 2016). As a result, there were perturbations in the previously documented strategic sophistication of cleaners [Triki et al., 2018]. This might explain the mismatch with what was previously documented [Wismer et al., 2014; Binning et al., 2017]. Despite these unfortunate environmental perturbations, social complexity emerged here to be a strong correlate of brain development. It is possible that functional changes through biochemical modulation of neural networks underpin the fast changes in cleaners' behaviour after the perturbations, whereas the structural changes can be relatively slower processes.

Since brains are energetically demanding [Mink et al., 1981; Navarrete et al., 2011], it is less costly to selectively enlarge key brain regions instead of enlarging the whole brain indiscriminately. Telencephalon and diencephalon together harbour several brain nuclei that have been documented to regulate social decision-making [Goodson, 2005; Amo et al., 2010; O'Connell, and Hofmann, 2011]. In fish, for instance, the forebrain has been repeatedly found to be directly linked to complex social behaviours such as learning, territoriality, mate choice, parental care, aggression [O'Connell, and Hofmann, 2011], fear conditioning [Portavella, 2004; Portavella et al., 2002; Portavella et al., 2003], and spatial learning [Salas et al., 1996a; Salas et al., 1996b; López et al., 2000]. In the current study, we have shown that cleaner social complexity correlates positively with the diencephalon, and to some extent with the telencephalon; where the diencephalon became 12 % larger when cleaner density doubled. In line with our predictions, socially complex habitats might have provided socially challenging environment for these cleaners, wherein larger forebrains were needed to process complex social cues. This is in favour of a social brain hypothesis with mosaic development, with a selective enlargement of a key brain region that plays a crucial role in regulating and fine-tuning complex social behaviours, such as: the link between neocortex size and group size in mammals [Dunbar, 1992; Shultz, and Dunbar, 2006], social learning in primates [Reader, and Laland, 2002], and social complexity in birds [Burish et al., 2004]. Nevertheless, further investigations of the neuronal density, connectivity and activity are necessary to bridge the gap between the size of key brain regions and their functionality [Herculano-Houzel, 2017; Logan et al., 2018]. Also, future research is still needed to uncover the direct link between social complexity, cognition and brain complexity.

3.5 Ethics

All work was performed under The Animal Ethics Committee of the Queensland government (DAFF) approval number (CA 2016/05/970).

3.6 Data availability

Data used in the study, the scripts for statistical analyses and figures are available in the repository Figshare (Data DOI: 10.6084/m9.figshare.6983906).

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3.8 Author's contributions

Z.T., R.B. and J.M. conceived the idea. Z.T. and R.B. designed the study. Z.T. and E.L. collected the laboratory data. E.L. and W.M. analysed the MRI scans. Z.T. analysed the data and generated the figures. Z.T. wrote the first version of the paper. Z.T. and R.B. finalised the paper with input from all authors. All authors declare no competing interests.

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3.10 Supplementary Information for Chapter III

Supplementary Methods of the cognitive tasks:

Cleaners are known to prefer clients’ mucus over ectoparasites, which constitute cheating [Bshary, and Grutter, 2005; Bshary, and Grutter, 2006]. Nevertheless, in the presence of an “image-scoring” bystander client, cleaners refrain from biting mucus from the current client and cooperate instead [Bshary, and Grutter, 2006]. Therefore, in the bystander effect task, cleaners were presented with Plexiglas plates (i.e., as surrogates for clients) with two types of food: a highly preferred food (i.e., prawn as a substitute for mucus) and a less preferred (i.e., fish flakes as a substitute for ectoparasites). The task consists of testing cleaners’ willingness to feed more against their preferences in the presence of an image-scoring bystander plate.

Cleaners in natural habitats prioritise clients with partner-choice to provide them with a cleaning service [Bshary, and Noë, 2003]. These clients with partner-choice are mainly visitor client that might seek a cleaning service simultaneously with a resident client. Cleaners’ optimal choice in such case, is to always service visitors first, then residents. The reason behind this strategy is that visitors usually leave if not serviced while residents are willing to wait [Bshary, and Noë, 2003; Triki et al., 2018]. Overall, we tested cleaners for their abilities to learn to prefer a visitor plate over a resident plate in a biological market task. The plates were of equal size and offering an equal amount of food, but they differ in their colour/pattern. The resident plate was always willing to stay in the aquarium until the cleaner would feed on it. The visitor plate, however, was an ephemeral food source that can be accessed if it is the first plate being inspected by the cleaner. The optimal solution is always to prioritise the visitor plate then the resident plate to double the food gain.

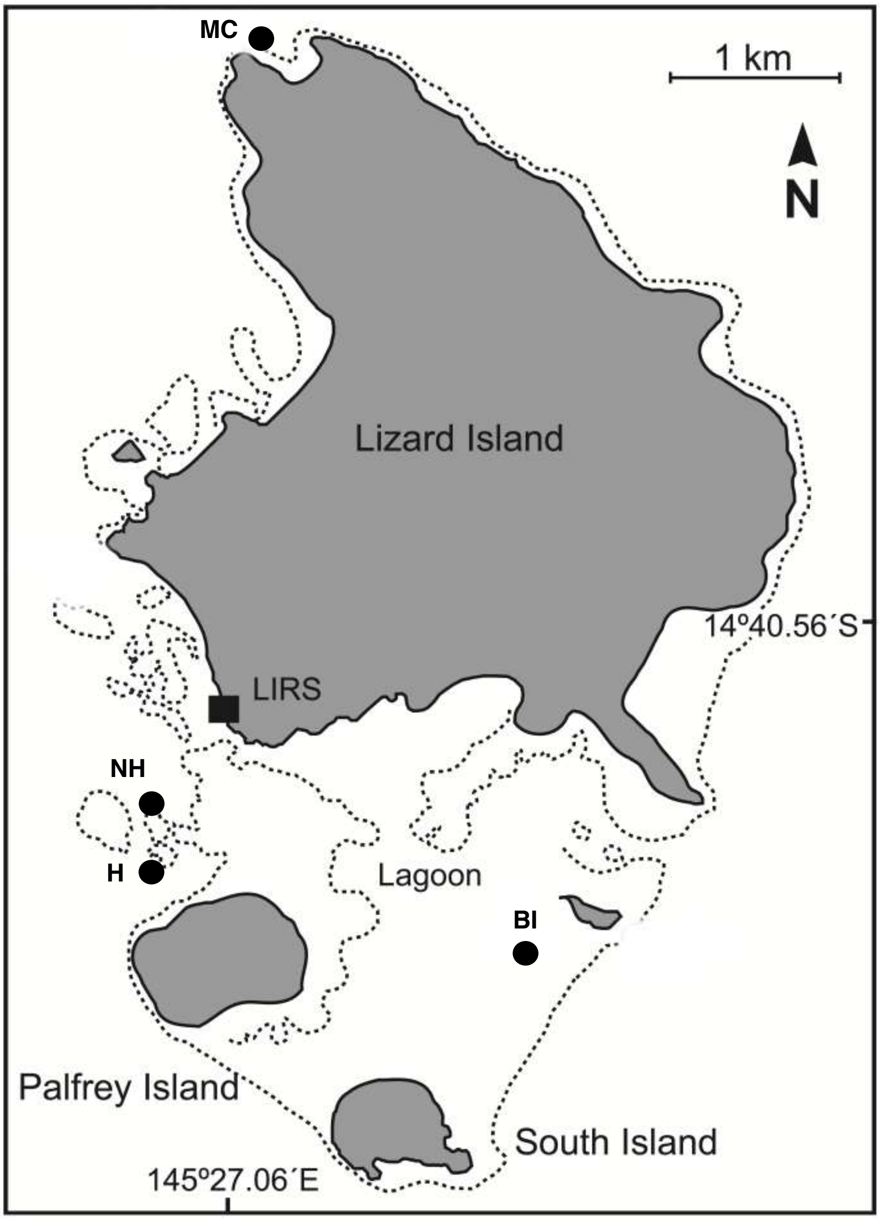


Figure S1. Map of Lizard Island group. The map is showing the detailed location of the four study reef sites at Lizard Island, wherein the filled circles refer to: Bird Islet (BI), Horseshoe (H), Mermaid Cove (MC), and Northern Horseshoe (NH). Depth at the study sites ranges from 1 to 16 m.

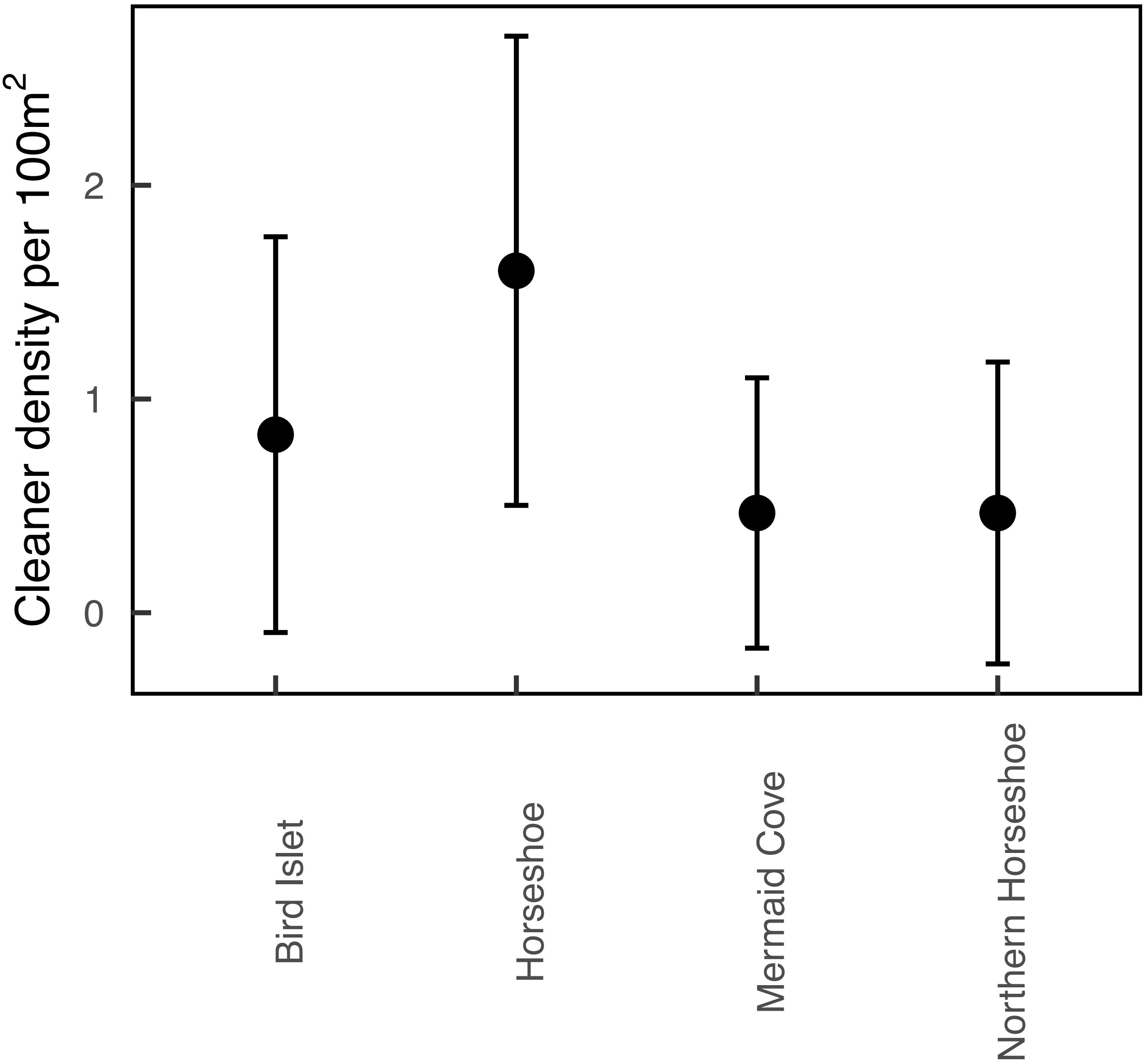


Figure S2. Social complexity of the studied sites. Mean and standard deviation of cleaner density per study site. There were (n=10) transect data points per site, except for Bird Islet where there were (n=8) due to its relatively small surface area. Number of cleaners sampled for brain tissue from each site was: (n=7) form Bird Islet, (n=7) from Horseshoe, (n=4) from Mermaid Cove, (n=1) from Northern Horseshoe.

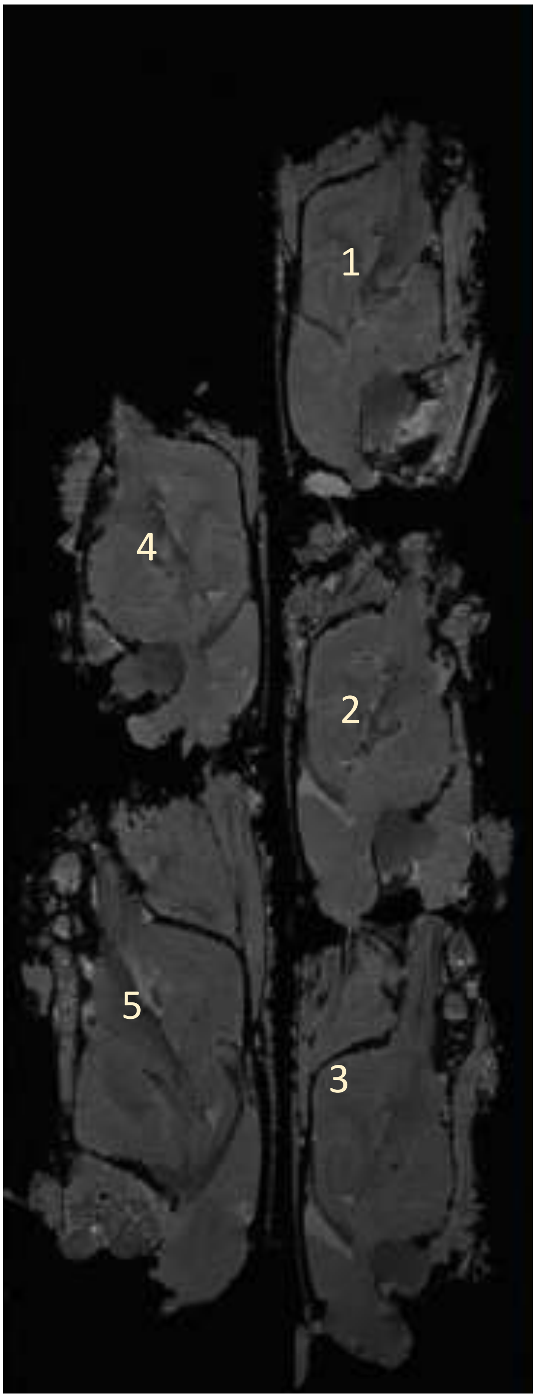


Figure S3. An example of one of the four group MRI scans. The spatial distribution of the five glued brains on the plastic sheet helped in identifying them later.

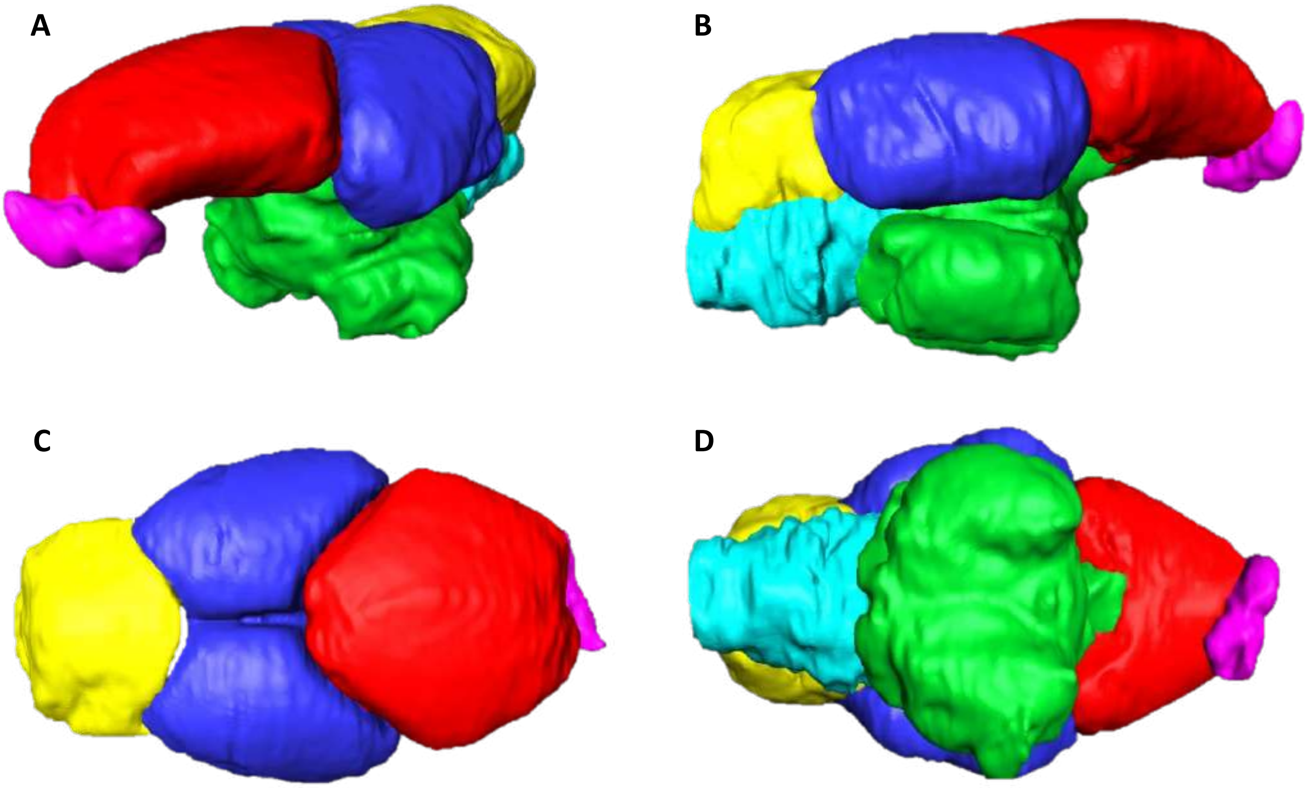


Figure S4. A 3D brain rendering showing the main brain areas. Brain segmentations was processed with the AMIRA software. The figure shows four different angles of the brain: **A** partial frontal view; **B** right view; **C** view from above; and **D** view from below. The brain is subdivided to six major brain areas, depicted in different colors: telencephalon (in red), olfactory bulbs (in pink), diencephalon (in green), mesencephalon (in blue), rhombencephalon (in yellow) and brain stem (in light blue).

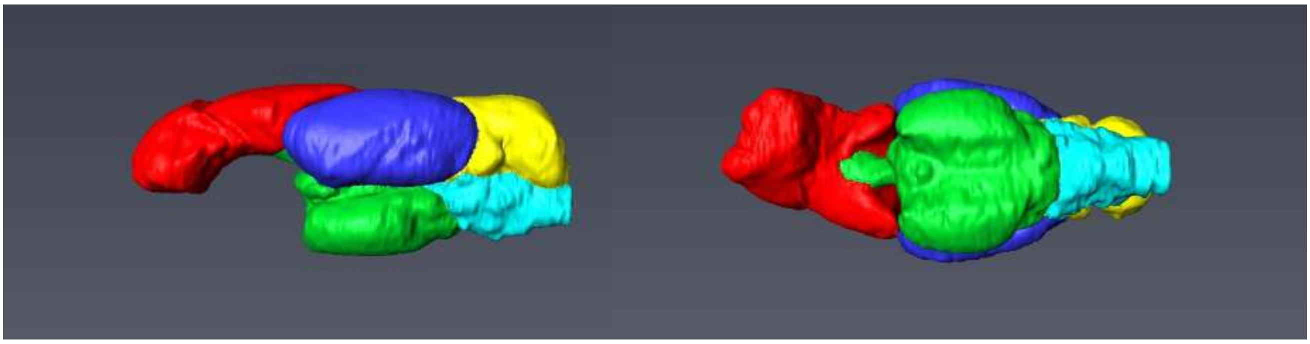
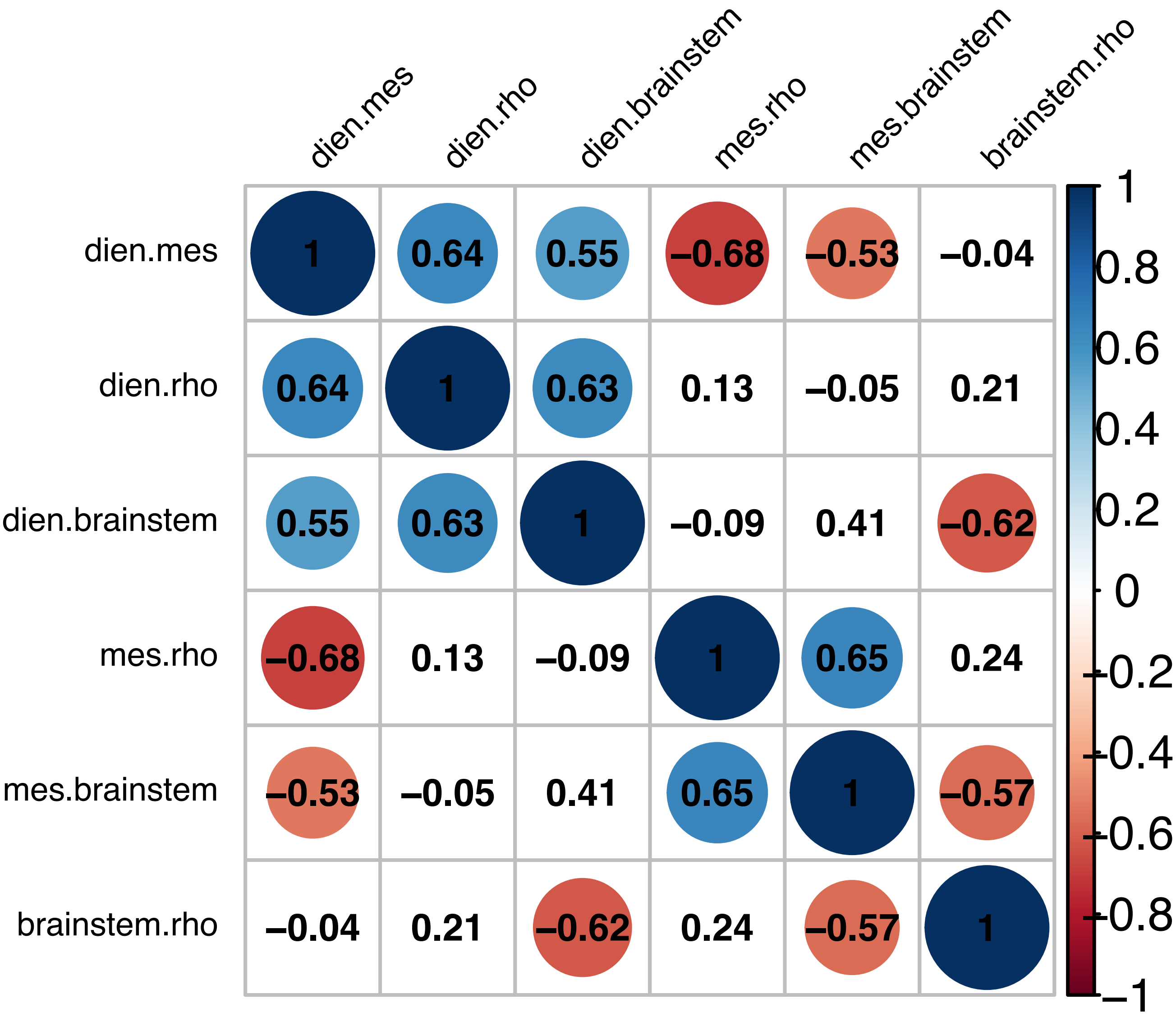


Figure S5. An example of distorted telencephalon (in red) due to brain dissection methods.



	dien.mes	dien.rho	dien.brainstem	mes.rho	mes.brainstem	brainstem.rho
dien.mes	1	0.64	0.55	−0.68	−0.53	−0.04
dien.rho	0.64	1	0.63	0.13	−0.05	0.21
dien.brainstem	0.55	0.63	1	−0.09	0.41	−0.62
mes.rho	−0.68	0.13	−0.09	1	0.65	0.24
mes.brainstem	−0.53	−0.05	0.41	0.65	1	−0.57
brainstem.rho	−0.04	0.21	−0.62	0.24	−0.57	1

Figure S6. Correlation matrix of the size ratio of brain main areas. Significance level was set at $|r| \geq 0.5$. This threshold was suggested by Krehbiel [2004], where a linear relationship might exist between two correlators if: $|r| \geq 2 / \sqrt{n}$ where "n" is the sample size, here (n=20). Dien: diencephalon, mes: mesencephalon, rho: rhombencephalon, brainstem: brain stem.

Table S1. Cleaners' performance in the two laboratory tasks. The table shows number of individuals either succeeding or failing the laboratory tasks from the four study sites. Success in the bystander effect task refers to cleaners' ability to adjust their feeding preferences by feeding more on less preferred food (i.e., flakes) in the presence of a bystander plate in comparison to a situation where the bystander plate is absent. Success in the biological market task refers to cleaners' ability to prioritize significantly the visitor plate over the resident plate.

Bystander effect task	Bird islet	Mermaid cove	Horseshoe	Northern horseshoe
failure	2	2	6	-
success	5	3	1	1
Biological market task	Bird islet	Mermaid cove	Horseshoe	Northern horseshoe
failure	3	-	6	-
success	4	1	1	1

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CHAPTER IV | Population densities and social competence predict forebrain size and neuron numbers in a fish (in cleaner fish)

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Abstract

Several "intelligence" hypotheses attempt to unravel the links between ecology, cognitive abilities and brain neuroanatomical traits [Dunbar, 1992; DeCasien et al., 2017; van Schaik et al., 2012; González-Forero, and Gardner, 2018], but tests based on comparative research remain contentious [Healy, and Rowe, 2007; Logan et al., 2018]. To reduce the effects of potentially confounding variables, it has been proposed to study the effects of naturally occurring variation in the ecology of a species on brain features [Logan et al., 2018]. Here, we show that in a wild fish species, the mutualistic cleaner wrasse *Labroides dimidiatus*, abundance of individuals as a proxy for social complexity in natural habitats correlates positively with the relative cell and neuron counts in the cleaner forebrain. Furthermore, forebrain relative size increased with the increase in the social complexity but only in the brains of individuals with high cognitive performance. Thus, within a species, a measure of cleaner abundance, as a proxy for both intra- and interspecific social complexity, correlated with forebrain complexity as a function of cognitive performance. The results thus provide evidence for the hypothesis that social challenges and their solution promote an increase in brain complexity.

4.1 Introduction

Vertebrate species show large variation in measures of brain complexity, most famously with respect to brain volume scaled to body size [Jerison, 1973]. Social and environmental complexity emerged as two competing hypotheses to explain this variation [Dunbar, 1992; Barton, 1996; Reader, and Laland, 2002; DeCasien et al., 2017; Street et al., 2017; González-Forero, and Gardner, 2018; van Schaik et al., 2012]. Typically, these hypotheses have been tested with correlative analyses of the neuroanatomical brain characteristics and proxies of the ecological complexity [Healy, and Rowe, 2007]. For instance, many comparative studies opted for group size [Dunbar, 1992; Dunbar, 1993; Barton, 1996; Kudo, and Dunbar, 2001; Beauchamp, and Fernández-Juricic, 2004; Shultz, and Dunbar, 2006; Street et al., 2017], mating systems [Pawłowski et al., 1998; Iwaniuk, 2001; Barton, 2006], social bonds [Dunbar, and Shultz, 2007; Emery et al., 2007] as the social factors, whereas others explored diet related aspects as environmental factors [Iwaniuk, and Nelson, 2001; DeCasien et al., 2017; Iwaniuk, and Nelson, 2001]. Although this research extrapolates that ecological complexity (i.e., social and/or environmental) drives high cognitive abilities, the quantification of cognitive performance is still important. Indeed, there have been studies that tried to establish the relationship between species cognitive performance with either neuroanatomical traits [Deaner et al., 2007; Shultz, and Dunbar, 2010; Herculano-Houzel, 2017; Krebs et al., 1989, 19; Slater, and Schreck, 1997; Lefebvre et al., 1998] or putative correlates of ecological complexity [Reader et al., 2011; Bond et al., 2003; Ashton et al., 2018]. Despite the continued efforts to produce large data sets of neuroanatomical characteristics and standardised cognitive test batteries to evaluate cognitive performance, the comparative approach has been criticised [Healy, and Rowe, 2007; Logan et al., 2018; Gonda et al., 2013; van Schaik et al., 2012]. This is mostly because the established measures are often crude, and many potentially confounding variables may not have been identified and hence not controlled for [Healy, and Rowe, 2007; Logan et al., 2018; Gonda et al., 2013; Thornton, and Lukas, 2012]. A complementary alternative is to generate validated data with the “bottom-up” approach that study variation at either the intraspecific level or in closely related species to test the ecological complexity hypotheses [Logan et al., 2018]. This can be achieved by identifying variation in an ecological variable of a potential interest, cognitive experiments that are linked to the ecological variable, and/or key brain regions. A classic example is the relationship between food-caching, spatial memory skills and relative hippocampus size in corvids and tits [Krebs et al., 1989]. Black-capped chickadees show seasonal plasticity in the hippocampus size as an adaptation for food-sorting needs [Smulders et al., 1995]. In humans, rich navigation experience of London taxi drivers correlates with enlarged posterior hippocampus [Maguire et al., 2000], while advanced musical skills are associated with an enlarged leftward cortex [Schlaug et al., 1995]. Furthermore, intraspecific artificial selection on larger brains in animals also documented the liaison between brain morphometrics and cognitive skills, that is, discrimination learning in mice [Wimer, and Prater, 1966], and reversal learning [Buechel et al., 2018] and numerical abilities in female guppies [Kotrschal et al., 2013].

Here we combined information of ecological variation and cognitive performance in ecologically relevant experiments as potential predictors of neuroanatomical traits of individuals of the cleaner fish *Labroides dimidiatus* (hereafter “cleaner”). Cleaners are an ideal model species to test aspects of the social brain hypothesis because in this species, foraging – a standard environmental cognitive challenge in most species – is a social challenge: cleaners obtain their food from interactions with client reef fish that visit them to have their ectoparasites removed [Randall, 1958; Losey Jr, 1979; Grutter, 1999]. Also, two relevant conflicts of interests exist in this mutualism. First, cleaners prefer to cheat clients by eating their mucus [Grutter, and Bshary, 2003]. Second, two or more clients may seek cleaning service simultaneously and hence compete over priority of access [Bshary, 2001]. These two conflicts can be featured in two laboratory-based cognitive experiments in which cleaners are challenged to make correct decisions such that their foraging success is optimised. Importantly, previous research provided the basic information necessary to test for potential links between social complexity, cognitive performance and brain features [Triki et al., *in review*]. Cleaner individuals of the same population, but from neighbouring reef sites are known to vary in their performance in these cognitive tasks [Wismer et al., 2014; Triki et al., 2018]. Most of the individual variance in performance can be attributed to variation in cleaner density and the highly correlated density of large client species [Triki et al. *In review*].

The two aforementioned laboratory-based cognitive experiments address complex cognitive abilities. The bystander effect task warrants reputation management (i.e., the increase of inhibitory control in the presence of an audience) [Bshary, and Grutter, 2005; Bshary, and Grutter, 2006]. The biological market task is a two-choice foraging task in which subjects need to prioritise an ephemeral food source over a more permanent one, a challenge in which cleaners outperform other vertebrate species such as primates [Salwiczek et al., 2012], rats [Zentall et al., 2017] and pigeons [Zentall et al., 2016]. Here, we tested a total of 40 cleaners from four different reef sites around Lizard Island, Australia (Supplementary Fig. S1), wherein fish survey data were collected to estimate population density of cleaner fish (Supplementary Fig. S2 and Fig. S3). We chose the 10 best and 10 worst performing individuals (Supplementary Fig. S4) to assess their neuroanatomical traits per brain part (i.e., brain dissected into five regions: telencephalon, diencephalon, mesencephalon, cerebellum, and brain stem; Supplementary Fig. S5). Since the social decision-making network is harboured in the forebrain (i.e., telencephalon and the diencephalon) in fishes (O'Connell & Hofman 2011), we predicted that neuroanatomical traits, such as size, neuronal and non-neuronal cells count, and densities of the forebrain would be selectively affected by social complexity and cognitive performance.

4.2 Results

Studied fish (N=20) measured (mean ± SD) 7.43 ± 0.61 cm body total length, and 3.52 ± 0.88 g body mass. Their brain cell counts (i.e., neuronal and nonneuronal cells) were 39'701'250 ± 7'515'848, where 4'860'656 ± 2'116'278 were neuronal cells, for a brain mass measuring 39.78 ± 7.02 mg.

Neuroanatomical measurements, such as size, cell and neuronal counts and densities, in the forebrain (i.e., telencephalon and diencephalon) were scaled to total brain (i.e., size, cell/neuron counts or densities of all five parts of the brain) and body size (see methods).

4.2.1 Brain region sizes

We found that forebrain relative size correlated significantly with cleaners' performance and cleaner density interaction (two-way ANOVA: N = 18, $F_{(1, 13)} = 11.384$, $p = 0.005$, adjusted-$R^2 = 0.88$, Fig. 1). *Post hoc* analyses showed that forebrain relative size had a positive linear relationship with cleaner density in cleaners exhibiting high performance in the cognitive tasks ($p < 0.001$), whereas there was no relationship between forebrain and cleaner density in the poorly performing cleaners ($p = 0.978$). Testing either telencephalon or diencephalon relative size individually lead only to a trend of a significant correlation relationship between the brain part relative size, performance and cleaner density (Telencephalon: N = 18, $F_{(1, 13)} = 4.210$, $p = 0.061$; Diencephalon: N = 18, $F_{(1, 13)} = 3.926$, $p = 0.069$) (Fig. 1).

4.2.2 Brain cell counts

The relative cell counts (i.e., neuronal and non-neural) in the forebrain, scaled to the whole brain, was a significant correlate to cleaner density (N = 20, $F_{(1, 15)} = 23.627$, $p < 0.001$, adj-$R^2 = 0.88$) but not to performance (N = 20, $F_{(1, 15)} = 0.015$, $p = 0.903$, adj-$R^2 = 0.86$) (Fig. 2a). Here, the relative cell counts in the forebrain increased by ~ 40 % in fish from a site with cleaner density ≥ 3 per 100 m^2 compared to a density of less than 0.2 per 100 m^2. While telencephalon cells correlated positively with cleaner density (N = 20, $F_{(1, 15)} = 6.244$, $p = 0.024$, adj-$R^2 = 0.63$, Fig. 2b), diencephalon cells showed only a trend (cleaner density: N = 20, $F_{(1, 15)} = 3.563$, $p = 0.078$, Fig. 2c). Furthermore, cell density (i.e., number of cells per 1 mg of brain tissue) in the forebrain correlated positively with cleaner density (N = 18, $F_{(1, 13)} = 5.313$, $p = 0.038$, adj-$R^2 = 0.11$), whereas performance showed no significant effect (N = 18, $F_{(1, 13)} = 0.083$, $p = 0.777$) (Fig. 2d). The analyses of cell density in either telencephalon or diencephalon separately did not show significant effects ($p > 0.05$, see Table 1) (Fig. 2e & 2f).

4.2.3 Neural cell counts

The relative neuronal cell counts in the forebrain showed a significant positive relationship with cleaner density (N = 20, $F_{(1, 15)} = 5.915$, $p = 0.028$, adj-$R^2 = 0.38$, Fig. 3a), with a ~ 42% increase from a site with cleaner density ≥ 3 per 100 m^2 compared to a site with a density of less than 0.2 per 100 m^2. This measurement of neuron counts in the forebrain did not show a significant correlation with performance (N = 20, $F_{(1, 15)} = 2.149$, $p = 0.163$) (Fig. 3a). Neither telencephalon nor diencephalon alone showed a significant correlation between neuron counts and cleaner density or performance ($p > 0.05$) (Fig. 3b & 3c). Finally, neurons density (i.e., number of neurons per 1 mg of tissue), in the entire forebrain or its parts showed no significant correlations with performance or cleaner density (see Table 1, and Fig. 3d, 3e & 3f).

4.2.4 Performance in the cognitive tasks and locally adaptive strategies hypothesis

High performance in the two laboratory-based cognitive tasks indicates that those cleaners can manage their reputation and prioritise visitor clients (i.e., ephemeral food sources as they can switch to alternative cleaners), also under natural conditions. However, such strategies can only be useful in high cleaner density areas, as visitor clients only readily switch between cleaners if the distance between cleaners (i.e., the cleaning stations) is relatively short [Triki et al. *in review*|. The concept of social competence [Taborsky, and Oliveira, 2012; Bshary, and Oliveira, 2015] would thus predict that high performing cleaners from high density sites and low performing cleaners from low density sites are the smartest (i.e., by applying locally adapted optimal strategies), and should hence have larger relative forebrains with more neurons. Here, we showed that if we encode for cleaners' strategies depending on whether it is in a socially rich (high cleaner density > 2) or poor (low cleaner density < 2) environment (i.e., a threshold adapted loosely from Fig. 1a & Fig. 3a), individuals making optimal strategies had a relatively larger forebrain (two-way ANOVA: N=18, $F_{(1,15)} = 15.637$, $p = 0.002$, adj-R^2= 0.89, 95% CI (0.06 0.21), Fig. 4a), with more neurons (two-way ANOVA: N=20, $F_{(1,17)} = 8.259$, $p = 0.012$, adj-R^2= 0.47, 95% CI (0.08 0.54), Fig. 4b) than in individuals adopting locally non-optimal strategies. *Post hoc* tests showed that the positive correlation relationship between either forebrain relative size or its neuron counts and cleaner density within every type of behavioural strategy was statistically significant (all $p < 0.04$, Fig. 4).

Table 1 | Relationship between brain neuroanatomical traits, cognitive performance and social complexity.

Neuroanatomical trait	Brain region	Correlate variable	n	F-value	*p*-value	95% CI	Figure
Relative size	**Forebrain**	performance	18	1.798	0.203	-0.03 0.13	Fig.1a
		Cleaner density	**18**	**6.983**	**0.020***	**0.06 0.19**	
		Performance x cleaner density	**18**	**11.384**	**0.005****	**-0.21 -0.05**	
	Telencephalon	performance	18	0.036	0.853	-0.18 0.22	Fig. 1b
		Cleaner density	18	1.722	0.212	0.02 0.35	
		Performance x cleaner density	18	4.21	0.061	-0.41 0.01	
	Diencephalon	performance	18	1.987	0.182	-0.05 0.23	Fig. 1c
		Cleaner density	18	1.631	0.224	0.01 0.24	
		Performance x cleaner density	18	3.926	0.069	-0.28 0.01	
Relative cell counts	**Forebrain**	performance	20	0.015	0.903	-0.12 0.13	Fig. 2a
		Cleaner density	**20**	**23.627**	**< 0.001*****	**0.07 0.29**	
		Performance x cleaner density	20	0.535	0.476	-0.18 0.09	
	Telencephalon	performance	20	0.009	0.927	-0.29 0.32	Fig. 2b
		Cleaner density	**20**	**6.244**	**0.025***	**-0.09 0.44**	
		Performance x cleaner density	20	0.003	0.954	-0.32 0.33	
	Diencephalon	performance	20	0.171	0.685	-0.37 0.23	Fig. 2c
		Cleaner density	20	3.563	0.079	-0.01 0.51	
		Performance x cleaner density	20	1.299	0.272	-0.49 0.15	
Relative cell density	**Forebrain**	performance	18	0.083	0.777	-0.18 0.13	Fig. 2d
		Cleaner density	**18**	**5.313**	**0.038***	**-0.08 0.17**	
		Performance x cleaner density	18	0.787	0.391	-0.09 0.23	

	Telencephalon	performance	18	3.199	0.097	-0.03 0.31	Fig. 2e
		Cleaner density	18	1.666	0.219	-0.14 0.14	
		Performance x cleaner density	18	1.136	0.306	-0.09 0.26	
	Diencephalon	performance	18	1.717	0.213	-0.52 0.13	Fig. 2f
		Cleaner density	18	2.038	0.177	-0.14 0.39	
		Performance x cleaner density	18	0.017	0.898	-0.36 0.32	
Relative neuron counts	**Forebrain**	performance	20	2.149	0.163	-0.09 0.40	Fig. 3a
		Cleaner density	**20**	**5.915**	**0.028 ***	**0.07 0.48**	
		Performance x cleaner density	20	2.843	0.112	-0.46 0.05	
	Telencephalon	performance	20	0.529	0.478	-0.32 0.61	Fig. 3b
		Cleaner density	20	1.129	0.305	-0.06 0.74	
		Performance x cleaner density	20	2.099	0.168	-0.83 0.16	
	Diencephalon	performance	20	0.47	0.503	-0.31 0.58	Fig. 3c
		Cleaner density	20	2.611	0.127	-0.06 0.71	
		Performance x cleaner density	20	1.102	0.311	-0.71 0.24	
Relative neuron density	**Forebrain**	Performance	18	0.386	0.545	-0.20 0.37	Fig. 3d
		Cleaner density	18	2.01	0.18	-0.10 0.37	
		Performance x cleaner density	18	0.185	0.674	-0.36 0.24	
	Telencephalon	performance	18	0.396	0.54	-0.35 0.64	Fig. 3e
		Cleaner density	18	0.114	0.741	-0.25 0.56	
		Performance x cleaner density	18	0.597	0.454	-0.70 0.33	
	Diencephalon	performance	18	0.024	0.879	-0.50 0.58	Fig. 3f
		Cleaner density	18	1.84	0.198	-0.23 0.65	
		Performance x cleaner density	18	0.059	0.813	-0.63 0.50	

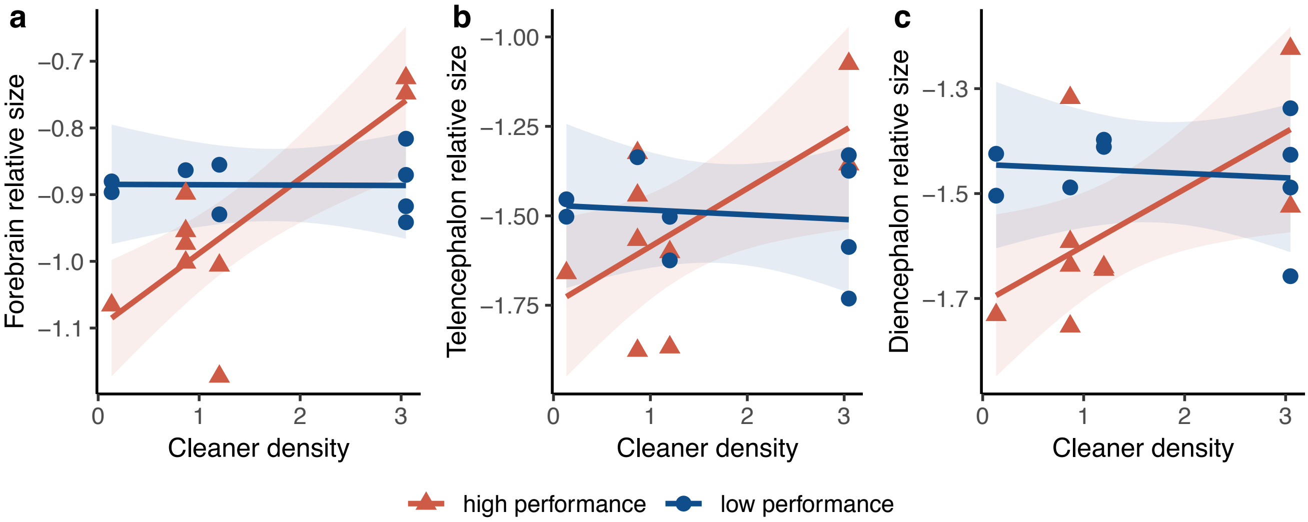

Figure 1 | Relationship between brain size, cognitive performance and social complexity. The relationship of **a** forebrain (i.e., telencephalon + diencephalon) **b** telencephalon, and **c** diencephalon sizes, scaled to total brain size and body size, with strategic performance in the laboratory-cognitive based tasks (i.e., bystander effect and biological market tasks) and cleaner density as a proxy for social complexity. Fitted regression lines and 95% confidence interval CI are generated with the function visreg() in R language.

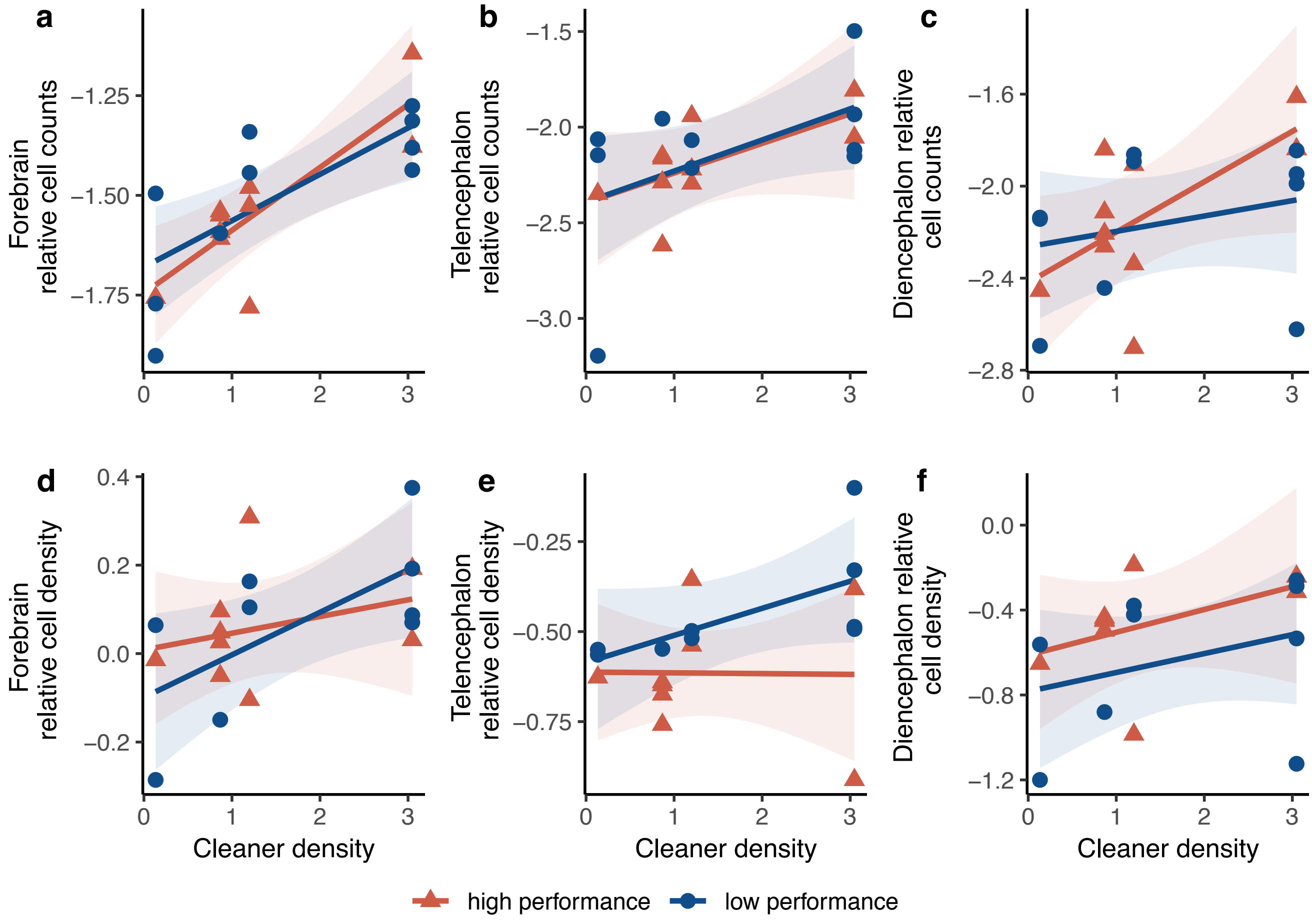

Figure 2 | Relationship between brain cell abundance, cognitive performance and social complexity. The relationship of **a** forebrain (i.e., telencephalon + diencephalon) **b** telencephalon, and **c** diencephalon cell counts, scaled to total brain cell counts and body size, with strategic performance in the laboratory-cognitive based tasks (i.e., bystander effect and biological market tasks) and cleaner density as a proxy for social complexity; while **d, e** and **f** are the cell density per 1 mg of tissue. Fitted regression lines and 95% CI are generated with the function visreg() in R language.

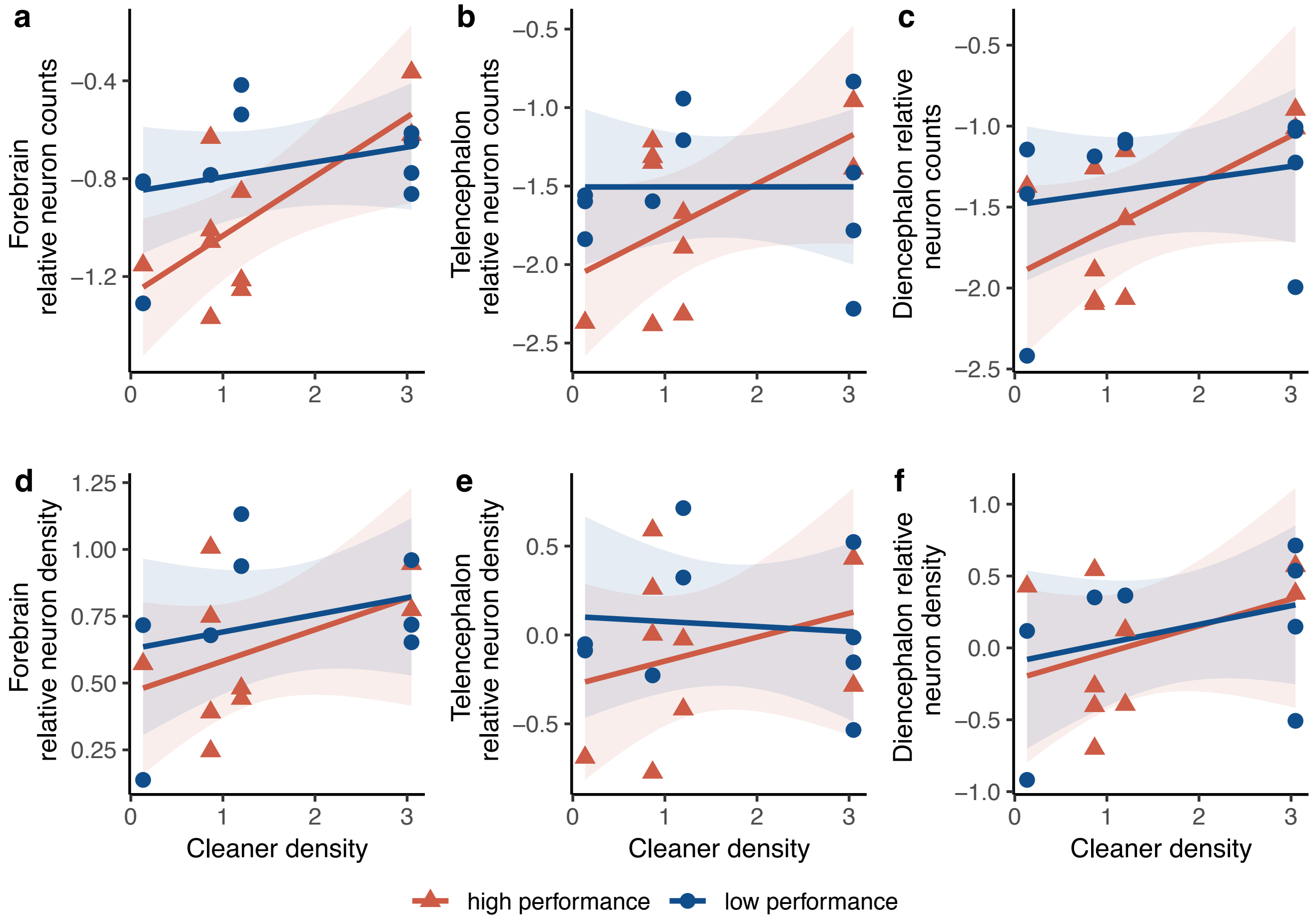

Figure 3 | Relationship between neuron abundance, cognitive performance and social complexity. The relationship of **a** forebrain (i.e., telencephalon + diencephalon) **b** telencephalon, and **c** diencephalon neuron counts scaled to total brain neuron counts and body size, with strategic performance in the laboratory-cognitive based tasks (i.e., bystander effect and biological market tasks) and cleaner density as a proxy for social complexity; while **d, e** and **f** are the neuron density per 1 mg of tissue. Fitted regression lines and 95% CI are generated with the function visreg() in R language.

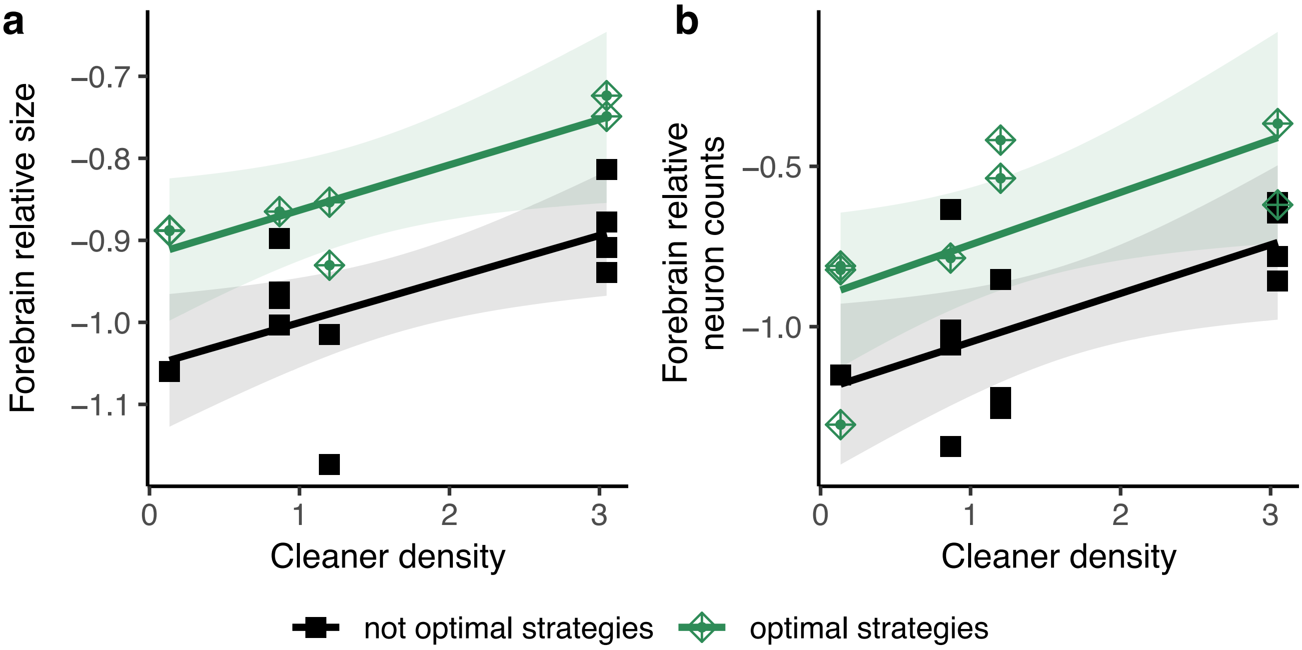

Figure 4 | Relationship between forebrain neuroanatomical traits and the local behavioural strategies. This graph is another way to visualise the data from **a** Fig. 1a and **b** Fig. 3a (see above), wherein we consider that high performance in the experiments reflects locally not optimal strategies in low density environments, while these strategies become locally optimal at high densities, while the reverse applies to low performance. Fitted regression lines and 95% CI are generated with the function visreg() in R language.

4.3 Discussion

Our findings support the social brain hypothesis on the intraspecific level of a fish species. In analogy to the neocortex ratio correlating positively with group size as an indicator of social complexity in primates [Dunbar, 1992; Dunbar, 1995], we provide the first evidence that both forebrain size ratio and its functional units (i.e., cell/neuron numbers ratios) increase as a function of cleaner fish and large client (i.e., visitors) species densities. Apparently, cleaners can adjust the processing power of the brain ontogenetically to the richness of intra- and/or interspecific social challenges.

The cognitive performance of cleaners in experiments capturing sophisticated aspects of cleaning interaction additionally explained variation in forebrain traits, though in less straightforward ways compared to various other studies [MacLean et al., 2014; Benson-Amram et al., 2016; Reader et al., 2011; Herculano-Houzel, 2017; Gronenberg, and Couvillon, 2010; Lefebvre et al., 1998]. Our experiments tested ecologically relevant features of strategies rather than more abstract aspects of general intelligence. This may explain why not absolute performance but performance indicative of suitable strategies at the site of capture predicted relative forebrain size and cell/neuron numbers. It has been evoked that cognitive performance should be interpreted with respect to the ecological relevance of the task to the studied species
[Shettleworth, 2009; Balda, and Kamil, 1989; Prétôt et al., 2016]. Our results highlight that the relevance of the task might even vary depending on the local ecological conditions that individuals experience in their natural habitats. Therefore, we need to integrate the concept of social competence [Taborsky, and Oliveira, 2012] to predict how an individual's performance in ecologically relevant social tasks relates to its cognitive abilities. Such integration will change our understanding of how ecological complexity contributes to the development of the brain and its key parts.

The findings suggest two phenomena occurring simultaneously: One follows the "social brain hypothesis" [Dunbar, 1992; Dunbar, 1995], that is, there is an ontogenetic variation in cleaner fish brain complexity between sites that vary with the variation in the social complexity degree. Also, the strong link found between social complexity and brain complexity, with no apparent overall effect of cognitive performance suggest that the extra brain tissue/neurons is probably to deal with social challenges beyond reputation management and partner service priority. The other phenomenon is the variation within sites, probably due to genetic divergence and/or selection on social competence.

4.4 Material and Methods

4.4.1 Study site and behavioural observations

The study was conducted at four different locations at Lizard Island (14.6682° S, 145.4604° E), Great Barrier Reef, Australia, between July and August 2018. To record cleaner fish natural interactions with client reef fish, scuba divers randomly selected (n=8) adult female cleaners at each site. The selected cleaners were followed, and video recorded for 30 min. Observers recorded cleaners' behaviour from a distance of ~ 2 m, to minimise disturbance, while warranting good video quality. All videos were recorded between 08:30 and 16:00 hours, using Canon G15® and G16® cameras. From the video encoding data, we extracted for each cleaner-client interaction the client species and the duration. From that, we estimated the total number of interactions per time unit (i.e., 30 min), and the total number of interactions with large clients per time unit.

4.4.2 Fish survey

To estimate cleaner fish densities and their clients, scuba divers conducted underwater fish survey at each study site (Supplementary Fig. S1). Observers counted fish abundance on transect lines. In total, a replicate of (n=10) transects of 30 m each was conducted at every study site, except at The Crest where (n=7) replicates were collected. Therefore, the transect line was either placed parallel to the reef crest (i.e., at Mermaid cove and The Crest), or parallel to the shoreline (i.e., at Northern horseshoe and Corner beach). On every transect line, observers recorded large-bodied fish, with total body length TL > 10 cm, along with cleaners on a 5 m width, whereas the small-bodied fish, with TL ≤ 10 cm, were recorded on a 1 m wide area along the 30 m transect. Fish counts were then scaled to densities per 100 m^2.

4.4.3 Laboratory experiments

For laboratory experiments, (n=40) adult female cleaner wrasse *Labroides dimidiatus* (total length TL: mean ± SD, 7.43 ± 0.61 cm) were collected from the four study sites. Scuba divers captured cleaners with barrier nets (2 m x 1 m, 5 mm mesh) and hand nets. At Lizard Island Research Station facilities, all fish were individually housed in glass aquaria (62 cm x 27 cm x 37 cm) and provided with PVC pipes (10 cm x 1 cm) as shelters. All fish were allowed an acclimation period of at least 14 days before proceeding with the laboratory experiments. Fish were fed daily with a paste of mashed prawn smeared on Plexiglas plates (8 x 15 cm). Gradually, fish were offered a mixture of mashed prawn and fish flakes, hereafter "flakes". All cleaners showed a successful feeding rate off the Plexiglas plates from the first two days of acclimation. For this reason, Plexiglas plates offering food items were used as a surrogate for clients reef fish [Bshary, and Grutter, 2006]. During Laboratory experiments, cleaners received food from the trials from 8:00 to 17:00.

Experiment 1: "Bystander effect" task

In this experiment, fish were presented with two types of food: one is highly preferred food and the other is less preferred. From previous research, we know that cleaners prefer eating a prawn food item (i.e., highly preferred food) over flake food item (i.e., less preferred food) in a choice test [Bshary, and Grutter, 2005]. Therefore, we used prawn and flake food items to mimic client mucus and ectoparasites, respectively [Bshary, and Grutter, 2005; Bshary, and Grutter, 2006; Wismer et al., 2014; Triki et al., 2018]. To ensure that fish distinguish between these two types of foods, fish were subjected to a training phase prior to testing them in the bystander effect task. In this phase, cleaners were trained to first eat less preferred food items (i.e., flakes) before eating a highly preferred food (i.e., prawn).

In total, we ran five learning trials over two days. In each single trial, an opaque and transparent partition were introduced together in the aquarium, dividing the latter in two compartments. This helped to confine the fish to one side of the aquarium, while the other side was left inaccessible to the cleaner. On the inaccessible side of the aquarium, a novel Plexiglas plate offering 12 flake items and two prawn items was introduced. The removal of the opaque partition was followed by the removal of the transparent one allowing the fish to see what is on the other side of the aquarium before granting it access. Cleaners were permitted to eat flake items. Consuming a prawn item, however, resulted in the withdrawal of the plate from the aquarium. The same plate with the remaining food items was reintroduced in the aquarium after 60 seconds. Again, the consumption of a prawn item lead to the withdrawal of the plate. At this stage, the trial would take end once no prawn item was left on the plate. During this training period, all fish experienced eating a flake item before eating a prawn item at least once.

When cleaners were tested in the bystander effect task, they were presented with two novel Plexiglas plates of similar size (12 cm x 7 cm) exhibiting different decoration of either yellow or white stripes. At this stage, every plate offered four food items in total: two flakes and two prawns. Similarly to the training phase, the plates would remain in the aquarium as long as the fish ate flake items only. Upon eating a prawn item, the plates would leave the aquarium. In order to maximize food intake, the optimal strategy is to feed on all available flake items before eating one prawn item. The bystander effect task had two conditions: "image-scoring" treatment and control. In the image-scoring treatment, whichever plate the cleaners are feeding first, makes the second plate an image-scoring bystander plate. In other words, cleaners can have access to the image-scoring bystander as long as they eat only flakes off the first plate. It aimed to reproduce natural conditions, where cleaners can have access to an image-scoring bystander client only if they are cooperative with the current client [Bshary, 2002; Pinto et al., 2011]. The control condition, on the other hand, is a "absence of an image-scoring plate" where cleaners are presented with a single plate.

The task was composed of several rounds of two trials each, one trial as control and one as treatment. An interval of 30 min was allowed between every two trials, and subsequently a 60 min time interval between two rounds. Randomization of the order of the two trials within each round for every cleaner was determined by flipping a coin. In total, cleaners were subjected to 9 rounds, over a period of two days. Previous research by Wismer et *al.* [2014] showed that this task is not a learning task. Therefore, the number of rounds does not have an impact on the cleaners' outcomes in this specific task. From here, we averaged cleaners' performance through the rounds: we subtracted the ratio of total flakes to total prawn items eaten by cleaners from the single plate in the control trials from the ratio of the first plate in the image-scoring treatment. Positive values, greater than zero, referred to induced cooperation by the mere presence of an image-scoring bystander. In other words, such values indicated that cleaners were feeding more against their preferences in the presence of an image-scoring plate than in its absence. Negative or zero values, on the other hand, indicated that cleaners' feeding preferences were not affected by the presence or absence of the second plate. Therefore, cleaners' ability to either adjust or not adjust their feeding preferences in the presence of a bystander was encoded as binary response. Positive values, and greater than zero referred to cleaners' ability to adjust their feeding preference in the presence of an image-scoring bystander a "yes", while negative and zero values referred to the absence of such ability "no". Furthermore, another variable was extracted from cleaners' performance in this task: the ability to access the bystander plate in the image-scoring condition was also recorded as a binary response. Access to the bystander plate was considered as "yes" while non-access as "no".

Experiment 2: "Biological market" task

Similarly to the bystander effect task, the biological market task was also an ecologically relevant task [Bshary, and Grutter, 2002]. In natural habitats, cleaners would prioritize clients with partner-choice to provide them with a cleaning service [Bshary, and Noë, 2003]. Clients with the partner-choice are mainly visitor clients. These clients might seek a cleaning service simultaneously with a resident client. Cleaners' optimal choice in such case, is to always service visitors first, then residents. The reason behind this strategy is that visitors usually leave if not serviced while residents are willing to wait [Bshary, and Noë, 2003; Triki et al., 2018]. Here, in the biological market task, we tested cleaners for their abilities to learn to prefer a visitor plate over a resident plate. Again, we used Plexiglas plates as surrogates for the clients. The plates were of equal size (10 cm x 7 cm), and offered an equal amount of food (i.e., one prawn item each). To facilitate visual discrimination of the two plates, we ensured that both plates had either vertical pink stripes or horizontal green stripes as decoration [Triki et al., 2018]. The plate with a resident role was always willing to stay in the aquarium until the cleaner would feed on it. The visitor plate, however, was an ephemeral food source. This plate is only accessible if it is the first plate given priority to for inspection by the cleaner. Otherwise, the visitor plate would be withdrawn from the aquarium if the cleaner inspects the resident plate first. Similarly to the bystander effect task, cleaners would be confined to one side of the aquarium by the two separations (i.e., opaque and one transparent) while the two test plates are placed on the other side of the aquarium. In total, we ran 200 trials per fish over a period of 10 consecutive days. To solve the task, a cleaner had to show a significant preference towards the visitor plate, which consisted of a score of either: nine or more successful choices out of a session of 10 trials; two consecutive eight successful choices out of a session of 10 trials; three consecutive seven successful choices out of a session of 10 trials. Upon solving the initial learning phase, a reversal phase took place with the reversed role of the two plates. The reversal task is meant to deal with potential bias of the cleaners towards a preferred plate. Also, the decoration, the spatial location (i.e., left or right) and the status of the plates (i.e., visitor or resident) were counterbalanced. This controlled for potential biases and guaranteed that cleaners' ability to solve the task is based on the status of the plate only. Two variables were recorded from this task: learning the initial task only and learning both initial and reversal. The outcome was set as a binary responses: "success" and "failure".

4.4.4 Study animals and brain sampling

In the laboratory experiments, we had a total of four cognitive variables: cleaners' response to bystander effect, cleaners' access to a bystander plate, cleaners' learning abilities of the initial task of the biological market test, and cleaners' learning abilities of the reversal task of the biological market test. Performing successfully in at least two out of the four of the aforementioned behavioural measurements was considered as "high" strategic sophistication, otherwise the performance was considered as "low" strategic sophistication (Supplementary Table S1). Among the 40 cleaners tested in these laboratory experiments, 20 cleaners were selected for brain sampling based on their performance (i.e., 10 cleaners with high strategic sophistication and 10 with low strategic sophistication). The 20 selected cleaners' performance in the two laboratory-based cognitive tasks correlated significantly (GLM: N=20, $X^2 = 5.894$, df = 1, $p = 0.015$, Supplementary Fig. S4b), wherein cleaners that solved the initial learning in the biological market task ate significantly more against their preferences in the presence of a bystander than in its absence (Fig. S4).

Selected cleaners were sacrificed by a rapid cervical transection. Immediately after, the upper part of the scales were removed to allow easy access to the brain tissue. We dissected the brain into five main brain parts under a stereomicroscope Zeiss steREO Discovery.V8 with a zoom set at 7:1. The five brain parts were: telencephalon, diencephalon, mesencephalon, cerebellum and brain stem (Supplementary Fig. S5). Brain tissue was then weighed and fixed for 24h in 4% paraformaldehyde (PFA) solution at 4°C. The brain tissue was then transferred to PBS 0.1% sodium azide solution and stored at 4°C. Three brain parts samples that belongs to two fish had an error while reading tissue weight, and as it was not possible try to weighing them again, their weight was recorded as "NA". Samples were shipped from Lizard Island to the Gulbenkian Institute in Lisbon for further brain tissue analyses.

4.4.5 Brain cells quantification

To count total cells and neuron numbers from the dissected brain parts, we followed the "isotropic fractioned" method suggested and validated by Herculano-Houzel and Lent [2005]. The experimenter ZT was blind to the identity of the samples during this part of the data collection. The method consisted of dissociating brain tissue using a tissue grinder. To facilitate dissolving brain cells membrane while simultaneously ensuring that nuclear membranes remain intact, we grinded the tissue in a saline detergent solution. Upon tissue homogenisation, samples were stained with diamino-phenyl-indol (DAPI). In a first step, cell counts were performed for every brain part with a haemocytometer (Neubauer improved counting chamber, Brand®, Blaubrand®) under microscope with fluorescence. Samples were then run through a heat-induced retrieval phase to prepare them for an immunocytochemical identification of brain neurons. In a second step, neurons in every brain part were counted by double labelling identification: DAPI staining and NeuN (i.e., neuronal protein) antigen (Anti NeuN rabbit antibody, Cy3 conjugate, Merck Millipore) labelling. Detailed protocol step-by-step procedure is on the public repository for laboratory protocols http://dx.doi.org/10.17504/protocols.io.wj8fcrw [PROTOCOL DOI].

4.4.6 Data analyses

All statistical analyses and figures were generated with the open source software R version 3.5.1 (2018-07-02). Neuroanatomical traits of telencephalon and diencephalon, either jointly (i.e., forebrain) or separately, were first scaled to total brain measurements, then log transformed and corrected with log body size (i.e., body mass and body length). All analyses were run with Linear Model (LM). Statistical tables were generated by the Anova () test type II sum of square test. The statistical test syntax was the following throughout all the statistical analyses (see statistical script along archived data) [log (brain region measure / total brain measure) / log (body mass) ~ performance level * cleaner density + log (body length)]. Statistical models' assumptions, such as normal distribution and homoscedasticity of the variance of the residuals were met.

4.5 Ethical note

The Animal Ethics Committee of the Queensland government (DAFF) approved the project under the number CA 2017-05-1063.

4.6 Data availability

Link available for the reviewing process: https://figshare.com/s/607187558db4bb5150ad; the DOI will be active once the data is made publicly available upon acceptance.
Data used in the study, the scripts for statistical analyses, and figures are available in the repository Figshare (Data DOI: https://10.6084/m9.figshare.7415576).

4.7 Acknowledgements

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4.8 Author's contributions

ZT and RB designed the study with input from RO. RB collected fish survey data. ZT and YE collected field data and ran the laboratory cognitive experiments. YE encoded the video observations. ZT collected fish brains, counted brain cells, analysed the data, generated the figures, and wrote the first draft of the manuscript. MT contributed to the cell quantification part. ZT and RB finalised the paper with input from all the authors. All authors declare no competing interests.

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Supplementary Information for Chapter IV

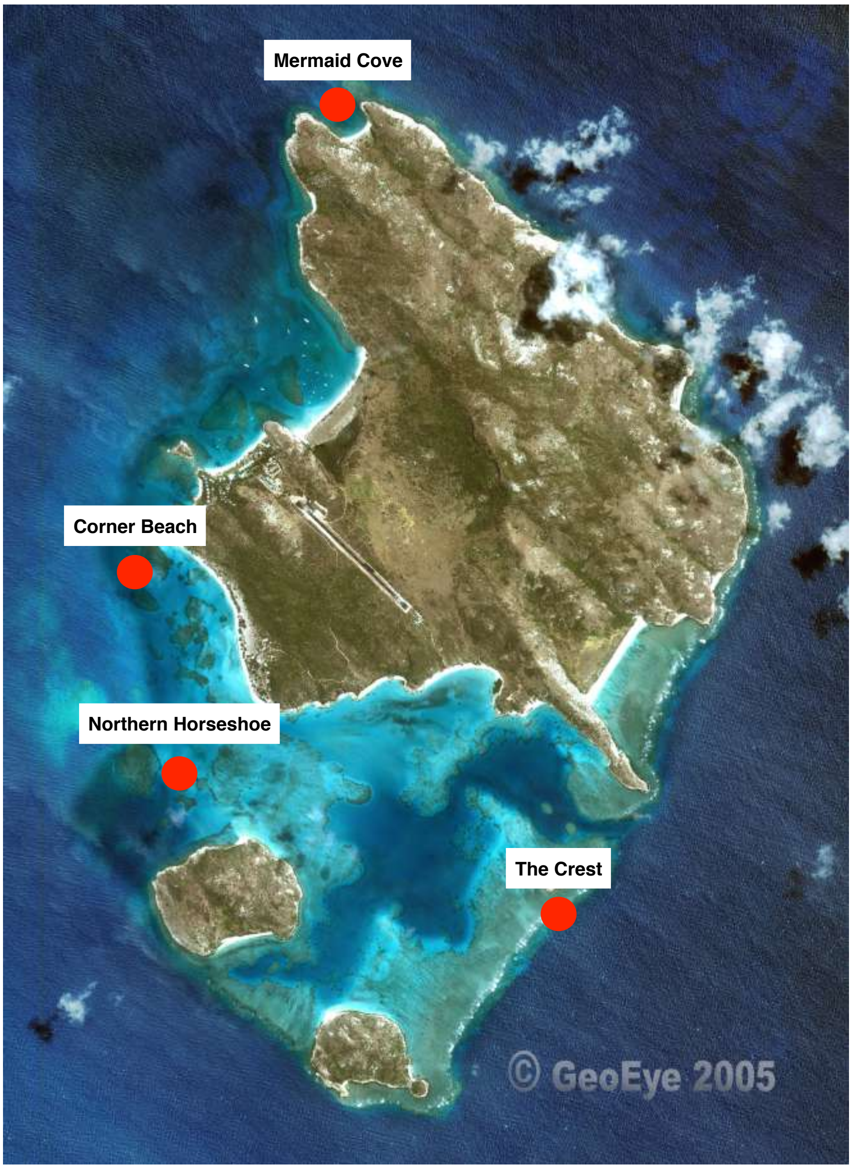

Figure S1 | Map of Lizard Island. Google maps satellite image showing the detailed location of the four study reef sites at Lizard Island. Credit *GeoEye2005*.

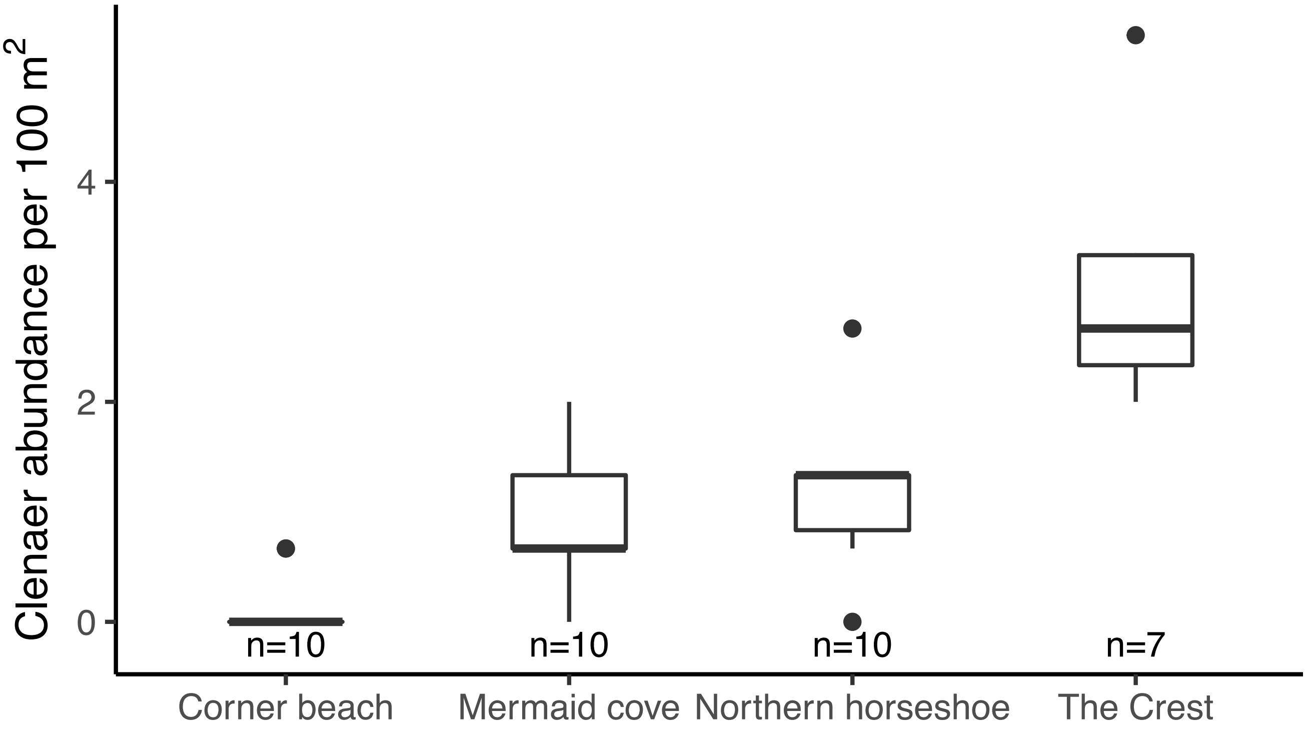

Figure S2 | Social complexity of the studied sites. Boxplot of median and interquartile of cleaner density per study site. "n" shows the number of transect replicate ran per site.

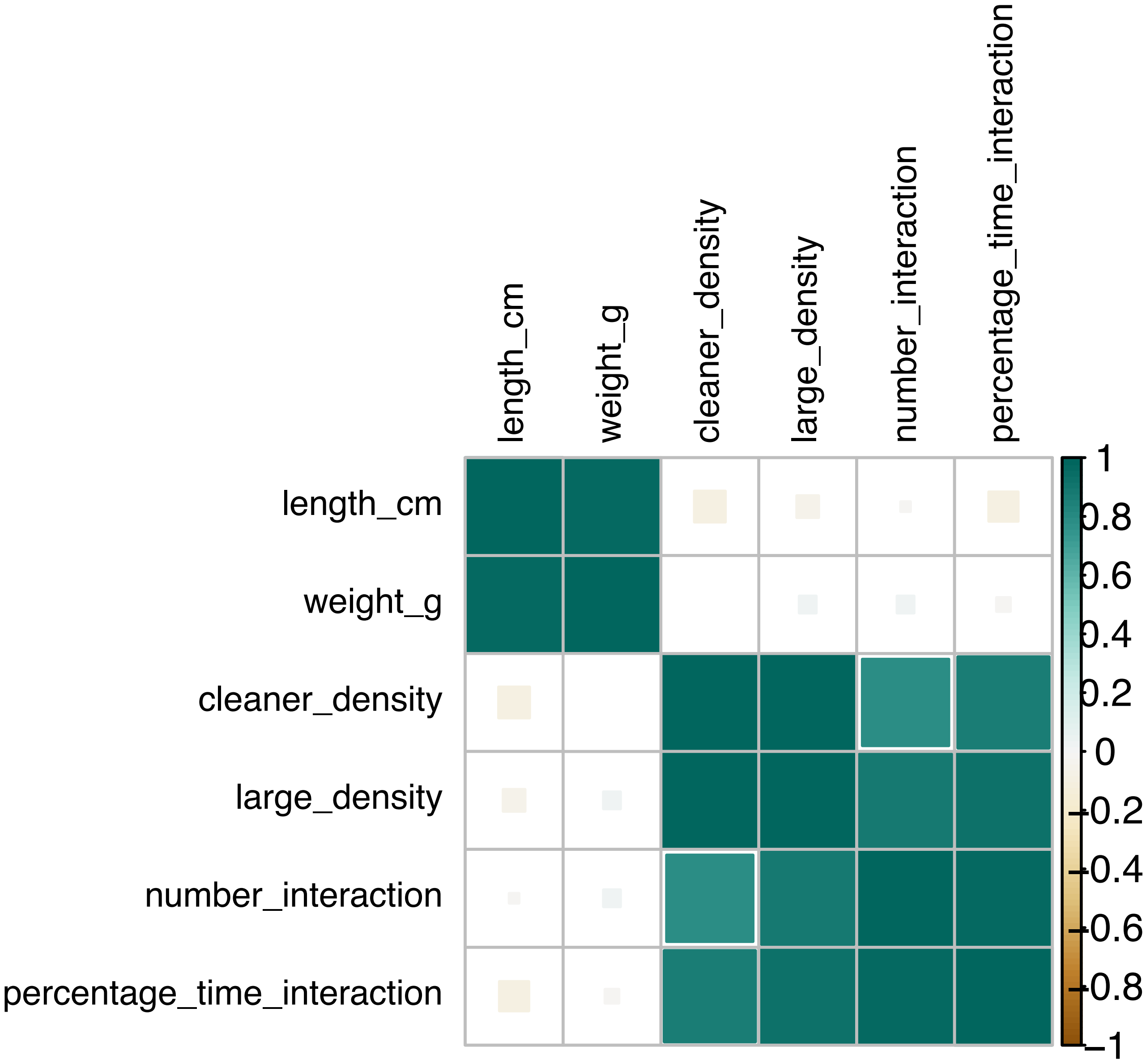

Figure S3 | Correlation matrix of fish survey data and behavioral observations. length_cm: body total length in cm; weight_g: body mass in g; cleaner_density: cleaner count per 100 m^2; large_density: large client count per 100 m^2; number_interaction: frequency of cleaner-client interactions per 30 min video observation; percentage_time_interaction: percentage of time spent by the cleaner interacting with clients per 30 min of video observation. The heatmap scale on the left shows the correlation coefficient.

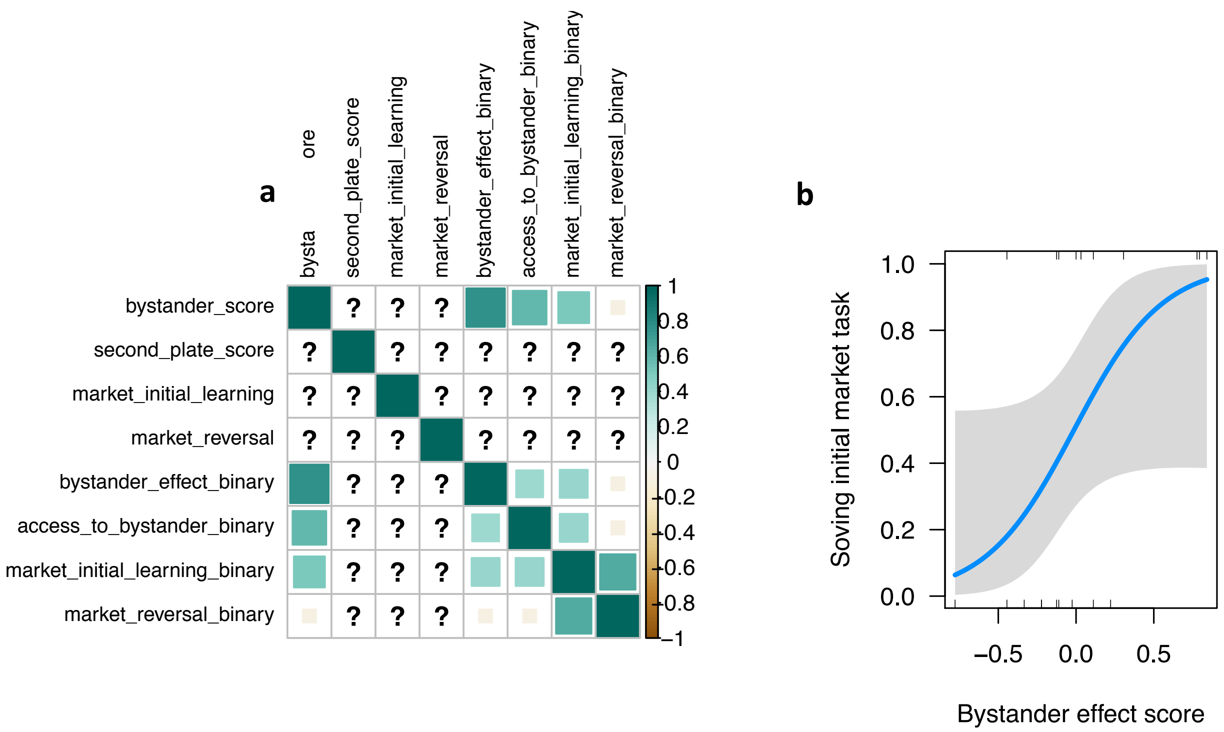

Figure S4 | Relationship between the cognitive performance measurements. a shows the correlation matrix of the recorded performances in the two laboratory-cognitive based tasks: bystander_score: we first calculate the total ratio of flake items to prawn items eaten by the cleaner over trials, then we calculate the difference between score when cleaners were presented with two plates (with a bystander) and when the cleaners were presented with single plate (no bystander. it shows if cleaners would adjust their feeding in presence of a bystander plate; second_plate_score: the ratio of flakes to prawn eaten from the bystander plate in the "image-scoring" condition from the bystander effect task. market_initial_learning number of trials needed to solve the initial test of the biological market task; market_reversal: number of trials needed to solve the initial and the reversal of the biological market task bystander_effect_binary: here we transformed the image_score data to binomial outcome: scores ≤ 0 were classified as "0", and scores > 0 were classified as "1"; access_to_bystander_binary "1" for the Cleaners who succeeded to feed from the bystander plate, while "0" for cleaners who failed; market_initial_learning_binary: binomial response in the initial learning of the biological market task, "1" cleaners solving the nitial test, while "0" is for cleaners who failed; market_reversal_binary: binomial response in the biological market task. "1" cleaners solving both the initial and reversal test, while "0" is for cleaners failed to solve initial and reversal in the biological market task. The heatmap scale on the left shows the correlation coefficient. **b** is the fitted line from the GLM model testing the relationship correlation between bystander effect score and ability of solving the initial biological market task (*$p \leq 0.01$).

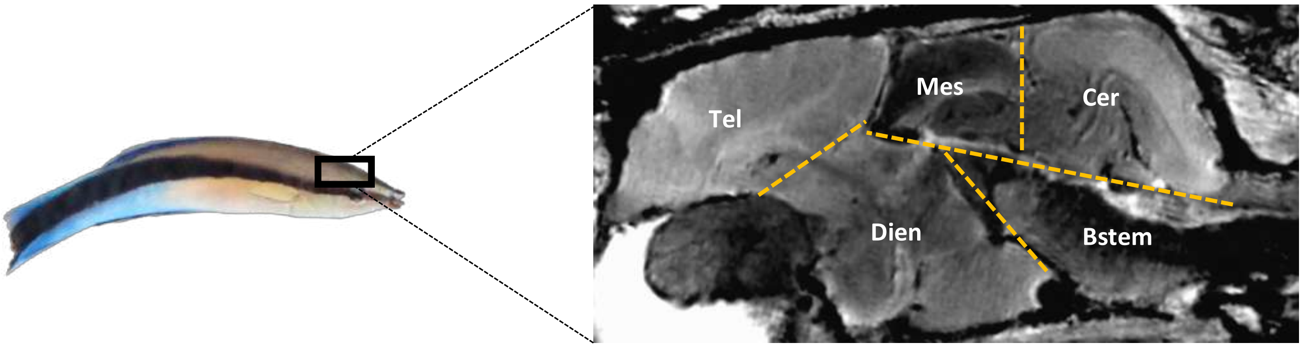

Figure S5 | An MR image showing the main five brain parts of the *Labroides dimidiatus* brain. A representative MR image of a transversal section of *L. dimidiatus* brain inside its braincase, the dashed lines show the delineation of the five brain parts followed during the dissection: Tel: telencephalon, Die: diencephalon, Mes: mesencephalon, Cer: cerebellum, and Bstem: brain stem. This MR image is provided here only as an example to show the anatomy of the cleaner fish brain. Images generated with OsiriX Lite$^{®}$ V. 9.5.2. Photo credit: *Zegni Triki.*

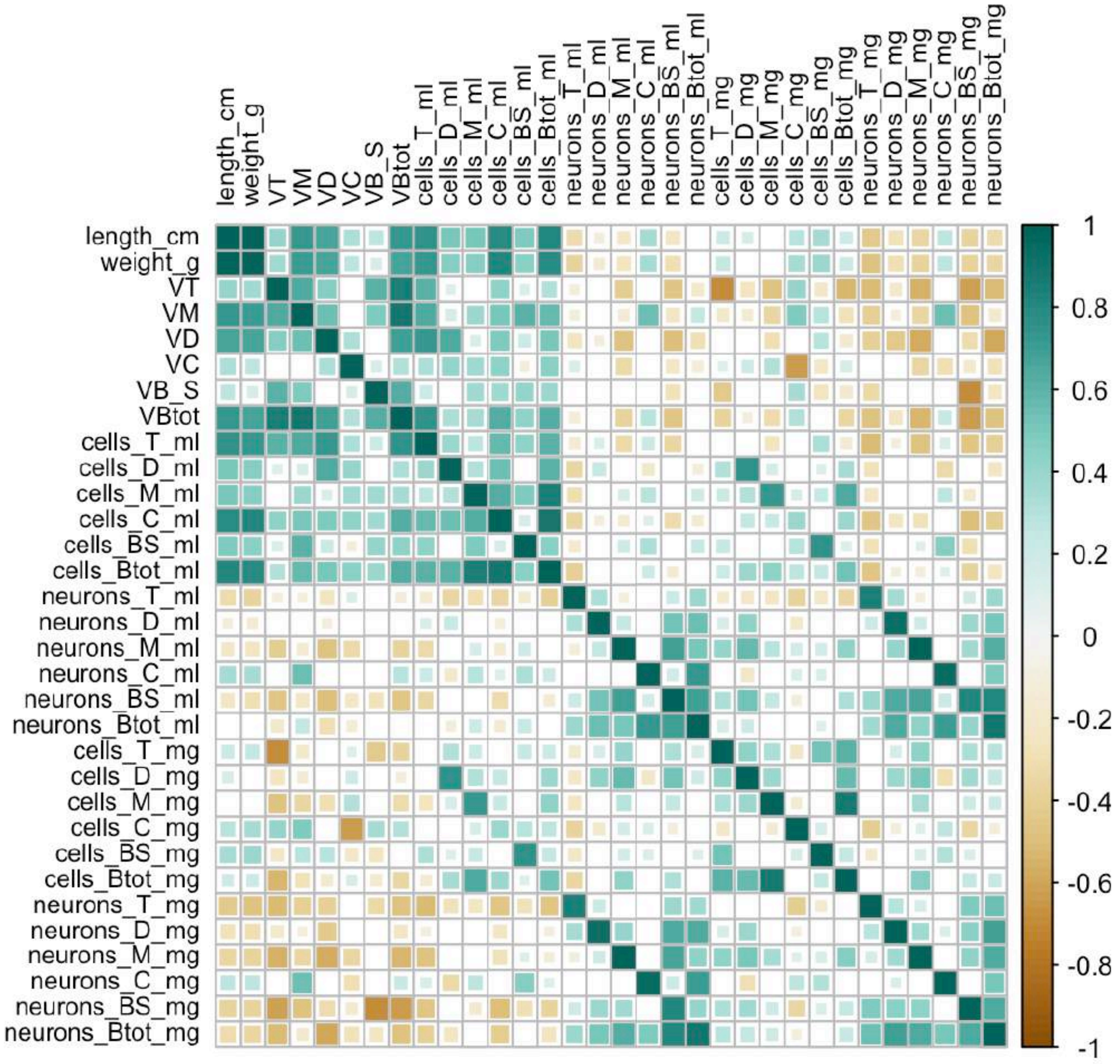

Figure S6 | Heatmap correlation matrix of all the brain measurements. The heatmap scale on the left shows the correlation coefficient. length_cm: body total length in cm; weight_g: body mass in g; VT: volume of the telencephalon in mg; VM: volume of the mesencephalon in mg; VD: volume of the diencephalon in mg; VC: volume of the cerebellum in mg; VB_S: volume of the brain stem in mg; VBtot: volume of total brain in mg; cells_T_ml: total brain cells of the telencephalon counted in 1 ml of an homogenized solution of the telencephalon tissue; cells_D_ml: total brain cells of the diencephalon counted in 1 ml of an homogenized solution of the diencephalon tissue; cells_M_ml: total brain cells of the mesencephalon counted in 1 ml of an homogenized solution of the mesencephalon tissue; cells_C_ml: total brain cells of the cerebellum counted in 1 ml of an homogenized solution of the cerebellum tissue; cells_BS_ml: total brain cells of the brain stem counted in 1 ml of an homogenized solution of the brain stem tissue; cells_Btot_ml: total brain cells of the whole brain; neurons_T_ml: total brain neurons of the telencephalon counted in 1 ml of an homogenized solution of the telencephalon tissue; neurons_D_ml: total brain neurons of the diencephalon counted in 1 ml of an homogenized solution of the diencephalon tissue; neurons_M_ml:

total brain neurons of the mesencephalon counted in 1 ml of an homogenized solution of the mesencephalon tissue; neurons_C_ml: total brain neurons of the cerebellum counted in 1 ml of an homogenized solution of the cerebellum tissue; neurons_BS_ml: total brain neurons of the brain stem counted in 1 ml of an homogenized solution of the brain stem tissue; cells_Btot_ml: total brain neurons of the whole brain; cells_T_mg: telencephalon cell density per 1 mg of telencephalon tissue; cells_D_mg: diencephalon cell density per 1 mg of diencephalon tissue; cells_M_mg: mesencephalon cell density per 1 mg of mesencephalon tissue; cells_C_mg: cerebellum cell density per 1 mg of cerebellum tissue; cells_BS_mg: brain stem cell density per 1 mg of brain stem tissue; cells_Btot_mg: whole brain cell density per 1 mg of whole brain tissue; neurons_T_mg: telencephalon neuron density per 1 mg of telencephalon tissue; neurons_D_mg: diencephalon neuron density per 1 mg of diencephalon tissue; neurons_M_mg: mesencephalon neuron density per 1 mg of mesencephalon tissue; neurons_C_mg: cerebellum neuron density per 1 mg of cerebellum tissue; neurons_BS_mg: brain stem neuron density per 1 mg of brain stem tissue; neurons_Btot_mg: whole brain neuron density per 1 mg of whole brain tissue.

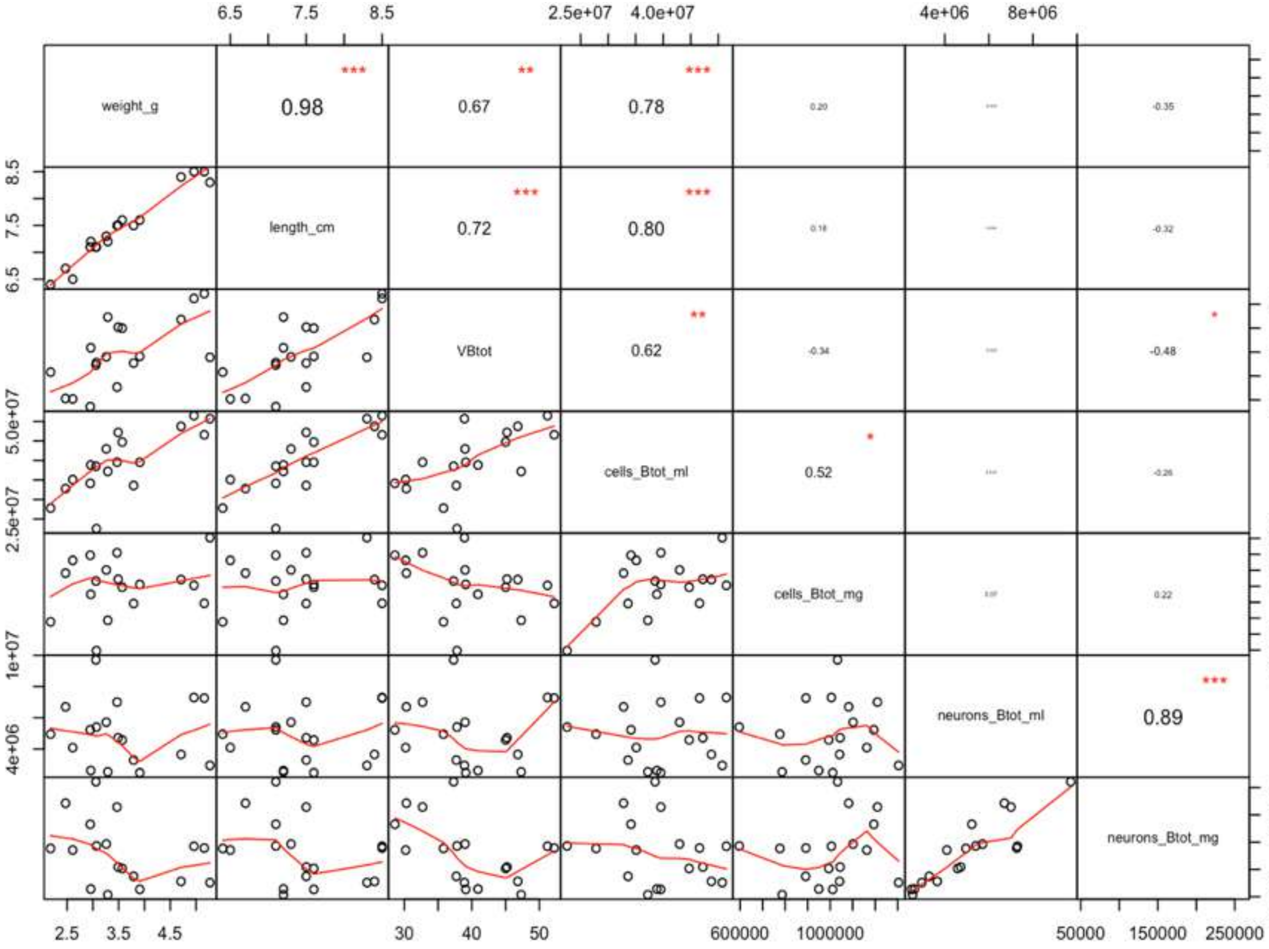

Figure S7 | Correlation matrix with scatterplots of the total brain measurements. see Fig. S6 caption for abbreviation information. Numbers refer to the correlation coefficient calculated “r” with the method “Pearson”: * $p \leq 0.05$; ** $p < 0.01$; ***$p < 0.001$

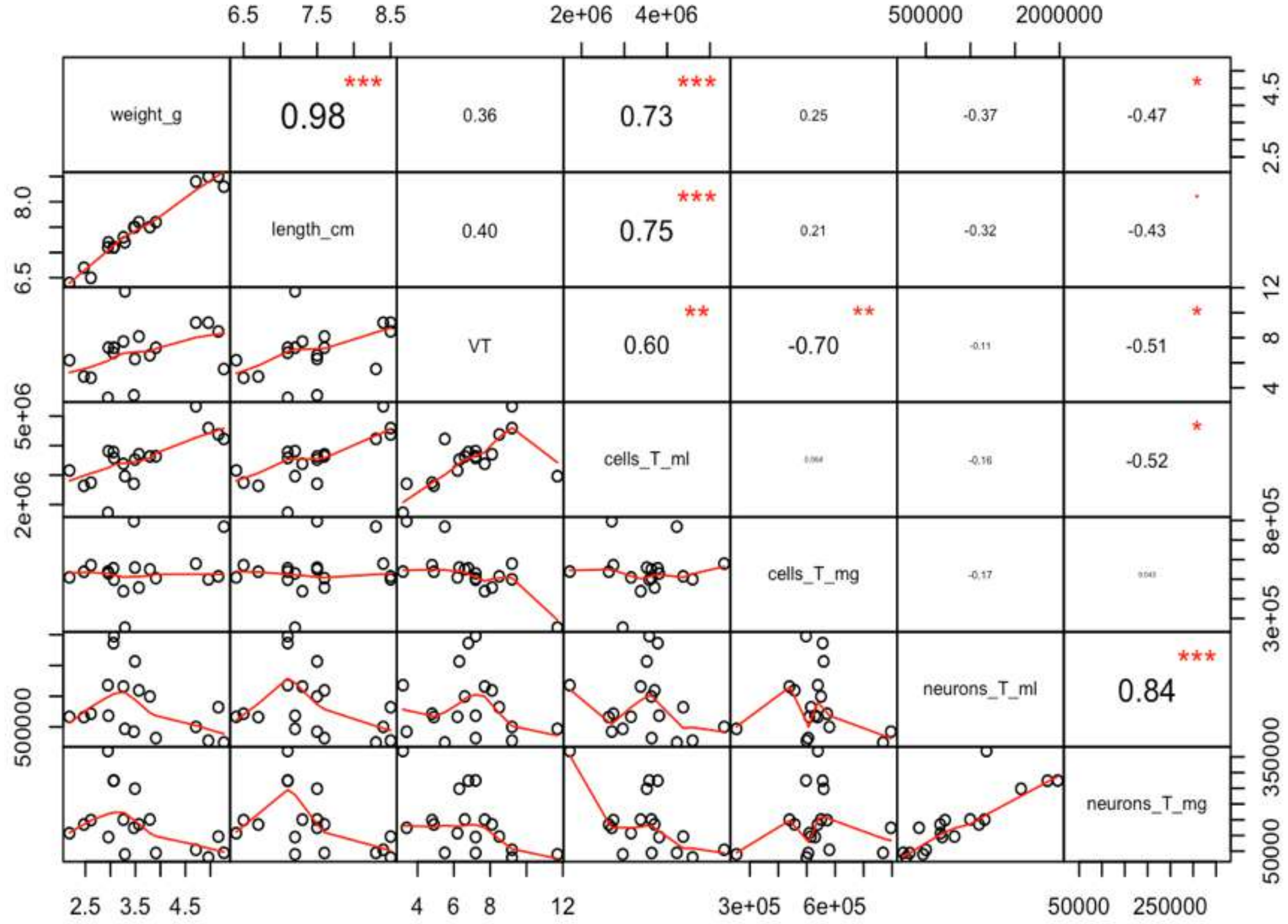

Figure S8 | Correlation matrix with scatterplots of the telencephalon measurements. Numbers refer to the correlation coefficient calculated “r” with the method “Pearson”: * $p \leq 0.05$; ** $p < 0.01$; ***$p < 0.001$

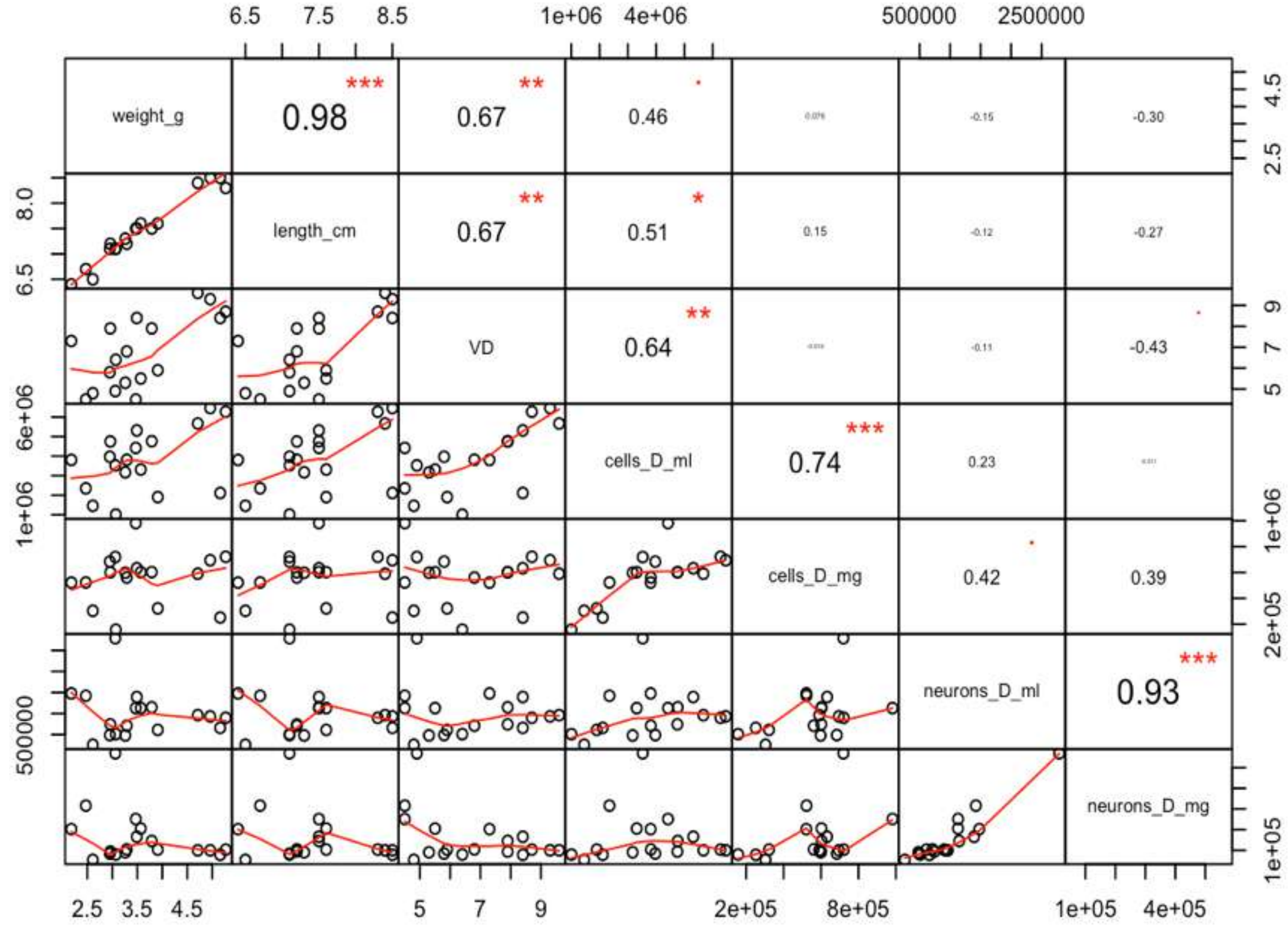

Figure S9 | Correlation matrix with scatterplots of the diencephalon measurements. Numbers refer to the correlation coefficient calculated "r" with the method "Pearson": * $p \leq 0.05$; ** $p < 0.01$; ***$p < 0.001$

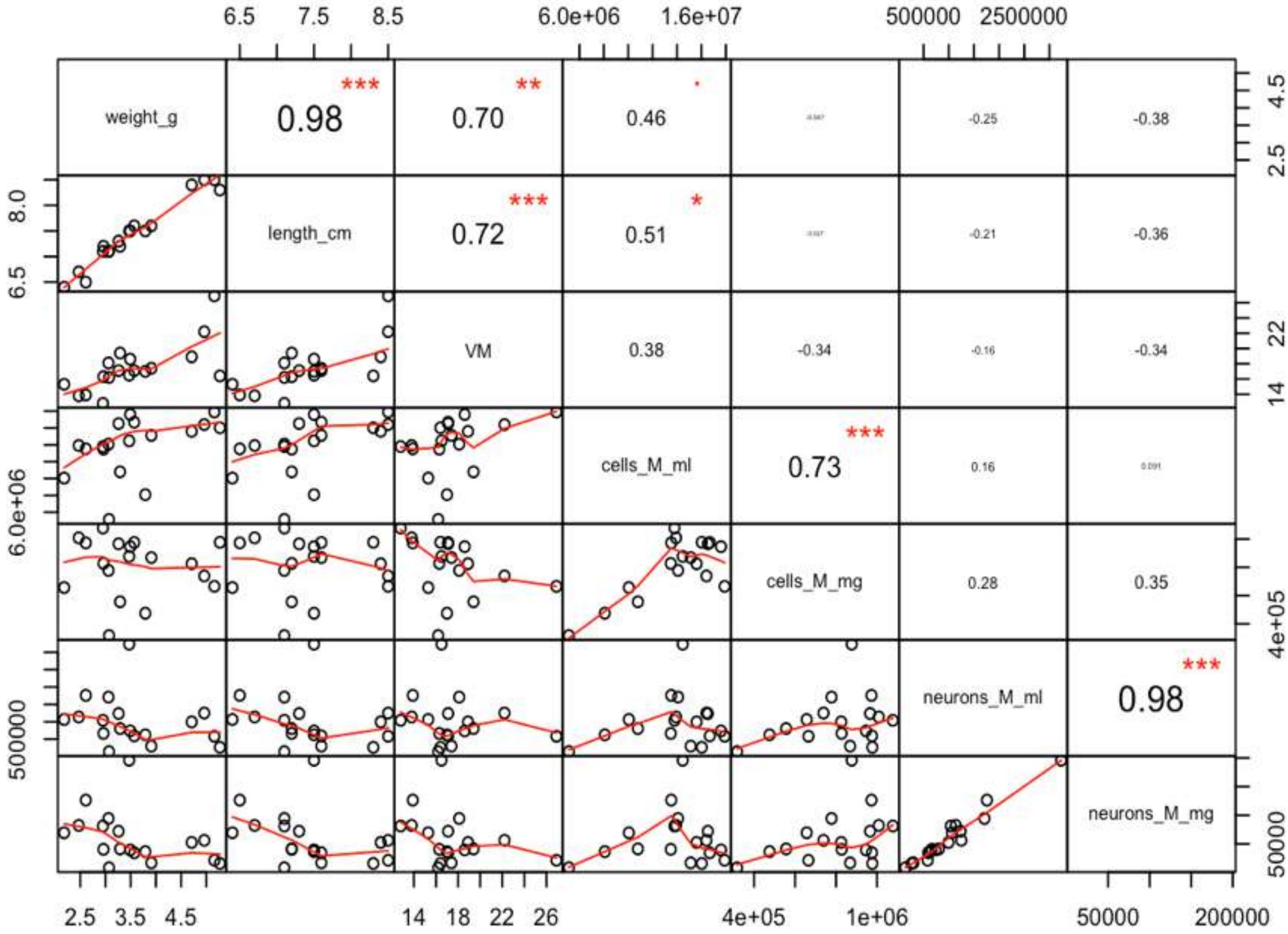

Figure S10 | Correlation matrix with scatterplots of the mesencephalon measurements. Numbers refer to the correlation coefficient calculated “r” with the method “Pearson”: * $p \leq 0.05$; ** $p < 0.01$; ***$p < 0.001$

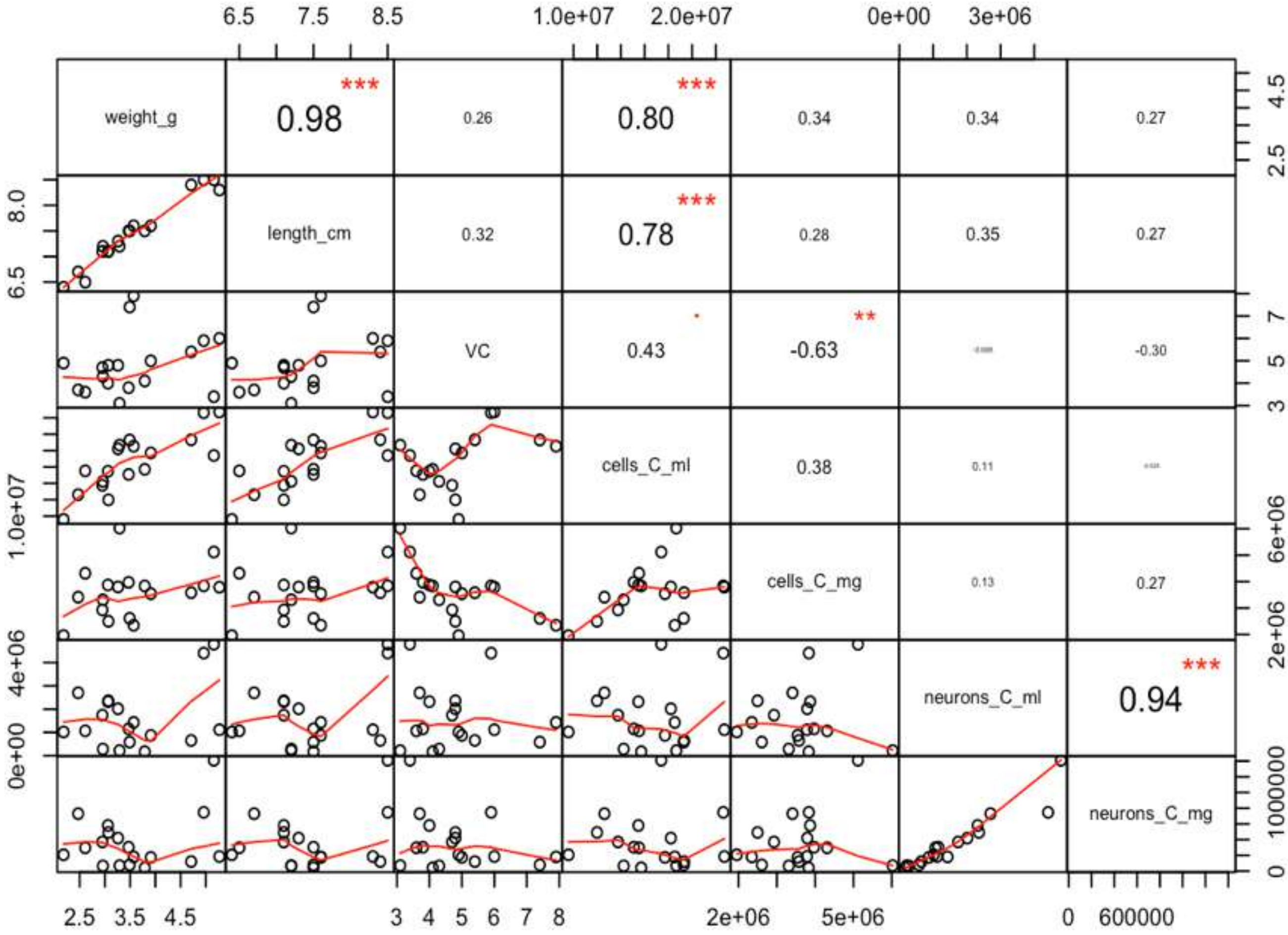

Figure S11 | Correlation matrix with scatterplots of the Cerebellum measurements. Numbers refer to the correlation coefficient calculated "r" with the method "Pearson": * $p \leq 0.05$; ** $p < 0.01$; ***$p < 0.001$

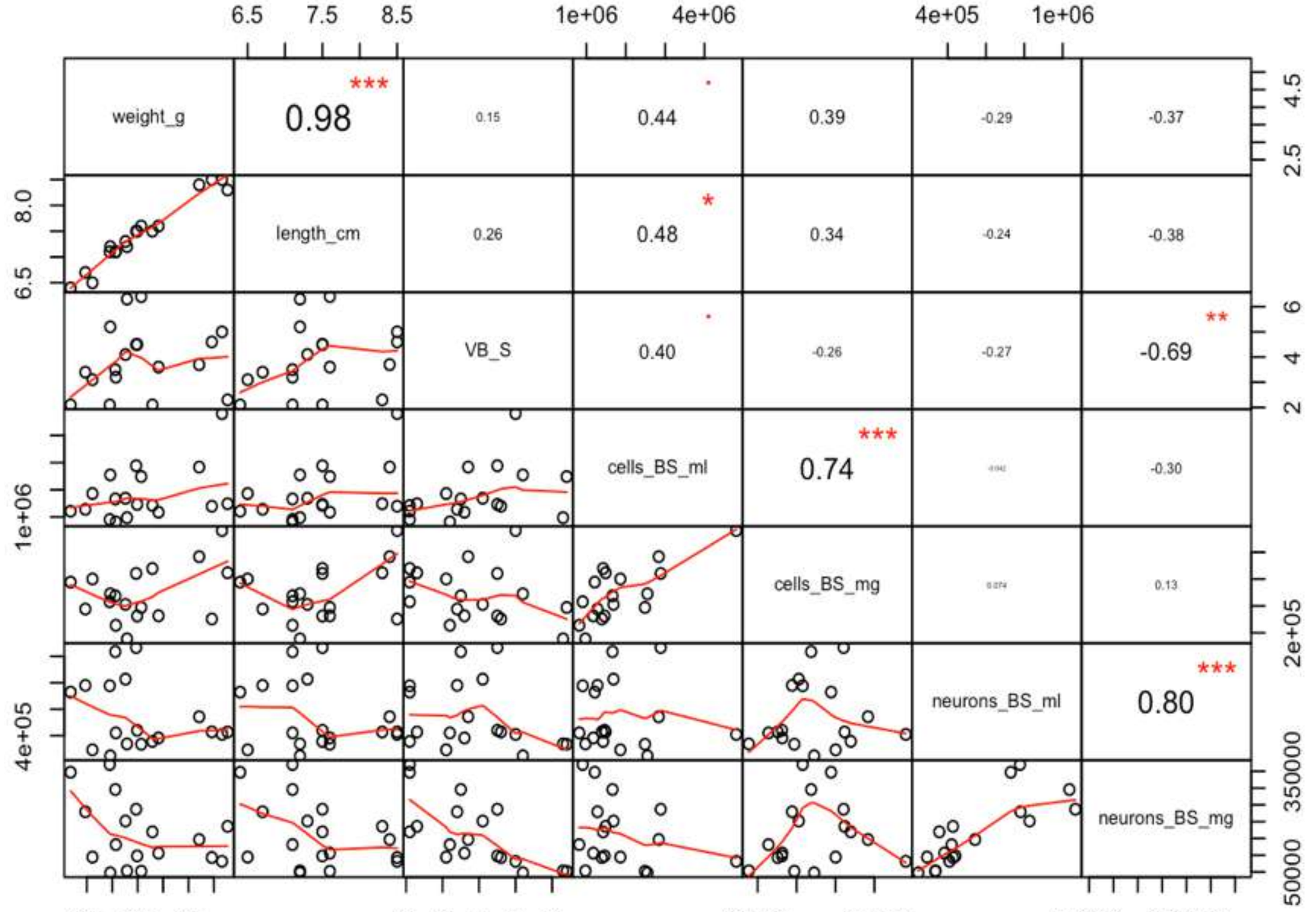

Figure S12 | Correlation matrix with scatterplots of the brain stem measurements. Numbers refer to the correlation coefficient calculated “r” with the method “Pearson”: * $p \leq 0.05$; ** $p < 0.01$; ***$p < 0.001$

Table S1. Summary table of the neuroanatomical traits measured in this study.

Variable	N	Mean	SD	Median	se	min	max
Body length (cm)	20	7.4	0.61	7.30	0.14	6.40	8.50
Body weight (g)	20	3.5	0.88	3.28	0.20	2.18	5.27
Telencephalon size (mg)	19	7.0	2.09	7.20	0.48	3.20	11.70
Diencephalon size (mg)	19	6.8	1.66	6.80	0.38	4.50	9.60
Mesencephalon size (mg)	19	17.4	3.14	17.10	0.72	12.80	26.90
Cerebellum size (mg)	20	4.8	1.26	4.75	0.28	3.10	7.90
Brainstem size (mg)	20	4.0	1.38	3.65	0.31	2.10	6.40
Total brain size (mg)	18	39.8	7.02	38.95	1.65	28.60	52.20
Telencephalon cell count per 1ml	20	3434375.00	953159.78	3606250.00	213133.01	1162500.00	5331250.00
Diencephalon cell count per 1 ml	20	3721250.00	1544496.81	3809375.00	345359.99	1006250.00	6462500.00
Mesecnephalon cell count per 1ml	20	14166562.50	3388396.46	14793750.00	757668.48	5137500.00	18518750.00
Cerebellum cell count per 1ml	20	16548750.00	3346757.19	16556250.00	748357.66	9581250.00	22718750.00
Brainstem cell count 1 ml	20	1830312.50	946110.19	1475000.00	211556.67	818750.00	4806250.00
Total brain cell count	20	39701250.00	7515848.00	39321875.00	1680594.70	22531250.00	51487500.00
Telencephalon neuron count per 1ml	20	815913.47	519009.87	674253.42	116054.13	246116.50	1978214.29
Diencephalon neuron count per 1 ml	20	967292.44	544176.41	855649.24	121681.55	258321.15	2788017.75
Mesecnephalon neuron count per 1ml	20	962981.78	689838.97	769769.98	154252.68	137407.13	3232657.66
Cerebellum neuron count per 1ml	20	1518168.63	1281731.75	1091409.24	286603.93	169559.35	4791796.88
Brainstem neuron count 1 ml	20	546510.69	241609.87	434415.05	54025.61	246936.76	1066316.27
Total brain neuron count	20	4810867.01	1995855.88	4650118.72	446286.94	2481241.06	9717886.00
Telencephalon cell density per 1mg	19	533158.97	113283.99	529513.89	25989.13	253205.13	795955.88
Diencephalon cell density per 1mg	19	558308.98	191362.44	597877.36	43901.56	157226.56	981944.44
Mesencephalon cell density per 1mg	19	819132.45	206988.01	868893.68	47486.31	317129.63	1077636.72
Cerebellum cell density per 1mg	20	3569672.88	955696.89	3682581.02	213700.32	1955357.14	6022177.42
Brain stem cell density per 1mg	20	479541.22	193167.84	421566.49	43193.64	154761.90	961250.00

Total brain cell density per 1mg	18	1004360.47	172682.63	1022095.46	40701.69	596064.81	1304145.24
Telencephalon neuron density per 1mg	19	134817.86	95225.24	124368.11	21846.17	30079.56	368553.54
Diencephalon neuron density per 1mg	19	155932.33	120571.50	103738.19	27661.00	53816.91	568983.21
Mesencephalon neuron density per 1mg	19	58756.53	45036.32	41413.31	10332.04	8481.92	195918.65
Cerebellum neuron density per 1mg	20	338571.08	330482.74	196866.80	73898.19	41355.94	1409352.02
Brain stem neuron density per 1mg	20	159904.68	95749.95	139110.18	21410.34	47487.84	370138.31
Total brain neuron density per 1mg	18	130698.02	59171.33	136932.90	13946.82	53640.99	260533.14

Table S2. Cleaners' performance in the laboratory experiments. The table show cleaners' performance in every laboratory task. It also shows the identity of the cleaners selected for brain sampled for brain cells count. High performance in at least two out of the four cognitive variables was considered as "high" strategic sophistication, otherwise it was labeled as "low" strategic sophistication.

Study site	Cleaner identity	Bystand-er effect	Access to bystander-plate	Initial learn-ing	Reversal learning	Brain sampl-ing	Strategic sophisticat-ion
Northern horseshoe	CL 01	yes	yes	success	failure	yes	high
Northern horseshoe	CL 02	yes	yes	success	failure	yes	high
Northern horseshoe	CL 03	no	yes	success	success	yes	high
Northern horseshoe	CL 04	no	yes	failure	failure	yes	low
Northern horseshoe	CL 05	no	no	failure	failure	yes	low
Northern horseshoe	CL 06	yes	yes	success	failure	no	high
Northern horseshoe	CL 07	no	no	success	failure	no	low
Northern horseshoe	CL 08	no	no	success	failure	no	low
Northern horseshoe	CL 09	yes	no	success	failure	no	high
Northern horseshoe	CL 10	yes	no	failure	failure	no	low
Corner beach	CL 11	no	no	success	success	yes	high
Corner beach	CL 12	no	yes	success	failure	no	high
Corner beach	CL 13	yes	no	success	failure	no	high
Corner beach	CL 14	yes	yes	failure	failure	no	high
Corner beach	CL 15	no	no	failure	failure	yes	low
Corner beach	CL 16	yes	yes	failure	failure	no	high
Corner beach	CL 17	no	yes	failure	failure	yes	low
Corner beach	CL 18	no	no	failure	failure	yes	low
Corner beach	CL 19	no	no	success	failure	no	low
Corner beach	CL 20	yes	no	success	failure	no	high
Mermaid cove	CL 21	no	no	success	failure	no	low
Mermaid cove	CL 22	yes	no	failure	failure	yes	low
Mermaid cove	CL 23	yes	no	failure	failure	no	low

Mermaid cove	CL 24	no	no	success	success	yes	high
Mermaid cove	CL 25	no	no	success	failure	no	low
Mermaid cove	CL 26	yes	yes	failure	failure	no	high
Mermaid cove	CL 27	yes	yes	success	success	yes	high
Mermaid cove	CL 28	yes	yes	success	failure	yes	high
Mermaid cove	CL 29	yes	yes	success	failure	yes	high
Mermaid cove	CL 30	yes	yes	failure	failure	no	high
The Crest	CL31	no	no	failure	failure	yes	low
The Crest	CL32	no	no	failure	failure	yes	low
The Crest	CL33	yes	yes	failure	failure	no	high
The Crest	CL34	no	no	failure	failure	yes	low
The Crest	CL35	yes	yes	failure	failure	no	high
The Crest	CL36	yes	no	success	success	yes	high
The Crest	CL37	yes	no	failure	failure	yes	low
The Crest	CL38	no	no	success	success	yes	high
The Crest	CL39	yes	yes	failure	failure	no	high
The Crest	CL40	yes	yes	success	failure	no	high

GENERAL DISCUSSION

D.1 Summary

This thesis aimed to unravel the mechanisms underlying the intraspecific behavioural variation of the cleaner wrasse previously documented by Wismer et al. [2014]. The results of that study identified several potential causes for the observed variation in strategic sophistication, namely client density and diversity, cleaner density, cleaner to client ratio, and the frequency of cleaner-client interactions. In Chapter I, the recent extreme weather events affecting the Great Barrier Reef, (i.e., consecutive cyclones and the 2016 El Niño event [Pizarro et al., 2017]) created a natural experiment to explore potential consequences of community changes on cleaner fish. After the perturbations, I found that cleaner densities were reduced by 80%, which was a disproportionate decrease compared to the variety of reef fish clients from which cleaners remove ectoparasites. Consequently, shifts in supply and demand yielded an increase in the clients' demand for cleaning. Therefore, clients became less selective towards cleaners, while cleaners were able to choose from a multitude of partners. In parallel, I found a significant decline in the ability of cleaners to manage their reputation and to learn to prioritise ephemeral food sources to maximise food intake in laboratory experiments. In other words, cleaners failed to display the previously documented strategic sophistication that made this species a prime example for fish intelligence. Taken together, low population densities may have caused various effects on cleaner/client individual behaviour, and as a consequence, interspecific interactions. At the same time, my data suggest that a recovery of population densities would cause a recovery of previously described interaction patterns and cleaner strategic sophistication within the lifetime of individuals.

Meanwhile, I have been involved in four side projects that are tightly linked to Chapter I (see Annex). First, I am a middle author on a study that compared how cleaners manage their reputation under natural conditions, depending on whether they live at a high density or a low density site [Binning et al., 2017]. Cleaners were injected with either cortisol or saline, wherein cortisol injections induce increased rates of cheating, which harms the cleaners' reputation. Previous research has documented that cleaners can selectively increase cheating of large visitor clients while providing more tactile stimulation to small residents to improve their reputation [Bshary, 2002; Soares et al., 2014]. Our study maintained these results for cleaners from a high density site and failed to find the same results for cleaners from a low density site [Binning et al., 2017]. I contributed by demonstrating that differences in reputation management could not be explained by differences in basal cortisol levels.

The second published paper investigated the cleaners' ability to count [Triki, and Bshary, 2018]. Cleaners had to learn to discriminate plates showing either 2 or 5 black squares, or 5 and 8 squares as only one plate offered a food reward on its back. Once individuals reached learning criteria, we ran various controls to confirm that the relevant information was indeed the number of squares (rather than total symbol surface area or density [Dadda et al., 2009; Agrillo et al., 2010]). Crucially for the topic of my PhD thesis, cleaners from high and low density sites performed equally well in this task.

The third manuscript in the Annex is accepted for publication [Triki and Bshary, *accepeted*]. I continued with the monitoring of fish diversities and density in the years following the cyclones and the El Niño event in order to analyse in more detail how these major perturbations affected different functional groups of fishes, like grazers, corallivores, piscivores, etc [Bellwood et al., 2004; Wilson et al., 2006]. The results show that the vast majority of functional groups was negatively affected, with piscivores being a major exception, at least within the time frame of the study. Data that compare fish communities before and after the perturbations are rare. As they are of interest for conservation management, we decided to publish the data though they do not fit our own research focus.

The fourth manuscript is the study by Wismer et al. [*in review*], wherein the authors investigated in more detail what cues might be salient in the biological market task, and how much high and low performing cleaners differ with respect to the cues they rely on. The study showed that both juvenile and adult cleaners, from low and high social complexity, perceived size as salient cue: In the absence of colour cues, these cleaners can solve the task if the visitor plate is larger and fail if it is smaller than the resident plate. However, when the colour (as the causal cue) and size (as a correlative cue) cues were combined, all subjects eventually performed above chance. In summary, cleaners from a high social complexity relied on colour, while cleaners from a low social complexity relied on size. My main contribution to this study was the analysis of behavioural video recordings of cleaner-client natural interactions, and quantifying the frequency of visitor clients leaving a cleaner if initially made to wait for the cleaning service. The results showed that cleaners from the high social complexity sites experienced such psychological punishment (client leaving) more frequently than cleaners from low social complexity sites.

The results from Chapter I still did not enable me to determine which ecological factors have causal effects on cleaning interaction patterns and hence on the cleaners' strategic sophistication. In Chapter II, I therefore explored in detail which market conditions may affect the complexity of social life and its cognitive challenges [Noë et al., 2001], and hence the strategic sophistication displayed in laboratory-based cognitive experiments. I found that cleaner strategic sophistication correlated with the degree of competition over access to client reef fishes. Only cleaners from sites with high outbidding competition, characterised by high cleaner and visitor densities, and visitor client behaviour enforcing such competition, consistently increased cooperation to gain access to an image-scoring audience, and/or learned to prioritise an ephemeral food source over a simultaneously offered permanent one. Thus, two hallmarks of cleaners' strategic sophistication are linked to strong market competition [Bshary, 2001; Bshary, and Noë, 2003].

The "social brain hypothesis" proposes a causal link between social complexity and either brain size or the size of key brain regions known to be involved in cognitive processing and decision-making [Dunbar, 1992; Dunbar, 1995]. Therefore, in Chapter III, I used the main outcome from Chapter II as the best proxy for the social complexity degree in cleaner's ecology, which is the cleaner fish local population density. This measure correlates well with the local population densities of large client fish (i.e., visitor clients). I then tested whether social complexity can predict cleaner fish key brain part sizes. The key brain parts were identified based on their previously documented role in underlying social behaviour in teleosts, such as the telencephalon and diencephalon that together forms the fish forebrain [O'Connell, and Hofmann, 2011; Goodson, 2005]. Here, I found a positive relationship between the local density of cleaners and the size of the cleaner's diencephalon. I employed three scaling methods that yielded similar results, wherein the relative diencephalon size correlated with local cleaner density. While telencephalon size estimates were noted as less reliable due to potential artefacts introduced during dissection and fixation, results are at least indicative of the same trend. In contrast, the other three brain region sizes (i.e., mesencephalon, rhombencephalon, and brain stem) were independent of the social complexity level of cleaners' habitats. Importantly, as the forebrain harbours various nuclei of the social decision-making network [O'Connell, and Hofmann, 2011], it appears that social complexity can be a driver of a selective enlargement of relevant brain regions [Dunbar, 1992; van Schaik et al., 2012].

In Chapter IV, as a follow up to Chapter III, I aimed to go beyond brain size and explore other neuroanatomical traits such as brain cell and neuron counts in addition to brain size. Importantly, I tested the effects of social complexity and performance in cognitive tasks. Here, I showed that in cleaner fish, the abundance of individuals as a proxy for social complexity in natural habitats correlates positively with the relative cell and neuron count in the cleaner forebrain. Furthermore, relative forebrain size increased with the increase in the social complexity but only in the brains of individuals with high strategic sophistication. Thus, within a species, cleaner abundance as a proxy for both intra- and interspecific social complexity, correlated with forebrain complexity as a function of cognitive performance. However, I suggest that high strategic sophistication can only be useful in high cleaner density areas, as visitor clients only readily switch between cleaners if the distance between cleaners (i.e., the cleaning stations) is relatively short [Chapter II, Triki et al. *in review*]. The concept of social competence [Taborsky, and Oliveira, 2012; Bshary, and Oliveira, 2015] would thus predict that high performing cleaners from high-density sites and low performing cleaners from low-density sites are the most socially competent (i.e., through applying locally adaptive optimal strategies), and should have larger relative forebrains with more neurons. Indeed, cleaners with optimal strategies had relatively larger forebrain and higher neuron counts. Furthermore these two forebrain neuroanatomical traits also correlated positively with the degree of social complexity, independent of performance in the experiments. Thus, the results in Chapter IV confirm the results of Chapter III: cleaner density appears to be a reliable predictor of relative forebrain size. The results thus provide evidence for the hypothesis that social challenges and their solutions promote an increase in brain complexity [van Schaik et al., 2012; Dunbar, 1992; Dunbar, 1995].

D.2 The big picture

D.2.1 The cognitive ecology approach to understand variation in strategic sophistication

The outcomes of this thesis have broader implications for studying the links between ecology, cognition and brain complexity. By applying the bottom-up approach [Logan et al., 2018] in this thesis dissertation, I examined both the ecological conditions and the neural mechanisms underlying strategic sophistication in a cleaner fish. I first studied what ecological conditions apply a selective pressure on cleaner's strategic sophistication. The results indicate that within a "biological market" framework, the magnitude of supply in the form of the cleaner fish local population density (i.e., a strong correlate of large/visitor client densities) select for cleaner's strategic sophistication in the two laboratory-based cognitive tasks employed throughout the thesis. Second, I integrated this ecological factor in the analyses of the link between neuroanatomical traits of the cleaner fish and its strategic sophistication. To the best of my knowledge, this is the first data that show a link between social complexity, performance and neuroanatomical traits at the intraspecies level. In analogy to the neocortex ratio correlating positively with group size as an indicator of social complexity in primates [Dunbar, 1992; Dunbar, 1995], my data provide rare evidence that brain complexity increases as a function of cleaner fish local population density. Cleaners can thus adjust the processing power of the key brain regions ontogenetically to the richness of social challenges.

It is important to point out that the cleaner fish has a pelagic larvae stage [Victor, 1986]. This implies that the documented variation is likely driven by ontogenetic effects. However, by looking closely at the outcomes one can observe that there were two phenomena occurring simultaneously: One follows the "social brain hypothesis" [Dunbar, 1992; Dunbar, 1995], that is, there is an ontogenetic variation in cleaner fish brain complexity between sites that vary with the variation in the social complexity degree. However, the strong link found between social complexity and brain complexity, with no apparent overall effect of performance, suggest that the extra brain tissue/neurons is probably to deal with social challenges beyond reputation management and partner service priority. Unfortunately, I did not measure the intraspecific aspect of cleaners' social life and the potential variation with cleaner local population density. This can be a promising line of future research to disentangle the effects of intra- vs interspecific social interactions and their impact on the brain complexity. Also, there is a potential for manipulating the ecology of the cleaner fish during ontogeny in laboratory conditions, then test for brain adjustments.

The other phenomenon is the recorded variation in brain complexity, but within the study site. As suggested in Chapter IV, this variation might be due to differences in the locally adaptive behaviour (i.e., social competence) of cleaners. However, whether this variation within sites is due to genetic divergence and/or selection on social competence, or other factors is still unknown. This can potentially be explored through selection lines on social competence, and then tests for brain adjustments.

D.2.2 The main findings and their implications

1. Biological market effects

Our hypothesis based on the outcomes of Chapter I suggested that market effects, such as cleaner fish and their client densities, especially the large/visitor client densities, were the best predictors of cleaner fish cognitive performance. These findings were then statistically confirmed in Chapter II. Cleaner density emerged as the best proxy for the cleaning market structure, and subsequently cleaner's performance. Although group size has been often suggested as a proxy for social complexity and the primary driver of brain complexity [Dunbar, 1992], it still does not explain how the social structure is linked to cognitive performance. Barret et al. [2003], and Noë et al. [2001] suggest that applying the biological market approach to understand social decision-making can frame social complexity better than group size in the studied system. Since social decision-making is context-dependent, the market effects are thus excellent factors in defining the context *per se*. In line with Barret et al. [2003], and Noë et al. [2001], I found that market conditions indeed appear to capture to some extent the degree of social complexity in the cleaner-client market. My results in Chapter I and II, in particular, confirm this by showing that the supply magnitude (i.e., cleaner density) drove the costs of partner choice option and cleaners to adjust their decision rules accordingly. According to Logan et al. [2018], the use of proxies for social complexity should be validated before implementing them as predictors for brain size to avoid uncertainties. Therefore, I can say that Chapter II is a validation that cleaner density is the best proxy for estimating important aspects of social complexity in this species, justifying the use of cleaner density in Chapter III and IV to predict brain complexity.

2. Is brain size as a reliable proxy for brain functionality?

Recent studies showing that neuron densities do not necessarily vary with variation in brain size or brain part sizes at the species level [Herculano-Houzel et al., 2015; Olkowicz et al., 2016], suggest that significance of brain size in terms of cognition is limited [Logan et al., 2018]. However, this argument by Logan et al. [2018] is somehow flawed as it is based only on neuronal densities without considering the neuron counts information. For instance, although the human brain has ~ 86 billion neurons, I found that *Homo sapiens* have a lower neural density than most of the mammalian species from a calculation I performed on the data tables provided in the study by Herculano-Houzel et al. [2015]. Furthermore, in Chapter IV, the relative neuron count and size of the forebrain yielded similar outcomes, whereas the neural density did not. Together, it suggests that neural density can be a piece of complementary information but what matters is the abundance of neurons in the brain/brain parts and not their density. Results from Chapter IV thus provide evidence that brain size can be a reliable proxy for brain processing power.

3. Methods and reproducibility of the results

The possibility to reproduce consistent outcomes in ecological studies provides strong support for the interpretations of the findings [Cassey, and Blackburn, 2006]. Here, the relationship between brain complexity and social complexity in cleaners was consistent throughout the two chapters, Chapter III and IV. Although the employed methods in estimating brain part sizes were not the same (MRI scans in Chapter III vs stereological dissection in Chapter IV), they still yielded consistent outcomes. Also, the two studies were carried out on different reef sites at Lizard Island, and in two different periods, 2016 and 2018. Together, the robustness of the results suggest that there are no major confounding effects of the employed methods.

D.3 Current and future research

D.3.1 Work in progress

1. Work designed to be part of this thesis but it is still in progress

I have an ongoing project with Dr Maryam Chaib De Mares, Dr Rebecca Young Brim, Dr Chelsea Weitekamp, Dr Hans A. Hofmann and Dr Redouan Bshary as co-authors. This project is a continuation of this thesis, wherein we used baseline neural state through gene expression and gene co-expression networks as a promising approach to assess brain functionality [Oliveira, 2012; Weitekamp, and Hofmann, 2014; Cardoso et al., 2015]. Recent studies suggest that key brain nuclei networks, namely the social behaviour network and the basal forebrain reward system that together form the social decision-making network, are highly conserved across vertebrates [O'Connell, and Hofmann, 2011;

O'Connell, and Hofmann, 2012]. In fishes, the network is spread over the telencephalon and the diencephalon. We assessed gene expression and gene co-expression networks in the telencephalon of cleaners from socially complex and poor habitats to investigate whether there are differences in brain functionality as an underlying mechanism of the strategic sophistication variation in cleaners.

Cleaners were caught in areas that differed in cleaner density, immediately brought to the water surface and the brains were prepared for later analyses on the boat. Gene counts from tissue samples from the telencephalon were obtained by mapping RNA sequenced reads to a reference assembly. The Ensembl annotation for *Oreochromis niloticus* (Tilapia) was used as a reference. Aligned reads were quantified using HTSeq to determine the expression level for each gene in each sample [Anders et al., 2015]. Principal component analyses showed that PC1and PC2 explain 16% and 12% of the variation, respectively (Fig. 1). The results showed that the neural gene expression varied significantly with variation in the social composition of the studied habitats on the PC1 (high vs low social complexity on PC1, student t-test, $n = 17$, $t = 4.579$, p-value = 0.0003, Fig. 1).
From the results presented in Fig. 1, we see that there are systematic differences between cleaners at low social complexity compared to cleaners from high social complexity throughout the chapters of this thesis and this current work. Cleaners do not only differ in their brain part sizes or cell/neuron count, but they also differ in their brain activity. Nevertheless, to draw more informed conclusions about telencephalon activity from these gene activity data, we are still working on co-expression gene network and candidate gene expression analyses.

Furthermore, I have frozen brain slices tissue from the brains used for the MRI scans in Chapter III. These brains were collected on purpose 30 min post-trial after testing cleaners in a bystander effect task. We aim to explore which telencephalon regions activity is significantly correlated with the performance in this task. I will be using the immunohistochemistry technic to determine neuronal activity with double-labelling methods [Weitekamp et al., 2017]. The technic is based on targeting an activity gene such as c-Fos and a candidate neurohormonal system. Positive double-labelled neurons indicate that these neurons were active around the time the fish had made its decision in the task. The candidate neurohormonal system will be chosen based on the outcomes of the candidate gene activity from the project described above.

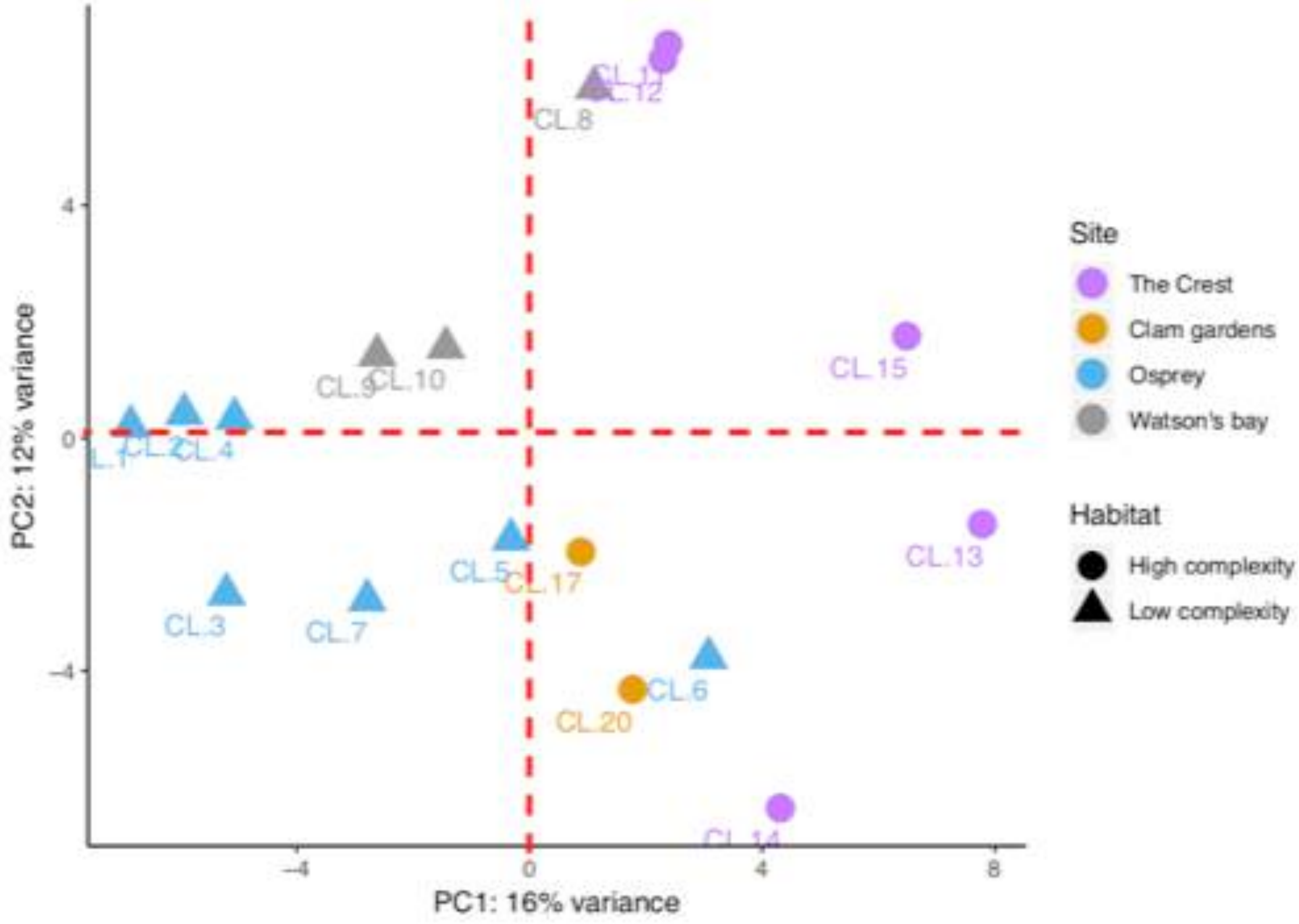

Figure 1. Principal Component Analysis of the neural gene expression. Study reef sites are indicated in the legend: filled circles are sites with low cleaner density, while filled triangles are sites with high cleaner density. There were (n=4) cleaners collected from Clam gardens, (n=6) from The Crest, (n=7) from Osprey, and (n=3) from Watson's bay at Lizard Island, Great Barrier Reef, Australia in July 2015.

2. Work directly linked to the thesis aims

Currently, I am supervising two masters thesis projects run by Virginie Staubli and Simon Niklaus. Virginie's project is based on testing cleaners' feeding preferences depending on their current satiation levels. The aim is to explore whether satiated cleaners are more willing to eat against their preferences or whether they choose highly preferred food instead. The tested cleaners were chosen from (n=10) high and (n=10) low social complexity sites to test whether systematic differences exist in this studied behaviour. These cleaners were tested with Plexiglas plates as surrogates of clients, but they were also tested with real clients for comparisons. Virginie is currently analysing the data already collected in July-August 2018.

Simon's project also had cleaners from (n=10) low and (n=10) high social complexity sites. His project aims to test whether partner control mechanisms applied by client fish to control cleaner fish cheating behaviour (i.e., punishment, and partner switching) operate through fear conditioning, with respect to the social complexity degree at their natural habitats. Here, we tested whether cleaners would adjust their foraging behaviour by feeding more against their preferences whenever a partner control mechanism is applied. Furthermore, we aimed to control the cleaner's fear conditioning through the blockade of adrenaline receptors. Simon is currently analysing the data already collected in July-August 2018.

D.3.2 Future Research

This thesis probably provided a basis for various research lines with the marine cleaning system. Many unanswered questions are arising from this thesis. For instance, the laboratory-based cognitive tasks used throughout the thesis chapters probably capturing only a small fraction of the cleaner fish cognitive abilities. When we look at Table 1 in the General Introduction, we see that cleaner fish have a rich behavioural repertoire that is worth exploring with respect to social complexity and/or brain complexity. Importantly, these links should be explored at the inter- as well as at the intraspecific level of the strategic sophistication and social decision-making. This would help to test whether it is the inter- or the intraspecific social interactions that would better explain the variance in brain complexity.

So far, some follow-up projects are already in place to explore the links between social complexity and brain neuroanatomical traits (i.e., size, cell/neuron count) with a wide range of laboratory-based cognitive tasks. The project will be led by Yasmin Emery (a new PhD student in the Behavioural Ecology laboratory). Yasmin will be collaborating with the current PhD student Mélisande Aellen. The Idea is to establish a "*g*" score for cleaner fish general intelligence [For general intelligence see Burkart et al., 2016]. Cleaners will be collected from sites differing in their social complexity degree and will be subjected to a battery of laboratory tests including number discrimination, inhibitory-control, feeding against the preferences, bystander effect task, as well as ecologically relevant tasks. At the end of the tasks, brain neuroanatomical traits will be established for each studied fish. I will be providing the necessary training for Yasmin to collect the brain measurements (i.e., brain dissection, brain cell/neuron counting methods).

D.3.3 Open questions

A potential open question is how to establish causality between social complexity and brain complexity and, as a consequence, cognitive performance/social competence. For instance, although there is a positive correlation, it is unclear whether cleaners with complex brains favour socially complex habitats, or whether social complexity causes the development of complex brains. Experimental manipulation projects are thus needed, wherein the translocation of cleaners from high social complexity sites to low social complexity sites, and vice-versa will be an appropriate manipulation. Although we tried to perform the translocation project in the past, Dr Sharon Wismer during her PhD in 2014 [Wismer, 2017] and myself in 2015, our attempts failed. In 2015, I found that a translocated cleaner was unfortunately exposed to predation due to its apparent stress behaviour after the release. Nevertheless, I think that by improving the translocation technic we might have a chance to succeed. For instance, a sophisticated translocation chamber can be constructed in a way that can be employed to protect the fish from predators and provide enough time for acclimation within the new reef habitat. Also, translocating cleaner couples might help in reducing their stress responses to the translocation.

To conclude, all this current and future research will help to increase our understanding of the links between cleaner fish social complexity and its brain development, and as a consequence, its strategic sophisticatio

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ANNEX | PARRALLEL RESEARCH LINKED TO THIS PhD

(Published or in review)

Title: Reputation management promotes strategic adjustment of service quality in cleaner wrasse

Authors: Binning, S.A., Rey, O., Wismer, S., **Triki, Z.***, Glauser, G., Soares, M.C. and Bshary, R.

***My contribution:** *I sampled, extracted and measured the baseline of the stress hormone "cortisol" levels.*

Status: *Published in 2017 in Scientific Reports*
Doi: 10.1038/s41598-017-07128-5
Link to the paper: https://www.nature.com/articles/s41598-017-07128-5

Abstract

Adjusting one's behaviour in response to eavesdropping bystanders is considered a sophisticated social strategy, yet the underlying mechanisms are not well studied. Cleaner wrasse, Labroides dimidiatus, cooperate by eating ectoparasites off "client" fishes, or cheat (i.e. bite) and eat client mucus. Image scoring by bystander clients generally causes cleaners from socially-complex (i.e. high cleaner and client abundance; high client species richness) habitats to increase levels of cooperation. However, some individuals may periodically provide tactile stimulation to small resident clients, which attract bystanders close that are bitten, a form of tactical deception. Cortisol injection can reproduce this pattern. Here, we tested whether cleaners from socially-complex versus simple habitats respond differently to cortisol injections in terms of their cleaning interactions with clients. We found that only cleaners from the socially-complex habitat respond to cortisol injection with strategies functioning as tactical deception: i.e. increased tactile stimulation to small clients and increased cheating of large clients relative to small ones. At the socially-simple site, where reputation management is less important, cortisol-treated fish increased their overall levels of cheating, especially of small clients. Thus, strategic adjustments to cooperative behaviour and tactical deception are likely context-dependent, forming part of general reputation management abilities in cleaner wrasse.

Title: Cleaner fish *Labroides dimidiatus* discriminate numbers but fail a Mental Number Line test

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Abstract:

Several species of primates, including humans, possess a spontaneous spatial mental arrangement (i.e., Mental Number Line MNL) of increasing numbers or continuous quantities from left to right. This cognitive process has recently been documented in domestic chicken in a spatial-numerical task, opening the possibility that MNL is a cognitive capacity that has been conserved across vertebrate taxa. In this scenario, fish might possess the MNL as well. Here we investigated whether cleaner fish *Labroides dimidiatus* show evidence for MNL in two experiments. In Experiment I we tested fish's abilities in number discrimination, presenting simultaneously either small (2 vs 5) or large (5 vs 8) continuous quantities where one quantity was systematically rewarded. Experiment II used a protocol of an MNL task similar to the study on chickens. We trained cleaners with a target number (i.e., 5 elements), then we presented them with an identical pair of panels depicting either 2 elements or 8 elements and we recorded their spontaneous choice for the left or right panel on each presentation. Cleaner fish showed high abilities in discriminating small and large numbers in Experiment I. Importantly, cleaners achieved this discrimination using numerical cues instead of non-numerical cues such as the cumulative surface area, density, and overall space. In contrast, cleaners did not allocate continuous quantities to space in Experiment II. Our findings suggest that cleaner fish possess numbering skills but they do not have an MNL. While similar studies on animals from various clades are needed to trace the evolution of MNL within vertebrates, our results suggest that this cognitive process might not be a capacity conserved across all vertebrate taxa.

Keywords: Continuous quantities; Counting; Learning abilities; Cognition; Fish; Domestic chicken

Introduction

Mathematical skills are key to the technological development of humans. For instance, later academic success is strongly linked to acquiring mathematical skills in early childhood (Claessens and Engel 2013). More recently, researchers tested a diverse range of species from distant lineages on their abilities to process numerical information. Mammals (Hauser and Spelke 2004), as well as birds (Rugani et al. 2008) and fish (see review by Agrillo and Bisazza 2014), can discriminate quantities. Indeed, the assessment of quantities offers important information in various natural contexts, such as optimal foraging and social affiliation (i.e., preferences towards social groups based on the size of the group). Various studies show that fish species can discriminate quantitative numbers based on numbering information only and not due to potential biases such as the cumulative surface area, density, or distribution of elements (Dadda et al. 2009; Agrillo et al. 2010; Piffer et al. 2013). The matching of mammals and fish performance in quantitative number discrimination tasks suggest that the underpinning mechanisms are rather similar (Bshary et al. 2014). Altogether, these results suggest that fish can provide an ideal system to study cognitive numbering abilities to determine which might be inherited from ancestral species.

In addition to counting abilities, humans' everyday use of mental arithmetic skills (i.e., calculations without the utilisation of any tools), are strongly linked to symbolic number-ordering capability (Lyons and Beilock 2011). Interestingly, this ability is not exclusive to humans' cognition. Nonhuman primates and other animal species can also learn to recognise and order numbers from small to large in exchange for a reward. For instance, nonhuman primates successfully order numbers, both when the numbers are symbols in Arabic digits (i.e., digital numbers), for instance 1, 2, 3 (Kawai and Matsuzawa 2000), or when the numbers are quantitative, for example varying numbers of dots (Brannon and Terrace 1998; Cantlon and Brannon 2006) (see Fig. S1). Fish, as well, possess number ordering skills. Studies on guppies show that they can locate an object based on its ordinal position in a sequence (Miletto Petrazzini et al. 2015b). While symbolic number-ordering skills are based on learned visual information, there is also an important abstract ordering skill called the mental number line (MNL). An MNL is a mental representation of quantities in space, where smaller quantities are placed on the left side of larger quantities (Feigenson et al. 2004). However, this concept remains controversial even for humans (Shaki and Fischer 2015), as it has been argued that associating numbers and space, in humans, needs further developmental number processing abilities such as reading. Furthermore, MNL is not a universal human cognitive ability as cross-cultural habits can have a significant role in shaping the MNL and its spatial direction (see review by Göbel et al. 2011; and Fischer and Shaki 2014). Nevertheless, a recent study by Rugani et *al.* (2015) showed that young domestic chicken *Gallus gallus* have an MNL. Based on their findings, the authors suggest that MNL is an innate capacity that may be highly conserved among vertebrates (Rugani et al. 2015).

We hypothesised that if the MNL is indeed a shared ability across vertebrates as suggested by Rugani. et *al.* (2015), it will be possible to demonstrate it in a fish as well. For this reason, we chose a species known for its sophisticated social strategies, the cleaner fish *Labroides dimidiatus*, to test it in two numbering experiments. *L. dimidiatus* is an obligate cleaner that feeds on ectoparasites, dead tissue, and scales off a variety of coral reef fish, so-called "clients" (Côté 2000). Cleaners show advanced strategic sophistication in nature and laboratory experiments, such as reconciliation with clients after a conflict (Bshary and Würth 2001), reputation management in the presence of an audience (Bshary 2002; Bshary and Grutter 2006; Pinto et al. 2011), and the ability to give priority to clients who are able to choose their partners and as such could potentially terminate cleaning sessions by leaving (Bshary 2001; Salwiczek et al. 2012). On a mechanistic level, there is evidence for cleaners recognising clients individually (Tebbich et al. 2002), for generalised rule learning (Wismer et al. 2016), and for some limited ability to learn about reverse reward contingency (Danisman et al. 2010). The fact that cleaners adapt well to the laboratory environment, feed easily off Plexiglas plates as surrogates of clients (Bshary and Grutter 2006), and are neophilic in comparison to other fish species (Gingins and Bshary 2016) makes them highly suitable for experiments designed to assess numbering abilities.

In the present study, we first established cleaners' abilities to discriminate small and large numbers of elements based on numerical information only, where we used continuous quantitative contrasts of 2, 5, and 8 elements (i.e., quantitative numbers). Then we tested cleaners' ability to associate the same numbers in space in a task similar to Experiment 1 from the study by Rugani et *al.* (2015). The contrasts, 2, 5, and 8 were chosen based on the study of Rugani et *al.* (2015). In the Rugani et *al.* (2015) study, chicks were tested for their ability to associate different amounts relative to a target with their spatial location. The authors' main question was whether, once trained that a panel of 5 elements offered a reward, the chicks would choose the location of subsequent amounts presented to them relative to the original reward panel (i.e. smaller amounts would be preferred on the left and larger amounts on the right). That is, after being trained on panels of 5 elements, chicks were presented two panels depicting 2 elements each, one on the left and one on the right of where the original 5 elements panel had been presented. In this case chicks approached the panel on the left more frequently. Whereas when they were presented with two panels with a larger amounts (8 elements each) they approached the panel on the right more frequently. Additional experiments to control for potential confounding variables like cumulative surface area, density, and overall space occupied by the elements still yielded similar results, indicating that chicks allocate space to increasing amounts from left to right and suggesting that they too have an MNL (Rugani et al. 2015).

For our number discrimination experiment we predicted that cleaners could learn to prefer the rewarding quantity, possibly based on numerical information only. This prediction was based on similar studies which provided evidence for number discrimination abilities in a variety of fish species (Agrillo et al. 2009; Dadda et al. 2009; Agrillo et al. 2010; Piffer et al. 2012; Agrillo et al. 2012). Also, if cleaners indeed possess an MNL, we expected that they would choose relatively smaller values on the left side and larger values on the right side of the position of the trained intermediate value in the MNL task.

Material and Methods

Experiment I "Number discrimination test."

Field site and animals

Experiments were conducted at Lizard Island Research Station, Great Barrier Reef, Australia. Experiment I was carried out in August 2017. Subjects were 20 adult females of *L. dimidiatus*. Fish were caught by scuba divers employing a barrier net (1 × 2 m net size; 0.5 cm^2 mesh size) and hand nets. Fish were housed individually in transparent aquaria (67 × 37 × 38 cm) provided with aerated running seawater. Cleaner fish were in the laboratory for 30 days before being tested in the numbers discrimination task. They were all trained to feed on mashed prawn smeared on Plexiglas plates (Bshary and Grutter 2006). Before the test, cleaners were subjected to two separate cognitive tasks that are linked to cooperative behaviour (for details, please refer to the supplementary material).

Procedure

In this experiment, cleaners were challenged with a visual discrimination task. Two plates of identical size (7 x 7 cm) and background colour (white), but with differing numbers of black squares were presented simultaneously, and cleaners had to choose one (Fig. S1 B&C). The correct choice offered an accessible food reward on the back of the plate while the wrong choice had an inaccessible food item on the back (to prevent olfactory cues being used in addition to the visual cues we were testing, Fig. S1 E). Cleaners were tested in one of two treatments that differed with respect to the number of black squares on each plate as cues to discriminate between the correct and wrong choice: 2 vs 5 squares (Fig. 1B) and 5 vs 8 squares (Fig. 1C). Ten fish were assigned to each treatment. We counterbalanced which number of squares was rewarded for each treatment across subjects. The panels were printed on regular paper and laminated to make them waterproof, and attached to Plexiglas plates of similar size by using Velcro stripes. The panels depicted a randomised distribution of the black squares among the white background. The distribution changed between trials to avoid cleaners learning the overall shape depicted by the squares.

Prior to the experiment, fish were offered one day of acclimation with the experimental paradigm. They were allowed to explore the two panels with number contrasts that corresponded to each cleaner's treatment. Also, the target panel during the training phase had a food reward. The configuration of squares on the panels used in training was not used again in the test. The next day we started with the experiment. The experiment consisted of two tests, an "initial" and a "control" test. The initial test was characterised by the fact that the size of each black square was invariably 1 cm^2. The position of squares was randomised. As a consequence, the task could be solved not only by counting the number of squares but also by discrimination between either total black surface area, spread, or density of the black squares (i.e., the interspace between the elements). Only cleaners that solved this initial task, by learning to prefer significantly the rewarding plate, were subjected to the control test (see below), named such because it controlled for alternative cues, leaving numerical discrimination as the only useful cue to solve the test. The cumulative surface area of the elements on all the control test panels was now 5 cm^2. Furthermore, 50% of the panels were controlled for the cumulative surface area and density, while the other 50% were controlled for the cumulative surface area and overall space, because density and overall space are negatively correlated. The alternation between these types of panels was used to preclude cleaners' use of density or spread as reliable cues. The cumulative surface area of the 2 and 8 elements in the small number test (2 or 8) was scaled to match the cumulative surface area of the 5 elements (Fig. 1 D&E). Therefore, each black square in the controlled panels of 2 elements had a size of 2.5 cm^2 and the new size of each of the black squares in panels of 8 elements was 0.625 cm^2, while the size of the squares on panels with 5 elements remained unchanged at 1 cm^2. Overall, we used 30 non-controlled panels in the initial test and 30 controlled panels in the control test (i.e., 10 panels for each contrast and test). Each random combination of two panels was used only once per trial and per session. Fish had 2 sessions per day of 10 trials each (10 trials in the morning, and 10 trials in the afternoon) between 7:00 and 17:00 for a maximum of 10 consecutive days.

For convenience, all trials were conducted in fish's home aquaria, to exclude the effect of disturbance and translocation on their performances during the tests (Grutter and Pankhurst 2000). For each trial, we introduced two barriers in the aquarium, one opaque and the other transparent. The fish were hence confined to one side of the aquarium, while the experimenter placed the panels for the trial at the other end of the aquarium. Once the panels were in place, the opaque barrier was removed first to let the fish see the setup. Three seconds later, the transparent barrier was removed so that the fish could access the panels. The presentation of the panels was controlled for side bias with counterbalanced presentations of the target panel on both left and right sides across 10 trials. The target panel had a prawn item as a food reward placed on the back of the plate, whereas the non-rewarding panel had an inaccessible food item placed on the back of the plate (see Fig. S1). Including the time of setting up the dividers and the panels in the aquarium, each trial lasted for 60 seconds on average. The time lapse between two trials was on average 11 minutes. All panels were designed with Keynote®.

The tests started with the panels showing squares of equal size. Upon reaching the learning threshold, fish were subjected to the control test. A fish was considered to have learned the task by reaching the following threshold: 7 correct choices in three consecutive sessions, or 8 correct choices in two consecutive sessions, or 9 correct choices in one single session. A choice was scored as correct once the fish swam behind the target panel and took the reward. Panels were retracted from the aquarium either after the cleaner had eaten the reward or after it had inspected the non-rewarding panel.

Experiment II "Mental Number Line test."

Field site and animals

Experiment II used the same field site, fish (*L. dimidiatus*), capture, and housing as in Experiment I. For this experiment, 40 adult females were tested, (n=12) in August 2015 and (n=28) in August 2016. Cleaners were in the laboratory for 20 to 65 days before the beginning of the number ordering abilities test. Before the experiment, we subjected cleaners to two separate cognitive tasks that are linked to cooperative behaviour (for details, please refer to the supplementary material).

Procedure

The number ordering abilities experiment was as similar as possible to Rugani and colleagues' study (2015). The panels were prepared as described in Experiment I (see above), following instructions in the supplementary section of the Rugani et al. (2015) study. Here we used panels depicting elements always of the same size of 1 cm^2. There were three types of panels with either 2 elements, 5 elements, or 8 elements. Fish underwent a training phase and a test phase. In the training phase, all presentations had panels of 5 elements. Each presentation had one panel of 5 elements, where the distribution of the 5 elements was randomised for each panel. Overall, in the training phase there were 20 panels of 5 elements presented to the fish with a food reward placed on the back of the plate (Fig. 2A). Similarly to the Rugani et al. (2015) study, we waited for the fish to complete 20 successful training trials. All training trials were completed on the same day, between 7:30 and 11:30. The plate was always placed at the middle of the aquarium side wall. Upon accomplishing the training trials, we then waited for 120 min before we started the test trials. During the tests, a pair of plates with identical panels were presented simultaneously without a food reward. One panel was placed on the left side and one panel was placed on the right side relative to the location where the training plate had been presented. Fish received two treatments, a small number test and a large number test, separated by 60 min, where the treatment order was counterbalanced. In the small number test, fish were presented with pairs of panels with a smaller number of elements than the target number of the training (2 panels of 2 elements each, 2 vs 2, Fig. 2B). In the large number test, fish were presented with pairs of panels with a larger number of elements than the target number of the training (2 panels of 8 elements each, 8 vs 8, Fig. 2 C). All panels had a randomised distribution of the elements on the white background. Fish received 5 trials in each test, where each pair of panels was presented only once per fish. A choice was scored once the fish swam behind a panel. Both panels were then retracted immediately from the aquarium. Including the time of setting up the dividers and the panels in the aquarium, each trial lasted for approximately 60 seconds, and the time lapse between two trials was 11 minutes. Overall, the experiment took one day, including the training phase and the breaks between tests.

Statistical analysis

Statistical analyses were generated using RStudio® (R version 3.3.1 (2016-06-21)). In Experiment I, we first tested the fish performance in the initial test to see whether they were able to learn to prefer the target panel successfully. Then, among fish that solved the initial test, we tested their ability to solve the control test. In both tests, the data was of a binomial order as success or failure in solving the tests based on the learning threshold adopted for the present study (i.e., three consecutive sessions with

7/10 correct choices, two consecutive sessions with 8/10 correct choices, or one session with at least 9/10 correct choices). For the cleaners that solved the initial test, we tested their spontaneous scores in the first session in the control test against the chance level of 50%. We tested their performance at the population level as well as at the individual level. We also tested their choices in each of the two controlled conditions (i.e., cumulative surface area and density, and cumulative surface area and overall space) separately against the chance level of 50%. These tests allowed us to explore which cues were used by the cleaners to solve the initial test, the cumulative surface area or the numerical information initially.

In Experiment II, for each fish and condition, we calculated the percentage of trials in which the left side was chosen. We also tested whether subjects had any side preferences as our main interest was to directly compare individuals' choices when faced with smaller vs larger numbers in a matched pair design. Data from 2015 and 2016, were pooled together in the analyses since we did not find significant differences between the years.

Results

Experiment I "Number discrimination test."

Fifteen of the twenty female fish tested in the number discrimination test successfully learned to prefer the target panel. Individual performance showed that cleaners are able to discriminate continuous quantities in the initial test (one-tailed Binomial Test, n=20, x=15, p=0.02, Fig. 3A). Among the cleaners that solved the initial test (n=15), three cleaners were not tested in the control test because they solved the control test by reaching the learning criterion at the maximum number of trials allocated to each fish (i.e., 200 trials). Therefore, from the (n=15), only (n=12) cleaners were subjected to the control test. Data analysis showed that cleaners could discriminate between continuous quantities based on numerical information only (one-tailed Binomial Test, n=12, x=10, p=0.019, Fig. 3B). Also, we found that cleaners' spontaneous choice in the first session of the control test was significantly higher than a chance level of 50 % of correct choices (M=69.16 %, SD=12.4 %; Wilcoxon one sample test, n=12, 2 ties, V = 55, p=0.005). To test whether the performance was due to cleaners using either density or overall space as cues we conducted two additional analyses. The significant results persisted when we calculated the performance of cleaners in trials in which cumulative surface area and density were controlled (M= 63.33 %, SD= 18.74 %; Wilcoxon one-sample test, n=12, V=64.5, p=0.042). Similarly, we obtained a significant result for trials in which cumulative surface area and overall space were controlled (M= 76.6 %, SD= 16.7 %; Wilcoxon one-sample test, n=12, V=78, p=0.002).

In the initial test, the probability of cleaners succeeding in the small (n=10, x=6) and the large (n=10, x=9) numbers treatments was not significantly different from each other (Fisher's Exact test, p=0.3). Also in the control test, the success rate was not significantly different between the small (n=5, x=5) and large (n=7, x=5) number treatments (Fisher's Exact test, p=0.47).

Experiment II "Mental Number Line test."

Overall, the fish had no significant preference for one side over the other (median 60% choice for the left side, Wilcoxon one-sample Test, n=40, V=166.5, p=0.83). Also, cleaners were not more likely to choose left in the small number test (2 vs 2) than in the large number test (8 vs 8; Wilcoxon two-samples test, n=40, V=233, p=0.77; Fig. 4). This lack of adjustment to test condition was also found when we tested the performance of the fish in the first trial in each situation, i.e. before the successive exploration of the alternatives may have masked their spontaneous preference (two-tailed Binomial Test, initial choice, n=40, 17 ties, remaining n= 23, x=12, p=1).

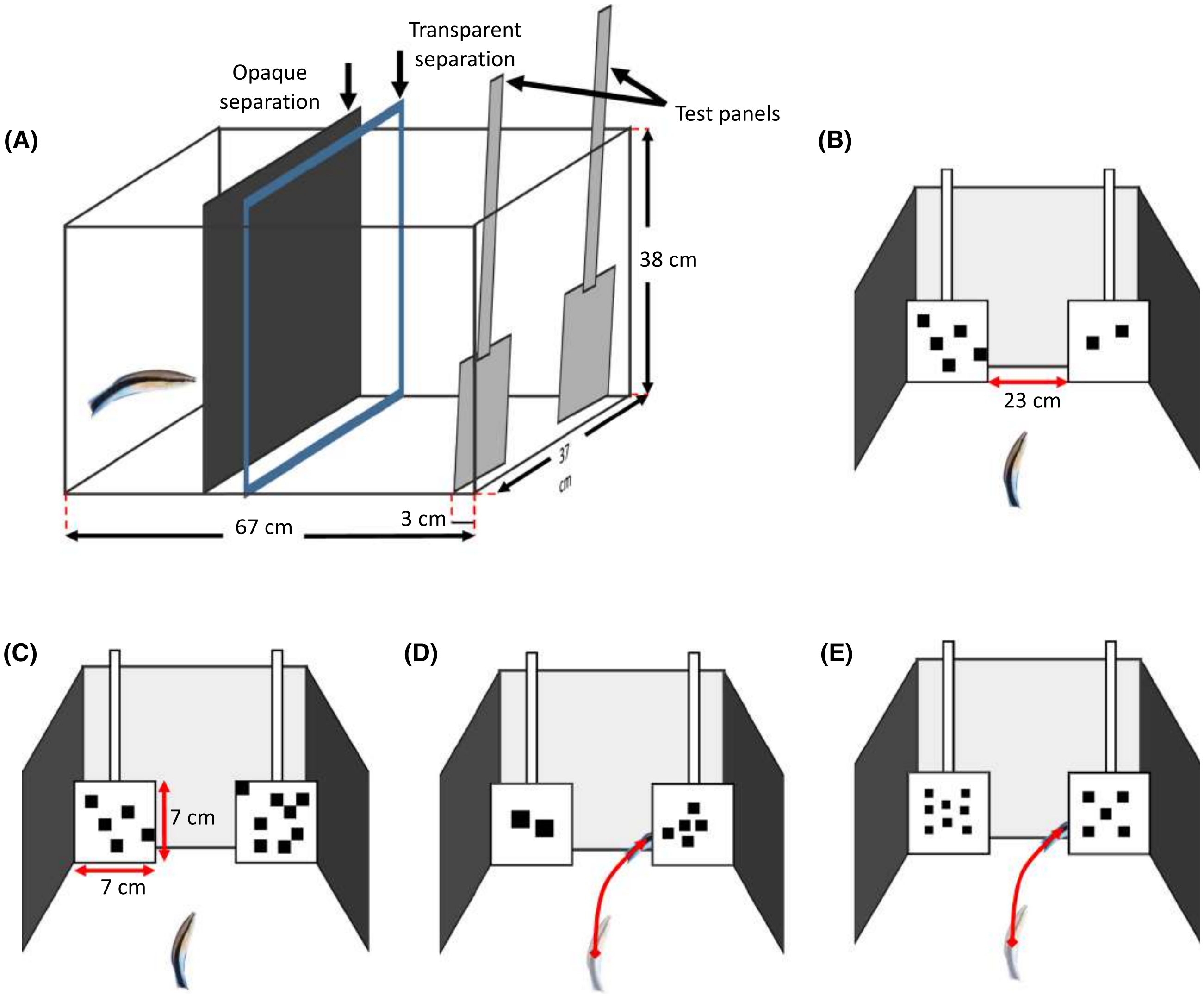

Figure 1. The experimental setup. **(A)** An experimental aquarium, for Experiment I and II, contains: the focal individual separated from the training/test panel(s) by two barriers. **(B)** An example of the set-up in the initial small number test (2 vs 5) in Experiment I. **(C)** An example of the set-up in the initial large number test (5 vs 8) in Experiment I. **(D)** An example of the set-up with controls for cumulative surface area and density of the small number test (2 vs 5) in Experiment I. **(E)** an example of the set-up with controls for cumulative surface area and overall space of the large number test (5 vs 8) in Experiment I. A choice was scored once the fish swam behind a panel (examples in **D** & **E**).

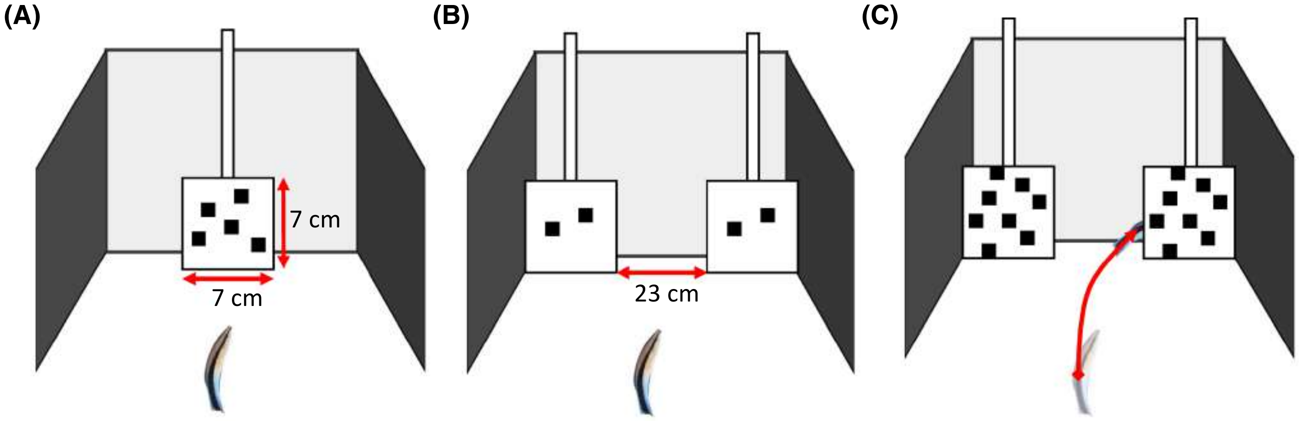

Figure 2. Examples of the set-up in Experiment II. **(A)** An example of the setup in the training part of the test with the target panels of 5 elements. **(B)** An example of the setup of the small number test (2 vs 2). **(C)** An example of the setup of the large number test (8 vs 8). A choice was scored once the fish swam behind a panel.

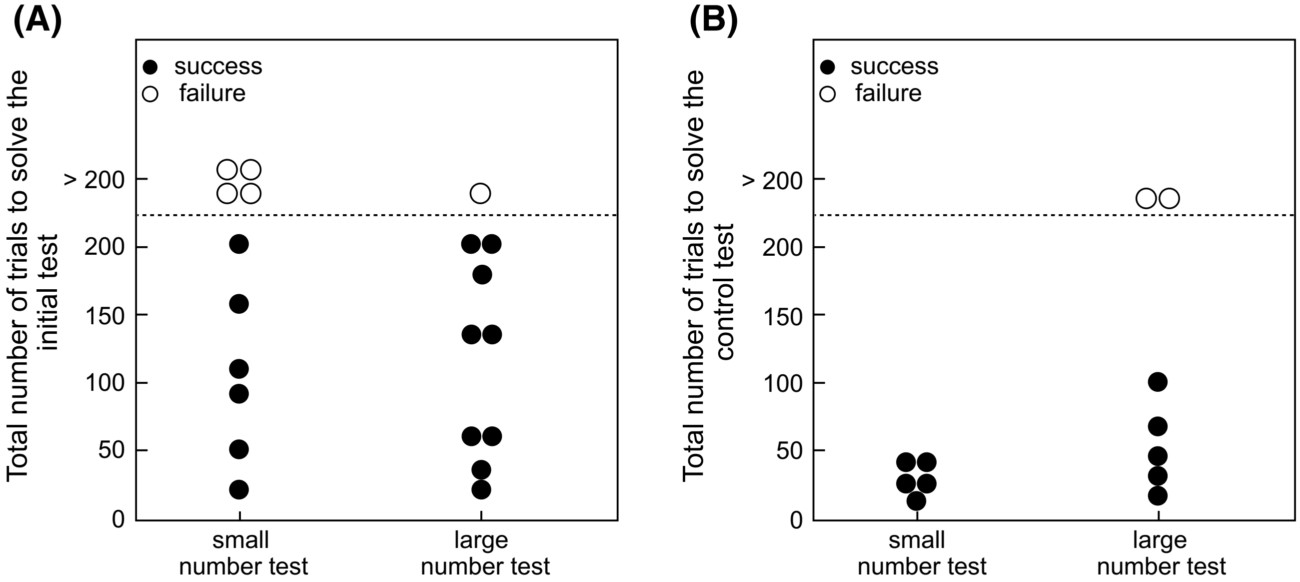

Figure 3. Scatterplot of individual performance, in the small number (2 vs 5) and large number (5 vs 8) discrimination tests, denoting the number of trials needed to complete the initial **(A)** and the control tests **(B)**. All individuals were offered a maximum of 200 trials for the whole task. Only individuals that succeeded in the initial test, within < 200 trials, were then tested with the control.

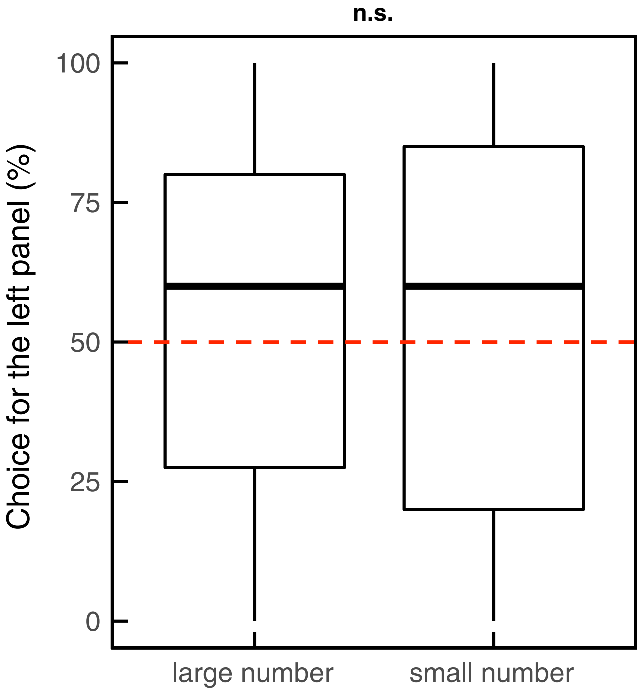

Figure 4. Boxplot of median and interquartile of the percentage of choice for the left panel in the small number test (2 vs 2), and in the large number test (8vs 8). The dashed line shows the random choice for the left side at 50% of probability. n.s.: statistically non-significant differences.

Discussion

Our main question was whether fish would spontaneously allocate spatial attributes to continuous quantities by placing smaller quantities to the left of larger quantities, in other words, whether they possesse an MNL. To test this hypothesis, it was crucial to establish also if the studied fish species could discriminate between continuous quantities, and which cues are used in such discrimination if it exists. Indeed, the first experiment showed that cleaners do discriminate continuous quantities, but we found no evidence for cleaners using an MNL in the second experiment using the same quantities.

Number discrimination

Our findings are in line with previous studies showing that fish can count, or use numerical information to discriminate between different quantities (Agrillo et al. 2008; Dadda et al. 2009; Agrillo et al. 2010; Piffer et al. 2012, 2013; Dadda et al. 2015). In Experiment I, cleaners performed equally well when distinguishing smaller (2 vs 5) and larger (5 vs 8) quantities. Also, their abilities in number discrimination are apparently due to the use of numerical information: as a population, cleaners spontaneously performed above chance when we controlled for the cumulative surface area, density, and overall space, and most individuals reached the learning threshold within the limited number of sessions accorded to them.

Several studies have been conducted on fishes' abilities to discriminate continuous quantities. However, the methods employed to test fishes' learning capacities in those studies varied. While some studies opted for the total time spent by each focal individual next to the target stimuli (Sguanci et al. 2010; Agrillo et al. 2012; Miletto Petrazzini et al. 2015a), others tested the correct overall choices against chance on the population level (Bisazza et al. 2014; DeLong et al. 2017). Here we aimed to test numerical discrimination abilities on the individual level and the population level. The performance criteria set in the present study were strict in the sense that conclusions were based on significant success on the individual and population levels. Most cleaners learned to choose the rewarding stimulus above chance levels in the initial task, and of those who succeeded most performed above chance in the control test where non-numerical cues were controlled. Thus, as a population, cleaners appear to be able to use numerical information. Importantly, their overall spontaneous choice in the control test (i.e., with panels corrected for the cumulative surface area, density, and overall space) suggests that cleaners were already using the numerical cues to solve the initial test.

The strong reliance of cleaners on numerical cues contrasts with results from various other studies in which species use the cumulative surface area as a cue to discriminate between continuous quantities, such as other fish (Agrillo et al. 2009, 2010), cats (Pisa and Agrillo 2009), and humans (Feigenson et al. 2002). On the other hand, previous research on fish numbering skills employed a multitude of different quantities used in the tests, from a few to hundreds of elements (see review by Agrillo et al. 2017). In our study, we tested the cleaners with few contrasts with smaller numbers (2, 5 and 8 elements), i.e., those that are key to interpret the results from the second experiment on mental number lines. As a consequence, our study only provides a preview of cleaner wrasse's numbering skills. Future studies are thus needed to establish the extent and limits of their counting abilities. Cleaners are a potentially interesting study species for this question as we do not see any particular ecological need for their counting abilities. In contrast, most other fish species tested in previous number discrimination tasks live in open-membership shoals. Benefits of shoaling (e.g., reduced predation risk and reduced costs due to food competition and coordination efforts) are a function of shoal size, which should lead to selection for the ability to assess the shoal size (Agrillo et al. 2010). In contrast, our cleaners live alone or in pairs, waiting for clients to visit. Client surface area correlates with parasite load (Grutter 1995), which seems to provide ecologically more relevant information than numbers as cleaners can only inspect one client at a time. Thus, cleaners' numerical discrimination abilities may reflect basic features of a fish brain rather than a specialised ecological adaptation.

Mental Number Line

From our findings in Experiment I, we conclude that the fish tested in Experiment II can discriminate between the test contrasts (2, 5, and 8 elements). However, our findings provided no evidence that cleaners use an MNL. The choice of a fish to seek the left or right panel was random as opposed to driven by the continuous quantities displayed on the panels. We restricted ourselves to the contrasts 2, 5, and 8 elements, and we did not proceed to other experiments with different contrasts as Rugani and colleagues did (2015). We thought that a follow-up experiment to Experiment II with larger contrasts would be a logical continuation only if the cleaners had shown significant preferences for either the left or right side. Importantly, the results from Experiment I mean it is unlikely that fish's chance-level performance in Experiment II was due to a lack of discrimination between the continuous quantities. This suggests an absence of spatial attribution instead of a lack of ability to discriminate quantities. In conclusion, we did not find evidence to support Rugani and colleagues' hypothesis that the MNL might be a shared ancestral ability among vertebrates.

There are various potential explanations for our negative results. First, we acknowledge some differences between our study and the Rugani and colleagues study (2015). Most importantly, we used adults rather than new-born individuals, and our individuals had been subjected to other experiments that involved food on Plexiglas plates. Also, cleaners approached the two panels only from the middle of the aquarium side, while the chicks could go round each panel from both sides. We find it currently unlikely that any of these differences can potentially explain success or failure in the experiment. Most importantly, conditions for cleaners were the same in Experiment I and Experiment II, and cleaners solved the task in Experiment I. Therefore, a lack of significant performance in Experiment II is likely due to factors beyond methodological differences. On the other hand, the fact that chicks chose panels on the left in the small number test and panels on the right in the large number test (Rugani et al. 2015) certainly indicates that MNL is not exclusive to mammals. One hypothesis that would reconcile all current evidence is that MNL possibly evolved only in tetrapods. Alternatively, as there are several lines of evidence that ecological needs may drive the convergent evolution of cognitive abilities (Kamil 1998; Shettleworth 2009), it is possible that the MNL may have evolved repeatedly and independently in various clades based on specific ecological needs. Further studies that vary species, age, and experience on various vertebrate clades will help us achieve a complete understanding of the distribution of MNL within vertebrates, and determine the degree to which natural or laboratory experience may shape this cognitive process.

Ethical approval

The Animal Ethics Committee of the Queensland government (DAFF) approved the project.

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Conflict of interest

All authors declare that they have no conflict of interest.

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Title: Fluctuations in coral reef fish densities after environmental disturbances on the northern Great Barrier Reef

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Abstract

Global warming is predicted to increase the frequency and or severity of many disturbances including cyclones, storms, and prolonged heatwaves. The coral reef at Lizard Island, part of the Great Barrier Reef, has been recently exposed to a sequence of severe tropical cyclones (i.e., Ita in 2014 and Nathan in 2015) and a coral bleaching in the year 2016. Reef fishes are an essential part of the coral reef ecosystem, and their abundance is thus a good marker to estimate the magnitude of such disturbances. Here, we examined whether the recent disturbances at Lizard Island had an impact on the coral reef fish communities. To do this, we examined fish survey data collected before and after the disturbances for potential changes in total fish density post-disturbance. Also, by sorting fish species into eleven functional groups based on their trophic level (i.e., diet), we further explored the density changes within each functional group. Our findings showed an overall decline of 68% in fish density post-disturbance, with a significant density decrease in nine of eleven trophic groups. These nine groups were: browsers, corallivores, detritivores, excavator/scrapers, grazers, macro-invertivores, pisci-invertivores, planktivores, and spongivores. The piscivores, on the other hand, were the only "winners", wherein their density showed an increase post-disturbance. These changes within functional groups might have a further impact on the trophodynamics of the food web. In summary, our findings provide evidence that the fish assemblage on the reefs around Lizard Island were considerably affected by extreme weather events, leading to changes in the functional composition of the reef fish assemblage.

Introduction

The recently observed increase in frequency and magnitude of extreme weather events is attributed to anthropogenic global warming (Cai et al., 2014; Cheal et al., 2017; Hughes et al., 2018). Such extreme events are a great threat to coral reefs worldwide (Hughes et al., 2017). Coral reefs are one of the world's most diverse ecosystems, with fish as an essential component. Losing live corals can thus have serious impacts on the diversity and stability of this ecosystem (Bellwood et al., 2006; Pratchett et al., 2008, 2011; Munday et al., 2008). For instance, one of the threats of extreme weather events to coral reefs is the prolonged El Niño cycles and the resulting coral bleaching. El Niño is a naturally occurring climatic event that brings warm water towards the Indo-Pacific. A recent prolonged El Niño event led to an increase in seawater temperatures (Cai et al., 2014; Hoegh-Guldberg & Ridgway, 2016). In these conditions, overstressed coral tissues expel their intracellular symbionts "zooxanthella" (i.e., symbionts from which corals gain their different pigmentations) which causes bleaching. The resulting bleached corals may die if they do not re-establish the symbiotic relationship with the zooxanthella within a range of six months post-bleaching (DiazPulido & McCook, 2002). In addition to the threat of coral bleaching, cyclones can also be destructive due to the formation of strong waves that can damage exposed coral reef fields (Cheal et al., 2017). Both cyclones and coral bleaching can thus result in environment degradation and habitat loss (Pizarro et al., 2017; Hughes et al., 2017).

Using fish assemblages, diversity, and abundance, researchers can evaluate the biological integrity and quality of a given habitat (Karr, 1981; Ganasan & Hughes, 1998). Several studies, for instance, showed that fish abundance can be negatively affected by environmental disturbances due to climate change, either directly through abiotic factors such as temperature and ocean acidification (Ferrari et al., 2011; Browman, 2016), or indirectly through habitat loss (Munday et al., 2008). Thus, changes in fish abundance should provide reliable information on the habitat quality.

Habitat degradation is known to have a negative impact on overall fish density (Munday, 2004; Wilson et al., 2008b, 2010). Bellwood et al. (2004) argue that further insights can be gained from analysing fish functional groups, but only in addition to knowing the cause and extent of the habitat degradation. Therefore, exploring potential changes at the level of fish groups that share the same function (i.e., functional group) might yield additional information about the mechanism and effect of the impact. For instance, three main functional groups displaying herbivore dietary traits (i.e., corallivores, excavator/scrapers, and grazers) can play an important role in coral reef recovery. The functional role of these three herbivores is complementary, and together their presence on the reef can play a role in its resistance to disturbances (Bellwood et al., 2004). In addition to the densities of herbivorous fishes, other factors also play a major role in coral reef resistance and recovery, such as the complexity of coral structure and water depth (Graham et al., 2015).

A suitable location to explore potential changes in fish abundance and functional groups after environmental disturbances is Lizard Island (Pizarro et al., 2017; Emslie, Cheal & Logan, 2017; Triki et al., 2018). The island is located in the northern Great Barrier Reef (GBR), Australia, within a marine reserve. The island was impacted by a sequence of extreme weather events three years in a row: In April 2014, Cyclone Ita hit Lizard Island (Pizarro et al., 2017), reaching an intensity of category 5 on the Australian scale (Puotinen et al., 2016). In April 2015, the island was again exposed to another severe cyclone, Cyclone Nathan, a severe category 4 cyclone (Pizarro et al., 2017). And finally, in February/March of 2016, the GBR was affected by a massive coral bleaching event, resulting in more than 60% bleached coral cover (Hughes et al., 2017).

In this study, we asked in to what extent fish communities would change as a function of environmental disturbances at Lizard Island. To do so, we compared fish densities before and after disturbances both overall and by functional group. We expected to find a decline in fish species that rely directly or indirectly on live corals for their diet (Wilson et al., 2006). In contrast, due to the colonisation of dead corals by microalgae (Cheal et al., 2010) we expected an increase in the abundance of various herbivorous fish species specialised on such algae (Randall, 1961).

Methods

Field site and fish census

The study was conducted on the reef around Lizard Island, Great Barrier Reef, Australia (14.6682° S, 145.4604° E). The study was carried out at two locations: Mermaid Cove and Northern Horseshoe reefs. Mermaid Cove forms a continuous fringing reef of approximatively 35,000 m^2 (i.e., estimated from maps: https://www.freemaptools.com/area-calculator.htm), with a depth range from 1 to 7 m. The reef is located in a small bay on the northern side of Lizard Island. The other location, Northern Horseshoe reef, is also a continuous reef consisting of a coral garden of approximately 17,000 m^2, with a depth range from 1 to 4 m. The reef is located on the western side of the island (see Figure 1). After the 2014 and 2015 cyclones, the reef at Mermaid Cove was heavily damaged. Northern Horseshoe reef, however, had been protected from these two cyclones due to its location within the lagoon (Pizarro et al., 2017; Lizard Island Research Station directors, personal communication). The coral bleaching event in 2016 affected all the reefs around Lizard Island including our two study sites.

We used underwater visual fish census methods based on earlier studies by Wismer et al. (2014) and Triki et al. (2018). Within each location, the observer swam ten replicates of a 30 m transect line on the reef flat. Due to the different shape of the reef at the two locations, the transect line was placed parallel to the reef crest at Mermaid Cove, whereas at Northern Horseshoe it was placed parallel to the shoreline (Following methods in Wismer et al., 2014). Along the 30 m transect line the observer first recorded the number of all large visible fish (i.e., species with body total length $TL > 10$ cm) on a 5 m wide area, then the number of small visible fish (i.e., species with body $TL \leq 10$ cm) on a 1m wide area. Each of the ten transect replicates, within each location, were sampled at least 10 meters apart from each other to minimise possible resampling of the same individuals. Only adult fish were surveyed, and their species was identified. Overall, there were 163 species identified in our survey (Table S1). All fish counts (i.e., large and small fish) were scaled per 150 m^2 to facilitate further statistical analyses.

The fish surveys from the two study locations were collected at the same time of day in a similar way between June and August within each year of data collection. Data were collected at Mermaid Cove in 2011 (in Wismer et al., 2014), 2016 (in Triki et al., 2018), and 2017. At Northern Horseshoe, the fish census was conducted in 2014, 2016 (in Triki et al., 2018) and 2017. We labelled the data collected in 2011 (i.e., from Mermaid Cove) and 2014 (i.e., from Northern Horseshoe), as fish survey before disturbances. Subsequently, the fish surveys in 2016 and 2017 were labelled as data after the disturbances (as in Triki et al., 2018).

Fish species were then categorised into functional groups based on the species' trophic level (Butterfield & Suding, 2013; Brandl et al., 2016). We sorted fish species into 11 trophic-functional groups (Table 1). The categorisation into dietary functional groups followed methods in studies by Wernberg et al. (2013) and MacNeil et al. (2015) (see Table S1 in the Supplementary material). For the few species for which trophic level was missing from these studies, we completed information from the FishBase (Froese & Pauly, 2016).

Statistical analyses

All data analyses and figures were generated by using the Software R version 3.5.1. All the recorded fish species were included in the present analyses. Fish counts on each transect represented fish abundance. Therefore, the transect line was the statistical unit in our sample size. Overall, we ran two statistical models. We fit the first model to test for the overall change in total fish abundance before and after the disturbances. It was a General Linear Model (GLM), with a negative binomial distribution. The model had fish abundance as the response variable, while the period before and after the perturbation was fitted as a predictor with data collection site as a covariate. The model had the following structure: fish abundance ~ period of data collection + site. The model assumptions were checked with visual plots with the function influencePlot() in R language.

The second model tested for potential changes in the abundance within the eleven trophic-functional groups. Here, we fitted a zero-inflated negative binomial distribution due to the presence of many zeros in the count data. The zeros refer to the absence of the some functional groups in the transects. The site identity was fitted as a covariate to control for potential differences between the two sites (R. Slobodeanu, personal communication). The model had the following function: fish abundance ~ functional group * period of data collection + site. As *post hoc* analyses for the second model, we ran least-squares means analyses with the function emmeans() from the package (emmeans in R language). The emmeans() function uses the Tukey method by default for multiple comparisons. The reported pseudo R-squared in the results are the Nagelkerke (Cragg and Uhler) values generated with the nagelkerke() from the package (rcompanion in R language) (see Liu, Zheng & Shen, 2008). For further details about statistical tests, R packages and script, please refer to our statistical script in the Figshare repository (DOI: 10.6084/m9.figshare.4990919).

Ethical note

The Animal Ethics Committee of the Queensland government (DAFF) approved the project (CA 2016/05/970 and CA 2017/05/1063).

Results

Fish census data showed that total fish abundance significantly declined after the environmental disturbances (ANOVA: $N = 60$, estimate = -1.239, $X^2 = 52.885$, $p < 0.001$, pseudo R-squared = 0.49, Figure 2), despite differences between the two study sites (ANOVA: $N = 60$, estimate = 0.519, $X^2 = 9.583$, $p = 0.002$). On the other hand, fish functional groups provided more details on where the decline in fish density occurred, with a significant interaction of the trophic-functional group and the period of data collection (ANOVA: $N = 660$, $X^2 = 68.899$, pseudo R-squared = 0.66, $p < 0.001$, Figure 3). *Post hoc* tests showed that ten out of the eleven functional groups went through a significant change in fish abundance after the disturbances, of which nine showed a decline (the contrast before – after): browsers (estimate= 1.534, $z = 3.427$, $p < 0.001$); corallivores (estimate= 2.099, $z = 2.418$, $p = 0.015$); detrivores (estimate= 15.784, $z = 3.610$, $p < 0.001$); excavator/scrapers (estimate= 8.904, $z = 4.308$, $p < 0.001$); grazers (estimate= 11.836, $z = 2.617$, $p = 0.009$); macro-invertivores (estimate= 3.580, $z = 2.195$, $p = 0.030$); pisci-invertivores (estimate= 1.133, $z = 2.337$, $p = 0.020$); planktivores (estimate= 102.06, $z = 4.340$, $p < 0.001$); and spongivores (estimate= 39.951, $z = 3.479$, $p < 0.001$). Only piscivores showed a significant increase in abundance (estimate= -0.662, $z = - 2.277$, $p = 0.022$), while micro-invertivores were the only functional group that did not show any significant changes (estimate= 6.956, $z = 1.152$, $p = 0.249$).

Table 1. Dietary functional trait used in sorting fish species into trophic-functional groups.

Trophic-functional group	**Diet**	**Example**
Browser	Macro-algae	*Naso unicornis*
Corallivore	Corals	*Chaetodon aureofasciatus*
Detritivore	Dead organic material "detritus"	*Ctenochaetus striatus*
Excavator/scraper	Remove reef substrate while looking for living material	*Chlorurus spilurus*
Grazer	Fast-growing macro-algae "turf algae"	*Siganus doliatus*
Macro-invertivore	Large invertebrates	*Balistapus undulatus*
Micro-invertivore	Small invertebrates	*Coris batuensis*
Pisci-invertivore	Fish and invertebrates	*Lethrinus olivaceus*
Piscivore	Fish	*Epinephelus merra*
Planktivore	Plankton	*Abudefduf sexfasciatus*
Spongivore	Sea sponges	*Pomacanthus sexstriatus*

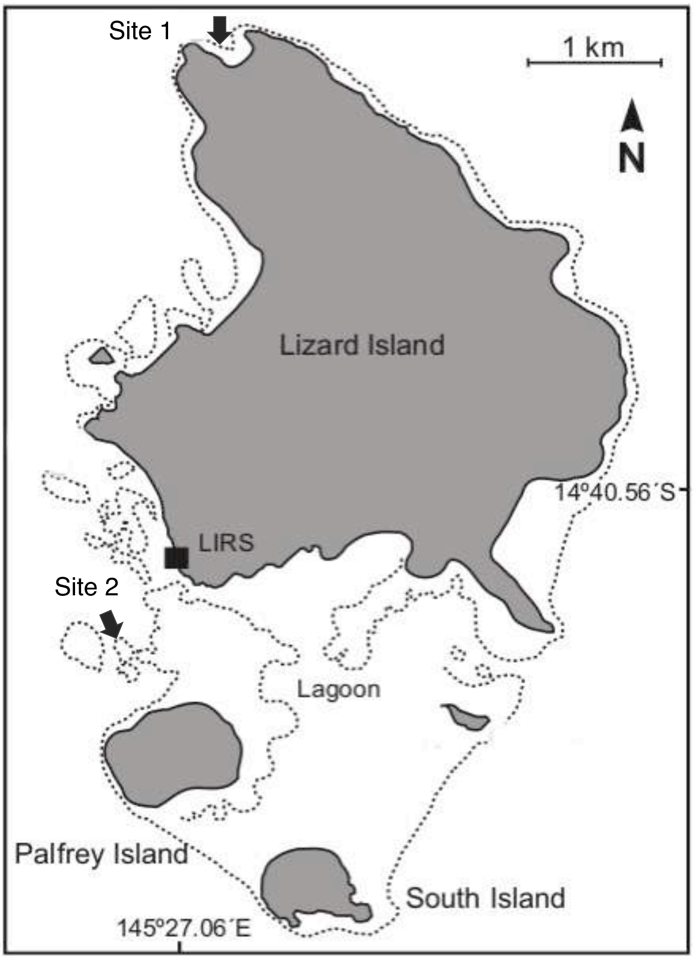

Figure 1. Lizard Island group map. The map is showing the two study sites: Mermaid Cove reef as Site 1 and Northern horseshoe reef as Site2. Modified from *Triki et al. 2018, Global Change Biology* (© 2017 John Wiley & Sons Ltd).

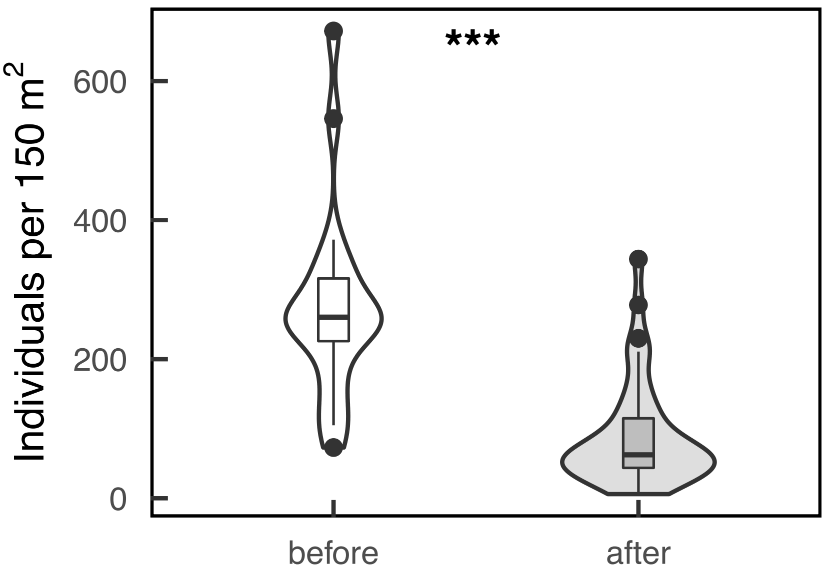

Figure 2. Total fish abundance. Boxplots are displaying median and interquartile of total fish abundance before (n=20) and after (n=40) the environmental disturbances (i.e., cyclones and coral bleaching). Negative binomial Generalised Linear Model: *** $p < 0.001$.

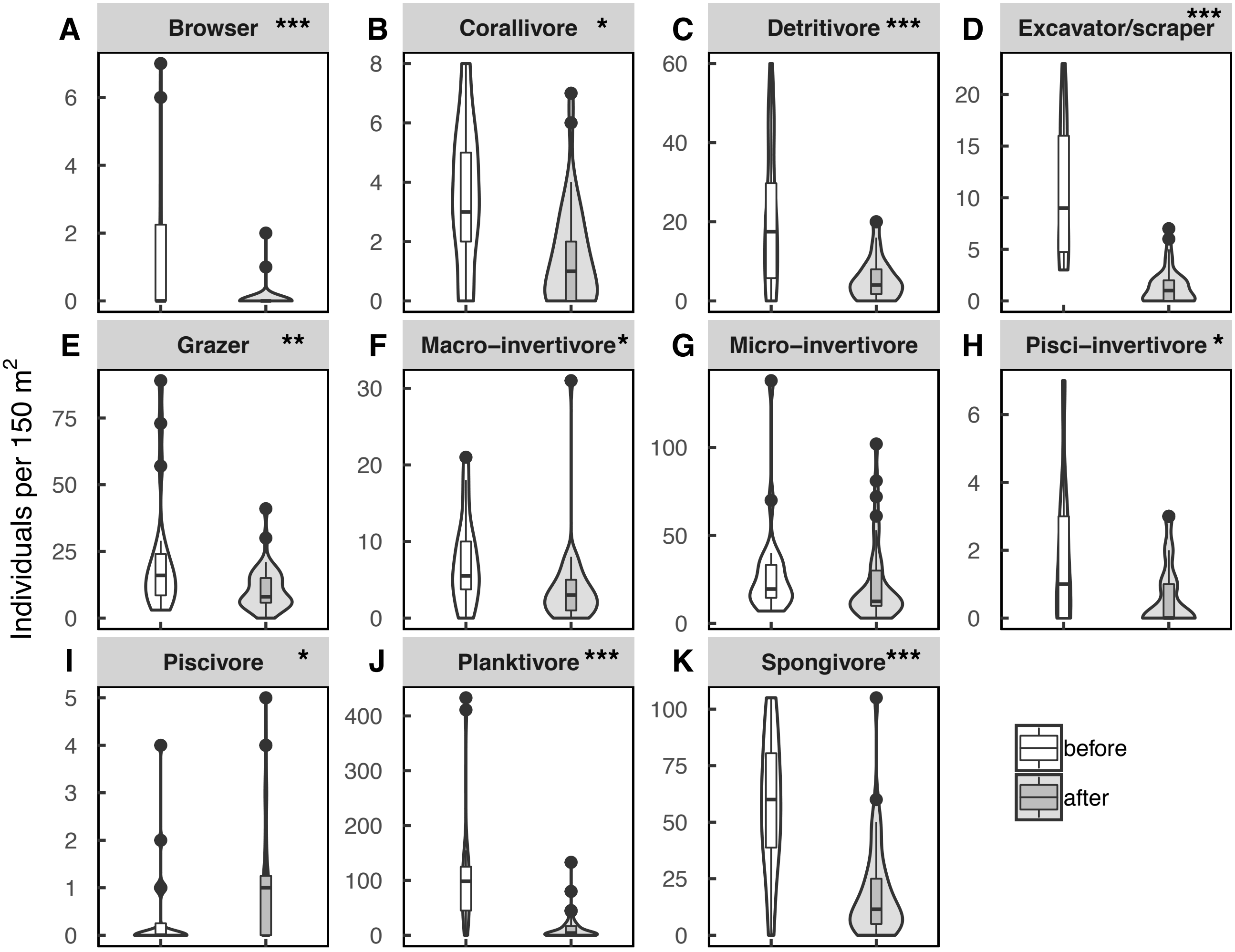

Figure 3. Fish abundance per trophic-functional groups. Boxplots are displaying median and interquartile of fish abundance within trophic-functional groups showing eventual changes from before (n = 20) to after (n = 40) the environmental disturbances (i.e., cyclones and coral bleaching). Note that due to the high variation in fish counts per functional group, the y-axes do not have the same scale. Post hoc analyses of a Zero-inflated negative binomial model showing differences between before and after the perturbations within each functional group: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Discussion

We identified a substantial decline in the density of reef fishes at Lizard Island following a sequence of severe tropical cyclones and coral bleaching. We documented a 68 % decline in fish densities; a percentage close to what Wilson et al. (2006) found in their meta-analysis of 17 independent studies on fish density after environmental disturbances, in which an average decline of 62 % was observed in fish density within three years after disturbances including cyclones and coral bleaching. These findings are in line with previous studies suggesting that the loss of coral cover would lead to a reduction in fish density (Jones et al., 2004; Russ & Leahy, 2017; Pratchett et al., 2018). It suggests that the recorded decline in the present study might be due to the loss of coral cover. Cyclones usually destroy the reef structure, which would impede corals from possible rapid recovery (Cheal et al., 2002). Also, coral recovery might be compromised if the reef is repeatedly exposed to tropical cyclones over short-time intervals (De'ath et al., 2012; Puotinen et al., 2016). In addition to damage from cyclones, bleaching can reduce coral cover (DiazPulido & McCook, 2002). Recently, Stuart-Smith et al. (2018) documented a 51 % coral cover loss at the GBR after the 2016 bleaching event. Subsequently, it is expected that habitat loss would lead to a decline in fish abundance (Pratchett et al., 2011; Brandl et al., 2016). We acknowledge an important caveat in the present study: we were unable to incorporate information about the benthic habitat structure and benthic communities. This information would provide more insight into the fluctuations in reef-dependent fish communities (Goren & Spanier, 1985; Holbrook, Schmitt & Stephens, 1997; Russ & McCook, 1999; Wismer, Hoey & Bellwood, 2009; Pizarro et al., 2017; Prazeres, Roberts & Pandolfi, 2017; Renfro & Chadwick, 2017). Also, due to the absence of data on the benthos, the significant effect of "site" in our model is virtually impossible to interpret (see supplementary Fig. S1 and Fig. S2). Nevertheless, the significant decline in fish densities recorded here can still inform us about the effect of extreme weather events on fish assemblages (Wilson et al., 2006).

Assessing fish density according to their trophic-functional groups showed a substantial decline in nine out of eleven functional groups. This suggests that most fish, regardless of their trophic affiliation, were susceptible to the disturbances. Nevertheless, the piscivore group was the only group to benefit from such disturbances. It is possible that due to the damage of reef structure and the resulting destruction of shelters, piscivores (i.e., reef-associated predators) would gain easy access to prey, from which they could benefit and thereby increase their numbers. Also, it is possible that bleached corals might no longer be suitable shelters for coral-dwelling species (Coker, Pratchett & Munday, 2009; Pratchett et al., 2011), nor appropriate camouflage background for small-bodied prey. As a consequence, predators would easily recognise their prey (Phillips et al., 2017), which would eventually change the assemblage structure of these predatory fishes (Emslie, Cheal & Logan, 2017). Nevertheless, such an increase might be transient in time and eventually be followed by a decline due to decreased numbers of prey. Also, the erosion of corals skeleton over time might result in a reduction of shelter and hunting options for ambush predators (Kerry & Bellwood, 2012).

Graham et al. (2011) predicted that micro-invertivores are one of the trophic-functional groups most vulnerable and macro-invertivores the least vulnerable to climate disturbances. Here, the micro-invertivores were the only group without apparent changes from pre- to post-disturbance. A potential explanation for this divergence is that micro-invertivores may show high functional redundancy, where losses in particular species can be replaced by population increases in other species that share a similar function (Micheli & Halpern, 2005; Brandl et al., 2016). Furthermore, we note that the decline in browsers, corallivores and pisci-invertivores, as well as the increase in piscivores documented in this study, differ from previous results also collected around Lizard Island (Ceccarelli, Emslie & Richards, 2016; Brandl et al., 2016). One potential explanation is that those previous studies used post-disturbance data collected in early 2015, i.e. only a few months after hurricane Ita hit the island, while we collected data 2-3 years after another cyclone and the El Niño event took place. A potential additional factor could be that the previous studies collected data in 3m and 9m depth, while our data include shallow areas of 1-2m depth.

The trophic-functional groups that were most abundant pre-disturbance, the planktivores and spongivores, also showed a decline in numbers post-disturbances (Figure 3). For instance, planktivores are mainly damselfish that are often highly coral-associated species (Feary et al., 2007; Wilson et al., 2008a), wherein habitat loss might explain the decline in their numbers. Such losses can be detrimental to the ecosystem balance, mainly because the planktivores play an important role in transferring nutrients from the pelagic environment onto the reef (Pace et al., 1999; Fisher et al., 2015). Spongivores also have a significant role in protecting corals by feeding on overgrowing sponges, thereby reducing coral-sponge competition (Hill, 1998). The decrease in fish density in the other functional groups: browsers, detritivores, grazers, and excavators/scrapers, can also have severe consequences on the health and resilience of corals after disturbances. These trophic-functional groups feed on macro-algae, which prevent the latter from over-colonising the corals. Their functional role is hence beneficial for coral resilience, coral settlement, and growth (Green & Bellwood, 2009; Cheal et al., 2010; Rasher, Hoey & Hay, 2013).

Conclusion

Environmental disturbances are expected to increase in frequency and magnitude due to global warming. Here we found that such environmental events were followed by reductions in fish densities across multiple trophic-functional groups around Lizard Island. These findings add to the data that shows that future coral reef fish communities are susceptible to significant changes on this island. Supported by the larger scale fish assemblage changes across the GBR shown by Hughes et al. (2018). It suggests that such losses can impact the functionality and stability of these communities (Green & Bellwood, 2009; Rasher, Honey & Hay, 2013).

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Title: Cue-based decision rules used by cleaner fish in a biological market context

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* **My contribution:** *I performed the video and data analyses of the behavioural observations.*

Abstract

In order to develop an evolutionary theory of social decision-making, we need to understand how individuals use environmental cues to form decision rules through associative learning. Here, we show that intraspecific variation in the performance of 'cleaner' fish in a learning task is based on variation in the relative salience of available cues, and correlates with variation in a cleaner's natural social environment. We exposed cleaners to a biological market task, where giving priority to an ephemeral ('visitor' client) food plate, over a permanent ('resident' client) plate, doubled the food reward. We manipulated two cues that cleaners potentially use to identify visitors and residents in nature: 1) size (correlative-cue: visitors typically > residents in size), and 2) colour-pattern (precise-cue: differentiates fish species). Without colour-pattern cues, cleaners generally exhibited a spontaneous preference for inspecting large plates. With both cues present, only cleaners collected from a socially-complex site, characterised by a higher frequency of visitor clients that leave cleaner territories when the cleaner is occupied with another client, settled on using the colour cue; therefore, outperforming cleaners from a socially-simple site. Variation in the relative salience of available cues may hence explain a portion of variation in cognitive performance and social behaviour.

Keywords: Cognition, Decision rules, Heuristics, Cooperation, Mutualism, reinforcement learning

Lizard Island