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What the cognitive abilities of the cleaner fish *Labroides dimidiatus* may tell us about vertebrate brain evolution

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Moorea
Lizard Island

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"What the cognitive abilities of the cleaner fish *Labroides dimidiatus* may tell us about vertebrate brain evolution"

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Résumé

La grande variation existant chez les vertébrés concernant la taille relative du cerveau en comparaison à la taille du corps a toujours été un challenge scientifique majeur ; les scientifiques ont tenté de comprendre quels facteurs y étaient impliqués, ainsi que l'implication d'avoir un plus grand cerveau, autrement dit est-ce que la taille du cerveau est liée aux capacités cognitives ? Plusieurs hypothèses comme celle de l'intelligence sociale (*social brain*), de l'intelligence écologique (*ecological intelligence*), ainsi que plus récemment celle de l'intelligence générale (*general intelligence*) présentent différents facteurs qui pourraient expliquer cette variation. Pourtant, les résultats obtenus jusqu'à maintenant peuvent souvent être interprétés comme soutenant différentes hypothèses, démontrant que des études supplémentaires sont nécessaires pour trouver quels facteurs sélectifs sont les principaux vecteurs de l'évolution cérébrale. Le fait que la taille cérébrale relative diffère en moyenne d'un facteur 10 entre les endo- et les ectothermes, c'est-à-dire qu'un ectotherme qui a le même poids qu'un endotherme possède un cerveau 10 fois plus petit, est particulièrement intriguant. Alors qu'autant les ecto- que les endothermes font face à des challenges aussi bien sociaux qu'environnementaux, nous devons comprendre quels aspects sont d'une différence si fondamentale qu'un facteur 10 apparaisse. C'est pourquoi les processus cognitifs sont étudiés dans différentes espèces animales, via des tâches écologiques ou abstraites pour tester leur relation à la taille cérébrale, et si ces processus cognitifs sont liés à l'intelligence générale.

Ma thèse de doctorat avait pour objectif d'enquêter si le poisson nettoyeur *Labroides dimidiatus*, une espèce avec une taille cérébrale moyenne chez les ectothermes, démontre des capacités cognitives dîtes avancées. Ce poisson nettoyeur interagit de manière mutualiste avec d'autres poissons du récif corallien, communément appelés 'poissons clients'. Il ôte les parasites externes (ectoparasites) se trouvant sur la peau des poissons clients, constituant ainsi leurs ressources alimentaires. De ce fait, les poissons clients sont nettoyés de leurs parasites. Cependant, le poisson nettoyeur a une préférence gustative pour le mucus des poissons clients par rapport aux ectoparasites ; dans ce cas l'interaction n'est plus considérée comme mutualiste n'étant plus bénéfique pour le poisson client, ce qui est considéré comme de la triche. À cause de ce comportement de triche, le poisson nettoyeur démontre de grandes stratégies comportementales sophistiquées, qui ont été documentées précédemment, comme par exemple l'effet d'audience, la gestion de sa réputation, et la priorisation des différents types de poissons clients afin de les maintenir dans leur territoire. Premièrement, les capacités cognitives étaient testées séparément puis avec ou sans pertinence écologique pour le poisson nettoyeur. Avec cette première approche, j'ai pu découvrir si le poisson nettoyeur était capable de démontrer des capacités cognitives uniquement dans un contexte écologique ou s'il était également capable d'en démontrer dans un contexte plus abstrait. Deuxièmement, j'étais intéressée de tester la présence d'intelligence générale chez les ectothermes en expérimentant cette fois-ci différents processus cognitifs. Quatre tâches cognitives n'avaient aucune pertinence écologique et une était liée à l'écologie du poisson nettoyeur en question.

Dans le premier chapitre, j'ai étudié le comportement des poissons dans un paradigme de gratification différée. Dans ledit paradigme, il a été démontré que les primates révèlent une performance élevée qui a été liée au haut niveau de maîtrise de soi, ainsi qu'à la compréhension de la remise de bénéfice dans le futur, affirmant ainsi la demande cognitive de cette tâche. J'ai travaillé avec deux espèces de poissons nettoyeurs (*L. dimidiatus* et *L. bicolor*), ainsi que deux espèces de poissons non-nettoyeurs de la famille des Labres (*Halichoeres trimaculatus* and *H. hortulanus*). Les deux espèces de poissons nettoyeurs diffèrent dans leur écologie : *L. dimidiatus* habitent dans des stations de nettoyage permettant les clients de décider dans quelle station ils veulent se faire nettoyer et par quel poisson nettoyeur. En revanche, *L. bicolor* ont un territoire plus grand et dépourvu de stations de nettoyage. Ce comportement dit errant permet à *L. bicolor* de tricher et ce plus souvent envers les poissons clients étant donné que ces derniers ne peuvent se remémorer par quel poisson nettoyeur ils ont été nettoyés. Les deux espèces de poissons non-nettoyeurs se nourrissent, quant à eux, de petits crustacés

qu'ils trouvent dans le sable. Je me suis demandée si l'écologie spécifique des poissons nettoyeurs augmenterait leur performance par rapport à celle des poissons non-nettoyeurs qui n'ont aucune raison écologique pour réussir autant bien que les poissons nettoyeurs. Toutes les différentes espèces de poisson testées dans ce paradigme ont réussi de manière égale à contrôler leur impulsion envers la nourriture pour en obtenir plus. En revanche, seulement les poissons nettoyeurs ont démontré une compréhension de la tâche en prenant préalablement la décision d'attendre ou non pour obtenir plus de nourriture, démontrant ainsi leur capacité d'anticipation.

Dans le second chapitre, *L. dimidiatus* a été testé selon une expérience abstraite sur l'apprentissage de concept, soit une approche classique pour tester si les animaux peuvent apprendre sans de renforcement spécifique (conditionnement). L'appariement à l'échantillon (*matching-to-sample*) a été utilisé comme expérience, dans sa forme la plus générale, par rapport à ce qui avait déjà été fait. Les poissons nettoyeurs ont rempli les conditions de réussite de cette tâche, nous démontrant ainsi qu'ils possèdent le processus cognitif de l'apprentissage de concept, autrement dit la capacité de raisonnement. De plus, un poisson a été testé dans un appariement à l'échantillon différé (*delayed matching-to-sample*) ; il a cependant échoué dans cette expérience. Du fait qu'un seul poisson a été testé dans cette dernière tâche, il est difficile de conclure que les poissons nettoyeurs ne possèdent pas le processus cognitif de la mémoire de travail (*working memory*).

Finalement, dans le troisième chapitre, *L. dimidiatus* ont été exposés à différentes tâches conçues pour tester l'intelligence générale. Les performances relatives à chaque tâche étaient respectivement évaluées et notées afin de vérifier la présence ou non d'un facteur d'intelligence générale dit '*g*' (*general intelligence factor*). Un aspect intéressant est que la variation intra spécifique dans les stratégies dites sophistiquées est liée à la densité de poissons indiquant une probable corrélation de la complexité intra- et interspécifique. Dès lors que ces stratégies étaient mises en relation à l'écologie du poisson nettoyeur, la question principale était de savoir si les performances des poissons nettoyeurs variaient dépendamment de la tâche écologiquement pertinente ou non. De ce fait, la moitié des individus venait d'une forte densité de poissons, tandis que l'autre moitié provenait d'une faible densité de poissons. Les poissons nettoyeurs ont démontré des capacités dans trois processus cognitifs sur quatre, c'est-à-dire en apprentissage et flexibilité, en inhibition, ainsi qu'en raisonnement quantitatif ; mais, ils échouèrent dans la tâche de la mémoire de travail (*working memory*). Quand bien même les poissons nettoyeurs ont démontré de bonnes performances dans ces différentes tâches, je n'ai pas trouvé une forme d'intelligence générale chez ce poisson ; les différentes tâches n'étant pas positivement corrélées entre elles dans une analyse de facteur de composant principal. De plus, la complexité de l'environnement, c'-est-à-dire la densité de poissons, n'a pas affecté la performance des poissons nettoyeurs et ce dans aucune des tâches.

En conclusion, cette thèse augmente l'évidence que les poissons nettoyeurs, comme représentants des ectothermes avec un cerveau relativement plus petit comparé aux endothermes, possèdent une variété de processus cognitifs considérés comme avancés, qu'ils peuvent appliquer à des situations non-écologiquement pertinentes. De ce fait, les résultats de cette thèse réfutent l'hypothèse que les majeures différences dans les capacités cognitives expliquent la différence d'un facteur 10 dans la taille relative du cerveau entre les vertébrés dits endothermes et ceux dits ectothermes. Cependant, malgré les capacités cognitives avancées des poissons nettoyeurs, ils sont dépourvus d'intelligence générale. Ce résultat nous amène à se poser la question de savoir si l'absence d'intelligence générale est un élément commun des ectothermes. Si ceci est confirmé, l'intelligence générale pourrait fournir une nouvelle explication quant à la division dans l'index d'encéphalisation entre endothermes et ectothermes.

Mots clefs: poisson nettoyeur; nettoyage mutualistique; capacités cognitives; écologie comportementale; gratification différée; apprentissage de concept abstrait; intelligence générale

General summary

Explaining the great variation in relative brain size in comparison to body size among vertebrates has been a great scientific challenge. Scientists have tried to understand what factors triggered this relative difference, as well as what it implies to have a bigger brain, i.e. how is brain size linked to cognitive abilities? Various hypotheses such as the social brain hypothesis, the ecological intelligence hypothesis, as well as most recently the general intelligence hypothesis propose different factors that could explain this variation. However, current results can often be interpreted as supporting various hypotheses, showing that additional studies are needed in order to disentangle which selective factors are the main driver of brain evolution. Particularly intriguing is the observation that relative brain size differs on average by a factor 10 between endo- and ectotherms, i.e. an ectotherm of the same weight of an endotherm possesses a ten times smaller brain. Thus, while both ecto- and endotherms face social and environmental challenges, we need to understand what aspects are of such fundamental difference that a factor 10 emerges. Therefore, cognitive processes are studied in various animal species via ecological or abstract task to test their relationship to brain size, and whether cognitive processes are linked to general intelligence.

My PhD thesis aimed to investigate whether cleaner fish *Labroides dimidiatus*, a species with a rather average ectotherm brain size, show advanced cognitive abilities. Cleaner fish engage in mutualistic interactions with so-called client fish. They remove the ectoparasites from their clients, constituting their food resources, and clients, in turn, get cleaned. However, cleaners prefer to eat mucus over ectoparasites constituting cheating. Because of this cheating behaviour, cleaners show great sophisticated strategies behaviour previously documented, such as audience effect, reputation management, and client type prioritization in order to retain their clients. First, cognitive processes were tested separately and with or without ecological relevance to the fish. With this first approach, I was able to disentangle whereas cleaner fish were also able to show cognitive abilities only in an ecological context or also in an abstract situation. Second, I was interested in testing the presence of general intelligence in ectotherms by testing this time different cognitive processes. Four cognitive tasks were lacking ecological relevance and one was related to cleaners' ecology.

In the first chapter, I tested fish in a delay of gratification paradigm. High performance in primates has been linked to high levels of self-control and low discounting of future benefits, proposing that the ability is cognitively demanding. I used two cleaner fish species (*L. dimidiatus* and *L. bicolor*), and noncleaner wrasse species (*Halichoeres trimaculatus* and *H. hortulanus*) were used. Cleaner fish species differ in their ecology as *L. dimidiatus* live at cleaning stations, allowing clients to decide whether to seek or avoid any particular cleaner. In contrast, *L. bicolor* are roving cleaners lacking cleaning stations. Such roving behaviour allows *L. bicolor* to cheat more towards their clients as these ones are not capable of following the cleaners bicolor individually. Both non-cleaner wrasses eat small crustaceans in the sand. I asked whether the special ecology of cleaners would increase their performance compared to non-cleaners which lack any ecological reason to perform well in this task. Fish species performed well at controlling their impulse toward food in order to get more. In contrast, only cleaners show an understanding of the task by making the decision early on to wait or not for a bigger food reward, showing the ability to anticipate.

In the second chapter, *L. dimidiatus* were tested in an abstract experimental design of concept learning, a classic approach to test whether animals can learn without specific reinforcement (conditioning). The matching-to-sample task was used in the most general form compared to previous experiments. Cleaners overall performed above chance in this task, suggesting that they possess the cognitive process of concept learning, i.e. reasoning ability. Furthermore, one individual was tested in a delayed matching-to-sample experiment but did not succeed. As only one individual was tested, it is rather difficult to conclude that cleaners lack working memory cognitive process.

Finally, in the third chapter, *L. dimidiatus* individuals were exposed to different tasks designed to test for general intelligence, and their performances in each task were ranked to check for the presence/absence of '*g*'. Interestingly, intraspecific variation in sophisticated strategies were linked to fish densities as a likely correlate of intra- and interspecific complexity. Since those strategies were related to cleaners' ecology, the main question was whether cleaners' performances vary depending on if the task was ecologically relevant or not. Therefore, half of the individuals were coming from a high fish density and the other half from a low fish density. Cleaners showed abilities in three out of four cognitive processes, i.e. learning-flexibility, inhibition, and quantitative reasoning. They failed in the working memory task. Even though cleaners performed well in the tasks, I found no evidence for general intelligence, i.e. the tasks were not positively loading on a single main principal component factor. Moreover, the complexity of the environment, i.e. fish density, did not affect cleaners' performance in any task.

In conclusion, this thesis increases evidence that cleaner fish, as representatives of ectotherms with relatively smaller brains compared to endotherms, possess a variety of supposedly advanced cognitive processes, which they can also apply to non-ecologically relevant situations. Therefore, the results from this thesis refute the hypothesis that major differences in the cognitive tool set may explain the 10-fold difference in relative brain size between endotherm and ectotherm vertebrates. However, despite cleaners' advanced cognitive abilities, they lack general intelligence. This result raises the question of whether the absence of general intelligence is a common pattern in ectotherms. If this was confirmed, general intelligence may provide a new explanation for the endotherm-ectotherm divide in the encephalization index.

Keywords: cleaner fish; cleaning mutualism; cognitive abilities; behavioural ecology; delay of gratification; abstract concept learning; general intelligence

General introduction

I.1 Cognition and brain size

Vertebrates are part of the animal kingdom (Ruggiero et al., 2015) and comprise different groups such as tetrapods including amphibians, reptiles, birds, and mammals as well as fishes (de Lamarck, 1801, 1822; Nielsen, 2012). The wide diversity of species, and thus body sizes, involved in the vertebrates' subphylum lead to great differences regarding brain size (van Dongen, 1998). Studies that focused on the relationship between brain size and body size emphasized that humans have a larger brain with respect to their body size (Striedter, 2005). When two body parameters, such as body size and brain size, are not proportionally scaled, this is called allometry (van Dongen, 1998). Here, body size is not proportionally scaled to brain size. Multiple studies focused on explaining this variation in brain size among vertebrates (Darwin, 1871; Gould, 1975; Jerison, 1973; Lande, 1979; Martin, 1981; Tsuboi et al., 2018). Overall, in vertebrates, it is clear that, when accouting allometry, human brains are the largest, and primates have generally larger brains than other vertebrates (Jerison, 1973; Martin, 1990; Passingham, 1985). Several scientists have stated that a relatively larger brain should provide some benefits such as 'better' cognitive abilities (Dunbar and Shultz, 2007; Kotrschal et al., 2013; Sol and Price, 2008). However, as the brain is an expensive tissue (Aiello and Wheeler, 1995; Harvey et al., 1987), some costs are induced and both these benefits and costs led to the great variation in brain size in vertebrates (Striedter, 2005). Therefore, the following question arises: what can a species with a relatively larger brain do that other species cannot? An answer could lie in the term 'cognition', defined by Shettleworth as the mechanism used by animals to perceive, learn, memorize, and make decisions by acquiring and processing information taken from the environment (Shettleworth, 1998, 2010). From cognition results cognitive processes, i.e. brain functions, like the basic processes of perception, attention, learning, and memory (Papini, 2008). From these basic processes, advanced cognitive processes can arise such as discrimination, categorisation, conceptualisation, flexibility, inhibition, planning, anticipation, working memory, spatial orientation, reasoning, problem-solving, metacognition such as theory of mind, social learning, and communication (Shettleworth, 1998, 2010, 2013) (Table 1). To test cognition in animals, cognitive tasks reflecting a cognitive process is carried by individuals and their performances measured. The cognitive tasks can be related to the ecology of the studied species, or not, depending on what cognitive process is being tested. So far, many studies found positive correlations between cognitive performance and brain size in diverse taxa, suggesting that cognition might explain larger brain size (Anderson, 1993; Barton, 1996, 2006; Benson-Amram et al., 2016; Bshary et al., 2016; Buechel et al., 2018; Burkart and van Schaik, 2010; Burkart et al., 2009; Chittka and Niven, 2009; Damerius et al., 2017; Deaner et al., 2006, 2007; DeCasien et al., 2017; Dunbar, 1995; Forss et al., 2016; Gibson et al., 2001; González-Forero and Gardner, 2018; GonzalezVoyer and Kolm, 2010; Gonzalez-Voyer et al., 2009; Harvey and Krebs, 1990; Healy and Rowe, 2007; Herculano-Houzel, 2017; Isler and van Schaik, 2009; Isler and Van Schaik, 2014; Kotrschal et al., 2013; Logan et al., 2018; MacLean et al., 2014; Pike et al., 2018; Pollen et al., 2007; Reader et al., 2011; van Schaik and Pradhan, 2003; van Schaik et al., 2017, 2012; Schuppli et al., 2016; Shumway, 2008; Street et al., 2017; Taylor and van Schaik, 2007; Whiten and Byrne, 1988, 1997).

Table 1: Definitions of cognitive processes and differences between the basic/complex ones (from Shettleworth, 1998, 2010, 2013). The definition of cognitive abilities is from Gottfredson, 1997.

	Definition	**Examples**
Cognitive processes	Brain functions allowing the process of information from the environment	Attention, sensation, knowledge formation, memory, working memory, reasoning, decision making, problem-solving, comprehension, language, metacognition
Basic cognitive processes		Attention, perception, memory
Advanced cognitive processes	Require the simultaneous use of multiple basic cognitive processes, i.e. use of knowledge leading to decision making	Planning, anticipation, reasoning (quantitative, by exclusion), problem-solving, inhibition, working memory, visual-spatial processing, flexibility, and metacognition (ToM, empathy)
Cognitive abilities / performances	General mental ability involving reasoning, problem-solving, planning, abstract thinking, and learning from experience	

I.2 Brain size evolution

The evolution of brain size is suggested to be driven by three main factors which could be social, ecological, or general intelligence. Several hypotheses have emerged from those factors to try to explain the variation in brain complexity, typically controlling for body size. First, the 'social brain hypothesis', which takes into account the social environment, i.e. group size, social system, mating system, deceptive behaviour, strength of social bonds, etc., of the animal species to explain brain size (Barton, 1996, 1999; Byrne and Corp, 2004; Dunbar, 1995, 1998; Humphrey, 1976; Shultz and Dunbar, 2007; Whiten and Byrne, 1988). A subversion of the social brain hypothesis is called the 'cultural intelligence hypothesis', which is the paralleled version of the 'Vygotskian intelligence hypothesis' applied uniquely to humans (Herrmann et al., 2007; Moll and Tomasello, 2007). This hypothesis takes into consideration culture to explain brain size in animal species in general (Boyd and Richerson, 1988; van Schaik and Burkart, 2011; Whiten and van Schaik, 2007; Wilson, 1985). Here, culture refers to the combination of social learning (Richerson et al., 2010), and general behavioural flexibility (Reader and Laland, 2002). Secondly, the 'ecological intelligence hypothesis' considered non-social ecological situations, i.e. foraging behaviour including finding food which depends on the food diet, to explain brain size (Clutton-Brock and Harvey, 2009; Clutton-Brock and Harvey, 1980; DeCasien et al., 2017; Gibson, 1986; Hutchins, 2010; Milton, 1981; Rosati, 2017). Thirdly, the 'general intelligence hypothesis' stipulates that a single general intelligence factor g might trigger cognitive performance which is linked to brain size (Burkart et al., 2017; Emery, 2004; Kolata et al., 2008; Matzel et al., 2003; Reader and Laland, 2003; Reader et al., 2011). General intelligence is defined as the capacity to relate different processes of cognition between each other. This means that when an individual performs well in a certain task, it might perform similarly well in another task, which evokes a different cognitive process. This positive correlation between different cognitive tasks is known as the positive manifold in humans (Burkart et al., 2017; Carroll, 1993; Spearman, 1904). Nevertheless, which hypothesis best explains the evolution of brain size is still up for debate (reviewed in González-Forero and Gardner, 2018). In fact, when taking the example of the social brain hypothesis, group size does not always

relate to advanced cognitive abilities, e.g. in birds, fish, and primates (Beauchamp and Fernández-Juricic, 2004; DeCasien et al., 2017; Pollen et al., 2007). A recent study has put forward the hypothesis that the relationship between cognitive performance and social relationships can be bi-directional, in the sense that cognitive performance might shape social relationships. Since the individual can acquire more information from its environment, that will help the individual to adapt more rapidly to social context, and therefore increase its social competence (Wascher et al., 2018). Further studies are needed to be developed in this direction. This shows how it is difficult to demonstrate causality, as social domains can be related to brain size and vice versa (Powell et al., 2017).

I.3 Endotherms vs ectotherms

So far, scientists have focused on advanced cognitive processes to explain the variation in brain size in animals (Barton, 1999; Gibson, 1986; González-Forero and Gardner, 2018; Healy and Rowe, 2007; Logan et al., 2018; Seyfarth and Cheney, 2002; Striedter, 2005). However, these scientists concentrated their effort mainly on primates or corvids, trying to explain why those two groups have larger brains relative to other mammals or birds, respectively (Bond et al., 2007; Clutton-Brock and Harvey, 2009; Delius et al., 2001; Emery, 2004; Emery and Clayton, 2009; van Horik et al., 2012; Lefebvre et al., 2004; Powell et al., 2017; Sol and Price, 2008; Zuberbühler and Janmaat, 2010). Whereas those studies all focused on endothermic species, a 10-fold difference in the cephalisation index exists between endo- and ectothermic species (Jerison, 1973). Is this difference linked to advanced cognitive processes or is it linked to something else? In fact, in the animal kingdom, a positive correlation between body weight and brain weight has been found (Harvey and Krebs, 1990; Jerison, 1955, 1969, 1973). As described in the previous paragraph, different hypotheses, and the studies coming from them, tend to explain what selection pressure caused brain expansion. As previously said, Jerison (1973) found and highlighted the cephalisation index, which is on average about 10 times bigger in endotherms (which were called 'higher vertebrates' by Jerison) compared to ectotherms (which were called 'lower vertebrates' by Jerison) (Figure 1). The terminology of endo- and ectotherms is used here to emphasize the difference between the two animal groups in their ability to maintain their metabolic body temperature. Endothermic organisms maintain their body temperature via internal bodily functions such as muscle contraction, e.g. when humans shiver when it is cold (Cannon and Nedergaard, 2004; Ohlberger, 2013). Mammals and birds are endotherms. Some cartilaginous fish such as some lamnid sharks, tuna, and billfishes (Block and Finnerty, 1994; Wegner et al., 2015) can be considered regional endothermic species, as they use endothermy only in specific organs allowing better mobility and senses (Willmer et al., 2005). Some snakes use facultative endothermy in order to incubate their eggs, so this endothermy is used only in particular conditions (Stahlschmidt and DeNardo, 2009). In contrast, the body temperature of ectothermic organisms follows the environmental temperature (Davenport, 1992; Ohlberger, 2013). Fish, amphibians and reptiles are part of this latter group. The link between ambient and body temperature, i.e. endothermy, could affect the brain system. Scientists have already shown that the brain is an expensive organ, in terms of energy (Armstrong, 1983; Mink et al., 1981), and have further reported a positive relationship between brain size and basal metabolic rate (Isler and van Schaik, 2006). Therefore, larger brains might allow higher basal energy costs. Individuals might be able to experience an increase in relative brain size, as long as they have access to either higher, or better, nutritional food values or decrease their actual energetic demands (Zuberbühler and Janmaat, 2010). If we relate the thermic management difference between the two animal groups, it seems that endothermic animals have a better chance to possess larger brains than ectotherms. This suggestion has been strengthened by two studies showing the positive effect of temperature on brain size in vertebrates, i.e. higher temperatures are related to bigger brains (Gillooly and McCoy, 2014; Yu et al., 2014). Furthermore, tropical fishes have larger brains than temperate fish species, which in turn have larger brains than polar fish species (Yu et al., 2014).

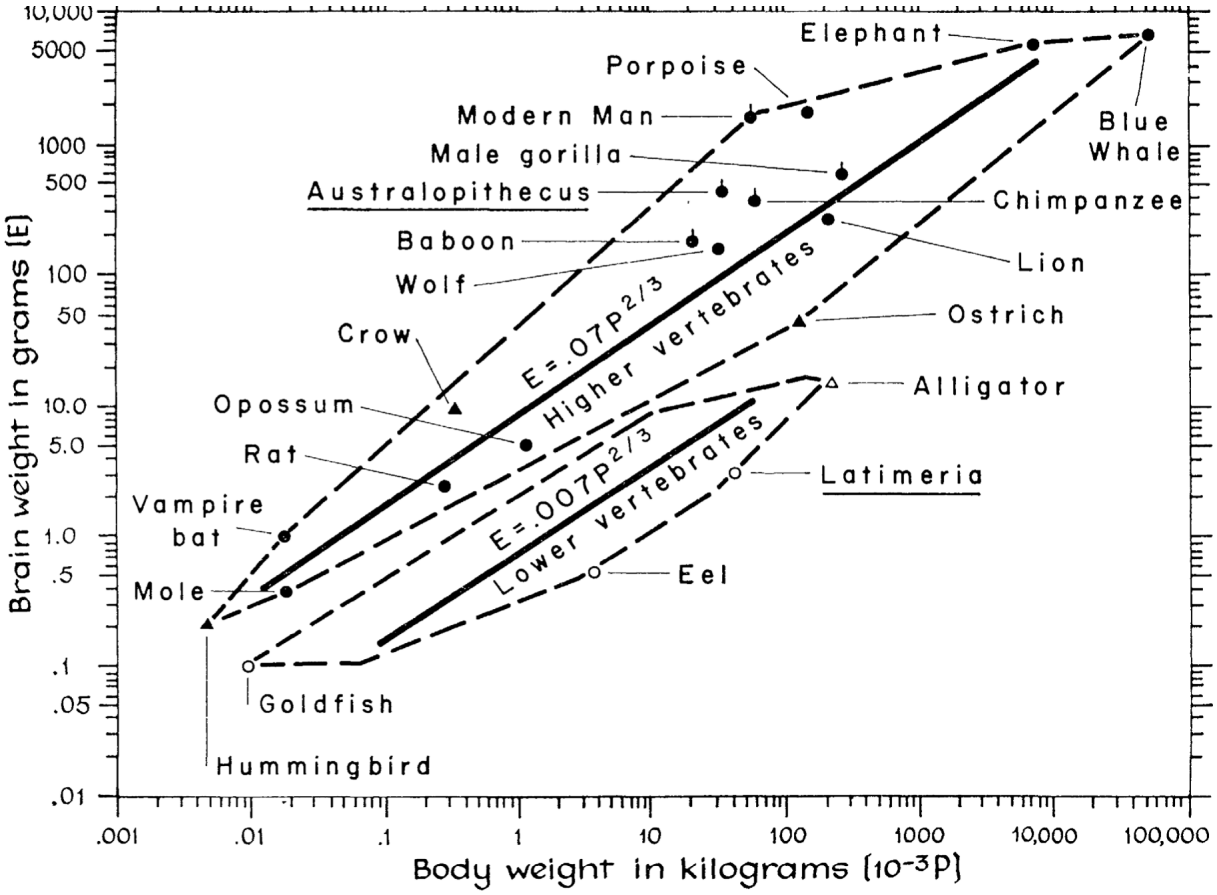

Figure 1: Figure taken from Jerison (1969). Illustration of the body:brain relations in familiar vertebrate species in 1969. Data on the fossil hominid, Australopithecus, and on the living coelacanth, Latimeria, were added. Minimum convex polygons are shown by dashed lines, enclosing visually fitted lines with slopes of two-thirds.

A key question might be whether endothermy removed a constraint (and hence opened the door for the evolution of larger brains) and/or whether ectotherms do not face selection on larger brains because their niches do not require larger brains. So far, no study could explain this 10-fold difference between endo- and ectotherms (Jerison, 1973). In principle, it is possible that a single process, which could be present in endotherms and absent in ectotherms, could cause a selection on larger brains. Another hypothesis might be that cognitive processes are additive in specific brain parts, and endotherms have more cognitive processes than ectotherms. The last hypothesis would be that endotherms have much larger memory capacities or obtain more precise or more multimodal information compared to ectotherms.

I.4 Comparative cognition study

Comparative cognition is the research of all cognitive processes, in all animal species, including humans (Shettleworth, 1993, 1998, 2010, 2013). Cognitive processes are compared between at least two animal species. Furthermore, this comparison highlights what processes permit animals to do what they do in their daily life. From an evolutionary point of view, this field of research leads, in a broader view, to the understanding of what animals share in terms of cognition, and specifically, what factors drive the evolution of these cognitive processes (Shettleworth, 2013). There are two approaches to comparative cognition: the phylogenetic approach, and the ecological approach (Bshary and Brown, 2014; Shettleworth, 1993). The phylogenetic approach is based on the comparison between closely related species sharing ancestry. Due to shared ancestry, this approach assumes similarities between related species. The other approach, the ecological, is based on natural selection, i.e. cognitive abilities of an animal species are related to its environment (social cues included) (Kamil, 1988; Shettleworth,

1993). In order to be able to compare different animal species, the same cognitive tasks must be addressed to each species. The tasks have to be adapted to the animal species in question. Depending on the question, the tasks could be ecologically relevant or not. To know the animal ecology may help interpret the results.

I.5 PhD thesis topic

The goal of my PhD thesis focused on testing advanced cognitive processes in the cleaner wrasse *Labroides dimidiatus* (hereafter 'cleaners'). On one hand, if fish possess advanced cognitive processes, the variation in relative brain size found in vertebrates cannot be explained by advanced cognitive processes. This first issue was approached with an ecological and an abstract (non-ecological) perspective. In the ecologically relevant approach (Chapter 1), the cognitive process of inhibition was tested with an experimental design that integrated ecological relevance to the fish. The abstract approach was used to reveal conceptual cognitive processes in cleaners, as well as working memory (Chapter 2). This time, no ecological relevance was present in the experimental design. On the other hand, the 10-fold difference in the cephalisation index between endo- and ectotherms suggests the possibility that larger endotherm brains are due to a reorganisation of the brain, which yields generalpurpose machinery, i.e. general intelligence. An abstract approach was used to investigate this second issue (Chapter 3). Here, several cognitive processes were tested such as learning-flexibility, spatial problem-solving, inhibitory control, quantitative reasoning, and working memory. Individuals' performances were ranked in each cognitive task and analysed to see whether cleaners possess general intelligence.

I.6 Study fish species and study sites

The bluestreak cleaner wrasse *Labroides dimidiatus* (hereafter 'cleaners') was first studied as it represents a model system to study interspecific mutualism. This little fish, measuring between 6-10 centimeters on average (even until 14 cm in French Polynesia), lives in a small territory called a cleaning station. Cleaners are well spread in the Indo Pacific Ocean, as well as in the Red sea (Froese and Pauly, 2010). They are protogynous hermaphrodites, or alternate hermaphrodites, which means that they start their life as females and when they are reaching a certain relative size they change into a male (Nakashima et al., 2000; Robertson, 1972; Sakai et al., 2001). In some cases, they might have to change sex again if the environment does not allow them to keep this rank. Males have a harem of one to several females (Robertson, 1972), each female living in one cleaning station. In their daily life, mutualism occurs due to their specific feeding behaviour. Indeed, cleaners feed on ectoparasites and dead tissues from the bodies of reef fish referred to as 'clients' (Randall, 1958). They typically have between 800 and 3000 interactions per day with their clients (Grutter, 1995; Triki et al., 2018; Wismer et al., 2014, 2019), where the cleaning can be procured by one cleaner or by pairs (couples) (Bshary et al., 2008; Grutter and Poulin, 1998; Losey, 1972; Losey et al., 1999). The removal of ectoparasites has been shown to be beneficial for clients in different ways, such as by increasing growth rate and size (Clague et al., 2011; Waldie et al., 2011), decreasing active immunity (and thus allowing resource allocation toward reproduction) (Ros et al., 2011), and by decreasing the rate of infection (Losey et al., 1999). In return, cleaners acquire energy intake via nourishment. However, cleaners prefer to eat mucus over ectoparasites (Grutter and Bshary, 2003), which constitutes a cheating behaviour from the part of the cleaner (Oates et al., 2010). Cheating behaviour can be quantified, as it provokes a jolt from the client (Losey, 1972; Randall, 1958). In order to obtain good service quality, i.e. cooperation from the cleaners eating ectoparasites instead of stealing a mucus bite, clients have various strategies to both prevent, and also respond to the cheating event (Bshary, 2002a). In return, cleaners have to establish sophisticated strategies in order to maintain a good relationship with their existing and future clients (Bshary, 2002b; Bshary and Würth, 2001; Bshary et al., 2007; Tebbich et al., 2002). Cleaners showed particularly high performances in tasks related to sophisticated strategies such as

audience effect, biological market, and generalisation (Bshary, 2001; Bshary and Noë, 2003; Bshary et al., 2014; Hammerstein and Noë, 2016; Noë and Hammerstein, 1994, 1995; Noë et al., 2001; Pinto et al., 2011; Wismer et al., 2016). Cleaners have even been shown to outperform monkeys and chimpanzees in a dichotomous choice task (Prétôt et al., 2016; Salwiczek et al., 2012). All the different findings on this particular fish showed how small brains do not directly imply poor cognitive performances when tasks are ecologically relevant, at least.

Cleaners' mutualistic interactions with their client fish are a natural illustration of the biological market, where two individuals exchange goods for their mutual benefits (Noë and Hammerstein, 1995). In fact, two main categories exist in client fish agreeing to their territory size, and thus the number of cleaning stations they have access to. This affects the decision in the choice of the partner. Clients with small territories, called 'resident' clients, generally have access to one cleaning station and thus wait their turn to be cleaned, as they do not have other options (Bshary, 2001). On the other hand, clients that live in large territories, called 'visitor' clients, have access to several cleaning stations and hence do not wait to be cleaned as they do have the choice between different cleaning stations (Bshary, 2001). Therefore, in order to avoid losing a foraging opportunity, cleaners give priority to visitor clients, and tend to give a better service quality to visitors in order to encourage them to come back to their stations (Bshary and Noë, 2003). This biological market was also experimentally tested in the laboratory, using an ephemeral plate (visitor client) which reacts by leaving the aquarium if not prioritized, and a permanent plate (resident client) which stays in the aquarium (Bshary and Grutter, 2002). Cleaners showed competency in this task by giving priority to the ephemeral plate consistently (Triki et al., 2019a; Wismer et al., 2019). Interestingly, a change in the environment, i.e. fish density, affects cleaners' performance in this task: when cleaners live in a poorer environment, i.e. lower fish density, they perform worse than the individuals coming from a more complex area, i.e. higher fish density (Triki et al., 2018; Wismer et al., 2014). These studies highlight how the environment is related to cognitive performance in cleaners. Additionally, the forebrain size of cleaners is positively correlated with their social group size in the sense that cleaners living in a higher cleaner density possess a larger forebrain relative to the rest of the brain (Triki et al., 2019b). As cleaner density did not affect the forebrain size scaled to body size, this suggests that cleaners develop a mosaic brain, i.e. depending on the social environment, certain parts of the brain will grow larger as it is positively correlated with social complexity (Triki et al., 2019b).

The different studies from my PhD project were conducted at two different field sites (Figure 2): the studies from the two first chapters were conducted on Moorea, French Polynesia, at the Gump Research Station. The advantage of this field site was the opportunity to work with two different cleaner fish species, *L. dimidiatus* and *L. bicolor*. Both cleaner fish species being abundant in this area, it was therefore easy to work with both species. Cleaners were caught in continuous reef around Moorea. The study from the third chapter was conducted at Lizard Island Research Station, Australia. For the third chapter, the second field site was more suitable as it providing enough space and appropriate accommodations for this study with a substantial number of cleaners.

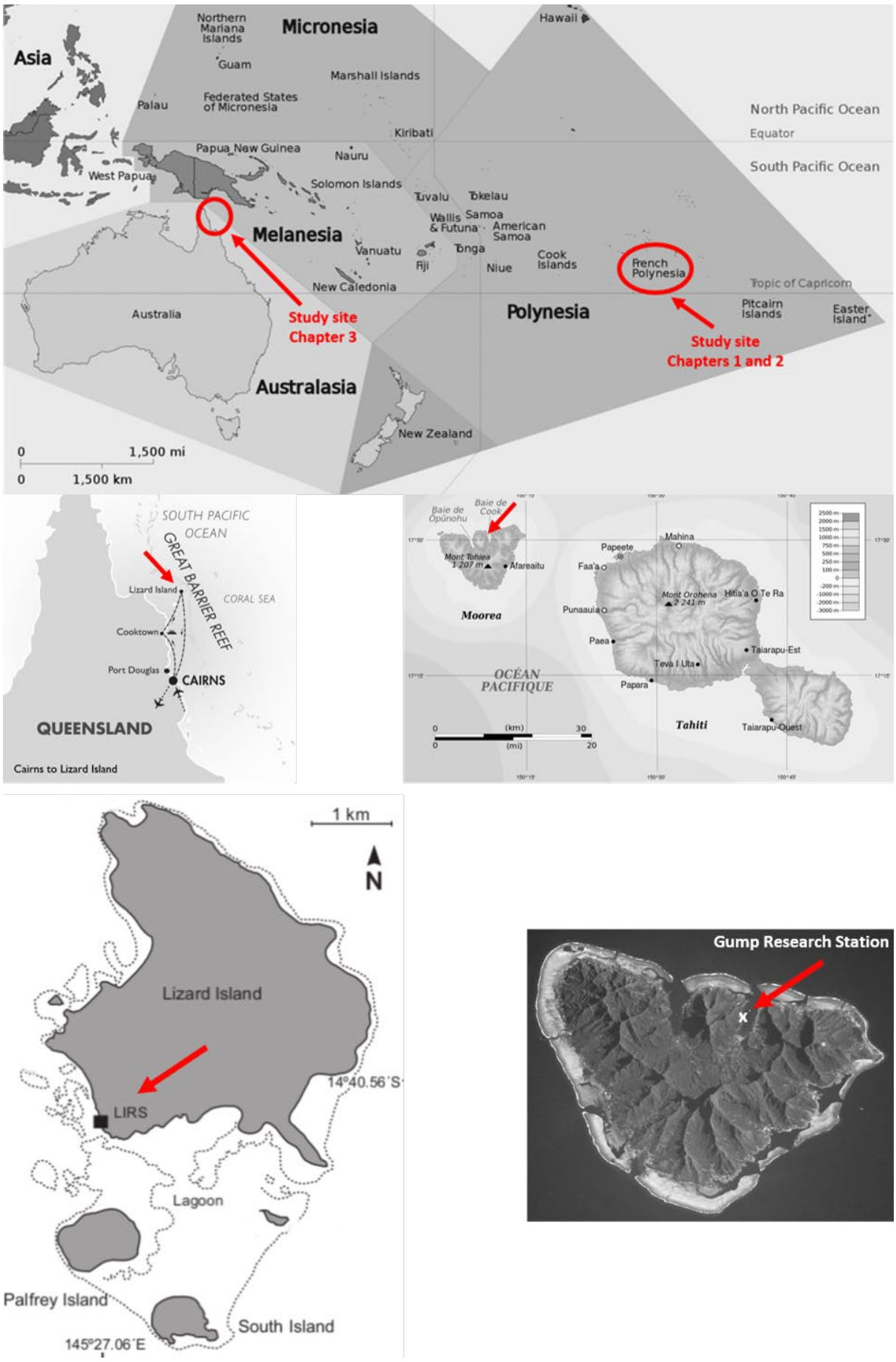

Figure 2: Study sites area map. The locations of Moorea, French Polynesia, and Lizard Island, Australia, are represented with both research stations at each site respectively.

I.7 PhD structure

As previously mentioned, I approached the cleaner wrasse *Labroides dimidiatus* cognition in two ways: the ecologically relevant and abstract approaches. For each approach, experimental tasks were determined to test specific cognitive processes. Furthermore, in the abstract approach, in addition to a specific cognitive process tested, correlations regarding performance between several cognitive processes were tested, i.e. general intelligence or simply *g*.

In the first chapter, I used the ecologically relevant approach. I tested *L. dimidiatus* in the delay of gratification paradigm where inhibition was the cognitive process tested. From previous studies on different animal species in this paradigm, inhibition was related to reciprocity, which is complex in that it supposedly warrants a large brain (Clutton-Brock, 2009; Connor, 2010; Hammerstein, 2003; Henrich and Henrich, 2007; Raihani and Bshary, 2011; Russell and Wright, 2009; Stevens and Hauser, 2005; Stevens et al., 2005). In order to disentangle whether a large brain is needed to perform well in this paradigm, and to determine whether ecology drives performance, cleaner wrasse seemed to be an appropriate species study. Indeed, as cleaners have a food preference, i.e. prefer mucus over ectoparasites, they have to show great self-control, i.e. inhibit their impulse, in order to cooperate with their client fish. The purpose of this first experiment was to see how much a cleaner fish would wait for either a 'tastier' or 'larger amount' of food (both called the 'better' food reward hereafter). The fish had to wait a certain amount of time, i.e. time-lapse, in order to get a better food reward. Within this experiment, I tested two different cleaner fish species, *L. dimidiatus* and *L. bicolor*, and two non-cleaner wrasse species, *Halichoeres trimaculatus*, and *H. hortulanus*. The two cleaner fish species differ in their home range size: *L. dimidiatus* live in so-called cleaning stations and wait for clients to visit for an inspection. Interactions with the same client are frequent, i.e. repeated interactions, and previous interactions are remembered, which promotes cooperation. In contrast, *L. bicolor* rove over large areas (400 m; Oates et al. 2010) and actively approach and pursue clients. Thus, *L. bicolor* can initiate interactions and it is rather unpredictable when they will meet any particular client again, i.e. one-shot interactions, except in the core area of their home range (Oates et al., 2010). From this environmental dissimilarity, the two cleaner fish species may differ in their ability to delay gratification. The non-cleaner wrasse species were used as control species to check whether they show similar abilities to cleaners, such a result would support the hypothesis that this is an ability of the wrasse family. If, on the other hand, strategic dissimilarities appeared between cleaners and non-cleaners, this would promote the hypothesis that ecological factors drive this ability.

In the second chapter, the abstract approach was used. I tested whether cleaners perform as well in cognitive tasks lacking ecological relevance. The cognitive processes of conceptualisation and of working memory were tested. In order to learn concept, individuals have to, for example, be able to categorize two stimuli as rewarding and consider those stimuli as having the same function (AvarguesWeber and Giurfa, 2013; Bodily et al., 2008; Cook et al., 2003; D'Amato et al., 1985; Giurfa et al., 2001; Herman et al., 1994; Katz and Wright, 2006; Katz et al., 2002; Lea, 1984; Newport et al., 2014; Zentall et al., 2008). I, therefore, tested *L. dimidiatus* in a concept formation task using the simultaneous matching-to-sample (MTS) task. In this experiment, cleaners had to integrate the concept of sameness, i.e. choose the same symbol as the sample stimulus (all stimuli have the same function = 'same'). Normally, this task is proposed with training and transfer trials (with sometimes pre-training trials also). The performance in transfer trials indicates whereas the animals understood the task in a conceptual way. Here, I organised the experiment differently by changing at each trial the symbols presented and no training trials were carried out. Additionally, one cleaner fish individual was tested in a working memory task with the delayed matching-to-sample (DMTS). A specific time interval, i.e. retention interval, was introduced between the sample presentation and the two stimuli (matched/unmatched). This retention interval insures that it activates the mental representation of the sample which had to be remembered in order to choose the correct stimulus (matching-to-sample)

(Carruthers, 2013; Fuster, 2015; Honig, 1978). These experiments help to elucidate whether cleaners, when tested for a single cognitive process, still performed well or whether they are unable to solve the task when lacking ecological relevance, i.e. use of abstract experimental design.

In the final chapter, the abstract approach was also used, but this time several cognitive processes were tested and all individuals performed the various tasks. The main interest in this last chapter was to measure whereas cleaner fish possess general intelligence. General intelligence, or *g*, is measured via numerous cognitive tasks, each reflecting a respective cognitive process. If all the tasks load positively in one dimension and this principal dimension explains most of the variance, then *g* is said to be present in the tested animal (Carroll, 1993; Jensen, 1999; Jensen and Weng, 1994; Spearman, 1904, 1928). From previous studies, *g* has been found in endotherms even if the bird studies are controversial. In fact, it seems that *g* bird studies are not consistent in the sense that tasks are not always all positively correlated. However, in the studies where *g* was absent, the number of individuals tested was rather small (< 50), and some were carried out in the field. These factors might have induced a bias in the outcomes. In this chapter, we chose different tasks from different cognitive processes and, more importantly, we chose tasks without any ecological relevance to test cleaner fish. Moreover, because Ashton et al. (2018) found that cognitive performance relates to social complexity, i.e. group size, in Australian magpies, half of the fish were caught in a complex environment, i.e. high fish density, and the other half were caught in a poor environment, i.e. low fish density. If results suggest no presence of general intelligence in fish, this will open the possibility that *g* is a major explanatory factor for the 10-fold difference in the cephalisation index (relative brain size) between endo- and ectotherms. Further studies are needed to test this hypothesis by testing various ectothermic species.

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1. Chapter I | Cleaner fish and other wrasse match primates in their ability to delay gratification

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1.1. Abstract

Humans are outstanding in their ability to achieve mutual cooperation for direct benefits. One oftenquoted factor driving our unique cooperation skills is our ability to delay gratification for future benefits, which is supposedly linked to our advanced cognitive abilities relative to other species. The established experimental paradigm used to test delayed gratification in primates and birds is to give subjects access to a small food reward, which can be exchanged for a larger (quantitative task) or better quality (qualitative task) food reward after a time delay. Here we use this paradigm to test two cleaners and two non-cleaner wrasses species for their ability to delay gratification. The specific ecology of cleaner wrasse makes them ideal candidates for this task: cleaners remove ectoparasites from visiting 'client' reef fish although they prefer to eat client mucus. This eating against preference is equivalent to delayed gratification as clients that have their ectoparasites removed (as opposed to their mucus) will both stay longer with a specific cleaner and return to it for later inspection. As predicted by an ecological approach to cognition, cleaners performed very well in the quantitative task, i.e. similarly to monkeys. However, they performed poorly if the delayed reward was of higher quality. Surprisingly, non-cleaner wrasse also performed well in the quantitative task, despite no evidence that they need such ability to cooperate in nature. However, only cleaners showed evidence for making decisions early on whether to wait or to directly eat the immediate reward. We conclude that the ability to delay gratification in fishes is not the product of advanced cognitive abilities, though the underlying mechanisms vary between species.

Keywords: anticipation; cognition; comparative approach; delay of gratification; self-control; wrasse

1.2. Introduction

Cooperation based on reciprocation, as illustrated with the iterated prisoner's dilemma game, has been argued to require advanced cognitive abilities (Clutton-Brock, 2009; Connor, 2010; Hammerstein, 2003; Henrich and Henrich, 2007; Raihani and Bshary, 2011; Russell and Wright, 2009; Stevens and Hauser, 2005; Stevens et al., 2005a). This is because cooperating in a prisoner's dilemma reduces immediate payoffs, and therefore basic reinforcement learning should lead to individuals defecting (Axelrod and Hamilton, 1981; Noë, 1990; Stephens, 2002a). Therefore, it has been argued that the ability to forsake immediate benefits requires maintaining a future perspective and low temporal discounting, i.e. the ability to evaluate a benefit earned after a certain amount of time (Stephens, 2002a; Stevens and Hauser, 2004). Evidence that humans are particularly good at forsaking immediate benefits in order to achieve a delayed reward in a monetary context (Rosati et al., 2007) has been used to explain why humans excel at cooperation based on reciprocity, while other animals supposedly rarely exchange reciprocal investments. This view has been repeatedly challenged, as grooming in primates typically involves delayed rather than immediate reciprocation (Schino and Aureli, 2008), and more generally experimental and observational evidence has accumulated indicating that reciprocity is more widespread in animals than previously assumed (Taborsky et al., 2016). However, such studies cannot address the degree to which variation in the ability to delay gratification may enhance reciprocal cooperation.

Assuming that temporal discounting is cognitively demanding, one may predict that this capacity correlates with brain size. Therefore, it seems most promising to look at large-brained species such as primates and corvids. The paradigm used to investigate delayed gratification involves giving an animal the choice between an immediate reward and a larger delayed reward (Mazur, 1987). In order to succeed and to be able to eat the larger delayed reward, the animal studied has to control itself by not eating the immediate reward. If it eats the immediate reward, the larger delayed reward is not given. The experiment starts with a short time-lapse which increases over sessions. As long as an individual succeeds in the task, i.e. it waits the requested amount of time in order to gain the larger delayed reward at least once within a session, the experiment continues. Once an individual fails to wait and consumes the immediate reward in all trials of a given session, it drops out of the experiment. This paradigm has already been used with several animal species. Various studies have shown that chimpanzees, bonobos, Tonkean macaques, long-tailed macaques, rhesus macaques, capuchin monkeys, marmosets, tamarins, crows, Goffin cockatoos, pigeons, and rats delay gratification to various extents in order to obtain a larger or tastier food reward (Auersperg et al., 2013; Beran et al., 1999; Dufour et al., 2007; Evans and Beran, 2007a; Green et al., 2004; Hillemann et al., 2014; Pelé et al., 2010, 2011; Ramseyer et al., 2006; Richards et al., 1997; Stevens et al., 2005b, 2011). However, species' performance varies, and it has proved challenging to relate variance to measures of brain complexity. For example, chimpanzees match humans when the reward is food (Rosati et al., 2007). Indeed, humans are much more willing to wait for money than for food (Rosati et al., 2007). Chimpanzees, capuchin monkeys, as well as Tonkean macaques adjust their decision to delay gratification to the amount of food to be gained, i.e. the larger the reward is, the longer they are willing to wait (Dufour et al., 2007; Pelé et al., 2011, 2011; Ramseyer et al., 2006). However, capuchin monkeys generally wait for shorter time periods despite their brain being relatively larger than the one of macaques (Dunbar and Shultz, 2007). In contrast, marmosets or tamarins wait even less (as predicted by their relatively smaller forebrains) and fail to show a correlation between the food ratio and the waiting time (Stevens et al., 2005b). In this line of thought, neither pigeons nor rats demonstrated the capacity to correlate food reward quantity and waiting time (Green et al., 2004; Richards et al., 1997). On the other hand, dogs showed also the ability to increase their waiting time in function of increased food reward (Leonardi et al., 2012). In the case of dogs, domestication may have had an important effect as dogs apparently outperform all other non-human species in the delayed reward task (Leonardi et al., 2012). For birds, crows and Goffin cockatoos as representatives

of large-brained clades perform poorly if they obtain more of the same food but are better at delaying gratification for better quality food items (Auersperg et al., 2013; Dufour et al., 2012; Hillemann et al., 2014). Overall, these results suggest that a species' ecology may play an important role in determining to what extent individuals may be able to delay gratification for different types of rewards.

The ecological approach to cognition predicts that performance in any task is a function of ecological relevance (Kamil, 1998; Shettleworth, 2010). Indeed, some of the above results have been linked to species' ecology. For example, the fact that cotton-top tamarins outperform common marmosets has been proposed to be a consequence of the former roving over broad distances in order to find their food, making them willing to wait longer for a food item than marmosets do, which have to travel less to find their food (Stevens et al., 2005c). Also, the dog's high performance can be linked to their specific ecology, i.e. the effects of domestication. Furthermore, birds are more willing to wait for better food rather than for more food. This result has been linked to either the quantitative reward used in the experiment with birds was too delightful leading to the rapid consumption of the delayed reward. Or birds attributing less value to quantity compared to quality when they have to wait for a delayed reward (Auersperg et al., 2013; Wascher et al., 2012). Here, we use the ecological approach to cognition to broaden our understanding of factors influencing the ability to delay gratification. As it stands, all previously tested species are endothermic vertebrates, i.e. species that have about ten times large brains relative to body size compared to ectothermic vertebrate species (reptiles, amphibians, and fishes; (Jerison, 1973; Tsuboi et al., 2018)). If brain size as a correlate of brain complexity is of major importance for the ability to delay gratification, one would expect that ectothermic species perform poorly when tested in the standard paradigm. In contrast, if ecology is an important driver of the ability to delay gratification, then it is of particular interest to study cleaner fish of the genus *Labroides* because of their rather unique ecology.

Labroides cleaners remove ectoparasites from so-called 'client' fishes (Côté, 2000; Randall, 1958). However, the cleaners prefer to eat clients' mucus, where its consumption constitutes cheating (Grutter and Bshary, 2003). Clients use various so-called partner control mechanisms, i.e. responses to cheating by cleaners that reduce the cheater's payoff, that make cleaners largely feed against preference (Bshary, 2010). Clients may simply terminate an interaction prematurely, they may in addition switch to a different cleaner for their next inspection, or they may punish a cleaner through aggressive chasing in response to being cheated (Bshary, 2010). Such actions indeed cause cleaners to feed against preference (Bshary and Grutter, 2005; Gingins et al., 2013). In other words, cleaners constantly face a peculiar challenge linked to delayed gratification: they need for every bite they take to forsake preferred food (mucus) and eat an ectoparasite for either almost immediate gratification (the interaction continues and another bite can be taken) or for delayed gratification (the client will return for its next inspection some 30 minutes later). As a consequence, the ecological approach to cognition would predict that cleaners should excel in the delayed reward paradigm. More specifically, cleaners should be good at delaying gratification if they receive more food as a reward as this corresponds more closely to their daily challenge. In contrast, waiting for a better reward does not fit their ecology: cleaners do not forsake to eat an ectoparasite in order to receive a piece of mucus. On the other hand, if relatively small brain size imposes constraints on the ability to delay gratification, cleaners should perform poorly on standard delayed gratification paradigms.

We tested two *Labroides* cleaner species, the bluestreak cleaner wrasse, *L. dimidiatus*, and the roving cleaner, *L. bicolor*. The species differ with respect to home range size, which in turn affects the course of cleaning interactions. Clients can decide whether to re-visit a territorial *L. dimidiatus* as a function of recent service quality they experienced (Potts, 1973; Randall, 1958). In contrast, *L. bicolor* individuals rove over large areas, preventing clients from seeking a cooperative individual for their next inspection (Mills and Côté, 2010; Oates et al., 2010a). Therefore, *L. bicolor* individuals mostly experience the immediate delayed gratification from feeding against preference (prolonging the interaction and obtaining more food by eating parasites rather than mucus), while the future benefits

of repeated interactions may be restricted to the core area of their home range (Oates et al., 2010b). If client decisions on repeated interactions are a key factor for the ability to delay gratification, we predict that *L. bicolor* is less able to delay gratification. We tested *L. dimidiatus* first in a quantitative and then in a qualitative delayed reward design. We only tested *L. bicolor* in the quantitative version of the task. In this version, we also tested two non-cleaner wrasse species, *Halichoeres trimaculatus*, and *H. hortulanus*, as controls. As these fish do not face the challenge of eating against preference in order to receive a delayed reward, we predicted that they would perform poorly, no matter whether the ability to delay gratification is mainly affected by a species' ecology or by its brain size. Additionally, we examined the quitting time of each individual from each species at each trial in the different timelapses in the quantitative delayed reward task, ratio 4:1 only. If fish are able to weigh up the costs and rewards of the food amount and take into consideration the factor of the time to wait to reach it, they can in principle decide in each trial whether they want to wait or not. In that case, they should either eat the immediate food reward early or wait the necessary amount of time to obtain the delayed better reward. Finally, we tested the two cleaner wrasse species also in a so-called self-distraction experiment in which additional objects may allow subjects to focus their attention away from the immediate reward. This paradigm has helped to improve the performance of chimpanzees (Evans and Beran, 2007b). We compare all our results explicitly to the published results on mammals and birds in order to evaluate the merits of the ecological approach to the ability to delay gratification.

1.3. Material and methods

1.3.1. Subjects

This study was conducted at the Gump research station on Moorea (French Polynesia) between 2014 and 2016. Cleaner wrasse from the Labridae family live in the Indopacific Ocean. Thirty-three cleaner fish (26 *Labroides dimidiatus*, and 7 *L. bicolor*) and four non-cleaner wrasses (3 *Halichoeres trimaculatus*, and 1 *H. hortulanus*) were caught with a barrier net (2m long, 1.5m high, mesh size 0.5cm) on nearby reefs. They were kept in glass aquaria of the minimal size of 40 x 50 x 40 cm and 50 x 30 x 35 cm. Cleaner fish were caught in October and November 2014 and tested in December, additionally, they were caught in March 2016 and tested in June. The acclimatization phase was at least 21 days. During the first field season in 2014, only cleaner fish *L. dimidiatus* were tested in the qualitative and the quantitative task of the delayed reward paradigm. Five individuals were tested in the qualitative task (ratio 1:1), and fourteen in the quantitative task: five with a ratio twice as large (2:1), six with a ratio 4 times larger (4:1), and three with a ratio 8 times larger (8:1). During the second field season, both cleaner fish species, *L. dimidiatus* (n=7) and *L. bicolor* (n=7) were tested in two experiments: the standard delayed reward and self-distraction both with a ratio 4 times larger (4:1). One *L. dimidiatus* individual had to be released before the self-distraction experiment, thereby only 6 *L. dimidiatus* individuals did both experiments. To counterbalance between both experiments, four individuals of *L. bicolor* and three individuals of *L. dimidiatus* started with the standard delayed reward experiment. The remaining three individuals of each cleaner fish species respectively started the opposite way with the self-distraction experiment first. Each individual had at least eight resting days between the two experiments. In addition, four *L. dimidiatus* were tested in the qualitative task after the previous two experiments, and two additional individuals were tested only in the qualitative task. They had fourteen days resting before being tested in this last experiment. As we did not expect *L. bicolor* to perform as well as *L. dimidiatus* in the delayed reward task, we decided later on during the second field season to add non-cleaner wrasse as control. Non-cleaner wrasses were caught in August 2016 and were acclimatized for about 15 days before being tested. As they were caught towards the end of the field season, we only had time to test them in the quantitative delayed reward task with a 4 times larger ratio (4:1). All fish were trained to feed small food items off Plexiglas plates where the item locations were marked with small dots. Prior to the experiment, cleaner fish performed other experiments unreported here.

1.3.2. Pre-experimental phase

For the cleaner wrasse *L. dimidiatus*, we used either prawn items (cleaners' preferred food, akin to mucus) or fish flakes mixed with mashed prawn (cleaners' non-preferred food, henceforth called 'flake', akin to parasites). During the first field season, we used either prawn or flake items in the quantitative task with *L. dimidiatus*. From the five individuals tested in the 2:1 ratio, three did the experiment with prawn items, whereas the other two did it with flake items. In the 4:1 ratio, three fish did the experiment with flake items, and the other three did it with flake items. From the three individuals tested with the 8:1 ratio, one individual did the experiment with flake items, and the remaining two individuals did it with prawn items (supplemental material: Table S1). As shown in Table S1 in the supplemental material, the two different types of food did not affect the performance of the fish in the task. During the second field season, only prawn items were used for all fish species: *L. dimidiatus*, *L. bicolor*, and the two non-cleaner wrasses *H. trimaculatus*, and *H. hortulanus*. The goal of this phase was to show the individual preference for the "better" plate. In the quantitative task, the "better" plate was the plate with a higher food amount. Whereas in the qualitative task, the "better" plate was the plate with tastier food i.e. prawn item. This pre-experimental phase was done in order to later test whether fish are able to wait for a bigger amount of food or tastier food. We exposed the fish to two plates: the delayed and the immediate reward. For the quantitative task (all fish species), the delayed reward was a higher amount of food (2, 4 or 8 times more) than the immediate reward which was one food item. As non-statistical significant difference was found in the performance of *L. dimidiatus* in the 4:1 ratio standard delayed reward between years, we, therefore, grouped the individuals from both years for this task (Figure S1). For the qualitative task (*L. dimidiatus* only), the delayed reward was one prawn preferred item whereas the immediate reward was one non-preferred flake item. We did four trials where the two plates were introduced in the aquarium and the fish ate what they wanted without removing plates. This step was done to acclimatize the fish to the procedure. These four trials were followed by 20 trials in which the plates were moved this time: when fish ate first the one item on the immediate reward plate, both plates were removed quickly so that the subject could not eat off the delayed reward plate (see Table S1 in the supplemental material). The fish were thus introduced to the plates, the different qualities/quantities, and the concept that the larger/better reward is not accessible if the item of the other plate is eaten. Previous research with a similar paradigm ('biological market task') showed that 20 trials are not enough to induce a preference for the ephemeral plate (Triki et al., 2019).

1.3.3. Experimental procedure

To avoid fishes' starvation before starting the experiment, they were fed by leaving a smeared mashed prawn plate in the aquarium for 5 minutes. This was done to match studies on other species in which standard food is available ad libitum outside experiments, thus lowering the probability of impulsive choices. Fifteen minutes after the smeared mashed prawn plate removal we started the experiment. Prior to the experimental session, 4 pre-trials were done in which we put the delayed reward plate behind a transparent Plexiglas barrier, waited for the time-lapse demanding during the session, then removed the barrier in order to let the cleaner have access to the delayed reward food item. This was done to show cleaners that after a certain amount of time waiting, the barrier was removed permitting them the access to the delayed reward. After these 4 pre-trials, we started an experimental session that was composed of 16 trials. At the beginning of each trial, a transparent Plexiglas barrier was inserted in the aquarium and the delayed reward plate placed behind. A stopwatch was started when the immediate food plate was inserted in the aquarium on the opposite side of the delayed reward plate. The position of the immediate reward plate was counterbalanced (8 times on the left side and 8 times on the right side). During the 16 experimental trials, whenever a fish was eating on the immediate reward plate before reaching the time-lapse demanded in this session, the delayed reward plate was removed from the aquarium and the trial ended. If the fish succeeded in waiting until the time-lapse demanded, we removed first the immediate reward plate and only then removed the

barrier to allow the fish access to the delayed reward plate. Individuals reached the criteria for success when they waited for the necessary time-lapse at least once over the 16 trials. This success allowed an individual to move on to the next time-lapse session. All individuals who did not attain the criteria for success were excluded from further trials. We planned to do a maximum of 8 sessions with increasing time-lapses before subjects could access the delayed reward: 2s, 7s, 15s, 30s, 1min, 2min, 4min, and 8min. During the first field season in 2014, only a few trials from time-lapses between 15s and 240s were recorded with a video camera (CANON) from the top of the aquarium. In the next field season in 2016, the experiment was recorded using a video camera (GoPro) outside the aquarium. We also recorded how long they waited before either failing or succeeding (quitting time). One session per fish was conducted per day.

1.3.3.1. Quantitative delayed reward

In total, we tested five *L. dimidiatus* on the 2:1 food ratio, thirteen on the 4:1 food ratio, 6 in 2014 and 7 in 2016, and three on the 8:1 food ratio (see Figure 1a-c). Furthermore, we added another cleaner species and 2 non-cleaner species to the 4:1 food ratio in 2016: 7 *L. bicolor* (cleaner fish) and 4 noncleaner wrasses (3 *H. trimaculatus* and 1 *H. hortulanus*).

1.3.3.2. Qualitative delayed reward

We only did this experiment with *L. dimidiatus* (n = 5 in 2014, and n = 6 in 2016). For the individuals from 2016, four were already tested in the delayed and self-distraction tasks. One individual started with the delayed task and the remaining three started with the self-distraction task. Two individuals were only tested in the qualitative task. We first allowed the cleaner to habituate to flake food (an unfamiliar food that was expected to become the less preferred food item). To do this, we inserted a plate with 14 food dots, half of which were prawn items, the other half were flake items. The flake items were a mixture of 20% of dried flakes of the mashed prawn quantity. We added a few drops of water to the dried flakes in order to obtain a paste that we mixed with mashed prawn. By doing this we let the cleaner get used to both tastes. We then demonstrated the individual preference for prawn over flake items by doing three trials of preference test (plate with 7 prawn and 7 flake items) and taking the mean as the percentage of prawn preference. We recorded the seven first food items that the cleaner ate. Cleaners were ready for the experiment when they showed a specific preference for prawn over flake items but also when they did not avoid eating flake items, meaning eating at least four prawn items on the seven first food items (at least 57.14%). Fishes that like prawn a bit less and flake a bit more may be less willing to wait for the delayed reward, it was thus important to measure food preference for each fish (Table S1 in the supplemental material). For the experiment, we had a 1:1 food ratio using a prawn item as preferred food (delayed reward) and flake item as non-preferred food (immediate reward) (see Figure 1d).

1.3.3.3. Self-distraction

This experiment was done with six *L. dimidiatus* which three were already tested in the delayed task and seven *L. bicolor* which four were already tested in the delayed task. The individuals who started with the self-distraction task were afterward tested in the delayed task. Only the food ratio of 4:1 was used. In addition, we added 3 objects in the aquarium: 1 surgeon model, 1 cleaner fish model, and 1 plain Plexiglas plate (see Figure 1e).

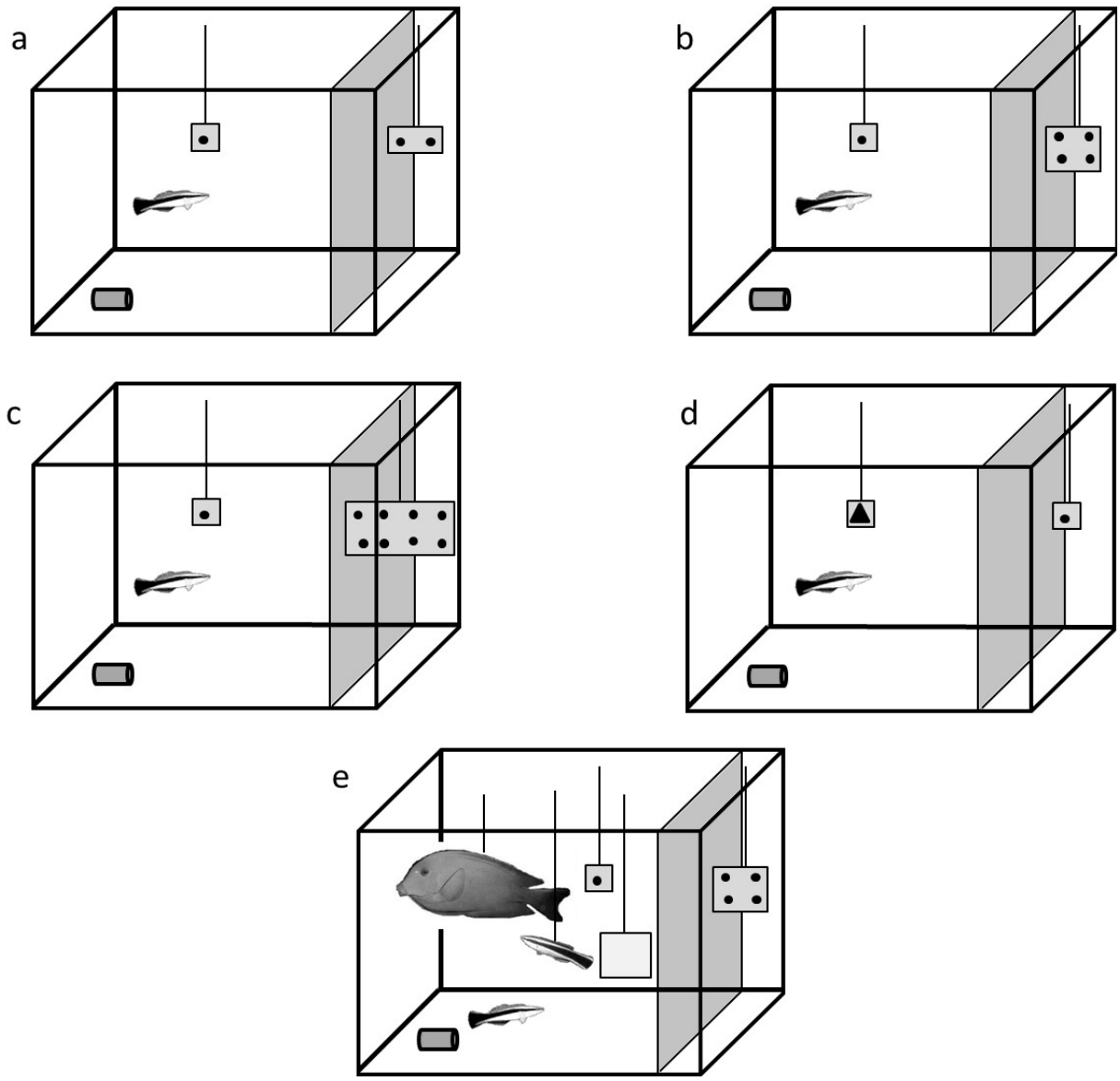


Figure 1: The experimental setup. a-c. Experimental aquarium for the quantitative delayed reward; 3 ratios are shown here: a) 2:1 food ratio, b) 4:1 food ratio and c) 8:1 food ratio. d. Experimental aquarium for the qualitative delayed reward; Prawn (circle shape) is used as delayed reward vs Flake (triangle shape) which is used as an immediate reward item. e. Experimental aquarium for self-distraction; with the immediate reward plate (on the left) stand 3 objects: 1 model client, 1 cleaner fish model, and 1 plain Plexiglas plate. The delayed reward plate displayed 4 prawn items (food ratio 4:1). In all the different experiments, a choice was made either to eat the immediate food reward before the end of the time-lapse demanded in the session or to wait and have access to the delayed food reward.

1.3.4. Statistical analysis

We ran Kruskal-Wallis and Wilcoxon tests, as well as a linear model using the program Rstudio © (R Version 1.2.5019, © 2009-2019 RStudio, Inc.) to answer our questions. First, we examined in ratio 4:1 if there was a difference between the fish species overall time-lapses using a Kruskal-Wallis test. We ranked each individual from the best performance, i.e. waiting the most (number 1), to the lowest performance, i.e. waiting the least (number 24). When two or more individuals scored the same, and, therefore, ties occurred, we looked at the percentage of success in the time-lapse before failing to distinguish between them. The individual with a higher percentage of success got higher rank than the one with a lower percentage of success in the last time-lapse where success occurred. If it happened that individuals failed at the same time-lapse, and, moreover, had the same percentage of success during the last time-lapse with success, they were assigned the average of the ranks that they would have obtained. Moreover, we discerned if there was any difference in the success rate of succeeding

individuals between the fish species and thus in three chosen time-lapses, 7s, 15s, and 30s, using a Kruskal-Wallis test. Additionally here, we analysed the quitting time of the four fish species. We compared the expected quitting time under the null hypothesis of a constant quitting time chance and the actually observed one (Dufour et al., 2007). This analysis tested whether fish had an estimate of the expected delay time and hence decide early on in a trial whether they were willing to wait for the delayed food reward (and then do so successfully) or not (and then eat the immediate reward without delay). We proceeded as Dufour et al. (2007) by using a Kaplan Meier survival analysis to calculate the observed probability to continue to wait during all trials. Both the successful and the failed trials were included in the analysis as censored data. We used then an adjusted Kolmogorov-Smirnov test (Haccou and Meelis, 1992) to compare the expected waiting time distribution with the observed one. We rejected the null hypothesis that an individual had a constant chance to quit, independently of the time passed in the trial when the difference between both distributions was statistically significant at $p < 0.05$. Apart from this individual-level analysis, we also tested whether subjects overall tended to eat the immediate reward faster than expected. Second, in the self-distraction task, we inspected if there was a difference in the performance between the two cleaner fish species overall time-lapses using a Wilcoxon test. We applied the same way to rank the individuals as previously done in the later experiment. Moreover, we compared the success rate of succeeding individuals between the two cleaner fish species at three time-lapses using a Wilcoxon test. This allowed discerning if there was any difference between the two cleaner species. Furthermore, we ran a linear model to test whether opportunities for self-distraction allow for longer waiting times than standard delayed reward experiments. Due to the distribution of data, we also ran a matched pair Wilcoxon test to confirm the robustness of the initial result with a non-parametric analysis. We calculated an index of success for each individual from both species. To be able to distinguish between the performance of each individual and to calculate the index per individual we took into consideration when they failed in the task (at which time-lapse) and added a value e.g. a value of 100 for the 2s time-lapse and increasing of 100 each next time-lapse ending with a value of 900 for the 960 time-lapse. Additionally to the failing time-lapse value, we added up the percentage of delayed reward chosen in the last successful timelapse. This addition permitted us to have at the end one index per individual reflecting their performance. We were then able to distinguish if a difference between the two experiments (standard delayed reward and self-distraction) occurred in both species taken into account, if a difference occurred between species, and if there was a difference within species between the two experiments. Third, we investigated if the quantity vs quality food items affected *L. dimidiatus* performance in the delayed reward task. We ranked each individual from the four different ratios (1:1, 2:1, 4:1, and 8:1) from 1 to 26. For the qualitative task, ratio 1:1, we took into account the five individuals from 2014, and only 2 out of the 6 individuals from 2016. As these four individuals were already tested in the previous delayed task, we excluded them in the qualitative task. Since they performed similarly as the others in this task, we maintained them in the plot, yielding to the difference in the number of individuals between the test and the plot. The number 1 did perform at best all ratios combined (Dimidiatus 7, failing at 960s time-lapse, ratio 4:1), whereas the worst performance was scored 26. If two or more individuals shared the same rank, we proceeded the same way as in the previous experiments. When we ran the analysis, we combined ratios 4:1 and 8:1 together, leading to the treatments: treatment 1 = ratio 1:1, treatment 2 = ratio 2:1, and treatment 3 = ratios 4:1 and 8:1. We investigated if there was a difference between the food ratios overall time-lapses using a Kruskal-Wallis test. Moreover, we compared the success rate of succeeding individuals between the two quantitative treatments at four time-lapses using a Wilcoxon test. This allowed perceiving if *L. dimidiatus* were more willing to wait for higher food ratios in the quantitative task.

1.3.5. Animal welfare

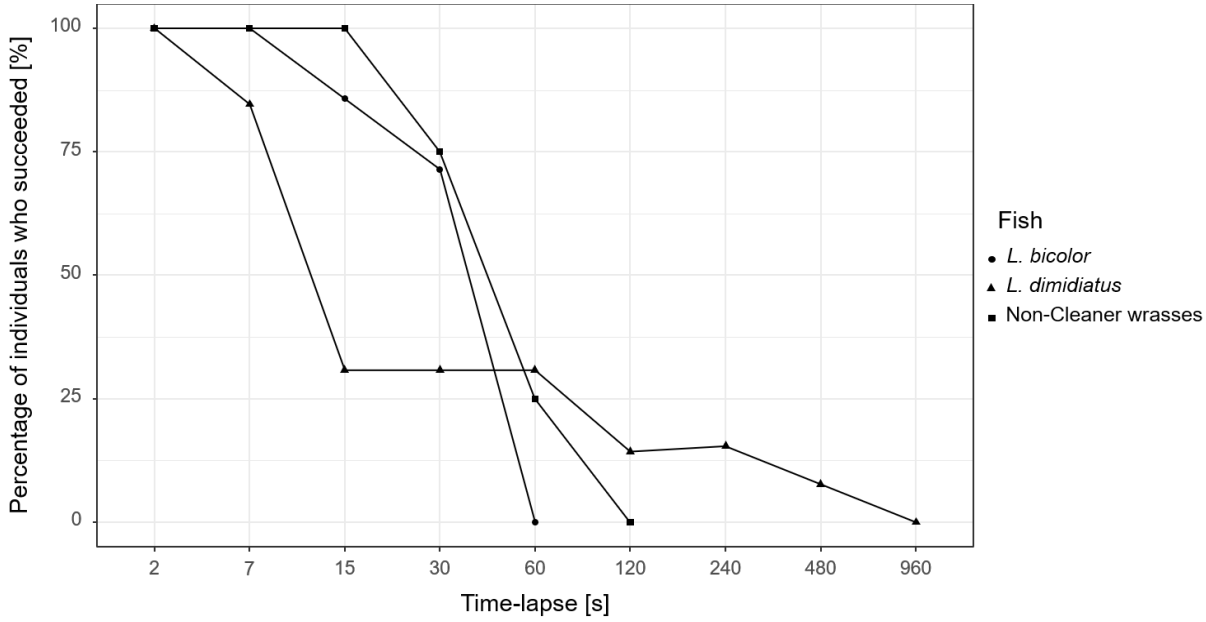
Our research study adheres to the ASAB/ABS Guidelines for the Use of Animals in Research. The study was approved by the French Polynesian authorities responsible of the program 'Délégation à la

Recherche' and by the Gump Research Station where the study was conducted in accordance with their rules and regulations for animal research.

1.4. Results

1.4.1. Comparison delayed reward ratio 4:1 in 4 fish species

In the delayed reward task using the 4:1 ratio, the longest waiting time for *L. dimidiatus* was 480 seconds, for *L. bicolor* 30 seconds, and for non-cleaning wrasses 60 seconds (Figure 2). On the other hand, proportionally less *L. dimidiatus* individuals passed the 30 seconds waiting criterion compared to *L. bicolor* and non-cleaning wrasses (Figure 2). A Kruskal-Wallis test revealed no significant difference between the fish species in the delayed reward task with a ratio of 4:1 ($X^2_2 = 1.95$, P = 0.377, $N_{L.bicolor} = 7$, $N_{L.dimidiatus} = 13$, and $N_{\text{NC wrasses}} = 4$; Figure 2).



N L.bicolor	7	7	6	5	0				
N L. dimidiatus	13	11	4	4	4	1	2	1	0
***N* NC wrasses**	4	4	4	3	1	0			

Figure 2: Mean of the percentage of individuals who succeeded in delaying rewards for the different time-lapse and with fish species as a variable (*L. bicolor N* = 7, *L. dimidiatus N* = 13 and Non-cleaner wrasses *N* = 4).

We ran a Kruskal-Wallis test to perceive if there was a difference between species with respect to the percentage of success at different time-lapses. We analysed the 7s, 15s, and 30s time-lapses only since in the 2s time-lapse all species succeeded, and in the 60s time-lapse only two species remained. We took into consideration in this analysis individuals who succeeded at least once and removed the individuals who totally failed in the according time-lapse. The analysis revealed that there was no significant difference in the success rate between species at those time-lapses (7s, $X^2_2 = 0.89$, P = 0.641, $N_{L.bicolor} = 7$, $N_{L.dimidiatus} = 11$, and $N_{\text{NC wrasses}} = 4$); 15s, $X_{22} = 5.46$, P = 0.07, $N_{L.bicolor} = 6$, $N_{L.dimidiatus} = 4$, and $N_{\text{NC wrasses}} = 4$), and 30s, $X^2_2 = 4.63$, P = 0.099, $N_{L.bicolor} = 5$, $N_{L.dimidiatus} = 4$, and $N_{\text{NC wrasses}} = 3$); boxplot in the supplemental material, Figure S2).

1.4.1.1. Quitting time

We first analysed the quitting times of all fish together at time-lapses 7s, 15s, and 30s, i.e. time intervals for which we had reasonable sample sizes for statistical treatment when each individual just

receives a ± sign depending on whether its average quitting time in its failed trials was above or below predicted quitting time. Overall distributions of quitting time are shown in Figure S3. Individuals most quitted earlier than the predicted quitting time (Table 1). In the 7s time-lapse, 13 quitted earlier vs 1 later (Binomial Test, x =1, P = 0.002, *N* = 14). In the 15s time-lapse, 8 quitted earlier vs 1 later (Binomial Test, x = 1, P = 0.04, *N* = 9). Finally, in the 30s time-lapse, 8 quitted earlier vs 0 later (Binomial Test, x = 0, P = 0.008, *N* = 8).

In a second step, we analysed whether any individual showed a significant deviation from the expected quitting time. We analysed the quitting times for all individuals who succeeded at least once at a given delay (starting with the 7s time-lapse). The rationale behind this analysis is to distinguish failures due to an inability to wait for long delays, from failures due to a decisional process such as "given the long delay ahead, I'd rather not wait". If fish anticipate the duration of the delay, they should decide early on whether to wait or not and quitting time should be occurring significantly earlier than predicted by their general ability to wait. In total, four individuals ever quitted significantly earlier than expected (Bicolor E at 15s and 30s, Bicolor D at 30s, Dimidiatus 4 at 60s and Dimidiatus 7 at 480s, Table S2). Giving up early could in principle also be the result of an extinction of the waiting behaviour, i.e. after early success, the fish gave up exceedingly faster as trials progressed. We hence plotted the waiting times as a function of trial number for each of the five significant results (Figure S4). Our reading of the graphs remains interpretive but the hypothesis that extinction of the waiting behaviour could explain early quitting time does not hold in (at least) two cases, for Bicolor E at 15s, and Dimidiatus 4 at 60s.

No non-cleaner wrasse individual ever showed a significant tendency to quit earlier than expected, opening the possibility that they have a lower understanding of the task, despite similar abilities to delay gratification. We hence evaluated the data in more detail. It is important to note that the power to detect a significant deviation from predicted values is dependent on the proportion of failed versus successful trials within a time-lapse; the more successful trials an individual achieves the lower is the power to test for significant deviations from predicted values in the failed trials. We, therefore, compared quitting time directly between individuals who succeeded at least once, grouped as either cleaners or non-cleaners. The two non-cleaner wrasse species generally showed a longer quitting time compared to the cleaner wrasse species (Figure S5), yielding significant differences in time-lapses 7s and 30s (Wilcoxon test: T = 5.5, P = 0.047, $N_{L.bicolor}$ = 6, $N_{L.dimidiatus}$ = 4, and $N_{\text{NC wrasses}}$ = 4 and T = 0, P = 0.036, $N_{L.bicolor}$ = 4, $N_{L.dimidiatus}$ = 1, and $N_{\text{NC wrasses}}$ = 3 respectively) but not in time-lapse 15s (Wilcoxon test : T = 5, P = 0.286, $N_{L.bicolor}$ = 4, $N_{L.dimidiatus}$ = 1, and $N_{\text{NC wrasses}}$ = 4).

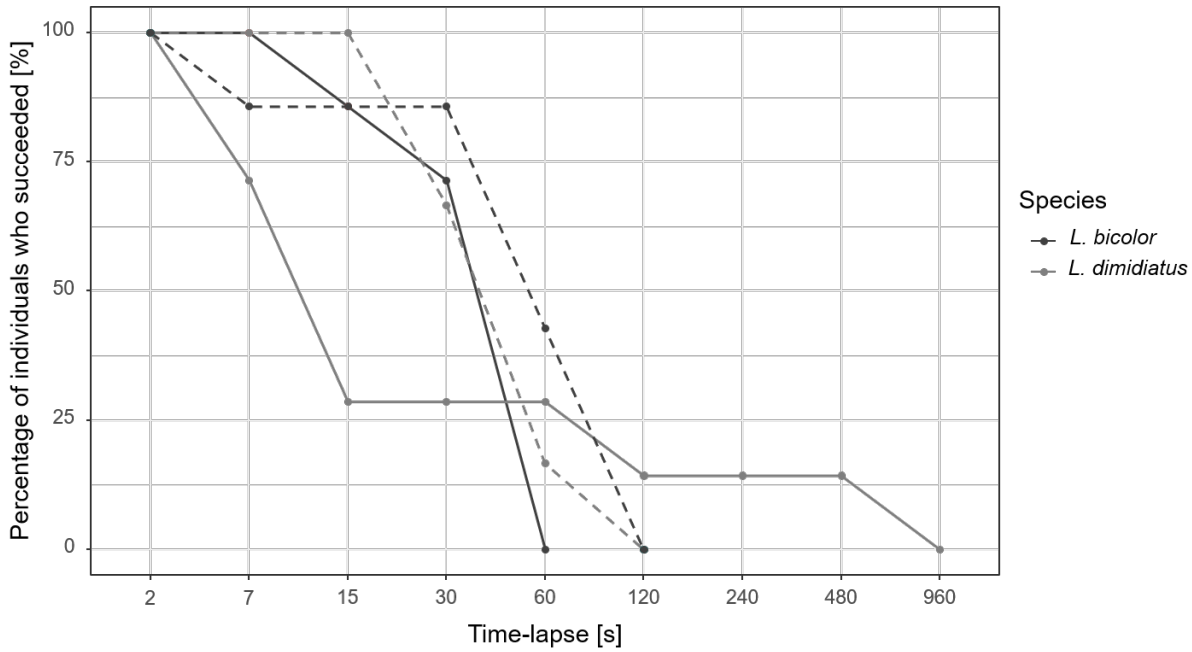
Table 1: Comparison between the expected and the observed percentage chance to wait in the quantitative delayed reward task of a ratio 4:1. The mean values are shown here. Additionally, the mean quitting time in second is shown with the number of failed trials in brackets on 16 trials in total (except for Dimidiatus 7 who ran 10 trials in the 15s time-lapse, and 8 trials in the 30s time-lapse). Three time-lapses are represented: 7s, 15s, and 30s. Only when the observed value is above the one expected, the box is coloured in grey. No coloured means that the observed value is below the one expected.

	7s time-lapse			**15s time-lapse**			**30s time-lapse**		
Individual	Observed (%)	Expected (%)	mean QT in sec (n° failed trials)	Observed (%)	Expected (%)	mean QT in sec (n° failed trials)	Observed (%)	Expected (%)	mean QT in sec (n° failed trials)
Dimidiatus 3	F	F	2.78 (16)	NT	NT		NT	NT	
Dimidiatus 4	93.8	94	3 (1)	S	S	-	S	S	-
Dimidiatus 5	40	44.4	2.95 (11)	F	F	2.53 (16)	NT	NT	
Dimidiatus 6	F	F	1.75 (16)	NT	NT				
Dimidiatus 7	S	S	-	90	90.5	7 (1/10)	48.2	56.1	5.4 (5/8)
Dimidiatus 8	17.2	31.6	2.43 (15)	F	F	2.13 (16)	NT	NT	
Dimidiatus 9	33.6	39.3	2.73 (13)	F	F	2.5 (16)	NT	NT	
Bicolor A	93.8	94	6.9 (1)	S	S	-	57.6	61.5	3.75 (8)
Bicolor B	93.8	94	2.5 (1)	93.8	94	2.5 (1)	41.7	50.5	5.08 (12)
Bicolor C	S	S	-	S	S	-	S	S	-
Bicolor D	81.7	82.3	3.17 (3)	71.3	72.5	4.8 (5)	39.2	49.2	4.04 (12)
Bicolor E	59.7	58.8	1.86 (7)	39.8	39.5	2.32 (11)	32.6	43.5	2.79 (12)
Bicolor F	28.1	32.9	2.25 (14)	25.9	36.3	2.47 (15)	F	F	2.06 (16)
Bicolor G	20.8	32.8	2.32 (14)	F	F	2.31 (16)	NT	NT	
Wrasse 1	93.8	94	4 (1)	49	52.4	5.1 (10)	36.9	42.6	6.43 (15)
Wrasse 2	53.1	54.8	3 (9)	59.2	61.1	5.3 (8)	33.8	40.2	7.73 (15)
Wrasse 3	87.9	88.3	4.5 (2)	63	64	5 (7)	58.8	60.5	14.93 (8)
Wrasse 5	82.4	83	4.17 (3)	33.8	40.9	3.7 (13)	F	F	5.81 (16)

Note. F = 100% failure; NT = not tested; S = 100% success; QT = Quitting Time.

1.4.2. Self-distraction: comparison between 2 cleaner fish species (*L. dimidiatus* and *L. bicolor*), and comparison between 2 experiments (standard delayed of gratification and self-distraction)

Overall, we saw that both cleaners were waiting for the same amount of time in this experiment (60 seconds; Figure 3). A Wilcoxon test revealed no significant difference between the two cleaner fish species in the self-distraction task with a ratio of 4:1 (T = 16, P = 0.52, N = 13 ($N_{L.bicolor}$ = 7, $N_{L.dimidiatus}$ = 6); Figure 3).



N L.bicolor	7	7	6	5	0				
N L. dimidiatus	13	11	4	4	4	1	2	1	0
N L.bicolor	7	6	6	6	3	0			
N L. dimidiatus	6	6	6	4	1	0			

Delayed Reward

Self-distraction

Figure 3: Mean of the percentage of individuals who succeeded in the self-distraction experiment (dashed lines) and the standard delayed reward ratio 4:1 (full lines) for the different time-lapses and with species as a variable (*L. bicolor N* = 7, and *L. dimidiatus N* = 7 in DR and *N* = 6 in SD).

Furthermore, success rates did not differ in any of the three analysed time-lapses (Wilcoxon test: 7s, T = 24.5, P = 0.33 (*N* = 12, with *N* = 6 per species); 15s, T = 26.5, P = 0.2 (*N* = 12, with *N* = 6 per species; and 30s, T = 16, P = 0.45 (*N* = 10, with *N* = 6 for *L. bicolor*); boxplot in the supplemental material, Figure S6). We took into consideration in this analysis the individuals who succeeded at least once and removed the individuals who totally failed in the according time-lapse.

Even though individuals did not manipulate the distractor objects, having distractors seem to increase the performance (Figure 4). However, the linear model with all individuals taking into account revealed no significant difference neither between species, nor between experiments (X^2_1 = 0.81, P = 0.37, and X^2_1 = 1.51, P = 0.22 respectively, $N_{L.bicolor}$ = 7, $N_{L.dimidiatus}$ = 7). No interaction term was significant between the two variables (X^2_1 = 0.39, P = 0.53). We note that one *L. dimidiatus* individual was an outlier, waiting until 8 minutes in the standard task. As this data point violates parametric assumptions, we also ran a simple non-parametric paired samples Wilcoxon test, either per species (Figure 4) or individuals (Figure S7) of both species combined to compare between experiments. Like with the model, we did not find a significant difference in performance between both experiments (*L. dimidiatus*: *N* = 6, P = 0.44; *L. bicolor*: *N* = 7, P = 0.4; combined: *N* = 13, P = 0.15; Figure 4, Figure S7).

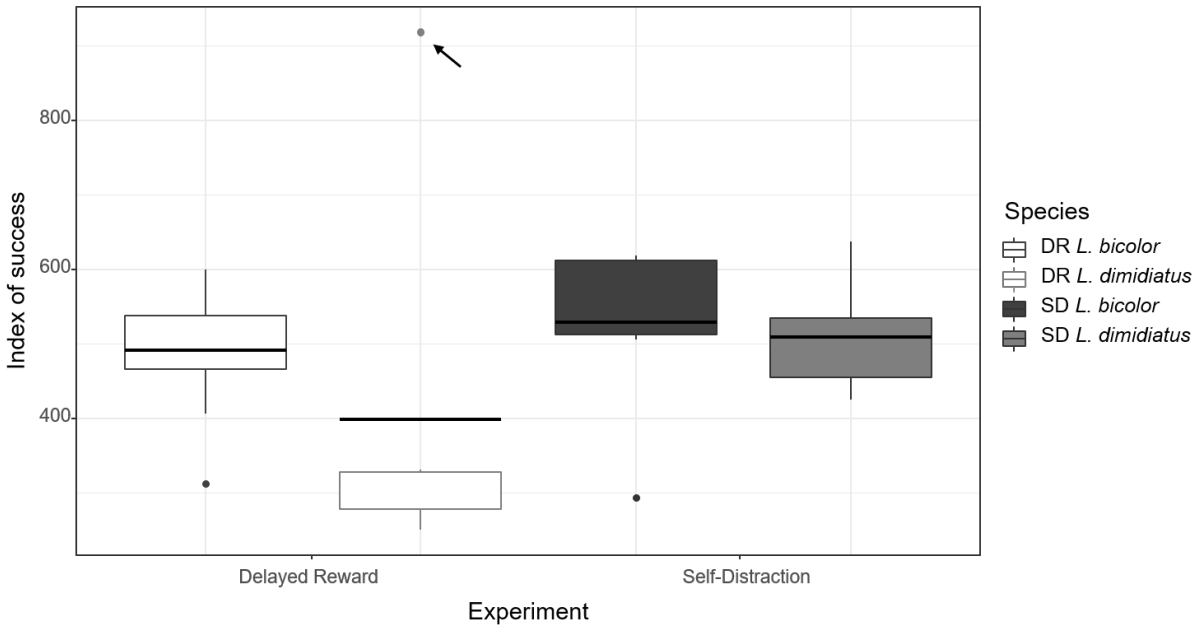
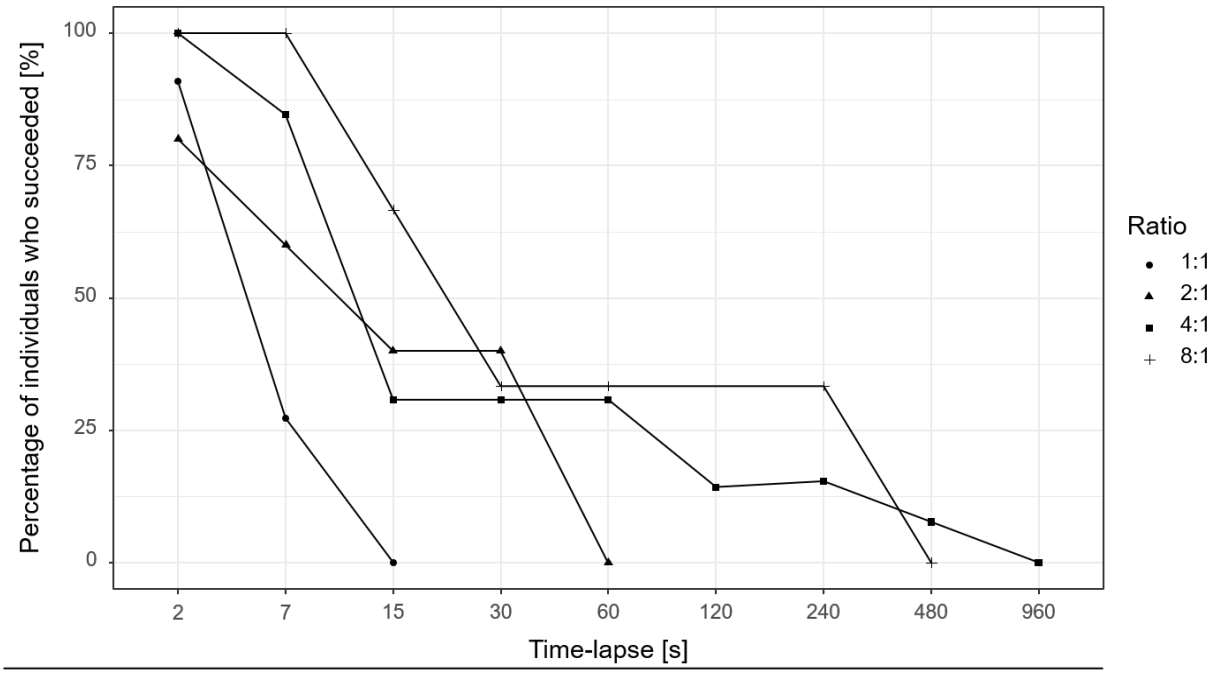


Figure 4: Boxplot of mean and interquartile range of the index of success of both experiments with species as a variable (DR *L. bicolor N* = 7, DR *L. dimidiatus N* = 7, SD *L. bicolor N* = 7, and SD *L. dimidiatus N* = 6).

1.4.3. Delay in rewards of quantitative and qualitative difference: *L. dimidiatus*

L. dimidiatus only waited up to 7 seconds for a higher quality food reward (Figure 5). In contrast, they waited up to 480 seconds for a higher quantity of food (Figure 5). A Kruskal-Wallis test was conducted to examine if a difference occurred between the qualitative (ratio 1:1) and the quantitative (ratios 2:1, 4:1, and 8:1) delayed reward task. As only three individuals were tested in the 8:1 ratio, we decided to combine ratios 4:1 and 8:1 before proceeding to the analysis. A significant difference (X^2_2 = 10.42, P = 0.006, *N* = 32; Figure 5) was found among the three treatments (treatment 1 = ratio 1:1, treatment 2 = ratio 2:1, and treatment 3 = ratios 4:1 and 8:1 combined). A post-hoc Dunn test revealed that only the 'high reward ratios' (treatment 3) was significantly different from the qualitative ratio (treatment 1) (z-value = 3.22, P = 0.001; Figure 5). *L. dimidiatus* performed better in the quantitative task with ratios 4:1 and 8:1 combined compared to the qualitative task (ratio 1:1) (mean rank of 10.72, and of 22.71 respectively). On the other hand, no significant difference was found neither between the 'low reward ratio' (treatment 2) and the qualitative ratio (z-value = 1.58, P = 0.11), nor between the 'low reward ratio' and the 'high reward ratios' (z-value = 1.04, P = 0.3).



Ratio 1:1	10	3	0						
Ratio 2:1	4	3	2	2	0				
Ratio 4:1	13	11	4	4	4	1	2	1	0
Ratio 8:1	3	3	2	1	1	NT	1	0	0

Figure 5: Mean of the percentage of individuals who succeeded in delaying rewards for the different time-lapse and with food ratio as variable (ratio 1:1 *N* = 11, ratio 2:1 *N* = 5, ratio 4:1 *N* = 13 and ratio 8:1 *N* = 3).

For the quantitative delayed reward task, we also tested how far the ratio between delayed and immediate reward affected the probability that individuals would succeed within each time-lapse. Here again, because we only had 3 individuals tested in the 8:1 treatment, we grouped them with individuals tested in the 4:1 treatment in order to compare these 'high reward ratios' with the 2:1 'low reward ratio'. Furthermore, for this analysis, we kept only the successful individuals according to the time-lapse. The analysis revealed that the probability of success was not significantly different between the 'high' and 'low' reward ratios for any single time delay (Wilcoxon test: 2s, T = 16.5, P = 0.15, N_{high} = 16, N_{low} = 4); 7s, T = 16.5, P = 0.61, N_{high} = 14, N_{low} = 3); 15s, T = 2, P = 0.21, N_{high} = 6, N_{low} = 2); and 30s, T = 0, P = 0.07, N_{high} = 5, N_{low} = 2); Figure S8). We note, however, that probability of success was invariably higher when the reward ratio was high (overall mean percentage success of successful individuals: low = 33% with *N* = 4, and high = 65.5%, with *N* = 16).

1.5. Discussion

We had asked how two species of cleaner fish and closely related wrasse perform in a delayed reward test. Based on the ecological approach to cognition, we had predicted that cleaners perform well, at least if they receive more food when waiting (quantitative delayed reward task); non-cleaning wrasse should perform poorly in comparison. A cognitive approach based on relative brain size would have predicted that fishes do not perform well in this paradigm. We found that fishes performed generally as well or even better than most mammals and birds tested so far. Furthermore, at least four individuals (2 *L. bicolor* and 2 *L. dimidiatus*), showed clear evidence that they make the decision early about whether to wait for the entire necessary time or to eat the available item immediately. Both *Labroid*es species did not improve performance in the presence of additional objects, which suggests that individuals did not use those objects to distract themselves. Additional experiments restricted to *L. dimidiatus* suggested that this fish species is consistently more willing to wait for a higher amount

of food, though the evidence remains descriptive at this stage. Moreover, *L. dimidiatus* performed poorly if the task was to wait for a same-sized but preferred reward (qualitative delayed reward task). Thus, the performance of fishes, in general, match primates in the delayed reward paradigm, and at least in cleaners the similar performance is apparently based on similar cognitive processes. Below, we discuss each aspect in turn.

1.5.1. Fishes are generally good at the quantitative task

The performance comparison of the four fish species in the quantitative delay task with a food ratio of 4:1 showed, surprisingly, that non-cleaner wrasses are also able to delay gratification as the other two cleaner fish species. Importantly, the good performance of fishes was not due to them being generally slow at approaching food. When the food plate was presented prior to experimental trials, individuals would start feeding within a second (MA, personal observation). This view is shown quantitatively with *L. dimidiatus* in the qualitative food paradigm, in which not a single individual waited 100% of trials even in the 2s time-lapse condition. To the best of our knowledge, the fact that non-cleaner wrasses are also good in this delayed gratification task does not have a ready ecological explanation in the context of reciprocity, i.e. in the social domain for which the test was used in primates (Dufour et al., 2007). We had assumed that cleaners are good at the task because their feeding ecology makes foraging a social task, eating against preference in order to obtain more of the same from cleaning client fish. The cleaners' intraspecific social life is largely based on competition that is resolved by a size-based hierarchy (Robertson, 1972; Soares et al., 2019), including in principle cooperative tasks like joint inspection of clients (Bshary et al., 2008). The other wrasse species have similar social organisations and otherwise forage in the sand looking for crustaceans, molluscs, and eat small fishes as well (Randall et al., 1997). The arising question is hence to what extent these wrasses forsake less profitable prey in order to focus on more profitable prey. Optimal foraging theory predicts that animals should be able to specialise on more profitable prey as long as encounter rates are high enough (Krebs and Davies, 1987), and various experiments confirm the value of the theory with crabs (Elner and Hughes, 1978), bluegill sunfish (Werner and Hall, 1974) and great tits (Krebs et al., 1977). Thus, any species with an ecology that selects for prey specialisation may be prepared to delay gratification for more and/or better food and hence perform well in the delayed reward task. More studies will be required to clarify the extent of these skills in fishes as well as other ectothermic vertebrates and invertebrates. An important arising question is to investigate to what extent mammals and birds experienced the exchange with humans as a social or as a foraging task. Potentially, if waiting in social tasks is more difficult (because the food has to be given to a potential competitor, i.e. the experimenter) than in a pure foraging context, endotherms may yet be shown to outperform fishes if the context is controlled for.

An alternative explanation for the generally high performance of fishes in the delayed reward test is that ectothermy plays a major role: the fishes' low metabolic rate compared to endotherms may enable them to wait for long time intervals even in the absence of cognitive adaptations. Furthermore, one aspect of our experimental design potentially made the task easier for fishes than for various other species tested so far: we presented the immediate reward on a plate, while primates, dogs or corvids had to hold the food either in their hand or even in their mouths/beaks. Thus, these other species faced an exchange task that may have required slightly more self-control compared to our fishes who were merely waiting.

We summarised all data from the different species that have been subjected to a similar quantitative delayed reward paradigm as the wrasses (Figure 6). The comparison shows that the fishes' performance equals the performance of primates. With respect to maximal waiting time, few *L. dimidiatus* cleaners matched even the performance of chimpanzees. With respect to mean performance, the fish species behaved like macaques, outperforming capuchins as well as crows and ravens. The results clearly show that a large brain is not needed to perform well in a delayed

gratification task. Dogs (Canis familiaris) also perform well in this task, which may be due to the fact that they have a special relationship with humans (domestication since the early Holocene (CluttonBrock, 1977)): the dog potentially wants to please its master or the experimenter by delaying the reward as much as it can (Leonardi et al., 2012). Capuchin monkeys and Tonkean macaques' overall performance was quite similar in this task, though capuchin monkeys were more impulsive in the sense that individuals failed more trials at a certain time-lapse compared to Tonkean macaques (Pelé et al., 2011). The authors suggested that social context might affect how animal species perform in this paradigm (Pelé et al., 2011). More specifically, the capacity of inhibition might be related to coping with the inconstancy of social interactions (Amici et al., 2009; Pelé et al., 2010). As Tonkean macaques do have more social interactions in comparison to capuchin monkeys, they might, therefore, perform better in the delayed gratification paradigm. Crows and ravens (Corvids) as well as parrots perform worst in this task and wait only 40 and 20 seconds respectively (Auersperg et al., 2013; Koepke et al., 2015; Wascher et al., 2012). However, birds perform well if the delayed reward is qualitatively better (see the section below).

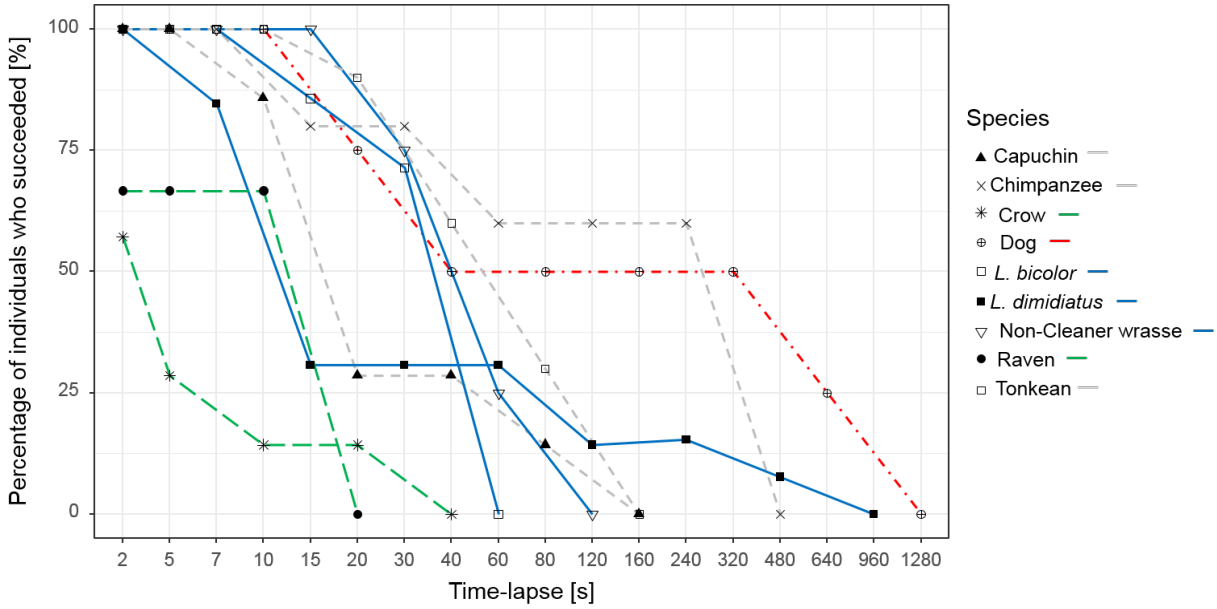


Figure 6: Mean of the percentage of individuals who succeeded in quantitative delayed reward for the different time-lapses and with species as variable (Capuchin *N* = 10, Chimpanzee *N* = 5, Crow *N* = 6, Dog *N* = 4, *L. bicolor N* = 7, *L. dimidiatus N* = 13, Non-Cleaner wrasse *N* = 4, Raven *N* = 5 and Tonkean macaques *N* = 7). Comparative results on ratio 4:1 except for corvids where all 3 ratios were combined (1:2, 1:4, and 1:8). Two different time-lapses applied depending on the species. For capuchin monkeys, Tonkean macaques, crows, ravens, and dogs the time-lapses used were as follows: 2s, 5s, 10s, and then doubled until 1280s. For chimpanzees and fish, the time-lapses used were as follows: 2s, 7s, 15s, then doubled until 960s.

1.5.2. Cleaner fishes can show evidence of understanding the task as seen by the individual quitting time analysis

All large-brained species tested so far, i.e. Tonkean and long-tailed macaques, brown capuchins, and chimpanzees for primates in quantitative task and crows and Goffin cockatoos for birds in qualitative task can anticipate the time they have to wait for a larger/tastier food reward and consequently decide in advance if the reward is worth waiting for (Auersperg et al., 2013; Dufour et al., 2007, 2012; Pelé et al., 2010, 2011). The one exception of large-brained species not anticipating for larger food is human children aged two years (Steelandt et al., 2012). Our results show that cleaner fish have the ability to anticipate as well as the other species cited previously (results for *L. bicolor* and *L. dimidiatus* in the supplemental material; Table S2 and Figure S4). This implies that also cleaner fish species are able to show anticipation in the task, deciding early on whether to wait or not for the delayed reward. The fact that cleaner fish can wait for a considerable amount of time as a function of the delayed reward and either wait or quite early in the task suggests that they can evaluate the relative amount of food provided. With this evaluation, they make an active decision whether to inhibit or not their

impulsiveness and thus during each trial. Interestingly, non-cleaner wrasses apparently lack this ability of decision making; at the very least they appear to be less able to make clear-cut decisions compared to the cleaners. Instead, it seemed that non-cleaner fish did not properly understand the task and produced waiting times in a rather random fashion. Thus, our results suggest that similar overall ability to delay gratification may be based on different cognitive processes within fishes: fish can wait but some species, i.e. cleaner fish, know why they wait and some probably not. The ability to make clear decisions is useful in a cleaning context, where cleaners have to decide for each bite whether they forego immediate gratification by searching for an ectoparasite to eat, or whether they scrape off a piece of preferred mucus. Taken together, our results indicate that ecological need may not only select for tweaking existing cognitive tools in order to increase performance but may also cause the evolution of new cognitive processes that enhance performance.

1.5.3. No difference in performance between the standard delay of gratification and self-distraction

Both cleaner fish species performed similarly in this task. In contrast to chimpanzees that regularly manipulate toys (Evans and Beran, 2007b), *L. bicolor* and *L. dimidiatus* rarely manipulated the objects in any way, like for example giving tactile stimulation to fish models with the pelvic fins – as cleaners do with clients (Potts, 1973) – to divert themselves during the experiment. For chimpanzees and children, extra objects apparently allow subjects to distract their attention away from the immediate reward food item and therefore to delay gratification for longer (Evans and Beran, 2007b; Miller and Karniol, 1976; Mischel et al., 1972; Toner and Smith, 1977). The lack of a positive effect of extra objects on cleaner fish performance could potentially be due to differences in experimental design: as mentioned before, cleaners did not have to hold the immediate reward in their hands or mouth, which means that they could avoid swimming near the food even in the absence of additional objects. Indeed, cleaners often focussed their attention on the delayed reward plate, staying in front of the transparent barrier in apparent attempts to move towards the plate. Another apparent coping strategy was to swim back and forth between the immediate and the delayed reward plates. The best performing *L. dimidiatus* behaved in a peculiar way, as it regularly inspected the immediate reward plate without eating the food item before swimming to the surface in a circle, and staying at the surface while looking towards the experimenter. Thus, cleaners did not need extra objects to distract their attention away from the immediate reward. Furthermore, in contrast to children and chimpanzees, in a recent paper on Fish intelligence, sentience and ethics (Brown, 2015), play behaviour is never mentioned as an argument why our treatment on fishes should improve. This lack of playfulness with objects may limit their ability to use self-distraction as a strategy with this particular setup.

1.5.4. *L. dimidiatus* shows evidence for caring about quantities

As predicted, cleaner fish *L. dimidiatus* performs well in a delayed gratification task with rewards of quantitative difference, reaching the time-lapse of 480 seconds (8 minutes) in the 4:1 ratio. On the contrary, they wait only 7 seconds when waiting for a preferred food item (qualitative task). We did both tasks (quantitative and qualitative) for the purpose of testing whether cleaner wrasse behave similarly when they have to wait for more identical food items or for a tastier food item. The cleaners' better performance in the quantitative task fits their ecological pressures: by forgoing to eat preferred mucus they can obtain more food in the long run, while not eating a parasite to obtain mucus with some delay has no relevance. Thus, their normal feeding behaviour may drive them to seek out higher quantities of food. Furthermore, cleaners showed a general tendency to wait more consistently as the delayed reward was particularly large relative to the immediate reward (4:1 or 8:1 compared to 2:1). Such a pattern has hitherto only been documented in chimpanzees and a few monkey species (longtailed and Tonkean macaques, as well as capuchin monkeys; Dufour et al., 2007; Pelé et al., 2010, 2011; Ramseyer et al., 2006). Larger sample size will be needed to evaluate whether our general tendency may also yield significant p values. It is not a basic skill for animals to increase the waiting

time as a function of reward size, as it was not found in other species such as marmosets, tamarins, rats and pigeons (Green et al., 2004; Richards et al., 1997; Stevens et al., 2005b).

The apparent similarity between *L. dimidiatus* and old-world monkeys and apes in paying attention to quantities should force us to reconsider the cognitive demands even regarding the details of this delayed reward task. As it stands, two mechanisms have been proposed to underlie decision-making in this task. Temporal discounting is the ability to evaluate a benefit earned after a certain amount of time (the initial value of the benefit will decrease over time) (Mazur, 1987, 2000; Stevens et al., 2005b). Alternatively, animals prefer to make an instantaneous choice i.e. short-sighted rules (Stephens, 2002b; Stevens et al., 2005c) that do not involve any evaluation of a delayed reward. This simple mechanism explains an animals' preference for instant food rewards compared to bigger/tastier delayed food rewards. Since the cleaners waited more often for a higher food reward, short-sighted rules are unlikely to explain their performance. Instead, cleaners may indeed use temporal discounting, as apparently do Goffin cockatoos, macaques, dogs, and chimpanzees (Auersperg et al., 2013; Dufour et al., 2007; Leonardi et al., 2012; Pelé et al., 2010, 2011; Ramseyer et al., 2006).

1.5.5. *L. dimidiatus* performs worst in the qualitative task

Corvids and the bluestreak cleaner wrasse revealed opposite results in the quantitative and qualitative tasks: corvids wait for better food but not for more (Hillemann et al., 2014), whereas cleaner wrasse *L. dimidiatus* waits for more food but not for better food (Figure 7). Environmental differences may explain this. In previous studies, researchers highlighted the fact that birds might be more willing to wait for a higher quality food compared to a bigger quantity of food as birds might attribute more value to quality compared to quantity in a delayed reward context (Auersperg et al., 2013; Wascher et al., 2012). Auersperg et al (2013), put forward as well the taste of the food reward that might have been of better/preferred taste than the one used in quantitative task yielding to quicker consumption of the delayed reward. Moreover, this dissimilar behaviour can suggest that birds in general encounter problems when they have to give value to a number of food items (Wascher et al., 2012). They can weigh the relative food value comparing the immediate low food reward and the delayed high food reward. When the delayed high food reward is considered worth the wait, then corvids are able to control their impulse and wait for the more valuable food reward. Not only other bird species displayed similar pattern to wait for food of higher quality as opposed to quantity, such as Goffin cockatoos (Auersperg et al., 2013; Dufour et al., 2012; Koepke et al., 2015; Wascher et al., 2012) but also brown capuchin monkeys (Drapier et al., 2005) performed better when waiting for food quality instead of food quantity. It has already been shown how feeding ecology impacts on temporal discounting and how this discounting function is context-dependent (Stevens et al., 2005c). As cleaners never forsake a less-preferred food item (parasite) in order to retrieve a delayed preferred food item (mucus) under natural conditions, the lack of ecological relevance of the qualitative task may explain their poor performance.

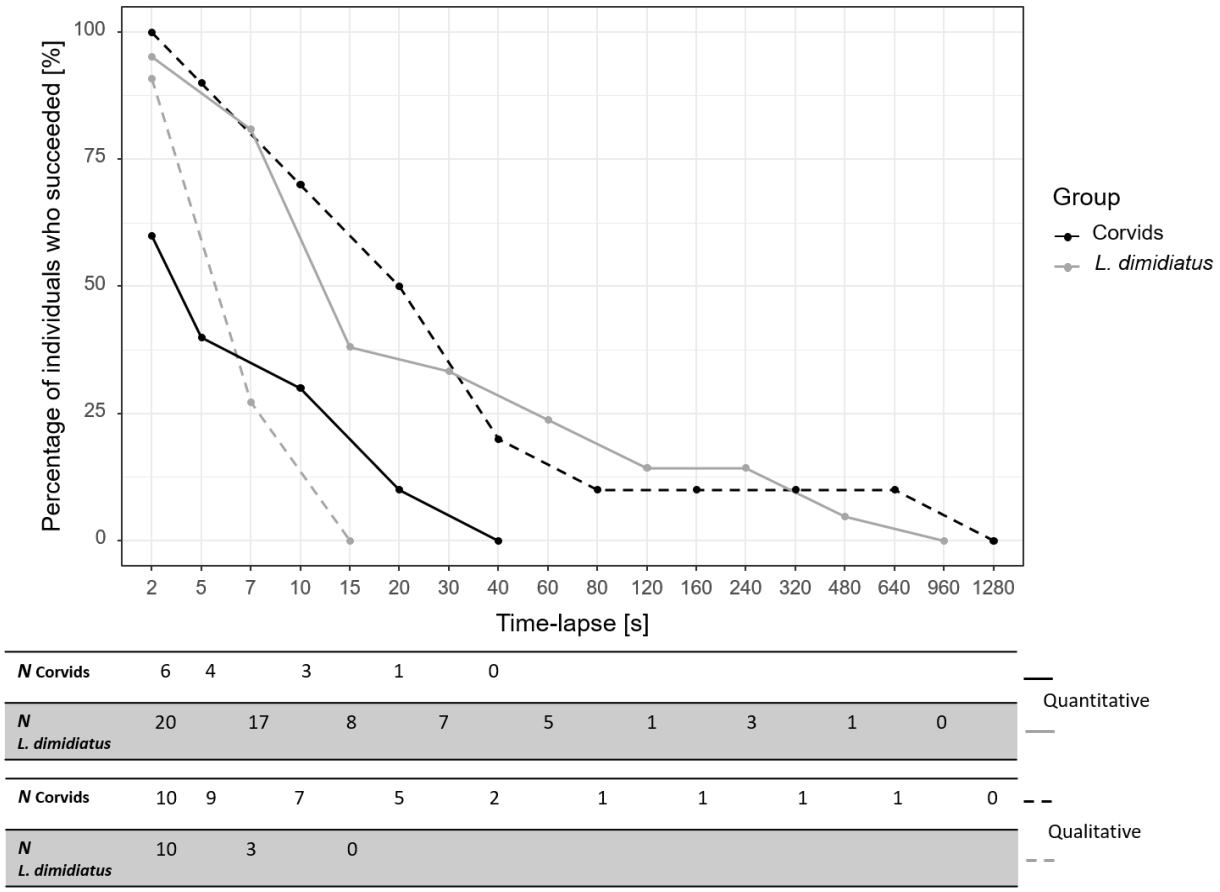


Figure 7: Mean of the percentage of individuals who succeeded in the qualitative delayed reward task ratio 1:1 (dashed-lines) and the quantitative delayed reward task where the 3 ratios 2:1, 4:1, and 8:1 were combined (full lines) for the different timelapse and with species as a variable (Corvids *N* = 10 and *L. dimidiatus N* = 11 for quality and *N* = 21 for quantity).

1.6. Conclusion

The outcomes of this study offer new perspectives on animal abilities to delay gratification. To delay gratification was assumed to be a cognitively demanding skill. Here we showed potentially controversial results, in that fish apparently match monkeys in their ability to delay gratification. While the ability to delay gratification is arguably one component involved in successful cooperation, our study emphasizes that this ability is not tightly linked to the presence of a large brain, and apparently not even to the ecological need to cooperate. Instead, the task as presented to the fishes may better be seen for what it is, namely an optimal foraging task rather than a social task. Potentially, being ectotherms may change the fishes' perception of time, making waiting easier compared to endotherms as mammals and birds. Interestingly, the strategic decision of whether or not to wait in any single situation may be more tightly linked to a cooperative ecology as the non-cleaning wrasse showed less ability in this respect. Thus, similar abilities to delay gratification may be based on different cognitive mechanisms within fishes. Finally, as the ecology of cleaners may prevent them from being able to wait for better food, it would be interesting to use the paradigm on fish species known to focus on highquality food to test how these perform in qualitative versus quantitative delayed rewards.

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1.9. Supplemental material

Table S1: Description of the species used for this study. Year of capture is noted, and information about which individual did which experiment is listed. The percentage of prawn preference when the fish were tested in the qualitative delayed reward task is illustrated. The 20 pre-trials before each experiment are shown with the percentage of time that fish individual chose the 'better' plate. Finally, the time-lapse when an individual failed appeared in the last column.

Year	*Species*	Individual	Experiment	Experiment order	Ratio	Food items	% prawn preference	% better plate chosen on 20 trials	When failed (0/16)
2014	*L. dimidiatus*	dimi1	DR	only DR 1:1	1:1	P vs F	57.14	30	2
2014	*L. dimidiatus*	dimi2	DR	only DR 1:1	1:1	P vs F	57.14	45	15
2014	*L. dimidiatus*	dimi3	DR	only DR 1:1	1:1	P vs F	71.43	25	7
2014	*L. dimidiatus*	dimi5	DR	only DR 1:1	1:1	P vs F	57.14	15	7
2014	*L. dimidiatus*	dimi6	DR	only DR 1:1	1:1	P vs F	57.14	5	7
2014	*L. dimidiatus*	dimi7	DR	only DR 2:1	2:1	P	NA	50	7
2014	*L. dimidiatus*	dimi8	DR	only DR 2:1	2:1	P	NA	50	15
2014	*L. dimidiatus*	dimi9	DR	only DR 2:1	2:1	P	NA	85	60
2014	*L. dimidiatus*	dimi10	DR	only DR 2:1	2:1	F	NA	60	2
2014	*L. dimidiatus*	dimi12	DR	only DR 2:1	2:1	F	NA	65	60
2014	*L. dimidiatus*	dimi13	DR	only DR 4:1	4:1	P	NA	65	480
2014	*L. dimidiatus*	dimi14	DR	only DR 4:1	4:1	P	NA	85	15
2014	*L. dimidiatus*	dimi15	DR	only DR 4:1	4:1	P	NA	80	15
2014	*L. dimidiatus*	dimi16	DR	only DR 4:1	4:1	F	NA	75	15
2014	*L. dimidiatus*	dimi17	DR	only DR 4:1	4:1	F	NA	75	15
2014	*L. dimidiatus*	dimi18	DR	only DR 4:1	4:1	F	NA	55	240

2014	*L. dimidiatus*	dimi19	DR	only DR 8:1	8:1	F	NA	85	30
2014	*L. dimidiatus*	dimi23	DR	only DR 8:1	8:1	P	NA	100	15
2014	*L. dimidiatus*	dimi24	DR	only DR 8:1	8:1	P	NA	80	480
2016	*L. dimidiatus*	Dimidiatus 3	DR	DR – SD – DR qualitative	1:1	P vs F	80.95	70	7
2016	*L. dimidiatus*	Dimidiatus 5	DR	SD – DR – DR qualitative	1:1	P vs F	100	65	15
2016	*L. dimidiatus*	Dimidiatus 6	DR	SD – DR – DR qualitative	1:1	P vs F	80.95	55	7
2016	*L. dimidiatus*	Dimidiatus 7	DR	SD – DR – DR qualitative	1:1	P vs F	100	65	15
2016	*L. dimidiatus*	Dimidiatus 10	DR	DR qualitative	1:1	P vs F	100	50	7
2016	*L. dimidiatus*	Dimidiatus 11	DR	DR qualitative	1:1	P vs F	90.48	50	7
2016	*L. dimidiatus*	Dimidiatus 3	DR	DR – SD – DR qualitative	4:1	P	NA	65	7
2016	*L. dimidiatus*	Dimidiatus 4	DR	only DR 4:1	4:1	P	NA	75	120
2016	*L. dimidiatus*	Dimidiatus 5	DR	SD – DR – DR qualitative	4:1	P	NA	70	15
2016	*L. dimidiatus*	Dimidiatus 6	DR	SD – DR – DR qualitative	4:1	P	NA	80	7
2016	*L. dimidiatus*	Dimidiatus 7	DR	SD – DR – DR qualitative	4:1	P	NA	95	960

2016	*L. dimidiatus*	Dimidiatus 8	DR	DR – SD	4:1	P	NA	70	15
2016	*L. dimidiatus*	Dimidiatus 9	DR	DR – SD	4:1	P	NA	70	15
2016	*L. dimidiatus*	Dimidiatus 3	SD	DR – SD – DR qualitative	4:1	P	NA	50	30
2016	*L. dimidiatus*	Dimidiatus 5	SD	SD – DR – DR qualitative	4:1	P	NA	60	60
2016	*L. dimidiatus*	Dimidiatus 6	SD	SD – DR – DR qualitative	4:1	P	NA	80	60
2016	*L. dimidiatus*	Dimidiatus 7	SD	SD – DR – DR qualitative	4:1	P	NA	80	60
2016	*L. dimidiatus*	Dimidiatus 8	SD	DR – SD	4:1	P	NA	85	30
2016	*L. dimidiatus*	Dimidiatus 9	SD	DR – SD	4:1	P	NA	70	120
2016	*L. bicolor*	Bicolor A	DR	DR – SD	4:1	P	NA	90	60
2016	*L. bicolor*	Bicolor B	DR	DR – SD	4:1	P	NA	75	60
2016	*L. bicolor*	Bicolor C	DR	DR – SD	4:1	P	NA	70	60
2016	*L. bicolor*	Bicolor D	DR	DR – SD	4:1	P	NA	55	60
2016	*L. bicolor*	Bicolor E	DR	SD – DR	4:1	P	NA	90	60
2016	*L. bicolor*	Bicolor F	DR	SD – DR	4:1	P	NA	80	30
2016	*L. bicolor*	Bicolor G	DR	SD – DR	4:1	P	NA	80	15

2016	*L. bicolor*	Bicolor A	SD	DR – SD	4:1	P	NA	70	120
2016	*L. bicolor*	Bicolor B	SD	DR – SD	4:1	P	NA	80	60
2016	*L. bicolor*	Bicolor C	SD	DR – SD	4:1	P	NA	90	120
2016	*L. bicolor*	Bicolor D	SD	DR – SD	4:1	P	NA	80	7
2016	*L. bicolor*	Bicolor E	SD	SD – DR	4:1	P	NA	65	120
2016	*L. bicolor*	Bicolor F	SD	SD – DR	4:1	P	NA	75	60
2016	*L. bicolor*	Bicolor G	SD	SD – DR	4:1	P	NA	80	60
2016	*H. trimaculatus*	Wrasse 1	DR	only DR 4:1	4:1	P	NA	80	60
2016	*H. trimaculatus*	Wrasse 2	DR	only DR 4:1	4:1	P	NA	80	60
2016	*H. hortulanus*	Wrasse 3	DR	only DR 4:1	4:1	P	NA	65	120
2016	*H. trimaculatus*	Wrasse 5	DR	only DR 4:1	4:1	P	NA	60	30

1.9.1. Data of years 2014 and 2016 not significantly different

In order to verify if the years were not different from each other, and consequently grouped them together for the ratio 4:1 with *L. dimidiatus* for the study we proceeded to a Wilcoxon test for the analysis. In the delayed reward task using the 4:1 ratio, the longest waiting time for *L. dimidiatus* was 240 seconds in 2014, and 480 seconds in 2016 (Figure S1). A Wilcoxon test revealed no significant difference between the different years in the delayed reward task with a ratio of 4:1 in *L. dimidiatus* (T = 14.5, P = 0.39, N_{2015} = 6, N_{2016} = 7); Figure S1).

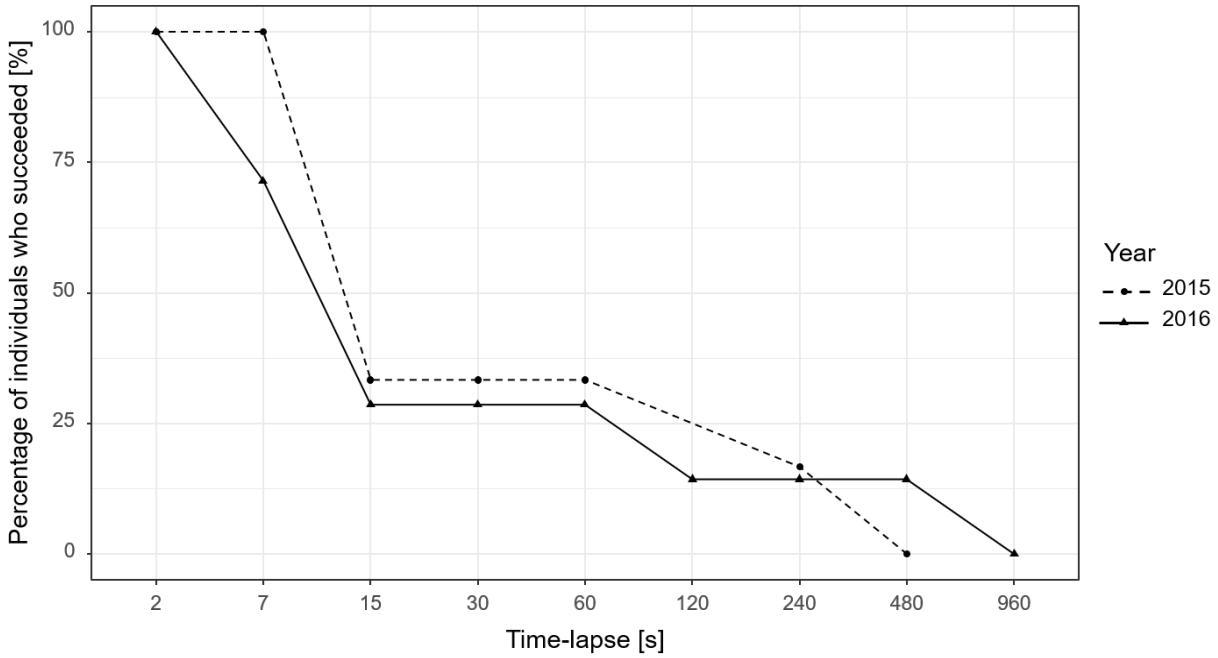
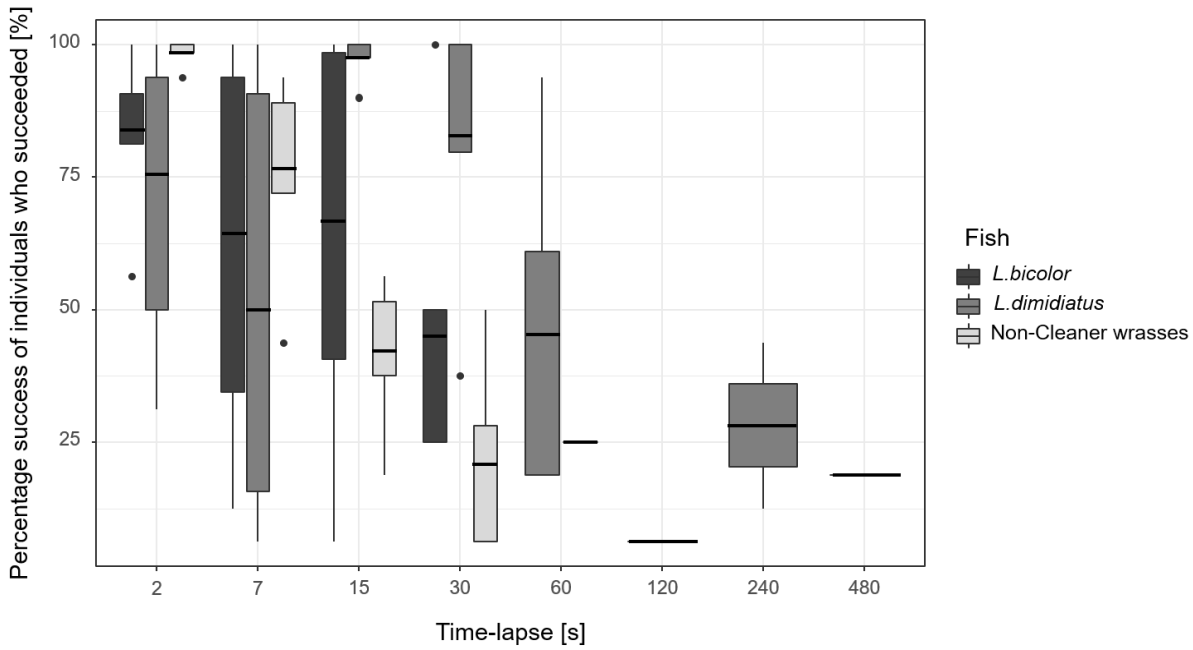


Figure S1: Mean of the percentage of *L. dimidiatus* individuals who succeeded in delaying rewards ratio 4:1 for the different time-lapse and with the year as a variable (Year 2015, *N* = 6, and Year 2016, *N* = 7).



N L.bicolor	7	7	6	5	0	0	0	0
N L. dimidiatus	13	11	4	4	4	1	2	1
***N* NC wrasses**	4	4	4	3	1	0	0	0

Figure S2: Boxplot of mean and interquartile range of the percentage success of individuals who succeeded in delaying rewards for the different time-lapses and with fish as a variable (*L. bicolor N* = 7, *L. dimidiatus N* = 13, and Non-Cleaner wrasses *N* = 4). 3 fish groups are shown here: at time-lapse 60s, only 2 fish groups are represented, *L. bicolor* being out, and at 120s, 1 fish species remained, *L. dimidiatus*, until 480s.

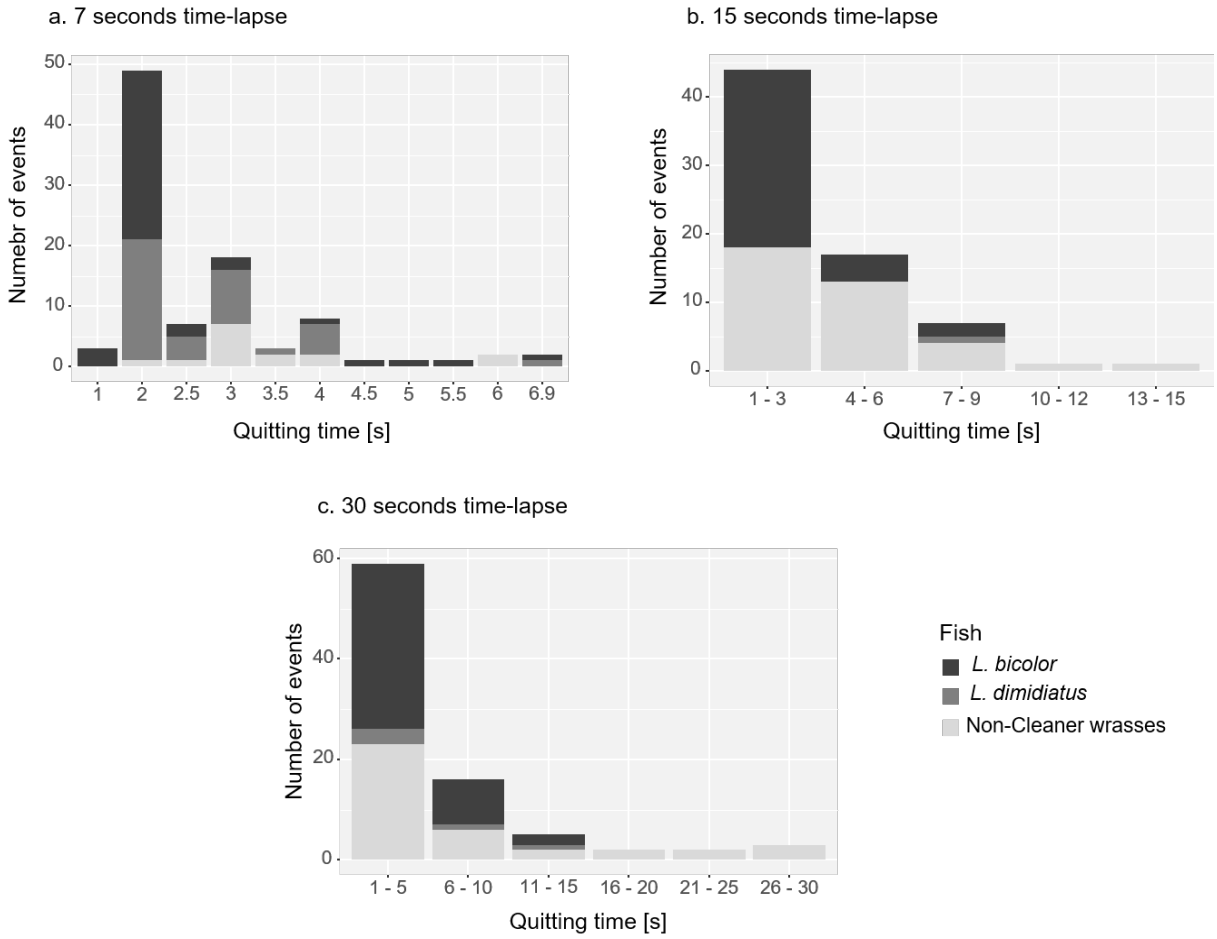


Figure S3: Number of events in function of the quitting time in seconds at 3 time-lapses (7s, 15s, and 30s; a-c) in the delayed reward experiment ratio 4:1. For the 15 seconds time-lapse (b) we grouped the quitting time by units of 3 seconds, and by units of 5 seconds for the 30 seconds time-lapse (c).

Table S2: Comparison between the expected and the observed percentage chance to wait in the quantitative delayed reward task of a ratio 4:1. Only significant values are shown here. Different time-lapses are represented: 60s and 480s for *L. dimidiatus*, and 15s and 30s for *L. bicolor*. The letter in brackets after each fish individual refers to Figure S4.

Individual	**60s time-lapse**			**Individual**	**480s time-lapse**		
	Time	Observed (%)	Expected (%)		Time	Observed (%)	Expected (%)
Dimidiatus 4 (a)	2	87.5	92.02	Dimidiatus 7 (b)	1	93.75	99.42
	2.5	75	90.12		2	87.5	98.84
	3	56.25	88.27		3	75	98.27
	4	43.75	84.67		4	68.75	97.7
	7.5	37.5	73.2		5	62.5	97.13
	20	31.25	43.52		18	56.25	90.04
	25	25	35.35		20	43.75	89
	54	18.75	10.58		31	37.5	83.47
	60	18.75	8.24		69	31.25	66.89
					266	25	21.23
					349	18.75	13.09
					480	18.75	6.1
Individual	**15s time-lapse**			**Individual**	**30s time-lapse**		
	Time	Observed (%)	Expected (%)		Time	Observed (%)	Expected (%)
Bicolor E (c)	1	93.75	89.63	Bicolor E (d)	1	56.25	92.48
	2	37.5	80.34		2	43.75	85.53
	6.5	31.25	49.09		2.5	37.5	82.25
	15	31.25	19.36		7.5	31.25	55.64
					12.5	25	37.64
					30	25	9.58
				Bicolor D (e)	2	75	86.73
					3.5	43.75	77.94
					7	37.5	60.74
					8.5	31.25	54.59
					10	25	49.06
					30	25	11.81

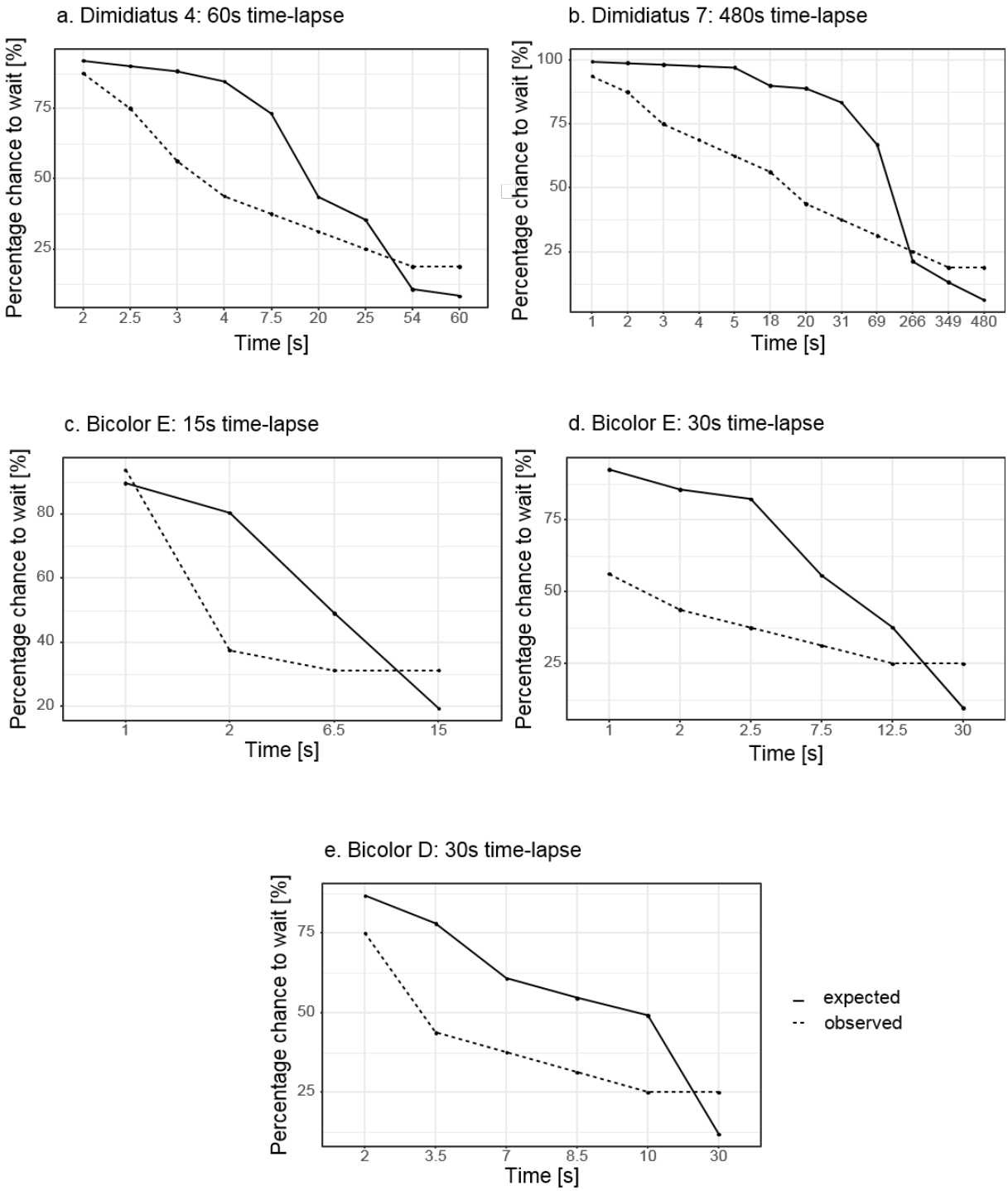
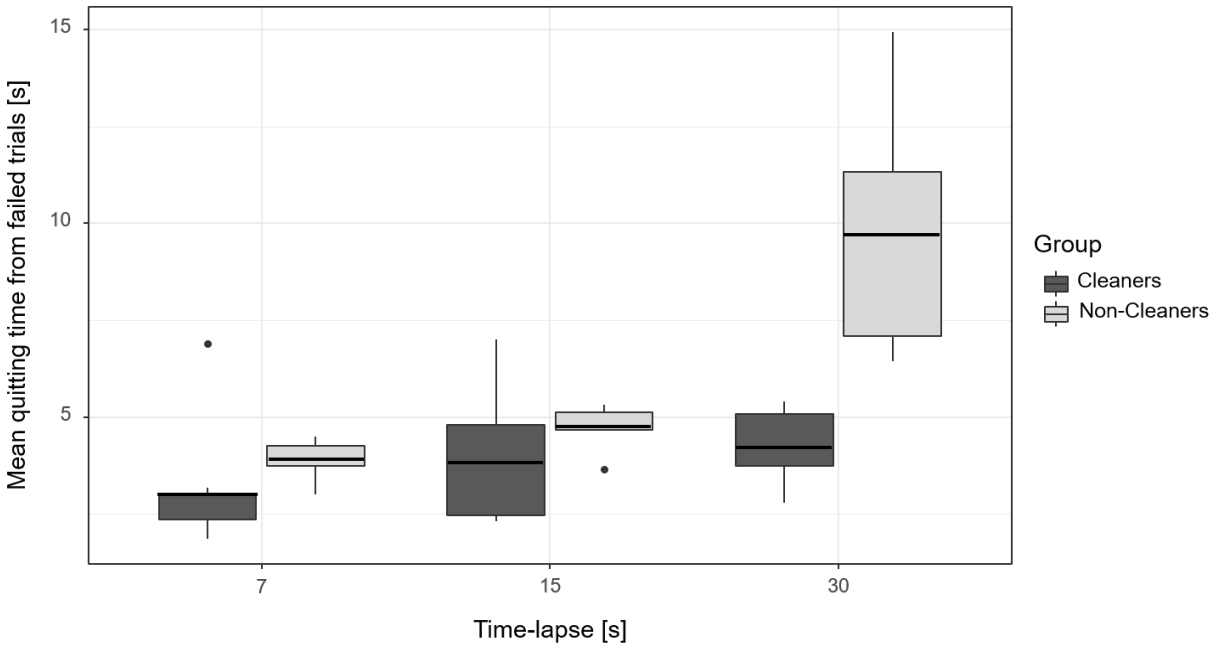
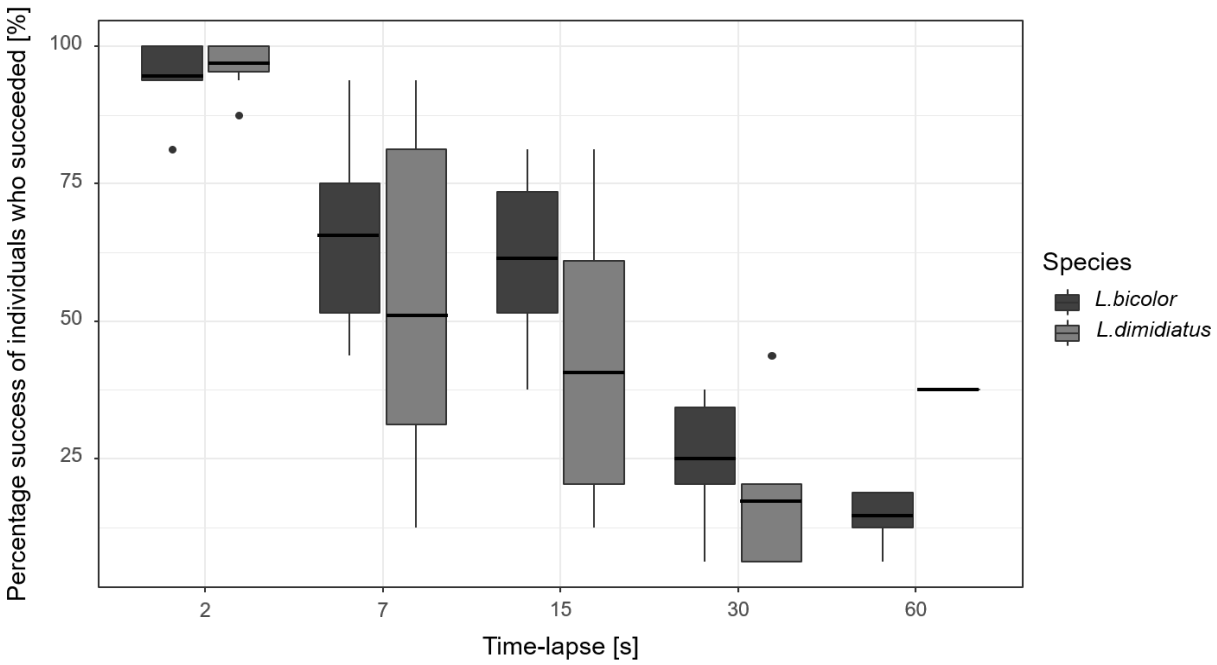


Figure S4: Difference between the percentage chance to wait of expected and observed error time at time-lapses where the difference was significant. Only four cleaner fish individuals were found to significantly quit earlier than expected and thus at different time-lapses (a-e).



***N* Cleaners**	12	8	7
***N* Non-Cleaners**	4	4	3

Figure S5: Boxplot of mean and interquartile range of the mean quitting time from the failed trials in the quantitative delayed reward ratio of 4:1 within the two fish's groups 'Cleaners' and 'Non-Cleaners'. The mean quitting time is represented in function of the 3 time-lapses: 7s, 15s, and 30s.



N L.bicolor	7	6	6	6	3
N L. dimidiatus	6	6	6	4	1

Figure S6: Boxplot of mean and interquartile range of the percentage success of individuals who succeeded in self-distraction for the different time-lapses and with species as a variable (*L. bicolor N* = 7, and *L. dimidiatus N* = 6).

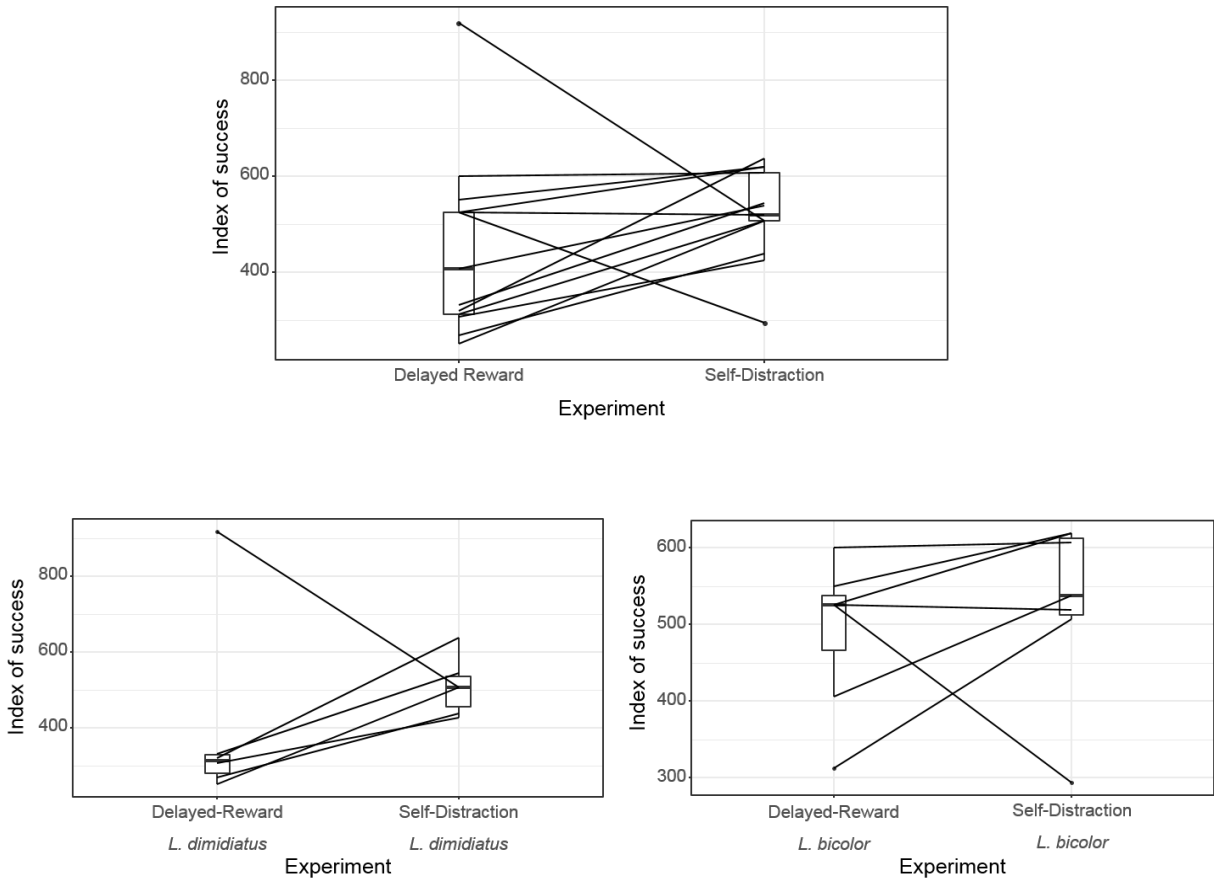
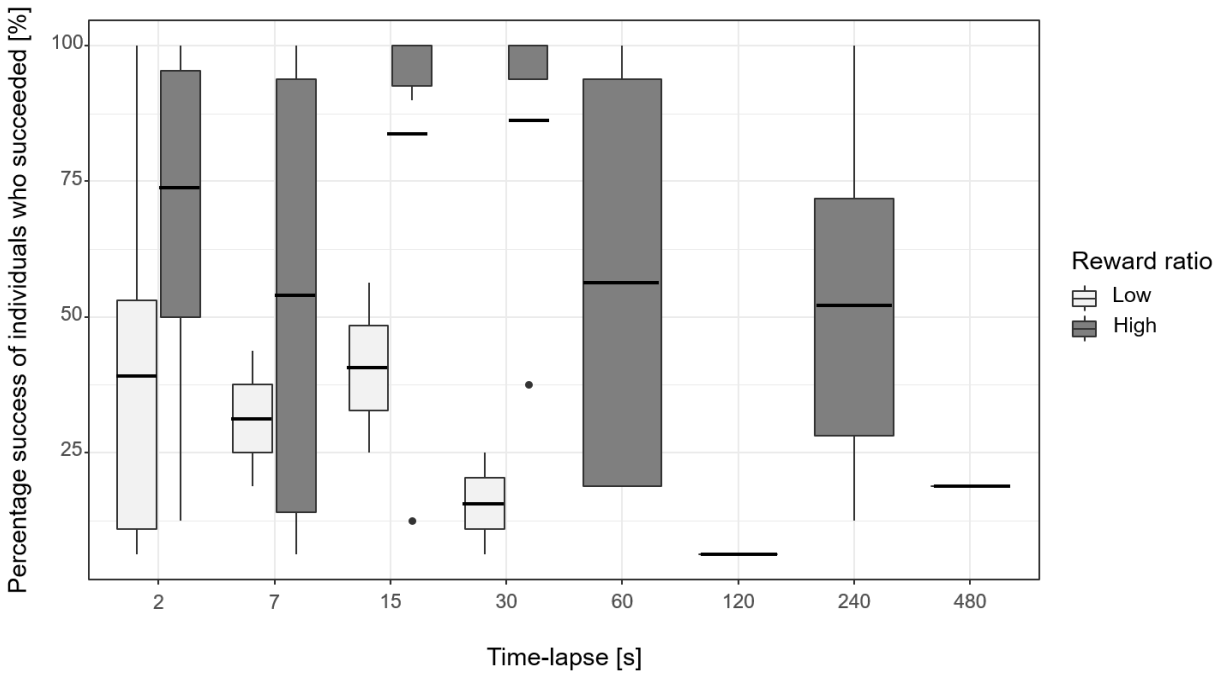


Figure S7: Paired-data plot of the index of success per individual in each experiment. On top: both species combined. Down: per species (*L. dimidiatus* on the left, and *L. bicolor* on the right).



***N* Low**	4	3	2	2	0	0	0	0
***N* High**	13	11	4	4	4	1	2	1

Figure S8: Boxplot of mean and interquartile range of percentage success of individuals who succeeded in delaying rewards for the different time-lapse and with reward ratio as a variable (reward ratio low = 2:1, *N* = 4, reward ratio high = ratios 4:1 and 8:1 combined, *N* = 13).

2. Chapter 2 | Cleaner wrasse *Labroides dimidiatus* perform above chance in a "Matching-to-sample" experiment

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MA collected the data and ran the analyses. All authors contributed to the writing.

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2.1. Abstract

Endothermic vertebrates have on average a 10 times larger brain to body ratio than ectothermic vertebrates. It is currently unclear what aspects of endotherm cognition are enhanced as a consequence, as there is plenty of evidence that ectotherms, including invertebrates, possess cognitive processes beyond basic classical and operant conditioning. One possibility is that ectotherms lack the ability to use higher cognitive processes outside ecologically relevant contexts. Here we tested this hypothesis on cleaner fish *Labroides dimidiatus*. It has been shown previously that cleaners show generalised rule learning in an ecologically relevant context; they generalise that any predator may provide protection from being chased by other fish. We tested for this ability in an abstract context, the matching-to-sample task. In this task, a sample is shown first, and then the subject needs to choose the matching sample over a simultaneously presented different one in order to obtain a food reward. We used the most general form of the task, using each stimulus only once in a total of 200 trials. As a group, the six subjects performed above chance, and four individuals eventually reached learning criteria. However, individual performance was rather unstable, yielding overall only 57% correct choices. A single highly performing individual was exposed to the delayed version of the task, where the sample was removed 30s before the two stimuli were presented. It failed in this version that tests for working memory capacities; nevertheless, any conclusion on this task must remain preliminary. Overall, the results add to the growing literature that ectotherms show the ability of abstract concept learning, though the lack of stable high performance may indicate quantitative performance differences to endotherms.

Keywords: abstract concept learning; *Labroides dimidiatus*; cognition; matching-to-sample; working memory

2.2. Introduction

Generalisation requires the ability to conceptualise a new environmental input based on available knowledge i.e. relating a new situation with previous experience (Emery & Clayton 2004). Because such cognitive flexibility is supposedly more complex than standard reinforcement learning, it has attracted considerable attention in animal cognition research (Wynne and Udell, 2013). As the ability to form abstract concepts is considered to be cognitively demanding, only large-brained animal species, like primates, were initially taken into consideration (D'Amato and Salmon, 1984; D'Amato et al., 1985; Premack, 1978, 1983a, 1983b). However, the ability to learn abstract concepts eventually turned out to be widespread in the animal kingdom, like in primates, dolphins, seals, birds, rodents, insects and fish (Penn et al., 2008; Wasserman and Young, 2010; review from Zentall et al., 2008). The standard approach is to allow subjects to learn an initial task by operant conditioning, and then to change stimuli (Wright et al 1988, Fechner 1860). If individuals are able to establish a link between what is learned and the new stimuli, they should show a rapid increase in performance in the transferred trials. Four different types of concept learning exist in which animal species showed competency, differing in the way individuals have to discriminate between stimuli (reviewed in Zentall et al., 2008). Discrimination can be based on forming classes, such as chair or flower, known as perceptual concept learning (Goldstone, 1994); discrimination to form categories by associating stimuli to another, such as an object with the word for that object, known as associative concept learning (reviewed in Zentall et al., 2014); discrimination via the relationship between or among stimuli, such as same/different, known as the relational concept learning (Thompson, 1995); and finally the discrimination via analogy, i.e. develop the relations between relations, such as a set of five same icons is similar to a set of five same but different icons and dissimilar to a set of five different icons, known as the analogical reasoning (French, 1995; Gentner and Medina, 1998; Vosniadou and Ortony, 1989). Overall, these concepts are represented by the relation of abstract rules to novel stimuli and, therefore, cannot be assigned to reinforcement learning (Katz et al., 2007; Premack, 1978; Thomas, 1980, 1996).

A well-known experiment to test for concept learning capacity in animals is the matching-to-sample task (hereafter MTS) (Clark and Sherman, 1970; Ferster, 1960; Skinner, 1950). This task is part of the relational concept learning where individuals have to understand the general concept of the task, which is to comprehend the concepts of 'same' and 'different' by relating stimuli to the sample stimulus in order to succeed, i.e. choosing the matched symbol over a simultaneously presented different one, and accordingly obtain a food reward (Truppa et al., 2011; Zentall et al., 2008). As a consequence, several stimuli can be used and the lack of conditioning is a prerequisite. Variation in the colour and shape of the stimuli might avoid any intrinsic preference that the individual can primarily have. The MTS task permits to perceive how individuals are able to associate rules with different stimuli (Skinner, 1950). In the simultaneous MTS, the sample stimulus is presented and then the matched and the non-matched stimuli are presented beside it (Konorski, 1959). Alternatively, in the delayed version (DMTS), the sample stimulus is shown alone (first exposure) and removed prior to the presentation of matched and non-matched stimuli (second exposure) (Blough, 1959).

Studies on MTS and DMTS, with an experimental design with two stimuli and one sample stimulus, showed variation in the performance of the different taxa tested but several studies yielded positive results (bottlenose dolphins (Herman and Gordon, 1974; Herman et al., 1989), California sea lions (Pack et al., 1991), capuchin monkeys (D'Amato et al., 1985; Truppa et al., 2010), chimpanzees (Finch, 1942; Nissen et al., 1948; cited by Oden et al., 1988), hens (Foster et al., 1995), honeybees (Giurfa et al., 2001), pigeons (Blough, 1959; Bodily et al., 2008; Wright et al., 1988), and rats (Iversen, 1993); summarized in Table S1 in the Supplemental Material). Different senses were used in those diverse studies, such as olfaction, audition, and vision. Overall, only a few studies have tested fish so far: goldfish (Goldman and Shapiro, 1979; Zerbolio and Royalty, 1983), Malawi cichlids (Gierszewski et al.,

2013) and archerfish (Newport et al., 2014). In those studies, all fish were tested via visual stimuli using either coloured light or forms and shapes. Evidence for simultaneous MTS was only found to work in goldfish (Goldman and Shapiro, 1979; Zerbolio and Royalty, 1983), but neither in cichlids (Gierszewski et al., 2013) nor in archerfish in simultaneous MTS and also DMTS (Newport et al., 2014). While Goldman and Shapiro (1979) did not run transfer trials, Zerbolio and Royalty (1983) demonstrated the success of goldfish also in transfer trials. A common point of all MTS studies is that animals were trained prior to the crucial test, using initially only two stimuli, alternating which one is also the sample stimulus, often running hundreds of trials before subjects reached learning criteria. After this training phase, experimenters conducted a transfer test using different stimuli from the training phase. If individuals performed well quickly under the new conditions, they were considered to have understood the task at a conceptual level ('match to sample') (D'Amato and Colombo, 1989; Katz et al., 2007; Thompson, 1995).

Given the mixed evidence for fishes regarding their capacity for conceptual learning, we tested cleaner fish *Labroides dimidiatus* in both the simultaneous MTS and the DMTS tasks, using different shapes and colour 2D images as stimuli. Cleaner fish are a suitable study species for an MTS task using an abstract paradigm as they are known to use generalised rule learning in an ecologically relevant context (Wismer et al. 2016). The ecologically relevant context is linked to conflicts between cleaners and 'client' reef fish that arise when cleaner feed on client mucus rather than on client ectoparasites (Grutter and Bshary, 2003). Clients sometimes respond to cleaners eating mucus with aggressive chasing (Bshary and Grutter, 2002). In such situations, cleaners may seek a passing-by predatory client, which functions as a social tool as its mere proximity makes the punishing individual stop its pursuit (Bshary et al., 2002). Within this context, Wismer et al. (2016) showed evidence for generalised rule learning in controlled laboratory experiments. In these experiments, cleaners were offered preferred and non-preferred food items on a Plexiglas plate. In response to cleaners eating a preferred item, the experimenter would start chasing the cleaner with the plate (manoeuvring a lever attached to the plate). However, there were two laminated pictures at the end of the aquarium, one showing a predatory client and one showing a harmless client. If the cleaner approached the picture of the predator, the pursuit with the plate stopped, while the pursuit continued if the cleaner moved towards the image of the harmless client. Once individual cleaners showed a significant preference for the predator picture, the two pictures were exchanged. In these follow-up tasks, cleaners reached learning criteria significantly faster, yielding evidence for generalised rule learning (Wismer et al., 2016). The results fit the prediction that animals typically excel in tasks that are presented in an ecologically relevant context (Kamil, 1998; Shettleworth, 2009). Solving ecologically relevant tasks may be achieved by any animal species, while more abstract presentations supposedly warrant relatively brains that allow flexibility beyond concise situations (Burkart and van Schaik, 2010; Burkart et al., 2017; Deaner et al., 2007; van Schaik et al., 2012). We, therefore, wanted to test if cleaner fish are capable of demonstrating the ability of concept learning even when the task has no ecological relevance, i.e. in an abstract MTS paradigm. In order to avoid any 'learn-how-to-learn' (Harlow, 1949) explanations as in the Wismer et al. (2016) study, and indeed all previous studies providing positive results on the MTS task, we opted against any repeated use of stimuli. Thus, in contrast to previous studies on MTS, we ran no training trials prior to testing. Instead, each symbol was used only once per fish. Under these circumstances, any performance above chance must be based on the formation of a general concept. We started with a simultaneous MTS task. Only if an individual reached learning criteria, we subjected it to a DMTS task, i.e. a task in which the sample is removed before its matched and non-matched alternative symbols are presented after a retention interval. The DMTS task can test for working memory as it adds the dimension of time. Working memory integrates the temporal dimension which is essential to activate long-term memory connections in order to plan in the near future (Fuster, 2015). The experiments should answer the question of whether cleaner fish show evidence for generalised rule learning only within a narrow ecologically relevant context or whether

they can form concepts also in more abstract circumstances. In the latter case, we would conclude that concept learning is a domain-general cognitive capacity of cleaner fish.

2.3. Materials and methods

This study was conducted from April until May 2017 at the Gump research station on Moorea (French Polynesia). Six cleaner fish (*Labroides dimidiatus*) between 7.5 and 8.6cm were caught with a barrier net (2m long, 1.5m high, mesh size 0.5cm) and using hand-nets in lagoons around Moorea. Cleaners were accustomed to plastic aquaria of a size of 69 x 50 x 43 cm (Figure S1), water level at 34 cm, with a small PVC pipe as a shelter for at least 19 days before experiments started. A pipe from the lagoon provided a continuous seawater flow.

2.3.1. Acclimation and pre-training

Cleaners were first trained to feed small items of mashed prawn off small dots painted on Plexiglas plates of a dimension of 10 x 7 centimetres. For the experiment, they were habituated to approach the experimental plate (20 x 20 cm) with a random training symbol in the middle (symbol drawn on a white square piece of paper of dimension 3 x 3 cm), and when they would touch or bite the training symbol they would receive a food reward, i.e. a piece of mashed prawn, by inserting the reward plate (3 cm width). As a first training step, a food item was placed directly on the training symbol itself. When fish were comfortable to approach the training symbol, the reward plate was used. Furthermore, subjects were familiarized with a dashed see-through barrier placed 10 centimetres from the aquarium wall with a sliding door inserted in the middle. The barrier created an "experimental" compartment into which the plate was introduced and a "resting" compartment to which the cleaner was initially confined. The door was opened once the experimental plate had been put into place (Figure 1). All cleaners readily approached the experimental plate before the trials began. The time it took each individual to be accustomed to the experimental setup and hence to start the experiment varied between 11 and 17 days. Consequently, not all individuals had the same amount of training days, and thus they did not start the experiment on the same day.

2.3.2. Procedure Experiment 1: simultaneous matching-to-sample (simultaneous MTS)

Cleaners were tested in their home aquarium. Once the fish was in the "resting" area, we fixed a GoPro camera using a clamp to clip it at the edge of the aquarium wall such that it filmed the trial. After 60 seconds, we introduced the experimental plate with the "sample" symbol placed in the middle of a 3x3 square matrix, for about 10 seconds (giving the fish time to look at the "sample" symbol; Figure 1a). The experimental plate, with hooks glued on the back, was hanged at the edge of the aquarium wall avoiding hands movement that might interfere with the fish choice. The plate was then removed to add the "match" and "non-match" symbols. To avoid the development of side biases, these two symbols could be placed on any of the eight remaining squares (Figure 1c), the exact locations predetermined to balance positions across trials. After 25-30 seconds, the experimental plate was reinserted with the three symbols (Figure 1b). The door was opened 3-5 seconds later so that the cleaner could make its choice. The fish had to be in physical contact with a symbol (either by giving tactile stimulation with its pelvic fins or by touching it with the mouth) so that we considered that a choice had been made. Only if the choice was the matching symbol, the cleaner received a small piece of mashed prawn as a food reward on the introduced reward plate. When it had finished eating, we gently removed both the rewarding and the experimental plate. If the "non-match" symbol had been chosen, no reward plate was introduced but instead, the experimental plate was quickly removed. We completed one trial with each fish before we conducted the next round of trials. As a result, the intertrial interval (ITI) was about twenty minutes for each fish. Each individual fish was exposed to a maximum of 200 trials, each one presenting two new symbols, so that the fish was confronted with a

maximum of 400 different symbols. The same set of 400 symbols was used for all fish but combinations, locations, and function ("match" and "not-match" symbols) were randomised (see Figure S2 for the first 20 symbols used).

2.3.3. Procedure Experiment 2: delayed matching-to-sample (DMTS)

Only cleaners that succeeded in Experiment 1 were to be tested in the DMTS task. The procedure was almost the same as in experiment 1, with one important difference: the sample symbol was only presented alone for around 10 to 15 seconds to allow cleaners to look at it, and then it was removed for the trials. Therefore, the fish faced only the "match" and the "non-match" symbols during trials after a retention interval of thirty seconds (see Figure S3 for the first twenty symbols used).

For both experiments, ten trials were conducted per individual per day. As long as cleaners did not reach the confirmation criteria (explained in the next paragraph), they were continuing in this experiment until the completion of 200 trials, i.e. 20 experimental days. On a few occasions, fish did not make a proper choice, i.e. they did touch both symbols with the body but randomly by swimming over them without stopping, and swimming back and forth in the aquarium but without making a choice by either biting or giving tactile stimulation to one of the symbols within the maximum exposure of 60s. These trials were discarded but not replaced. As a consequence, few individual daily data sets contained only nine data points. We directly recorded the individuals' choices (correct/incorrect) but all trials were also video recorded and can be requested. Fish were fed ad libitum for five minutes 20 minutes prior to the start of the experiment. When the experiment was finished for the day, fish were fed ad libitum about two hours.

2.3.4. Learning and confirmation criterion

An individual was considered to have learned the task if it performed significantly above chance levels (based on a sign test table) in either one session (9 or 10 out of 10 trials correct), two successive sessions (twice at least 8 out of 10 trials correct) or three consecutive sessions (three times at least 7 out of 10 trials correct). In order to be conservative, we exposed any individual that would reach a learning criterion to another 10 trials and only considered the individual to have succeeded if it chose correctly at least 7 out of 10 trials. Such an individual would then be tested in the DMTS paradigm. Failure to achieve at least 7 out of 10 correct choices meant that the individual continued with the simultaneous MTS experiment. The same criterion was applied for the DMTS task.

2.3.5. Statistical analyses

Apart from the individual learning criteria, we also asked whether our six cleaners as a group performed above chance levels (i.e. > 50%). We ran a generalized linear mixed-effects model with the statistical program R Rstudio © (R Version 1.2.5019, © 2009-2019 RStudio, Inc.), with the choice as our response variable and session/Individual as a random factor.

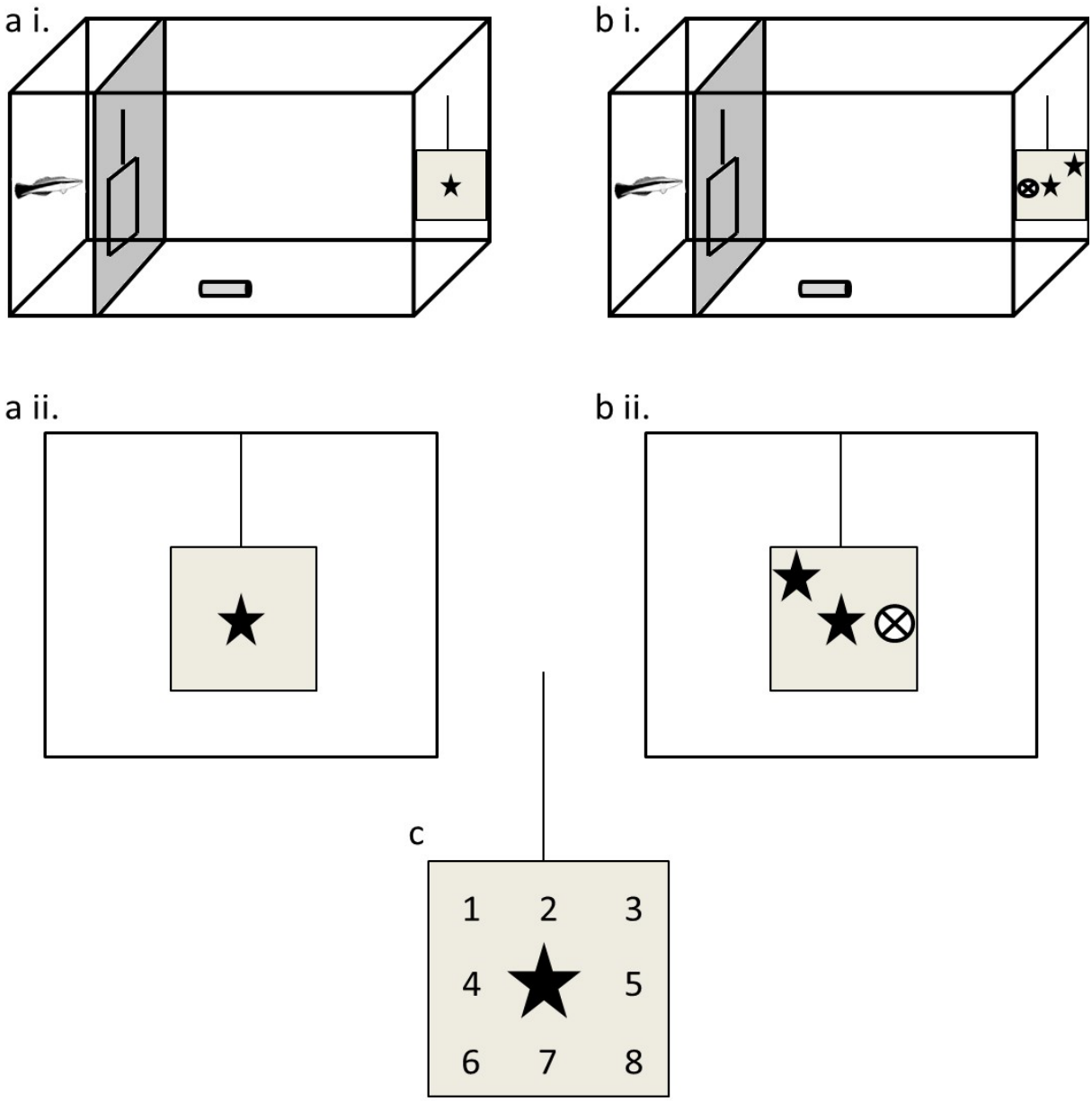

Figure 1: Exposition 1 (a) and 2 (b) of the experimental plate. i. Set up, ii. Fish view. We used eight different positions around the central sample symbol as emplacements for the matched and the non-matched symbols (c).

2.4. Results

2.4.1. Simultaneous MTS

Four out of the six cleaners eventually reached the learning criterion. Two of these four cleaners also reached the confirmation criterion in the next session, while the other two failed and never reached the learning criterion again until the completion of the 200 trials (Figure 2). Of the two successful cleaners, one completed the task in 40 trials (including the confirmation session), while the other one needed in total 180 trials. Only the former was subjected to the delayed MTS experiment. We ran into time constraints for the latter and hence let it complete the 200 trials for the MTS task. It lost its high performance during the final next two sessions (Figure 2).

Combining individual performances, cleaners succeeded significantly above chance as a group (estimate = 0.16, std. error = 0.062, z-value = 2.58, p = 0.01; Figure 2). The effect size is small (n=6, mean=57.21%). Individual mean performance varied between 48.78% and 75%. This latter high value

was due to the individual reaching criterion within only 40 trials. Even without that individual, cleaners still performed above chance as a group (estimate = 0.13, std. error = 0.064, z-value = 2, p = 0.046).

Table 1: Mean percentage of correct choice (=success) of each individual throughout all sessions tested.

	Bouchon	Chip	Georges	Leon	Merlyn	Titi
Mean % success	48.78%	57.24%	53.25%	75%	51.83%	57.17%

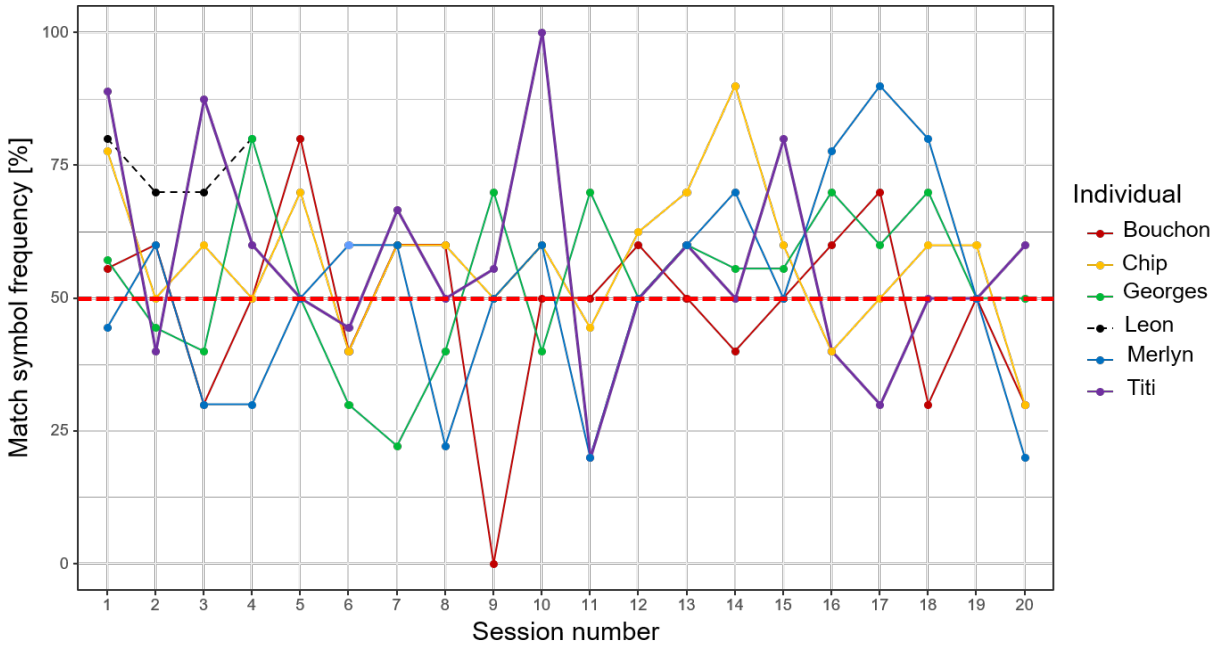

Figure 2: Simultaneous matching-to-sample experiment. The frequency when the matched symbol was chosen is represented in function of the session number (1 session = 10 trials; n = 6). The red dashed horizontal line indicates chance expectation (50% correct choices).

2.4.2. Delayed MTS

The only cleaner that was subjected to the DMTS task did not succeed within 200 trials (20 sessions). While it reached 70% twice in a row and once 80%, it also performed below 50% in various sessions, yielding overall 49.94% correct choices (Figure 3).

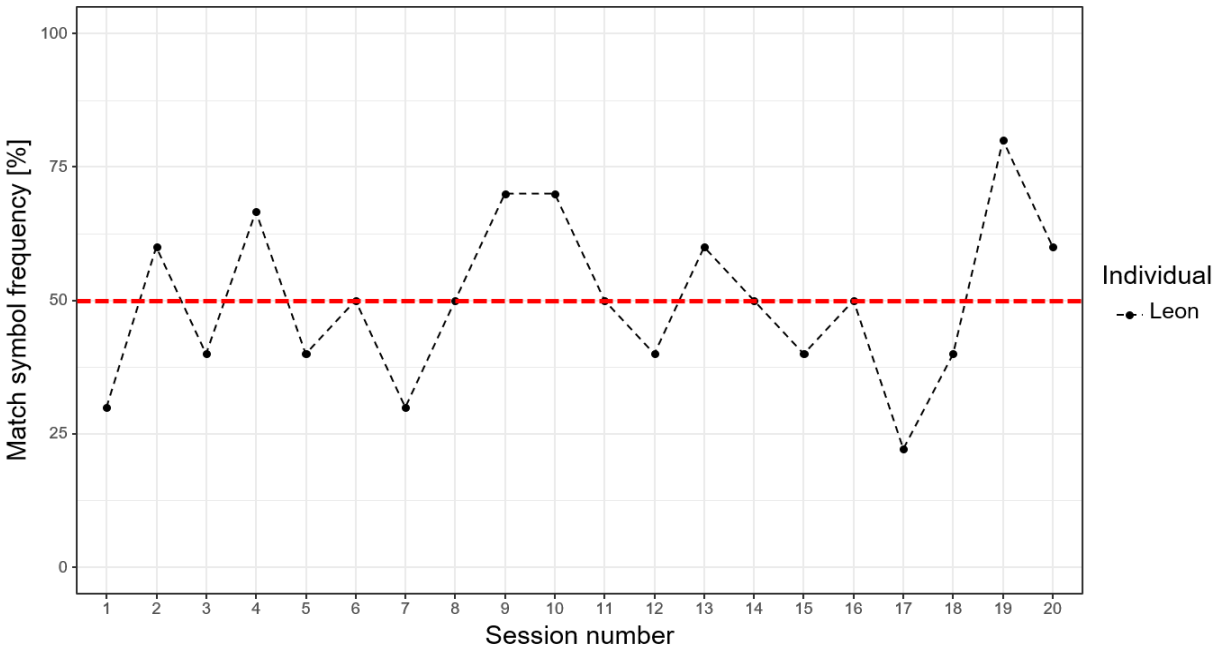

Figure 3: Delayed matching-to-sample experiment. The frequency when the matched symbol was chosen is represented in function of the session number (1 session = 10 trials; n = 1). The red dashed horizontal line indicates chance expectation (50% correct choices).

2.5. Discussion

We had asked to what extent cleaners are able to learn an abstract general rule without any prior training, using the MTS task. According to our results, cleaners have the potential to learn an abstract rule from scratch as they performed above chance as a group. Four out of six cleaners eventually reached learning criteria that are commonly used in cognitive experiments (Fisher, 1970; described in Bshary and Grutter, 2002b; Salwiczek et al., 2012; Triki and Bshary, 2017), and two of these cleaners even passed a more conservative criterion. However, the cleaners' performance was also quite unsteady, with two individuals returning to chance levels after reaching the initial criterion and one returning to chance levels after passing the additional test. This contrasts with other experiments in which cleaners generally continued to perform well in sessions after reaching learning criteria (Wismer et al., 2019; Salwiczek et al., unpublished data). It also contrasts with the cleaners' consistent ability to use generalised rule learning in an ecologically relevant task (Wismer et al., 2016). Taken together, cleaners overall expressed some understanding, but with only a minority performing properly above chance. Single individuals excelling at specific tasks is a widespread phenomenon in cognitive studies (Beran et al., 1999; Herrmann and Call, 2012; Leonardi et al., 2012; Aellen et al., in prep), highlighting the importance to understand intraspecific variation in cognitive performance (Thornton and Lukas, 2012).

To the best of our knowledge, the procedure that we used, i.e. the simultaneous MTS task in its most general form, was never tested before. Most previous studies started with a training phase that consisted of using two stimuli that alternated as matched and non-matched symbols, referred to as the 'standard MTS paradigm'. When an individual reached learning criteria, another set of stimuli known as the transfer stimuli was presented. This transfer task was introduced to rule out the possibility that subjects had learned two configurations rather than the rule 'match to sample'. If individuals had learned the rule, the number of trials needed to reach criteria with those second transfer stimuli should decrease compared to the training stimuli. While this approach yielded many positive results (Table S1), a kill-joy explanation would be that subjects merely learned how to learn rather than understand the concept (Harlow, 1949; Penn et al., 2008; Wasserman and Young, 2010; Zentall et al., 2008). There is limited evidence that the general version is more challenging than the

standard version of the MTS task. Training pigeons with 76 trials per day, Wright et al. (1988) exposed two subjects to the standard version (2 stimuli alternating roles) and 2 subjects to 76 fixed pairs of stimuli (that would be used again over consecutive days, alternating roles as well). The two pigeons exposed to the standard version reached criteria after 1216 trials, while it took the other two pigeons 27'360 trials. On the other hand, the latter performed very well in transfer trials while the former did not. In the light of these results on pigeons, the overall positive performance of cleaners in only 200 trials becomes more impressive, given their relatively much smaller brains. Nevertheless, given that small differences in experimental design may cause major differences in performance, we consider it important that more studies will use our design on a variety of species belonging to different clades.

The fact that we obtained overall performance above chance levels despite using new stimuli at every trial favours the interpretation that cleaner wrasse are indeed capable of abstract concept learning (Augier and Thibaut, 2013; Thibaut and Witt, 2015). However, we note that the fish did not improve their performance from the first 100 trials to the second 100 trials, which should have been expected. One possibility is that the increment of the number of stimuli used during the experiment increased the information load that the fish were exposed to (Augier and Thibaut, 2013). Therefore, the experimental design may have caused an overload of information, which increased the cognitive difficulty of the task and prevented a continued improvement of performance over the trials. It has been documented before that more information does not necessarily lead to better performance (Thibaut and Witt, 2015; Thibaut et al., 2010). Alternatively, cleaners might have a small perception bias that causes them to slightly favour the symbol that they see twice. To rule out that potential explanation, future experiments should reward the symbol that is the 'Oddity from sample' (Wright and Delius, 2005). If cleaners perform above chance level in this opposite reward scheme, perception biases could be ruled out as an explanation for cleaner performance.

Until now, positive evidence for abstract rule learning by fishes in the MTS task was restricted to goldfish (Goldman and Shapiro, 1979; Zerbolio and Royalty, 1983), the proper controls conducted with a negative reinforcer (electric shock) (Zerbolio and Royalty, 1983). In contrast, archerfish (Newport et al., 2014) and a cichlid (*Pseudotropheus sp.*) failed at MTS tasks (Gierszewski et al., 2013). The current study with cleaner fish thus provides additional evidence that fish can learn an abstract concept. We cannot propose an explanation for the positive results on goldfish and cleaners and the negative results on the other two species. The key idea of the MTS task is that it tests for abstract concept learning in a context that lacks ecological relevance. Given that neither goldfish nor cleaners appear to have particularly large brains compared to other fish species (Allen et Kuiter, 1999; Cuvier et Valenciennes, 1839; Fowler et Bean, 1928; Lacépède, 1801; Randall, 1981; Bleeker, 1855; all cited in Chojnacka et al., 2015; Kotrschal et al., 2013), there is also no neuroanatomical explanation for the species differences. In mammals, the neocortex is linked to intelligence and behavioural flexibility (Dunbar, 1998), and it is known to be related to conceptual learning in humans (Binder and Desai, 2011; Martin, 2007). However, as also indicated by the MTS study on honeybees (Cope et al., 2018; Hammer, 1997), the ability of abstract concept learning is apparently not linked to the evolution of a highly derived brain structure. What is currently unclear is what ecological factors may select for the ability of abstract concept learning, yielding positive results in bees and in some fish species but not in others.

With the second experiment (delayed MTS), we aimed to investigate how far cleaners endowed working memory to solve an abstract task. Working memory in humans is defined as "the domaingeneral subsystem of the mind that enables one to activate and sustain (sometimes via active rehearsal) a set of mental representations for further manipulation and processing" (Carruthers, 2013).

In experiments with animals, working memory is involved in the memorizing of a stimulus and thus in either inter- or intra-trial intervals (Honig, 1978). In order to test animals and verify if they endowed

such memory, the DMTS task is one of the most common tasks to test it, as the sample stimulus is presented during the trial's first exposure and has to be remembered before being recognized in the trial's second exposure. Previous studies with mammals and birds showed success in this task with decrement of performance when the retention interval increased (as in bottlenose dolphin (Thompson and Herman, 1981), capuchin monkeys (D'Amato and Colombo, 1985), and hens (Foster et al., 1995); review of DMTS studies in Lind et al., 2015). Newport et al. (2014) tested archerfish in the DMTS task but without any success. In our study, only one individual was tested in this task and it failed similarly to the archerfish. This latter result may suggest that cleaners have difficulties using their working memory (this links to their failure in object permanence, unpublished data). Our retention interval was relatively long (30s), which may have led to the poor performance of our fish (Nelson and Wasserman, 1978). Nevertheless, because only one fish individual was tested in this task, current conclusions can only be preliminary.

2.6. Conclusion

The cleaner wrasse *L. dimidiatus* can solve the MTS task but individuals are not particularly good at it, at least in the general version we used. We cannot compare their performance to other species because of the new design. In the future, cleaners should be tested in the old design and other species in ours. Based on the limited data on pigeons being exposed to many different stimuli, the performance of cleaners within the 200 trials presented here was rather good. Thus, generalised rule learning in cleaners does not seem to be restricted to ecologically relevant contexts, indicating that abstract concept learning is a general cognitive tool present in this species.

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2.9. Supplemental material

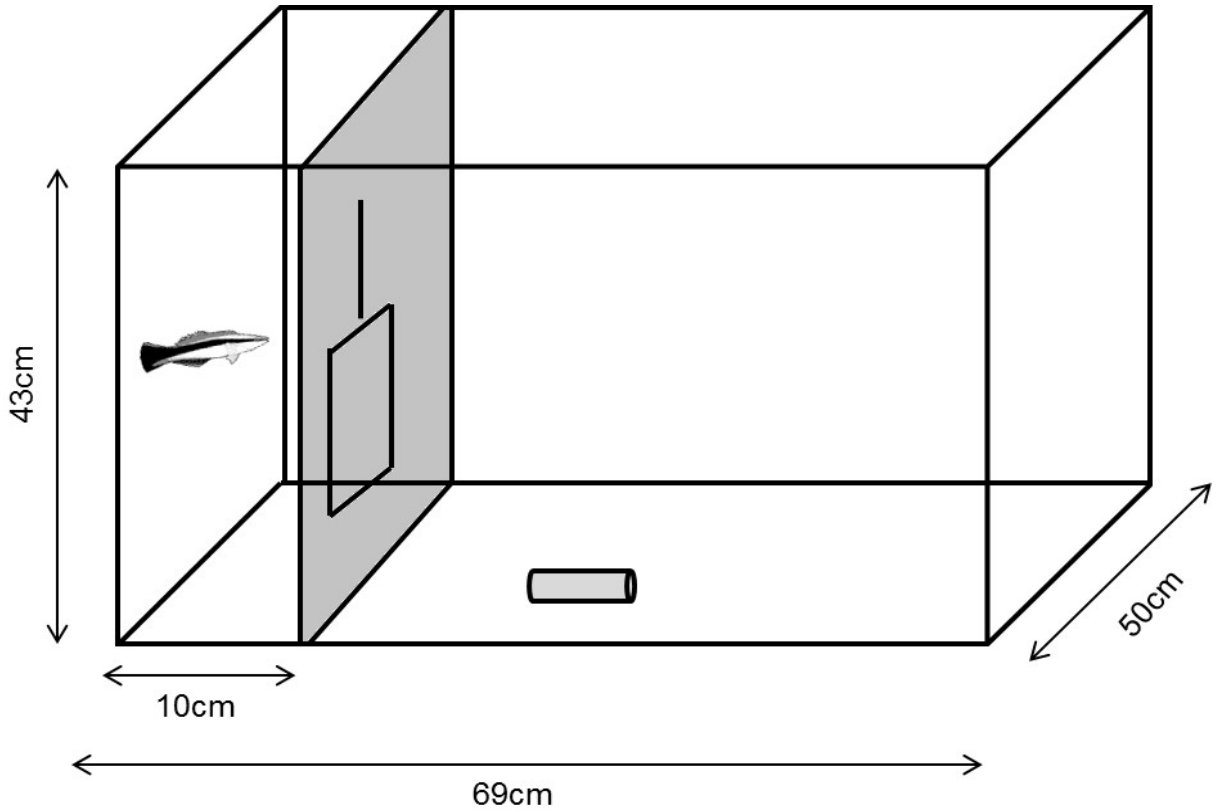

Figure S1: Experimental aquarium design. The “resting” area is on the left where the fish is waiting behind a see-through grid barrier with a door in the middle. On the right is the “experimental” compartment where the experimental plate is displayed during the trial.

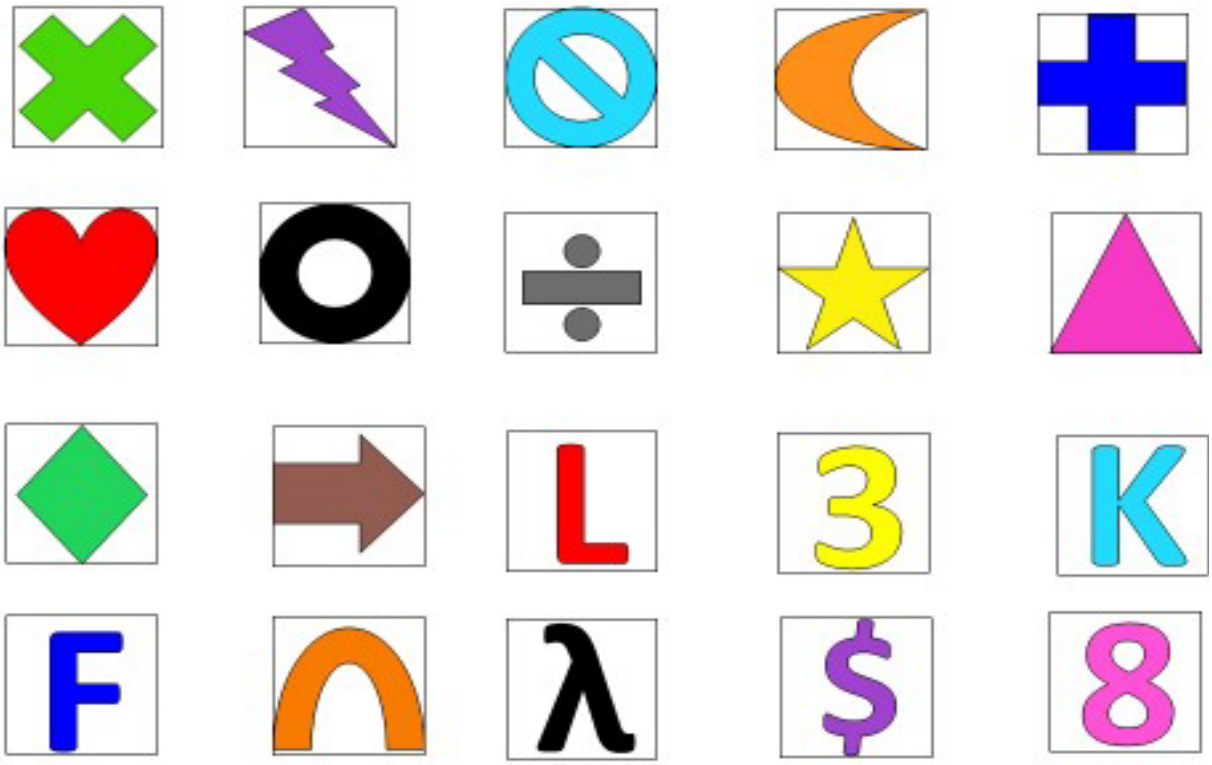

Figure S2: Representation of the twenty first symbols used in the matching-to-sample task. The symbols were drawn on a 3 x 3cm white paper sheet.

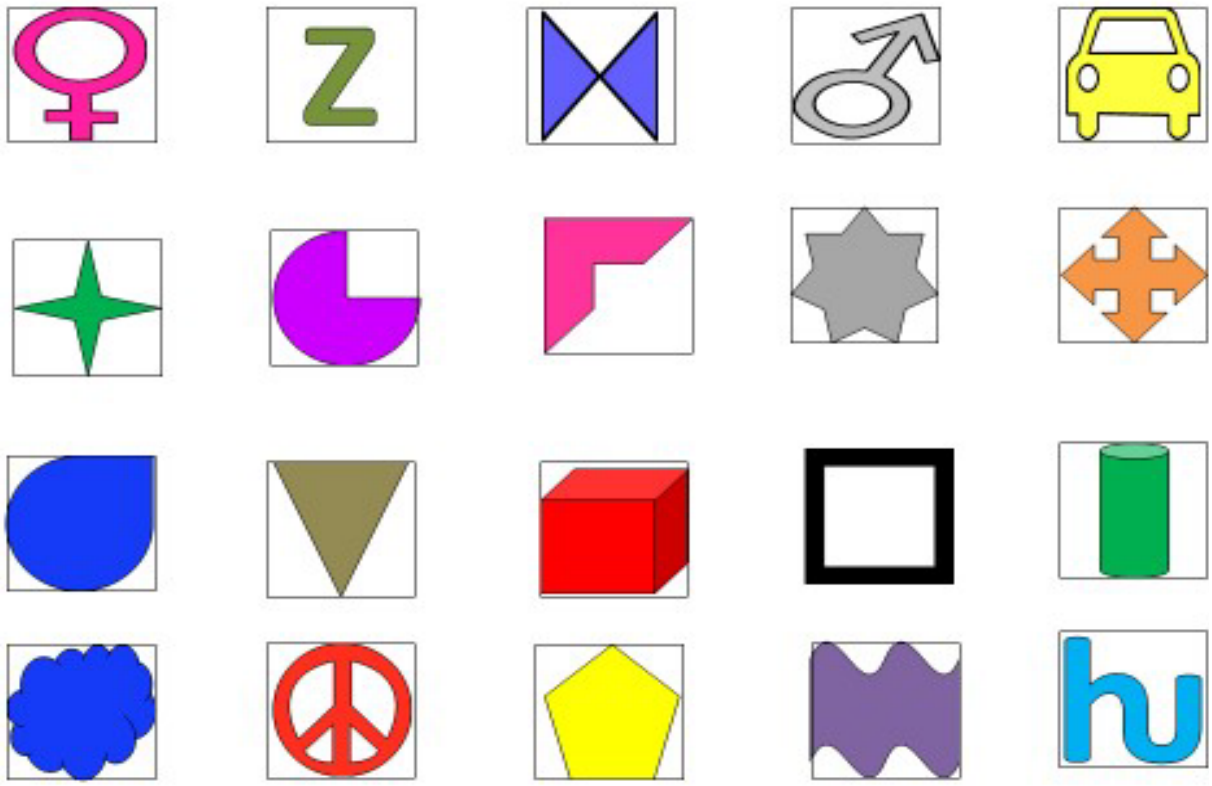

Figure S3: Representation of the twenty first symbols used in the delayed matching-to-sample task. The symbols were drawn on a 3 x 3cm white paper sheet.

Table S1: main results of MTS and DMTS studies.

Reference	Organisms (n)	Task	Stimuli	Pre-training and/or training	N° training trials	Transfer test	N° transfer trials	Success/ fail
Finch, 1942 (by Oden et al., 1988)	Chimpanzee (4)	MTS	?	two training stimuli	600 – 1000	new stimuli	similar to Oden et al, 1988: 24 trials	success
Nissen et al., 1948 (by Oden et al., 1988)	Chimpanzee (4)	MTS	?	two training stimuli	600 – 1000	new stimuli	similar to Oden et al, 1988: 24 trials	success
Blough, 1959	Pigeons (4)	MTS	light	two training stimuli steady light / flicker light	500 – 1000	new stimuli (for 20 presentations)	8000 90% correct	2/4 succeed ed
		Delay MTS				delay: 0, 0.6, 1, 1.2, 2, 5 or 10; only 4 delay were presented per individual	5s: 60-70% correct	2 stable decrease with time 2 unstable
Herman et Gordon, 1974	Bottlenose dolphin (1)	Delay MTS	auditory	2 stimuli Training: 21 different pairs stimuli 9 days trained in delay auditory stimuli	934	tested on 346 unique delayed matching problem 5 - 10 trials same pairs delay randomized: 1.5, 3, 4, 6, 9, 10, 12, 15, 18, 20, 24, 30, 35, 40, 60 9 blocks of 5 delays with between 4 and 25 trials each	20 – 524 not under 79.3% correct choice	success

Goldman and Shapiro, 1979	Goldfish (5+5)	simultaneous MTS (n = 1)	light	Pre-training: white light in the middle, white light both side, touch and receive food	300 – 500	same stimuli	8400 60%	success
				Training: 3 stimuli: red, green and blue light	8400 60%			
Zerbolio et Royalty, 1983	Goldfish (24+24)	MTS (n = 4)	light	12 with red/green 12 with blue/yellow 30 days 40trials/day 5days/week	840 on 1200 trials total	2x 8 (n = 2) new stimuli 2x 8 (n = 2) same stimuli 30 days 40trials/day 5days/week	600 on average	success
D'Amato et al, 1985	Capuchin monkeys (8)	MTS	symbol	pre-training: sample - 1 stimulus showed		3 visual stimuli: coloured disk red, yellow and blue	240 - 624	success
				MTS training: simultaneous MTS procedure, only 2 stimuli used (shape) criterion: 2x 90% on 2 successive blocks of 48 trials	624 – 4673			
Wright et al, 1988	Pigeons (4)	MTS	symbol	pre-training: 2 stimuli (arrow + apple)				
				training: 35 trials (1st session), grain placed on correct comparison stimulus before choice				
			Trial unique	152 different stimuli presented once daily 76 trials per day --> 75% over 360 training sessions (18 months; 360 presentations)	27360	transfer: 10 trials/session 2 novel stimuli used at each trial 25 warm up trials // 10 transfer trials incorporating in remaining 51 training trials + 40 stimuli (2 consecutive transfer sessions)	7000 60%	success (n = 2)

						second transfer test with 40 test stimuli		
			2 stimuli	train with 2 stimuli 75% 16 sessions (1216 presentations)	1216	Same as above	7000	fail (n = 2)
				76 trials criterion: reach 75% or more then tested to transfer				
Oden et al., 1988	Infant chimpanzees (4)	MTS	objects	Pre-trained to handle 2 objects (cup and lock) when put object into a baking tin rewarded via hugs and congratulations	642 – 1000	2 new stimuli session of 12 trials reward with food and congratulations even when incorrect choice to keep them motivated 6 transfer tests so 12 novel objects in total	24 mean % correct 67.7 - 85.4 %	success
				training: give one object have to put it in baking tin, then two alternative objects were placed 20cm apart and equidistant from the pan session of 12-24 trials / ITI = mean of 30s / 1-3 times daily 5 times a week criterion level: at least 10/12	816 (mean)			

Herman et al., 1989	Bottlenose dolphin (1)	MTS	objects	4-5 stimuli 16-20 trials per session 2 sessions per day simultaneous MTS as training: 72 trials over 6 days	432 81%	Same stimuli 2-4 sessions for a total of 36-63 trials overall 83.3% correct NO transfer trials	564 83.3%	success
		Delay MTS	objects	zero-delayed matching training: 350 trials over 11 days	350 72.1%	same stimuli as in MTS part 1: delay 1, 3, 5, 10, 15, and 20s balanced blocks of 6 trials 13 sessions session of 30 trials, final session of 36 trials delayed randomized in the 6 trials	396 performance decrease with time increase	success
						part 2: delay 1, 3, 10, 20, 30, and 40s balanced 6 trials blocks 16x 24 trials + final session of 12 trials	396 until 30s above 94% correct	success
						part 3: delay 1, 3, 20, 40, 60, and 80s blocks same as part 2	396 constant decrease from 30s to 60s then steep decrease between 60 - 80s	success
							73% correct at 80s	
Pack et al, 1991	Sea lion (1)	generalized MTS	symbol	MTS training with 2 stimuli 24 trials per session criterion 2x 90% in a row	269	Same stimuli transfer test: add 1 novel stimuli compared to the 2 previous one from training phase criterion 2x 90% in a row	72	success
				with 3 stimuli	503			
		Delay MTS	symbol	Pre-training: 120 trials 5 sessions 3 stimuli	600	novel pair of objects criterion 2x 90% in a row		

Iversen, 1993	Rats (3)	MTS	light	pre-training: steady / blinking sample in the middle, rat press it, stimulus (same) on one of the side, press , food 2x 100 trials	200	Same stimuli 80% after 25 sessions 90% after 50 sessions for Rat 1, 60 for Rat 2 and 4 for Rat 3 NO transfer trials	1000 – 1500 60%	success
				MTS training: simultaneous MTS / same stimuli as in pre-training 1 session = 100 trials + number of repeated trial	5000			
Foster et al, 1995	Hens (6)	Delay MTS	light	2 stimuli: green and red light light in the centre, peck 5 times within 5sec, tone 0.5s, centre key light out, 0.01s two side light 62 sessions: 28 sessions increase time from sample to stimuli from 0.25 to 1.25 for 42 sessions	as pigeons	Same stimuli 30 trials per day, if do not reach it stop after 40 min	as pigeons	success
Giurfa et al, 2001	Honneybees (360)	MTS	Colour grating	2 stimuli (colour) Y-maze, entrance encountered sample stimulus then stimuli A and B have to choose in decision chamber	60	2 new stimuli (grating)	60	success
Bodily et al., 2008	Pigeons (5)	simultaneous MTS	cartoon images	3 pre-training stimuli 84 trials/session	2352	transfer trial with novel stimuli each time 4 consecutive transfer sessions conducted 48 novel pairs	384	success

			comput er	3 same stimuli as pre-training training: 96 trials/day 12 combinations configuration >85% correct on 2 consecutive sessions with CP --> without CP: >85% correct in 1 session	672			
Truppa et al., 2010	Capuchin monkeys (6)	Simul taneo us MTS	symbol	2 stimuli used 12 consecutive correct trials no more than 3 corrections	10852	10 transfer tests composed of 4 sessions	1812 - 48 (decreasing over test)	success
						final transfer test single 100 trials session with new stimuli	59%	
Gierszewski et al, 2013	Cichlids (9)	Simul taneo us MTS	symbol	2 stimuli presented 10 trials per session, 2 per day criterion 3x 70% in a row 35-40 sessions	350 – 400	using cross and circle	within 400 trials	fail
Newport et al, 2014	Archerfish (7)	Delay MTS	symbol	10 stimuli	380	3 new stimuli	200	fail
		Simul taneo us MTS	symbol	As Goldman and Shapiro, 1979 Pre-training: white light in the middle, withe light both side, touch and receive food Training: 3 stimuli: red, green and blue light		3 stimuli	800	fail

3. Chapter 3 | No evidence for general intelligence in a fish

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3.1. Abstract

Endothermic vertebrates, i.e. with stable internal body temperature, have, corrected for body mass, roughly 10 times larger brains than ectotherms, i.e. with internal body temperature varying with the environmental temperature. However, the cognitive advantages of larger brains remain unclear since in specific cognitive tasks, fish often perform similarly to endotherms, such as primates. In endotherms (or at least primates), the best cognitive correlate of brain size is general intelligence rather than performance in specific cognitive domains. We, therefore, tested if ectotherms might lack general intelligence (psychometric *g* factor) and tested 69 cleaner fish from the Labridae family (*Labroides dimidiatus*). We tested those fish in four non-ecologically and one ecologically relevant cognitive tasks. Different cognitive domains were highlighted, such as learning – flexibility, spatial problem-solving as well as inhibitory control, quantitative reasoning, and working memory. Furthermore, because general intelligence has been shown to be linked to group size, we compared cleaner fish in low vs high-density environments. Overall, fish performed similarly to primates in some of the specific tasks, but we did not find evidence for the factor *g*, i.e. performance across tasks was not correlated. Moreover, cognitive performance was not linked to the environment. This suggests that – i) larger brain size of endotherms correlates with general intelligence (leads to this working hypothesis), ii) *g* is not an inevitable by-product of brains, iii) cleaner fish perform equally to primates in some cognitive tasks but do not need general intelligence to do so.

Keywords: general intelligence; *Labroides dimidiatus*; ectotherm vertebrate; cognition; brain evolution

3.2. Introduction

Shettleworth (2010) defined cognition as the ability of animals to acquire information from their environment (perception) and to process and store (memory) those pieces of information in order to behave appropriately (decision-making). Such cognitive abilities can be studied to evaluate general intelligence in animal species, including humans (Spearman, 1904, 2000). In humans, general intelligence in its broad definition involves various domains of cognition as it implies multiple processes such as reasoning, planning, problem-solving as well as learning from experience (Gottfredson, 1997). To have this capacity of general intelligence, i.e. to be able to make the connection between the knowledge from different cognitive domains, will lead to more flexible behaviours, to understand abstract concepts, and have conceptual thoughts. A good proxy for general intelligence in humans is the psychometric factor *g*, also known as the general intelligence factor *g*. *g* results from the positive manifold, i.e. the well-established finding that in humans, performance across tasks is correlated: individuals performing well in one cognitive task or domain usually also perform well in other tasks (Carroll, 1993; Jensen, 1999; Jensen and Weng, 1994; Spearman, 1904, 1928). Factor analyses of performance across such tasks will thus result in a first factor on which all tasks load positively, and this general factor is referred to as *g* (Spearman, 1904).

The presence of general intelligence in non-human animal species has been largely debated in the last decades as, at first, it was suggested to be unique to the human species. Recent reviews (Burkart et al., 2017; Chabris, 2007; Matzel et al., 2011a; Searcy and Nowicki, 2019; Shaw and Schmelz, 2017) highlight the increasing evidence for a *g* factor in non-human animals such as bowerbirds (Isden et al., 2013), chimpanzees (Hopkins et al., 2014; Woodley of Menie et al., 2015), cotton-top tamarins (Banerjee et al., 2009), dogs (Arden and Adams, 2016), mice (Galsworthy et al., 2002, 2005; Kolata et al., 2005, 2007, 2008, 2010; Light et al., 2008, 2010, 2011; Locurto and Scanlon, 1998; Locurto et al., 2006; Matzel et al., 2006, 2003, 2008, 2011a, 2011b, 2017; Sauce et al., 2014; Wass et al., 2012), New Zealand Robins (Shaw et al., 2015), orangutans (Damerius et al., 2018), rats (Anderson, 1993), and rhesus macaques (Herndon et al., 1997). The general pattern from all these different studies is that the different tasks loaded positively on the first PCA factor and explained at least 17% of the variance. Contrariwise, other studies found no evidence of such a general intelligence factor *g* in similar species previously listed such as chimpanzees (Herrmann and Call, 2012; Herrmann et al., 2010), mice (Locurto et al., 2003), and satin bowerbirds (Keagy et al., 2011). Other studies on species like black-capped chickadees (Guillette et al., 2015), European starlings (Farrell et al., 2016; Nettle et al., 2015), and song sparrows (Boogert et al., 2011) also lack evidence of the factor *g*. In those studies where the evidence of g is lacking, not all tasks loaded positively, and/or the dominant dimension only explained less than 20% of the variance. That performance between tasks is not positively correlated seems to be a recurrent pattern in bird *g* studies (Anderson et al., 2017), except in Isden et al. (2013) with bowerbirds. Furthermore, in general, in *g* studies, except for humans *g* studies where the positive manifold is ubiquitous throughout studies (Carroll, 1993), positive correlations between different tasks are found but tend to be weak (Boogert et al., 2011). Though in those different studies where *g* was not found, few individuals were tested and sometimes the tasks were tested in the field. These two features could have skewed the results as only courageous individuals might have participated in the experiment. This highlights how important the number of individuals tested is, as well as how individuals are tested, i.e. in which condition.

The broad definition of general intelligence for non-human animals suggests that an individual is able to use knowledge that it gains through previous experiments, from physical and social milieus relations, to establish meaningful behaviour. The individual will apply its knowledge in new as well as in habitual situations which implies flexibility and reasoning (Byrne, 1994; Rumbaugh and Washburn, 2003; Yoerg, 2002). It remains unclear if there is any link between the general intelligence factor *g*, i.e. the psychometric *g*, and the large definition of general intelligence in non-human animal species.

Numerous studies went in the direction of a single variable explaining the variation of cognitive performance in several tasks, which support the idea of the existence of a psychometric *g* in nonhuman animal species (Damerius et al., 2018; Deaner et al., 2006; Reader et al., 2011). Overall, many studies find evidence for a general intelligence factor *g* in animal species, and animals studied were endothermic species (mammals and avians). Furthermore, it has been shown that cognitive performance is correlated with brain size (Anderson, 1993; Benson-Amram et al., 2016; Deaner et al., 2007; Jerison, 1973; MacLean et al., 2014; Reader et al., 2011; Roth, 2015; Rumbaugh et al., 1996). Moreover, laboratory experiments revealed, when manipulating the increment of brain size in individuals via artificial selection, i.e. pairing males and females with larger or smaller relative brain size together until fourth generations (Kotrschal et al., 2013), this brain magnification directly affected cognitive abilities, i.e. increasing the performance (Buechel et al., 2018). It is then inevitable to relate this to intelligence, as the bigger the brain, the better the cognitive abilities (Cox et al., 2019; Ritchie et al., 2015; Roth, 2015). After all, *g* might be an inevitable by-product of brains as all the previous studies suggest.

It has been largely suggested that intelligence, as well as self-control, which has also been found to be related to *g* in chimpanzees (Beran and Hopkins, 2018), correlates with brain size (Deaner et al., 2006; Hopkins et al., 2019; Jerison, 1955; MacLean et al., 2014; Reader et al., 2011). Already in 1973, Jerison studied the relation between brain weight and body weight in vertebrates using the logarithms of each measure. Three main conclusions came out of this study: 1) from the mammal class, humans do not possess the heaviest brain, 2) there is a strong correlation between brain size and body weight, and 3) there are two groups that massively differ in brain size, namely the lower vertebrates group, including fish and reptiles, and the higher vertebrates group with birds and mammals (primates included). The last point emphasizes the fact that reptiles and fish that weigh the same as a bird or mammal, have a 10 times smaller brain. To further test this link, we wanted to study a fish, which, as mentioned, would have a 10 times smaller brain compared to a same weight mammal or bird. As previous studies showed that the general intelligence factor *g* is correlated to brain size, we should find little evidence of *g* in fish since fish have smaller brains. However, if *g* is a mere by-product of having a brain, then we would find *g* in fish as well (Bartholomew et al., 2009, 2013; Kovacs and Conway, 2016; Rabaglia et al., 2011; Thomson, 1916; Van Der Maas et al., 2006). To proceed to this study, we chose a particularly smart fish, the cleaner wrasse *Labroides dimidiatus* that demonstrates similar cognitive performances than primates. Therefore, if *g* is not found in this cleaner wrasse species, it seems rather unlikely that it would be found in other fish species.

Until now, fish were never tested in any general intelligence test battery. We, therefore, chose to implement a test battery on a cleaner fish, *Labroides dimidiatus*, to inspect whether a general factor *g* is present in fish or not. Cleaner fish are a suitable species to run lab experiments as they are easy to catch, acclimate easily in captivity and can be maintained for up to 3 months in an aquarium. Because of the complexity of their environment and the strategies developed as a by-product, plenty of studies demonstrated the performance of this specific fish species in various cognitive tasks and its similar performances to other endothermic species such as primates delay of gratification (Aellen et al. in prep), generalisation (Wismer et al., 2016), and foraging decision rules (Salwiczek et al., 2012)). Moreover, it showed particular cognitive skills in its understanding of the environment in general (reputation, audience effect (Pinto et al., 2011), Machiavellian intelligence (Bshary, 2011)). All these abilities highlight how environmental structure/condition shapes cognitive capacities, at least in the example of the cleaner fish *Labroides dimidiatus*, even though it remains unclear how this fish manages to do this.

From an evolutionary point of view, testing fish instead of other vertebrates will broaden the scope of the discussion of general intelligence. We could investigate questions such as when did it appear, what selective pressures helped in its evolution, and if certain cerebral structures are needed to acquire it. The goal of this study was to identify whether a general intelligence factor *g* is present or absent in

the cleaner fish *Labroides dimidiatus* using a large number of individuals. To achieve this, we tested each individual in four different cognitive tasks. The tasks were chosen in such a way as to avoid any transferable knowledge from one task to the other. The four tasks were from different cognitive domains and involved learning – flexibility, spatial problem-solving as well as inhibitory control, quantitative reasoning, and memory. We used a reversal learning task for the learning – flexibility domain, a detour task for spatial problem-solving and inhibitory control, a numerical task for quantitative reasoning, and finally, an object permanence task for working memory. We specifically chose these experiments because they are usually used to test endothermic animals, i.e. tasks without any social relevance and here non-ecologically relevant indication to the fish (Burkart et al., 2017). For the numerical discrimination task, as it has already been shown that cleaner fish use numerical cues to succeed in a task (Triki and Bshary, 2017), we tested for a generalised numerical task, i.e. fish will receive a food reward if they chose the plate with the greatest number of squares. Furthermore, as general intelligence was revealed to be related to group size (Ashton et al., 2018) and has been shown in cleaner fish that client fish density affects cognitive abilities of cleaners (Triki et al., 2018; Wismer et al., 2014), we also compared female cleaner fish from two different sites: a high-density and a lowdensity client area. We chose to test only female cleaners to avoid any sex bias since it was shown that in guppies (*Poecilia reticulata*), relative brain size affects cognitive performance in females but not in males (Kotrschal et al., 2013). We could then compare performances from individuals from both sites and test whether or not we could find any cognitive difference, at the level of specific tasks as well as at the level of a potentially arising *g* factor.

3.3. Materials and methods

3.3.1. Cleaner fish: *Labroides dimidiatus*

The cleaner wrasse, *Labroides dimidiatus*, is a protogynous fish and lives in a small territory called a cleaning station (Randall, 1958, 2005; Randall et al., 1997). It lives in harems and males can have up to five females in their territory comprising several cleaning stations (one per female) (Robertson, 1972). It is widespread in the Indopacific ocean and can also be found in the red sea (Randall, 1958). It lives for up to four years (Randall et al., 1997). In its daily life, it feeds on other reef fish called clients by removing ectoparasites from them. Cleaner fish have around 2000 interactions per day (Grutter, 1996). As cleaner fish prefer to eat mucus (called cheating) over ectoparasites (Grutter and Bshary, 2003), this leads to different strategies. Different behavioural strategies can be developed by either the client, in order to still have a good service from the cleaner wrasse (chase, flee) (Bshary, 2002a), or tactics from the cleaner fish to keep the client around in its cleaning station despite the cheating behaviour (tactile stimulation) (Bshary and Wurth, 2001; Soares et al., 2011).

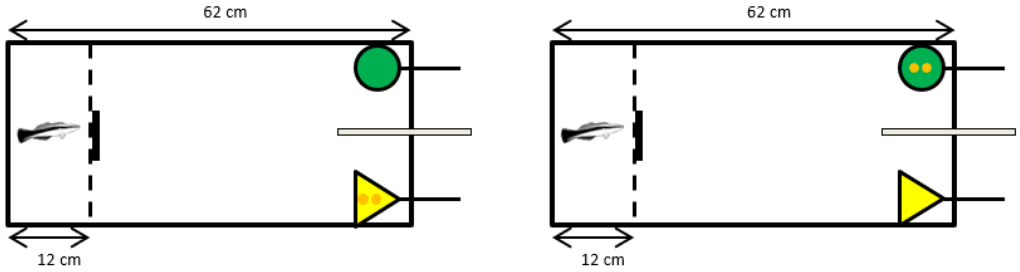
3.3.2. Capture, individuals housing, and acclimation

This study was conducted at Lizard Island Research Station in Australia in February - May 2018 and 2019. To control any sex bias in our study we captured only adult females. Eighty female cleaner fish were caught with a barrier net (2m long, 1.5m high, mesh size 0.5cm; 40 fish each year) and handnets on nearby reefs. Forty individuals were from high-density client fish area (Birds Islet crest; 20 each year; Figure S1), and another forty were from a low-density client fish area (Birds Islet lagoon; 20 each year; Figure S1). Each individual was housed in a glass aquarium (62 x 27 x 38 cm). Each year, the forty individuals were caught at the beginning of the field trip but were split into two experimental sets of 20 individuals each. We ran the experiment with twenty fish at a time. Thereby, we had for the entire experiment running a total of four experimental sets within the two years. Cleaner fish were acclimatized for at least 12 days before being subjected to experiments for the 1st and 3rd experimental set and 44 days minimum for the 2nd and 4th experimental set. Cleaner fish were acclimated to feed on Plexiglas plates, mimicking client fish in the captive environment. We provided mashed prawn as food that we smeared on the Plexiglas plate. When fish were well accustomed to

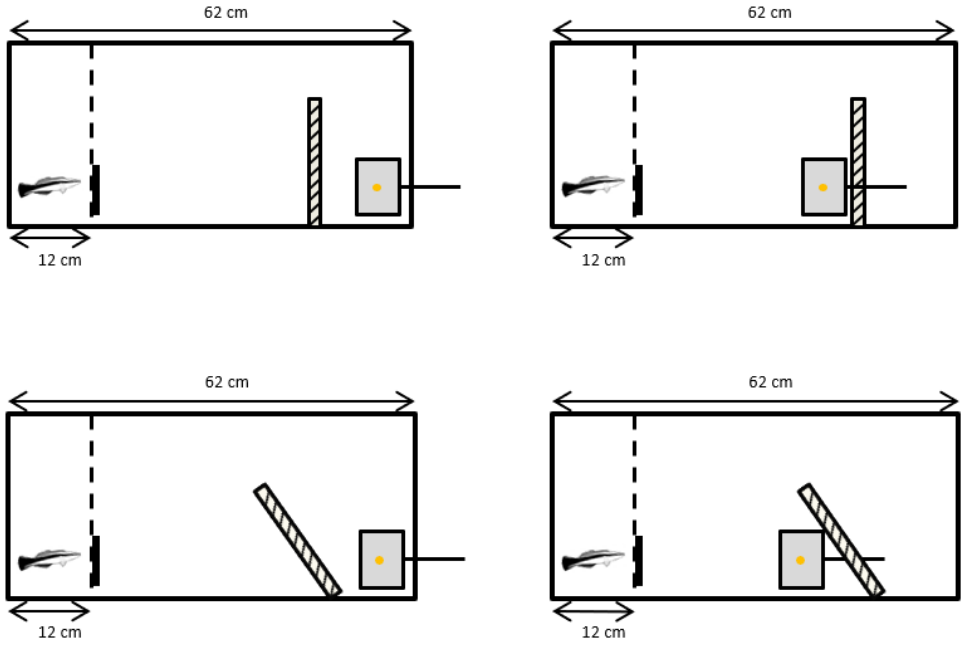
their feeding plate, which took two to three days, they were trained to eat small pieces of mashed prawn placed on dots drawn on another feeding plate. Once fish were eating invariably well on the feeding plate with and without dots, they were habituated to the different plates and barriers that we used during all the different experiments (door dimension 7x18cm that could be in the middle of the barrier or on the side for experiment 2). Before the experiments, the fish were fed ad libitum every day, introducing the feeding plate in the morning and removing it at the end of the day. During experimental days, fish were fed ad libitum at the end of the day after the experiment was completed. One day off was kept between each experiment. Fish conducted the experiment in their home aquarium. All experiments were video recorded using a GoPro that was mounted on the forehead of the experimenter.

3.3.3. Experimental setups

1. Reversal learning



2. Detour task



3. Numerical discrimination

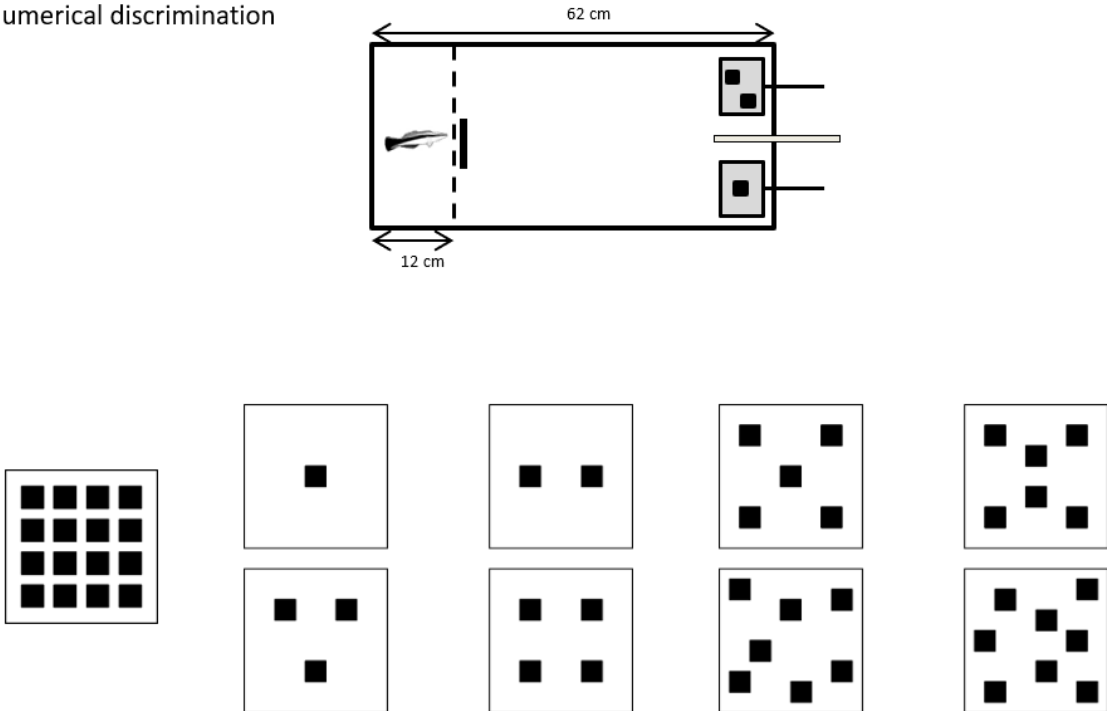


Figure 1: Experimental setup of the 3 first tasks from general intelligence study in fish.

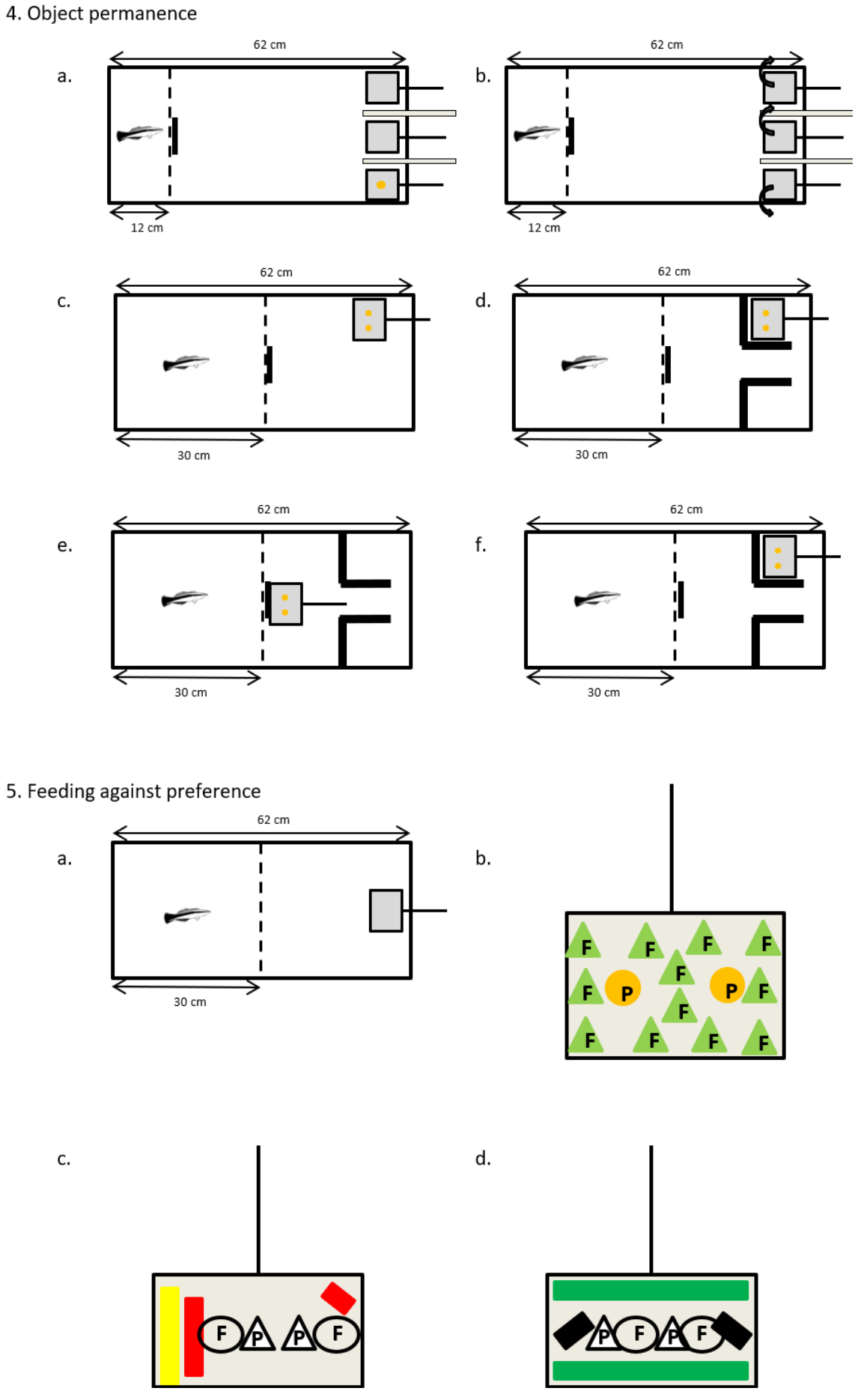


Figure 2: Experimental setup of the 2 last tasks from general intelligence study in fish.

3.3.3.1. Experimental protocol

3.3.3.1.a. Reversal learning: learning – flexibility

For this task (Figure 1.1), we first trained the fish to learn that between two plates, only one was rewarded. When they reached the criterion of success, i.e. a single time 10/10 or 9/10 correct choices, two consecutive sessions of 8/10, or three consecutive sessions of 7/10, we inverted the rewarding plate. With the second phase of the experiment (reversal), we were able to measure fish flexibility. We took the measure of the second phase (reversal) into consideration in the analysis.

3.3.3.1.a.I. Training before experiment 1a: Associative learning – training

Cleaner fish were exposed to the experimental setup 10 days prior to the start of the experiment. Fish were placed behind a transparent grid barrier with a see-through door on one side of the aquarium. The barrier was placed 12cm from the aquarium wall forming the resting area. A yellow triangle shape Plexiglas plate (8cm wide and 7.5cm height) was placed on the left side of the aquarium with two pieces of prawn located on the back. On the other side, a green round shape Plexiglas plate (8cm wide and 7.5cm height) without food was positioned. We positioned the 2 plates on each side of an opaque separation (10cm wide) and let the individual enter the experimental area by opening the door. We coded the choice of the fish when the head passed the opaque separation and the fish was located in one of the compartments. Every morning, we inserted the 2 plates at the same location, removed the door and recorded where they were going first (towards which plate). We did 2 trials on the same day. Fish were fed mashed prawn ad libitum during the day (after the 2nd trial). The interval between the two trials was around 30 – 45 minutes. Within those days, cleaner fish were well acclimated to the apparatus and associated that there was food available on the yellow triangle shape Plexiglas plate placed on the left side since 64 individuals chose the yellow plate in 100% of the time during the last training days (two trials) and only 5 individuals chose it in 50% of the time.

3.3.3.1.a.II. Procedures experiment 1a: Associative learning – experiment

The fish were placed in the resting area prior to starting the trial (Figure 1.1.a). In the experimental area, we positioned the 2 plates on each side of an opaque separation (10cm wide). The trial started when we let the individual enter by opening the door. We counted the choice of the fish when the head passed the opaque separation. Each fish performed 20 trials per day (2x10 trials). The experimenter tested each fish once before moving on to the next fish leading to an intertrial interval of about 30 minutes. Once each fish had been exposed to one trial the next round of trials began. Twenty trials could be completed within a day, starting at 6:00 and finishing around 17:00. In order to succeed in this task, fish had to satisfy a performance criterion of once achieving 10/10 or 9/10 trials correct choices, 8/10 trials on 2 successive sessions, or 7/10 trials on 3 consecutive sessions. When fish reached the criterion, they started the reversal learning the next day.

3.3.3.1.a.III. Procedures experiment 1b: Reversal learning

Fish were fed as in the previous experiment and the same procedure was followed, but we reversed the reward location (Figure 1.1.b). Thus, two pieces of mashed prawn were now placed behind the green round shape Plexiglas plate on the right side of the aquarium. Each fish performed 20 trials per day and was tested the same way as described in experiment 1a. The criterion was the same as in experiment 1a and experiment 1b ended when this criterion was reached. We could run 5 days of the experiment, which is the equivalent of 100 trials. When fish reached 100 trials in the reversal learning without reaching the criterion, we added an extra 5 trials preventing them to go on the yellow plate by inserting in front of it a see-through barrier. We did add those five extra trials to make sure that the individuals were aware that food was also available on the other green round plate. We ensured

this way to measure flexibility in those individuals, were they completely stuck or could they modify/shift their behaviour. On the 6th day, we ran another 20 trials to see whereas after these extra 5 trials they could reach the criterion or not. At the end of the experiment, we were able to measure fish flexibility by the number of trials they needed to succeed in the reversal experiment. The lowest number of trials needed to reach criterion, i.e. same criterion as in Associative learning, got the first rank (best performance) and the highest number of trials until reaching criterion got the highest rank (lowest performance).

3.3.3.1.b. Detour task: inhibitory control

We tested here if cleaners were able to detour an obstacle to get a food reward (Figure 1.2). We started with a straight obstacle 15 centimeters in front of the food plate reward and an aperture 5 centimeters wide either on the left or the right side. As cleaners were able to swim around the obstacle, we increased the difficulty by putting the obstacle at an angle. This task permitted to measure inhibitory control in fish as well as spatial problem-solving. The number of bumps when the obstacle was positioned at an angle was used as a measure of inhibitory control, assuming that fish with better inhibition would bump less into the transparent obstacle.

3.3.3.1.b.I. Day prior to the experiment

We did two rounds with the experimental plate with 2 prawn items, making sure that fish were not afraid of it. We were confident that fish were not scared of the plate when they swam normally around it and came directly to feed on it. Also, we acclimated them to the obstacle by inserting it in the aquarium for 45 minutes twice during the day (once on the right side and once on the left side).

3.3.3.1.b.II. Procedures detour task experiment 2

The fish was placed in the resting area prior to starting the trial. One transparent grid obstacle (19cm wide) was placed in the aquarium 15cm away from the experimental wall side where the reward plate was placed. On the first experimental day, we ran 10 trials in the morning with the obstacle on the right side. The reward anthracite Plexiglas plate (10 x 5cm) was inserted and randomly placed either in front of the obstacle (no inhibition) or behind it (inhibition), under the constraint that the reward plate could not be more than twice in a row either in front or behind the obstacle. With this alternation, we were able to quantify a strong measure of inhibition and avoid simple conditioning. The reward plate displayed 2 pieces of mashed prawn. In the afternoon, we changed the obstacle position and put it on the left. As cleaner fish went around the obstacle quite easily, we counterbalanced the next day the obstacle's side (50% on the right side and 50% on the left side; following the same rule as for the reward plate, i.e. the obstacle could not be more than twice in a row on the same side). They had to flexibly adjust their decision on every trial (Figure 1.2.a-b). On the third day, we increased the difficulty of the task by putting the obstacle in a 45 degrees angle, increasing the inhibition requirement because fish had to swim away from the food in order to access it (Figure 1.2.c-d). The alternation of the reward plate location and the side remained. When the reward plate was correctly located, we removed the door and let the fish swam toward the reward plate. Only in the trials when the reward plate was placed behind the obstacle (Figure 1.2.b and d), we started a stopwatch when the fish passed through the door of the grid barrier and stopped it when the individual reached the reward plate. Additionally, each individual did have 1 minute to reach the reward plate; if it exceeded this time we stopped the trial and went to the next individual. We recorded the success and failure of each individual, how much time they needed to go behind the obstacle in order to reach the reward plate, and the number of bumps they did against the obstacle before reaching the reward plate. Each fish performed 20 trials per day. The experimenter tested each fish once before moving on to the next fish leading to an intertrial interval of about 30 minutes. Once each

fish had been exposed to one trial the next round of trials began. Twenty trials could be completed within a day, starting at 6:00 and finishing around 17:00.

3.3.3.1.c. Numerical discrimination: quantitative reasoning

This task tested quantitative reasoning in fish in its general form (Figure 1.3.a). General in the sense that different number combination pairs were used each day and the presentation was randomized each day as well. In a previous study, cleaner fish were shown to use numerical cues, but they only used one pair in their study, lacking the general pattern (Triki and Bshary, 2017). Therefore, we went further and tested if fish still succeeded in a generalisation form of numbering. We had 20 different combinations of numbers represented with 1.1 centimeters black squares (Figure 1.3.b). In order to get a food reward, the general rule for the fish was: go for the larger one. Since fish had to learn the concept of the experiment on their own, we let them 80 trials before taking the mean of the percentage of correct choices over the last 80 trials for each individual. This measure was used in the analysis.

3.3.3.1.c.I. Day prior to the experiment

We acclimated the fish to the 10 squares plate by smearing mashed prawn on it during acclimation in the tank to make sure they were not afraid of it. We placed 2 prawn items on the back of the plate and did two rounds.

3.3.3.1.c.II. Procedures numerical discrimination experiment

The fish was placed in the resting area prior to starting the trial. Then the 2 plates were inserted and placed 10 cm apart on the same side of the aquarium in the experimental area. The plates were positioned on each side of an opaque separation (10 cm wide). The side of the 2 plates was counterbalanced (50% on the right side and 50% on the left side; following the rule that the reward plate could not be more than twice in a row on the same side). When plates were correctly located, we removed the door and let the fish choose a plate. When the fish swam toward the higher amount of squares displayed on the Plexiglas plate (white square plate 7,4 x7,4cm), 2 pieces of mashed prawn (reward) were accessible behind the correct plate. If it swam to the plate that displayed fewer squares it would only find an inaccessible piece of mashed prawn behind. This inaccessible piece of prawn avoided fish to use their olfactory cue to find the food reward. Hence, they did have to pay attention to the plates in themselves to make their choice. Whatever the choice, the other plate was removed and hence inaccessible. Twenty different combinations were used: 5:1, 6:3, 6:2, 6:4, 4:3, 3:2, 2:1, 4:1, 5:2, 3:1, 5:3, 7:3, 7:4, 7:2, 9:3, 8:3, 8:4, 8:5, 9:4, and 9:5. The numbers were represented by 1.1 centimeters black squares. We randomized 8 times the order of those 20 combinations (1 session of 20 trials per day). The position of the squares changed each time (the fish had not seen twice the same disposition of the same square number). In order to receive a food reward, fish had to choose the highest number of squares. Each fish performed 20 trials per day. The experimenter tested each fish once before moving on to the next fish leading to an intertrial interval of about 30 minutes. Once each fish had been exposed to one trial the next round of trials began. Twenty trials could be completed within a day, starting at 6:00 and finishing at 17:00. We ran 8 days of the experiment, which was the equivalent of 160 trials.

3.3.3.1.d. Object permanence: working memory

With this task, object permanence was tested, i.e. whether a subject was able to retrieve an object after it was moved behind an opaque barrier (Figure 2.4.a-f). This task measured if fish had a mental representation of the object permitting them to find an object back even without seeing it anymore. Three different methods were used. The first method was implemented during the first field season

with the first experimental set of fish. Three plates were presented but only one offered a food reward. The plates were placed each one in a different compartment. After a few seconds, the plates were flipped back. The fish had to retrieve the plate where the food reward was displayed. The setup was intimidating for the fish, and as they were scared they were not paying much attention to the rewarding plate and developed a side preference (right, left, or middle). We then decided to change the procedure and to use only one rewarding plate with the next experimental sets. With the second experimental set during the first field season, we displayed one rewarding plate on either the right or the left side of the aquarium. After a few seconds, we added in front of both sides an opaque barrier, one barrier hiding the reward plate. The fish had to retrieve the rewarding plate behind the right barrier. For the second and last field season, we changed the procedure once more for the last two experimental sets. We still had one rewarding plate, but this time the opaque barriers were place beforehand the presentation of the plate. The plate was displayed between the two opaque barriers already in place, and after a few seconds, was moved behind one of the two barriers. This last procedure was more ecologically relevant compared to the other ones. We used the percentage of correct choices overall the experimental trials as measurement. Since we changed the procedures three times, and the number of fish that could have been taken into account differed from the other tasks, this task was excluded from the analysis.

3.3.3.1.d.I. Object permanence v.1: three plates flipped back (experimental set n°1, 2018)

Day prior to the experiment

We did two rounds with the experimental plate with the 2 prawn items to make sure that fish were not afraid of it (Figure 2.4.a-b). Moreover, we acclimated them to the 2 separations structure twice 15 minutes during the day.

Procedures object permanence v.1 experiment

The fish was placed in the resting area prior to starting the trial. Then 3 grey Plexiglas plates (4.5 x 4.5cm) were inserted and placed 15cm apart from each other in the experimental area. Two opaque separations divided the experimental area in 3 compartments where each of the experimental plates were placed. One of the 3 plates would display two pieces of mashed prawn as a reward. The side of the reward plate was counterbalanced (33% on the right side, 33% on the left side and 33% in the middle; following the rule that the obstacle could not be more than twice in a row at the same place). When fish paid careful attention to the plates and we were sure that it had seen where the reward plate stand, we flipped the plates over making the reward invisible. We removed the door and let the fish choose on which plate it wanted to go first. When the fish swam toward the reward plate it succeeded in the task (correct choice). On the other hand, the 2 other plates led to the failure of the fish in the task. Each fish performed 20 trials per day. The experimenter tested each fish once before moving on to the next fish leading to an intertrial interval of about 30 minutes. Once each fish had been exposed to one trial the next round of trials began. Twenty trials could be completed within a day, starting at 6:00 and finishing around 17:00. We ran 4 days of the experiment, which was the equivalent of 80 trials.

3.3.3.1.d.II. Object permanence v.2: one rewarding plate covering (experimental set n°2, 2018)

Day prior to the experiment

We did two rounds with the experimental plate with the 2 prawn items to make sure that fish were not afraid of it (Figure 2.4.c-d). We acclimated them also to the two white barriers twice 15 minutes during the day.

Procedures object permanence v.2 experiment

The fish was placed in the resting area prior to starting the trial. Then one grey Plexiglas plate (4.5 x 4.5cm) with 2 green bands and 2 black dots was inserted and placed 30cm away from the transparent barrier dividing the aquarium into two areas: resting and experimental. The side of the plate was counterbalanced (50% on the right, and 50% on the left side; following the rule that the plate could not be more than twice in a row on the same side). When fish paid careful attention to the plate, we inserted 2 white barriers on each side, one covering the plate where the food was displayed, making the reward invisible. We removed the door and let the fish swam behind one of the two barriers. When the fish swam toward the right barrier (reward plate), it succeeded in the task (correct choice). Each fish performed 20 trials per day. The experimenter tested each fish once before moving on to the next fish leading to an intertrial interval of about 30 minutes. Once each fish had been exposed to one trial the next round of trials began. Twenty trials could be completed within a day, starting at 6:00 and finishing around 17:00. We ran 4 days of the experiment, which was the equivalent of 80 trials.

3.3.3.1.d.III. Object permanence v.3: one rewarding plate hiding (experimental sets n°3 – 4, 2019)

Day prior to the experiment

We did two rounds with the experimental plate with the 2 prawn items to make sure that fish were not afraid of it (Figure 2.4.e-f). Two white barriers were placed twice 15 minutes during the day in the aquarium to acclimate the fish to it.

Procedures object permanence v.3 experiment

The fish was placed in the resting area prior to starting the trial. Two cover barriers were installed in the experimental area of the aquarium. Then 1 grey Plexiglas plate (4.5 x 4.5cm) with 2 green bands and 2 black dots was inserted between the 2 cover barriers (behind the transparent grid barrier placed 30cm away from the experimental area aquarium wall) to make sure the fish pay attention to the plate. The plate was then hidden behind one of the two cover barriers (the side of the reward plate was counterbalanced: 50% on the right and 50% on the left side; following the rule that the plate could not be more than twice in a row on the same side). We removed the door and let the fish choose between the 2 cover barriers. When the fish swam behind the right cover barrier it succeeded in the task (correct choice). Each fish performed 20 trials per day. The experimenter tested each fish once before moving on to the next fish leading to an intertrial interval of about 30 minutes. Once each fish had been exposed to one trial the next round of trials began. Twenty trials could be completed within a day, starting at 6:00 and finishing around 17:00. We ran 4 days of the experiment, which was the equivalent of 80 trials.

3.3.3.1.e. Feeding against preference: inhibitory control (ecologically relevant task)

This task is linked to the Detour task as it measures inhibitory control in fish, but with ecological relevance rather than in a more abstract way (Figure 2.5.a-b). We wanted to see if there was any correlation between ecological and non-ecological tasks within the same domain, i.e. inhibitory control. Feeding against preference is part of the daily life in cleaner fish as they do prefer to eat mucus over ectoparasites. We presented to fish one plate with the same amount of prawn (preferred food items) and flake (unpreferred food items). The flake items were mimicking ectoparasites in the natural environment, which was the less-preferred food for the fish, and the prawn item was considered as fish mucus (=preferred food). We took the mean number of flake items eaten before eating the prawn as a measure of inhibitory control for the analysis.

3.3.3.1.e.I. Day prior to the experiment

We smeared mixed fish flakes and mashed prawn (20% concentration flakes) on feeding plate instead of mashed prawn only to ensure that fish were eating the new type of food properly.

3.3.3.1.e.II. Procedures feeding against preference experiment

Day 1: training (Figure 2.5.a-b)

We did six training trials using a 15x10 cm plate with 14 items displayed on it (12 flake and 2 prawn items). One trial was composed of two plate expositions: first, we introduced the plate in the aquarium, when the fish ate a prawn item we quickly removed the plate and noted how many flake items were eaten before the prawn item. We waited 30 seconds before reintroducing the plate and removed it when the second prawn item was eaten. We noted again how many flake items were eaten before the ingestion of the second prawn item. Food items were placed on dots drawn on the plate. The intertrial interval was of one hour.

Day 2: experiment (Figure 2.5.a; c-d)

We used two experimental plates (10 x 6 cm) with 2 flake and 2 prawn items that were presenting different patterns. We alternated between trials with 1 plate and trials with 2 plates. In the trials where 2 plates were exposed, we removed the plate when fish were eating one prawn item and removed the second plate 0.5s after. In the one plate trial situation, only one plate was removed. We did 12 repetitions per situation. We randomized the two plate situations as well as the 2 different patterns of the plate in the 1 plate situation. For the analysis, we took into consideration the mean of the number of flake items eaten before the prawn item only in trials from the 1 plate situation.

3.3.4. Measurements and data analysis

3.3.4.1. Measurements

In each experiment, we ranked each fish by its performance (Table 1). If two or more individuals obtained the same results, we made the total of the ranks and divided by the number of individuals competing for the same rank. For example, if 3 individuals were competing for the same rank, we totalled the rank number and divided it by the number of individuals ((15 + 16 + 17)/3 = 16). In this example, all individuals would have rank number 16.

For Reversal learning (hereafter RL), we took into consideration the number of trials needed until reaching the criterion, i.e. 10 or 9 correct choices over 10 trials once, 8/10 correct choices on 2 successive sessions of 10 trials, or 7/10 correct choices on 3 consecutive sessions. In the Detour task (hereafter DT), we measured the mean of the number of bumps the fish did until they went around the obstacle positioned at an angle and reached the food reward plate. The mean of the percentage of correct choices during the 80 last trials was used for the Numerical discrimination (hereafter ND) task, as the fish had to learn something from the experiment before we took into consideration their performance. We also used the mean of the percentage of correct choices for the Object permanence task (80 trials). As we changed the protocol three times for this task and only 52 fish were available for the PCA analysis, we decided to exclude this task though from the analysis. All four cognitive tasks were considered as non-ecologically relevant to the fish. We added one ecologically relevant task to this test battery with Feeding against preference (hereafter FAP). In this task, we took the mean of the percentage of flake items eaten in the 1 plate situation (12trials). The fish ranking is summarized in Table 1.

Table 1: Explanations of how fish were ranked in the different tasks depending on their performance.

Task	How rank
Reversal learning (RL)	N° of trials to reach criterion
	Small n°, low rank (best performance)
Detour task (DT)	Mean n° of bumps against an obstacle positioned at an angle
	Small n°, low rank (best performance)
Numerical discrimination (ND)	Mean % correct choices during the 80 last trials
	High %, low rank (best performance)
Object permanence v.2 and v.3 (OP)	Mean % correct choices over 80 trials
	High %, low rank (best performance)
Feeding against preference (FAP)	Mean n° of flake items eaten before a prawn item in 1 plate exposition
	High n°, low rank (best performance)

3.3.4.2. Individuals retained for the analysis

From the eighty fish caught for this study, we were able to run the analysis with 69 individuals. Two died after the first night in captivity, and one after 3 days of captivity, the reason is still unclear. For the eight other individuals, they either refused to do the task as they were too scared of the experimental setup: four were scared of the reversal reward plate in RL, one was scared of the DT, and one was scared of the ND. Or they sadly passed away: one jumped out of the aquarium at night before the ND task, and one had to be euthanized after the RL task as it was not able to eat anymore. From the 69 individuals' performance used in the analysis, 36 individuals were from high-density client fish area (Birds Islet crest), and 33 were from low-density client fish area (Birds Islet lagoon).

3.3.4.3. Statistical analysis

First, we examined variation in the performance of the cleaners in each task using descriptive statistics (means with standard deviation, and maximum – minimum values; Table 2). For the ND and OP tasks, we verified if cleaners as a group performed above chance using a non-parametrical test-related samples Wilcoxon signed-rank test in IBM ® SPPS ® Statistics version 25.0.0.1. Second, we looked at the correlation between the different tasks using the Spearman-Rho correlation test. A 0.0125 Bonferroni correction was carried out (α/number of tasks = 0.05/4). Third, the performance ranking of each individual in each task was used to perform a principal component analysis (PCA) with the three retained tasks that were non-ecologically relevant, i.e. RL, DT, and ND. The PCA allowed assessing if the performance in each task was loading positively on the first PC factor. Fourth, we investigated whether non-cognitive variables such as the site of capture, the year, the experimental set, as well as body length could have had an effect on the variation found in the PCA using a linear model (package 'lme4') (Bates et al., 2015). The Spearman-Rho correlation test, the PCA, as well as the linear models were carried out in the statistical program R Rstudio © (R Version 1.2.5019, © 2009-2019 RStudio,
Inc.).

3.3.4.4. Ethical note

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

3.4. Results

3.4.1. Variation in the performance

Before proceeding to the PCA analysis, it was important to make sure that fish individuals as a group had understood the tasks. For RL and DT, those two tasks had their own criterion of success where fish showed that they understood the principle of each task by reaching criterion in RL and swimming around the obstacle positioned at an angle in DT. In the case of ND, we had to verify that cleaners as a group performed above chance within the 80 last trials, which they did (Wilcoxon-test, $p = 0.001$). Even if fish seemed to perform randomly in the OP task, they performed above chance as a group (Wilcoxontest, $p = 0.034$, $n=52$ as the first experimental set was not included). We had to remember that the two versions of OP differed in the experimental design. When we looked if individuals as a group still performed at a significant level above chance, taking version 2 and 3 separately, then none of the versions remained significantly above chance (Wilcoxon-test, $p = 0.119$, $n=18$ in OP v.2, and $p = 0.153$, $n=34$ in OP v.3). Moreover, because we did not have the same amount of fish performing in this task, we excluded the OP task from the analysis. No ceiling effect was identified in the other tasks, which was another crucial point for the analysis (Table 2).

Table 2: Description of the tasks and their measurements. The mean, maximum and minimum values are from the 69 individuals taking into consideration in the PCA. Exception made for the OP task in which the data of 52 individuals came from experimental sets 2 – 4. * indicates the significance above chance in the DR and OP tasks.

Cognitive process	Task	Performance measurement	Measurement description	Mean (SD)	Max – Min	N
Learning – flexibility	Reversal learning	Quantity	Number of trials needed to reach criterion	51.02 (26.72)	125 - 20	69
Inhibitory control – spatial problem solving	Detour task	Quantity	Number of bumps done when obstacle positioned at an angle	8.49 (5.91)	35.1 - 0.2	69
Quantitative reasoning	Numerical discrimination	Percentage	Percentage of rewards retrieved in the 80 last trials	51.54 * (3.67)	62.5 - 40	69
Working memory	Object permanence v.2 and v.3	Percentage	Percentage of rewards retrieved in 80 trials	51 * (3.92)	60 - 41.25	52
Inhibitory control (ecologically relevant)	Feeding against preference	Quantity	Number of flake items eaten prior prawn in trials with 1 plate exposition	0.65 (0.22)	1.25 - 0.25	69

3.4.2. Correlations between the different tasks (n=69)

We did a pairwise comparison between the different ranks of each task. The only significance that we found was between DT which negatively correlated with FAP (Spearman-Rho r = -0.283, p = 0.018 which remained significant after the Bonferroni correction p = 0.03). No other significant correlation was found between the other tasks (Table 3; Supplemental material: Figure S2).

Table 3: Correlation matrix of all four tasks (n=69). * means the significant correlation.

Correlation	Reversal learning	Detour task	Numerical discrimination	Feeding against preference
Reversal learning	1	0.026	-0.137	-0.158
Detour task		1	-0.051	-0.283 *
Numerical discrimination			1	0.120
Feeding against preference				1

3.4.3. Principal component analysis: 3 cognitive tasks (n=69)

We ran a Principal Component Analysis (PCA) to investigate if the three tasks would all load positively on the first dimension. Dimension 1 is explained by 38.53% of the variance in performance over all fish individuals (Table 4; Figure 3). Divergence occurred between the three tasks: two loaded positively (RL and DT), whereas the other one loaded negatively (ND). These results revealed no evidence of a psychometric *g* in the fish species *Labroides dimidiatus* as not all three tasks.

Table 4: Principal component matrix with component scores and item loadings of the three cognitive tasks in the three dimensions.

Item	**Item loadings**		
	g (PC1)	*g* (PC2)	*g* (PC3)
% of variance	38.53	32.78	28.69
eigenvalues	1.16	0.98	0.86
Reversal Learning	0.701	-0.331	0.631
Detour Task	0.357	0.925	0.13
Numerical Discrimination	-0.733	0.133	0.667

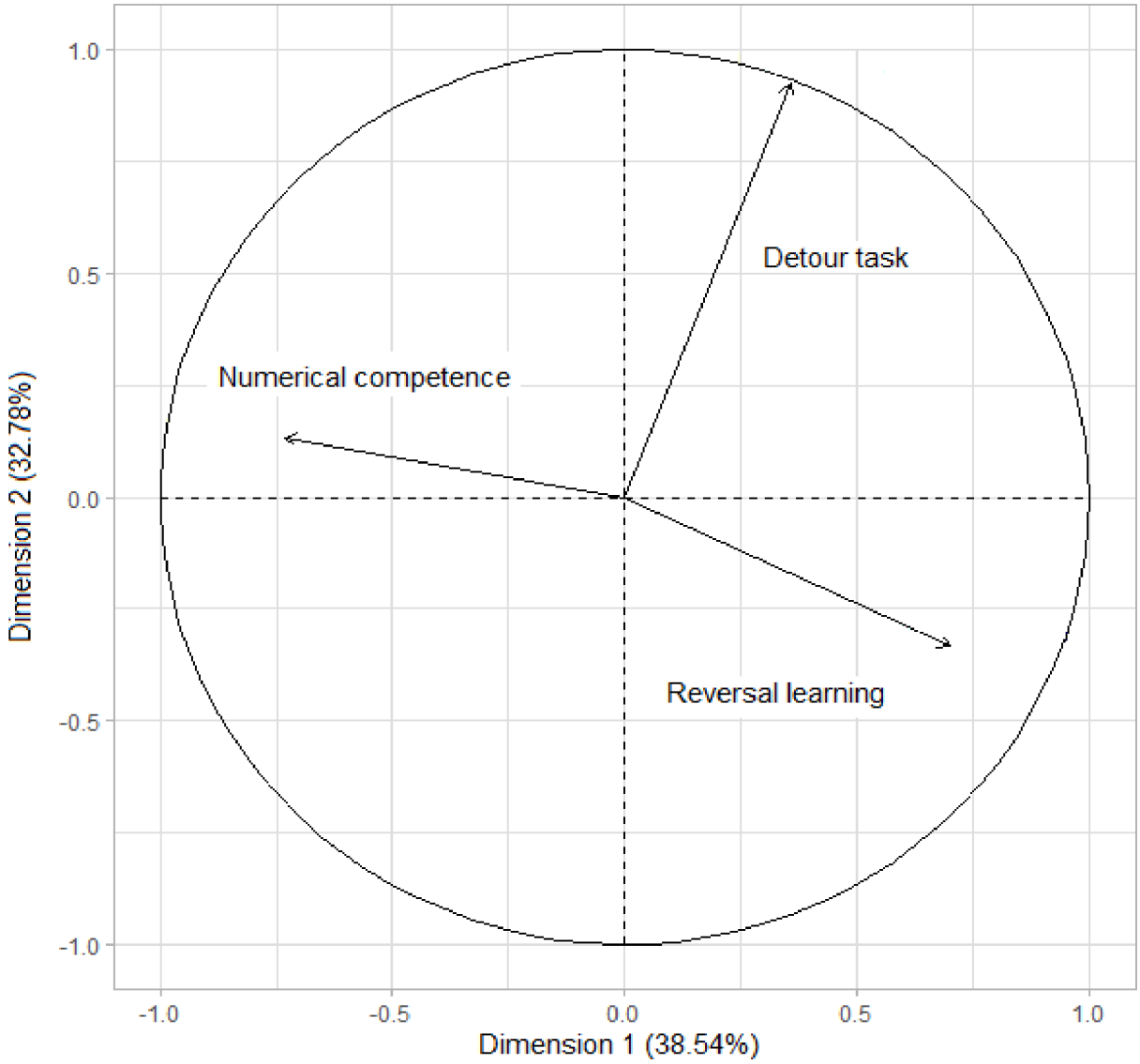


Figure 3: Principal Component Analysis (PCA) of the three cognitive tasks. Dimension 1 explained 38.53% of the variance in performance over the entire fish individual (n=69; eigenvalue 1.16), and dimension 2 explained 32.78% of the variance (n=69; eigenvalue 0.98) are represented. The three cognitive tasks are represented.

3.4.4. Non-cognitive variables effect on PC1 and PC2

We investigated if the environment – capture site (high density = crest / low density = lagoon), the year (2018 – 2019), the experimental set (1 – 2), and the body length had an effect on the performance of the individuals overall the tasks. One linear model was run and we found that the variable year had a significant effect on the PC1 (Table 5), whereas the variable experimental set was significant in the PC2. The fact that PC1 is explaining 38.53% of the variance and the PC2 explained 32.78%, we cannot conclude that those variables are mainly affected the PCA in general. However, this showed that the environment has no effect on the performance of the fish individual in different cognitive tasks, neither the body length.

Table 5: Linear model of PC1 and PC2, n = 69.

PC1	Estimate	Std. Error	p-value		PC2	Estimate	Std. Error	p-value
(Intercept)	0.579	0.452	0.205		(Intercept)	0.822	0.418	0.054
Capture site	0.006	0.259	0.983		Capture site	-0.299	0.24	0.763
Year	-0.663	0.26	0.013 *		Year	0.073	0.241	0.763
Experime-tal set	-0.172	0.256	0.506		Experimen-tal set	-0.483	0.239	0.047*
Body length	0.08	0.159	0.618		Body length	-0.176	0.148	0.239
F-statistic: 1.987 on 4 and 64 DF, p-value: 0.107					F-statistics: 1.859 on 4 and 64 DF, p-value: 0.128			

3.4.5. Non-cognitive variables effect on each tasks' performance

Additionally, we examined if the non-cognitive variables as previously mentioned had an effect on the performance of the individuals and thus in each task. We ran four linear models, one for each task separately using their rank, and tested if the four non-cognitive variables had any effect on each task. Two of the non-cognitive variables, the capture sites, and the body length, did not have a significant effect on the individuals' performance in all four tasks ($p > 0.1$; Figure S3). The year was found to have a significant effect on the RL task only indicating that the individuals from the second year showed higher performance (2019, $p = 0.006$; Figure 4A). The year did not have any effect on the three remaining tasks in the individuals' performance (DT, ND, and FAP, $p > 0.3$; Figure S4 A – C). The experimental set, whether being tested after 12 days acclimatisation or after 44 days acclimatisation, did have an effect on the performance of the fish in the DT and the FAP tasks. In the DT task, fish performed better when they were more acclimated to the captivity condition ($p = 0.02$; Figure 4B), whereas in the FAP the effect was reversed compared to the DT, i.e. fish performed better when they were tested after less time spent in captivity ($p = 0.02$; Figure 4C). The two remaining tasks, RL and ND, were not significantly affected by the experimental set ($p = 0.38$; Figure S4 D – E).

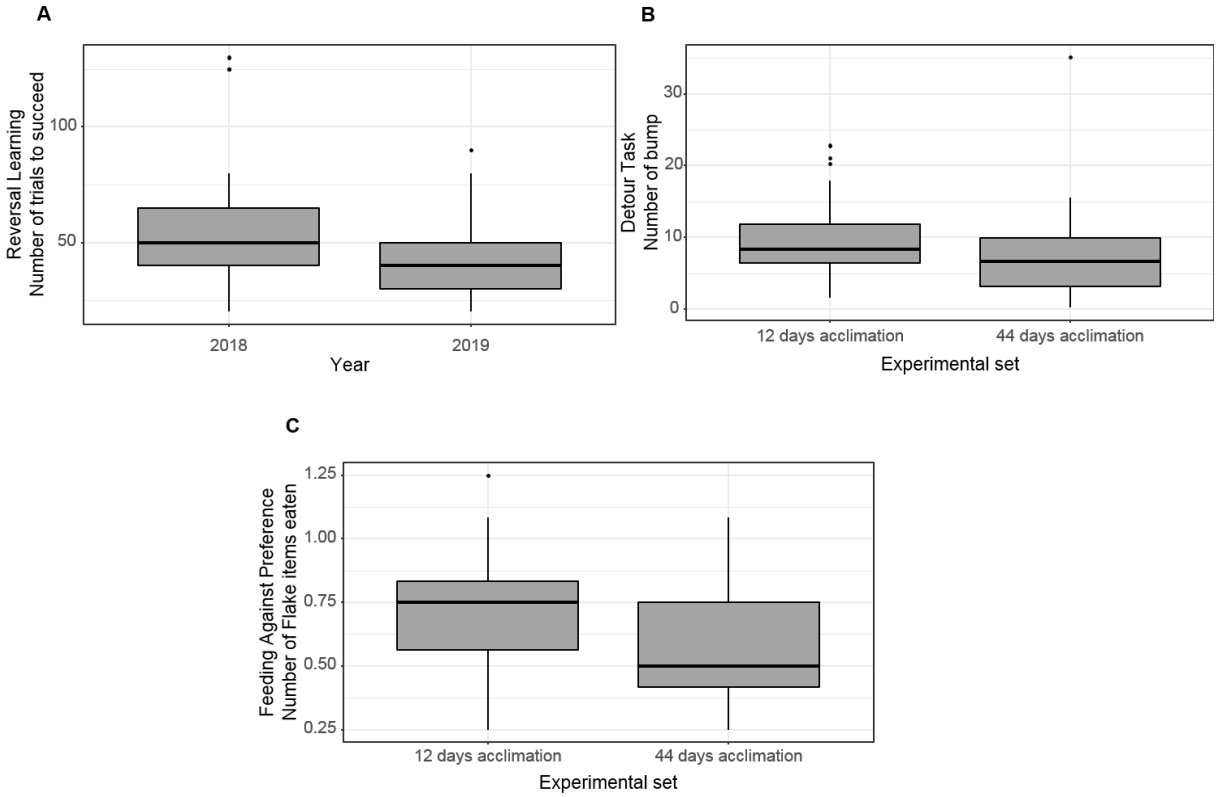


Figure 4: Performance in Reversal Learning comparing the two different years (2018 on the left, and 2019 on the right). A significant difference was found between years and the performance in RL (A) (linear model, p = 0.006). Performance in Detour Task and Feeding Against Preference comparing the two different experimental sets (12 days acclimation on the left, and 44 days acclimation on the right). A significant difference was found between the experimental sets and the performance and thus in both tasks DT (B) (linear model, p = 0.02) and FAP (C) (linear model, p = 0.02).

3.5. Discussion

This study aimed to test whether a fish species would show evidence of psychometric general intelligence. In the presence of general intelligence, we would expect that individual performances in each task were positively correlated with one another, known as the positive manifold (Carroll, 1993; Jensen, 1999; Jensen and Weng, 1994; Spearman, 1904, 1928). Furthermore, a first principal component should have been found to explain a significant amount of the variance with all tasks loading positively. These two conditions combined would be consistent with a psychometric *g* (Burkart et al., 2017), which so far has been found in humans and several mammals. In birds, results are more ambiguous as a positive manifold is inconsistently found, raising the question of whether general intelligence is present in birds (Searcy and Nowicki, 2019). However, many of the bird studies were carried out with few individuals and sometimes in the field. Conducting the experiments in the field might induce bias in the data as it may encourage only audacious and/or more motivated individuals to participate in the experiment. Here in our study, no *g* factor was found in *L. dimidiatus*. This is a first result suggesting the absence of general intelligence in an ectothermic organism, and, therefore, rejecting the idea that a positive manifold is an automatic consequence of having a brain (Bartholomew et al., 2009, 2013; Kovacs and Conway, 2016; Rabaglia et al., 2011; Thomson, 1916; Van Der Maas et al., 2006). Nonetheless, compared to other *g* studies where several tasks were tested and sometimes from the same cognitive processes, only three tasks were available for the analysis coming from three cognitive domains in our study, the working memory process being removed due to a lack of understanding of the task. As the three tests were from different cognitive domains, and a total of sixty-nine individuals were included in the analysis, we are confident in the validity of our results.

While looking at the correlation between the different tasks, the two inhibitory control tasks (DT and FAP) were unexpectedly found to be significantly negatively correlated. We would have expected that

because they both rely on the same cognitive process, i.e. inhibition, they would correlate positively. The fact that one of the tasks was linked to ecology, while the other was not, affected the performance of the fish. However, because of the captive environment, it might have been smarter for the fish, in this particular situation, to feed on prawn (preferred food) instead of flake (less-preferred food). Since we provided the fish with food on a daily basis, no matter their behaviour, they might have to get used to it and as a result, ate their preferred food items. Therefore, we would have obtained a positive correlation between the two inhibitory tasks. Contrariwise, this negative correlation between the two tasks from the same cognitive process but differing in their origin, i.e. non-ecological and ecological, suggests that the tasks are solved with different cognitive mechanisms.

Overall, these negative results potentially raise the possibility that g might generally be absent in fishes and other ectotherms as they share similar brain anatomies. More studies are needed in this line of thought. The next studies should be focused on endo- and ectotherms to see whether a general pattern emerges that *g* is restricted to endotherms. Not finding general intelligence in a fish compared to endotherms where *g* is present, raises the question of whether *g* is specific to endothermic organisms which have, in general, a 10 times larger relative brain size compared to similar body-sized ectotherms, known as the cephalisation index by Jerison (1969, 1973) or more recently, encephalisation. The difference in relative brain size between the two organism types might be explained by the general cognitive domain of intelligence present in endotherms and requiring an increase in brain size permitting the relation between the different cognitive processes. While, in fish and ectotherms, no general cognitive domain appears present but rather a domain-specific organisation with each cognitive process working without any relation to the others (Burkart et al., 2017). This is not the only difference that appears between these two organism types. Tsuboi et al. (2018) showed that the ontogenetic brain-body allometry, i.e. variation among developmental stages, is bi-phasic in endotherms whereas it is constant in ectotherms. This difference can be explained by parental provisioning where a positive correlation is found between the extra energy input from paternal care towards the young and the mother, and the evolutionary brain size expansion (Heldstab et al., 2019). The expensive brain hypothesis stipulates that brain size increment induces costs, which need to be supported via 1) the increment of energy input via diet quality and avoidance of starvation as it has been shown that hibernators have smaller brains (Heldstab et al., 2018; Veitschegger, 2017); 2) the allocation of the energy differently by reducing the energy provided for other functions, e.g. locomotion, rate of development, and fertility (Isler and van Schaik, 2009).

This difference in brain size and brain organisation between endo- and ectotherms does not mean that fish are not able to achieve high performance in specific cognitive tasks. High strategic sophistication driven by ecology such as clients' recognition (Tebbich et al., 2002), generalised rule learning (Wismer et al., 2016), image-scoring and reputation management (Binning et al., 2017; Bshary, 2002b, 2002b; Bshary and Grutter, 2006; Pinto et al., 2011), as well as most recently the mirror test (Kohda et al., 2019) is shown in cleaners. In general, fish behave intelligently (Brown, 2015; Brown and Laland, 2003; Brown et al., 2011; Bshary, 2011; Bshary and Brown, 2014; Bshary et al., 2014). Ectotherms are thus capable of high cognitive performance but this performance is not linked across domains. Despite their lack of *g*, at least from this single study, cleaners performed highly in two tasks here: reversal learning and detour task. It is even more interesting to observe that both tasks RL and DT were going in the same direction in the first dimension of the PCA. In a previous study, behavioural flexibility as measured with RL, and inhibitory control as measured in the DT, were said to reflect similar cognitive mechanisms (Bond et al., 2007). That could be an explanation as to why they are going in the same direction in the PCA even without showing any correlation between the two tasks.

The cleaners did not perform well in the object permanence task, mainly performing at a chance level, even though it was significantly above chance when taking two different versions together. This task was a measure of the cognitive process of working memory. During this specific task, cleaners were not paying much attention to what was going on which is a problem in this kind of task. We consider

this lack of attention problematic, as it has been argued that attention is primordial in the process of working memory (Cowan et al., 2005). We will have to investigate more on this topic with cleaners to come to the conclusion of whether these fish have object permanence or not. As this cognitive process has been shown in groupers (Vail et al., 2013), i.e. staying up to twenty minutes in front of a caveat where a prey hides, working memory is present in fish taxa. In everyday life, cleaners do not need to remember where a client fish is as they are surrounded by them. Conversely, cleaners remember when they had an interaction and with whom (Salwiczek and Bshary, 2011). This might be a reason why we would not find working memory in cleaners as it is not triggered by their environment. Further studies are still required to disentangle whether cleaner fish have object permanence or not.

We were interested in testing if the performance in each task was influenced by non-ecological variables such as the year when the fish were captured, and the experimental set, i.e. the difference in the number of days spent in captivity before testing (12 vs 44 days). The year only had an effect on the RL task, increasing the performance during the second year. We could argue that it might be possible that we caught the same individuals from a previous year, but firstly, we should have found this effect on all tasks, and secondly, it has been shown that cleaners do remember traumatic events, such as capture, and thus until eleven months, consequently it did not seem possible that we caught the same fish from 2018 in 2019 (Triki and Bshary, 2019). We, therefore, do not have an explanation for this result but it was mainly important to see that year did not have a major impact on our study. On the other hand, the experimental set did seem to affect the DT and the FAP tasks. Cleaners that were more acclimated to the captivity environment performed better than those less acclimated. This showed how habituation in a new environment occurs quickly (within a month and a half), and how it helps in a task where spatial orientation is tested. In the FAP task, individuals that were less acclimated to the laboratory condition were more willing to eat against their preference compared to those who spent more time in captivity. This outcome raises the question of whether cleaners stop eating against their preference after spending time in captivity or if they lose motivation as the FAP task was the last one tested, i.e. after 72 days in captivity for this experimental set. Hence, it is interesting to see how the time spent in captivity inversely affects these two tasks and thus how it is important in cognition.

We were also interested in verifying whether the environment had an effect on cognitive performances, as it has previously been shown in Australian magpies how group size, and thus environment, affects performance in cognitive tasks (Ashton et al., 2018). Ashton et al. (2018) highlighted the fact that sociality does have an impact on cognition. In the case of cleaner fish, intra- and interspecific complexity correlates with each other, at least in terms of fish densities (Triki et al., 2019). If cleaners followed the pattern found by Ashton et al. in 2018, we should have found that cleaners from the high fish density site were better performing compared to the fish from the lower fish density. We did not find any relation between performances and capture sites in any of the tasks. Therefore, we do not come to the same conclusion as Ashton et al. with Australian magpies. The fact that cognitive performance in the different tasks remains the same, independently of the environment where the fish come from, excludes the conclusion that cognitive abilities are generally dependent on the interspecific social complexity at least not in the light of general intelligence and within the cleaner wrasse *Labroides dimidiatus*.

Additionally, we checked if the environment influenced the performance of the fish in the different cognitive tasks separately. We did not find any difference in performance when the fish were coming either from a high fish density site or from an adjacent low fish density site. It has already been shown in previous studies that the environment can influence sophisticated strategies of this specific cleaner fish (Triki et al., 2018; Wismer et al., 2014). When testing cleaner fish in non-ecological tasks, the fact to come from a high or low-density fish area does not affect fish performance.

Since the review from Burkart et al. in 2017, multiple studies focused on testing the presence or absence of *g* in animals but differences between test batteries, as well as differences in the analyses,

prevented from reaching a general consensus (*g* studies since 2017 in Table S1). Burkart et al. (2017) suggested that efforts should be done in order to find a proper methodology to test for the presence of *g*. In our study, we presented a methodology that can be applied to multiple species, and for the first time, the presence of *g* was tested in fish. The big picture is that endotherms, with few exceptions in bird studies, show evidence for general intelligence: the psychometric *g*. With our study on fish we expected to fill the gap of the cephalisation index between lower, ectotherms, and higher, endotherms, vertebrates in the phylogenetic tree of vertebrates and determine where general intelligence appeared and how. Are specific brain structures needed to display general intelligence? After several studies (Jerison, 1961, 1963, 1969, 1982; Jerison and Barlow, 1985), the main conclusion emphasised that mammals or birds of the same weight as reptiles or fish have a 10 times bigger brain. We hypothesized that this 10-fold difference in body weight between ectotherms (lower vertebrates) and endotherms (higher vertebrates) might be explained by the presence or the absence of general intelligence: the presence of psychometric *g* in the endotherms, and absence of psychometric *g* in ectotherms. Therefore, small-brained animal species (ectotherms) lack general intelligence in comparison to all mammals and birds tested so far: endotherms with larger brains.

3.6. Conclusion

The lack of evidence of general intelligence in fish is revealed by the absence of a positive manifold, i.e. tasks were correlated positively with each other, and by the absence of a psychometric *g*, i.e. not all tasks loaded positively on the first factor explaining most of the variance (Carroll, 1993; Jensen, 1999; Jensen and Weng, 1994; Spearman, 1904, 1928). The most astonishing result was the negative correlation between the two tasks normally assigned to express the same cognitive process: inhibitory control. It may suggest that behavioural inhibition is achieved differently depending on if the information is ecologically relevant or not. Furthermore, the performance in the four different tasks was not influenced by the environment in contrast to what was found in Australian magpies by Ashton et al. in 2018. This might be explained by the fact that the two capture sites were not isolated from each other, i.e. cleaners from both sites could swim around and end up in the other site, so a cleaner individual caught in the low-density fish area could have come from a high-density fish environment, and vice versa. The fact that we did not find evidence of *g* in cleaner wrasse might be explained by the 10-fold difference in cephalisation index that Jerison emphasised between endotherms (mammals, birds) and ectotherms (fish, amphibians, and reptiles) (Jerison, 1973). The absence of *g* in fish provides an additional step in understanding the evolution of general intelligence: when did it appear and what is the capital gain that endotherms obtain from such general ability. More studies focusing on ectotherms as well as endotherms should be carried to increase the data set on such big inquiry, permitting in the future to address the questions of what selective pressure led to the emergence of general intelligence.

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3.9. Supplemental material

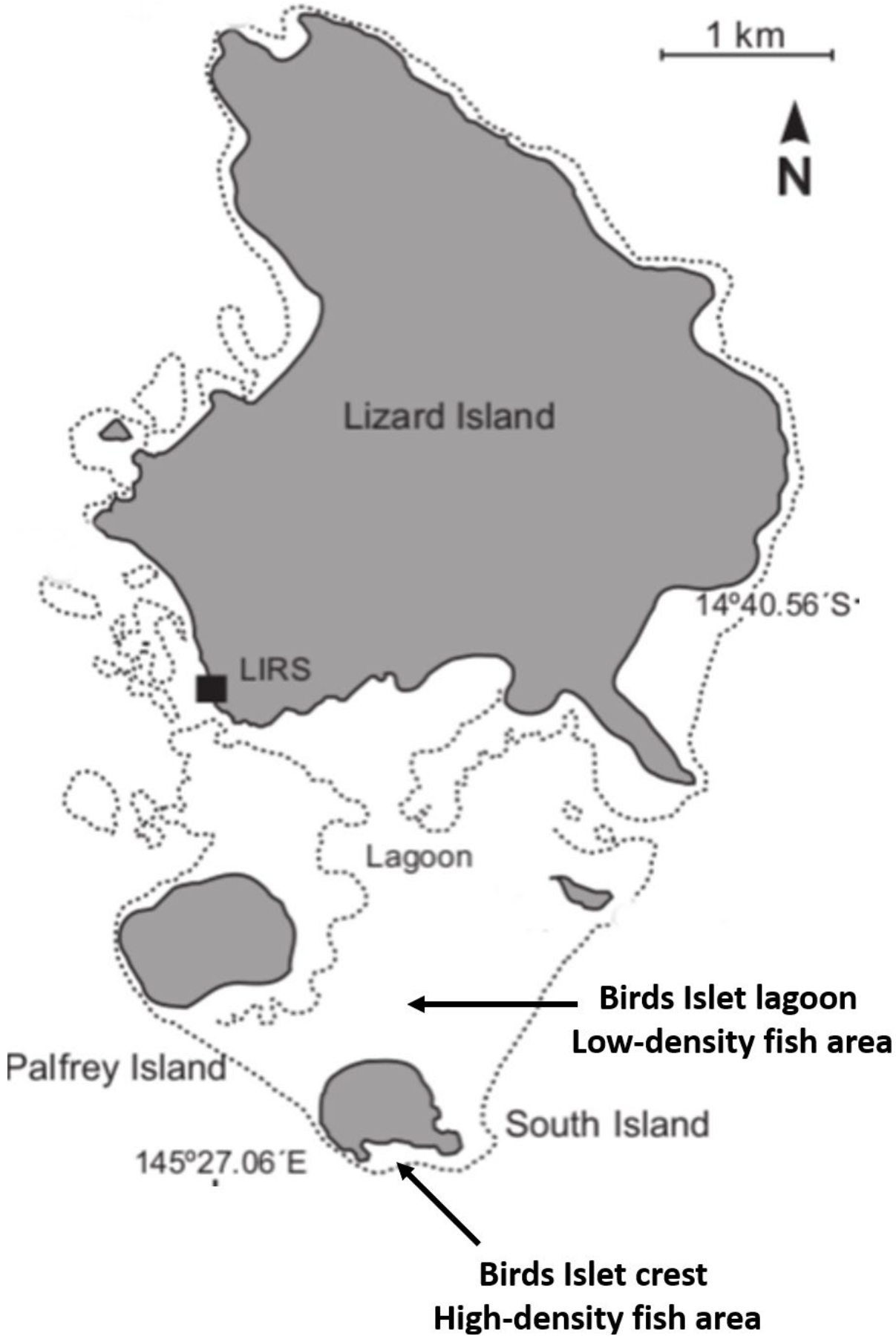

Figure S1: Study sites area.

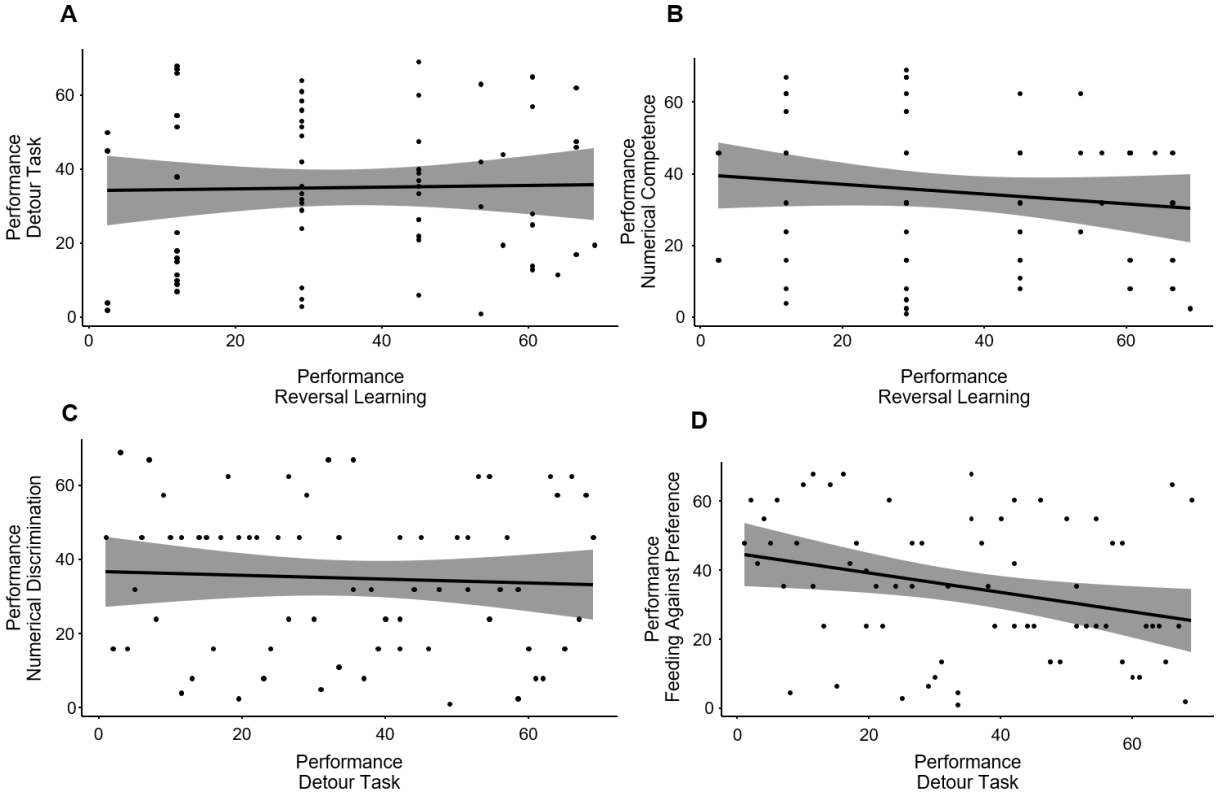

Figure S2: Correlations between the different tasks. The only negative correlation was found between DT and FAP (SpearmanRho r = -0.283, p = 0.02 (p = 0.03 with Bonferroni correction) (D)), the two tasks from the same cognitive processes: inhibition. No other correlations were found significant between the remaining tasks: (A) RL – DT, Spearman-Rho r = 0.026, p = 0.8; (B) RL – ND, Spearman-Rho r = -0.137, p = 0.3; (C) DT – ND, Spearman-Rho r = -0.051, p = 0.7). The correlations between RL, DT, and ND were not tested with FAP as those tasks were not from the same cognitive process, lacking any relevance.

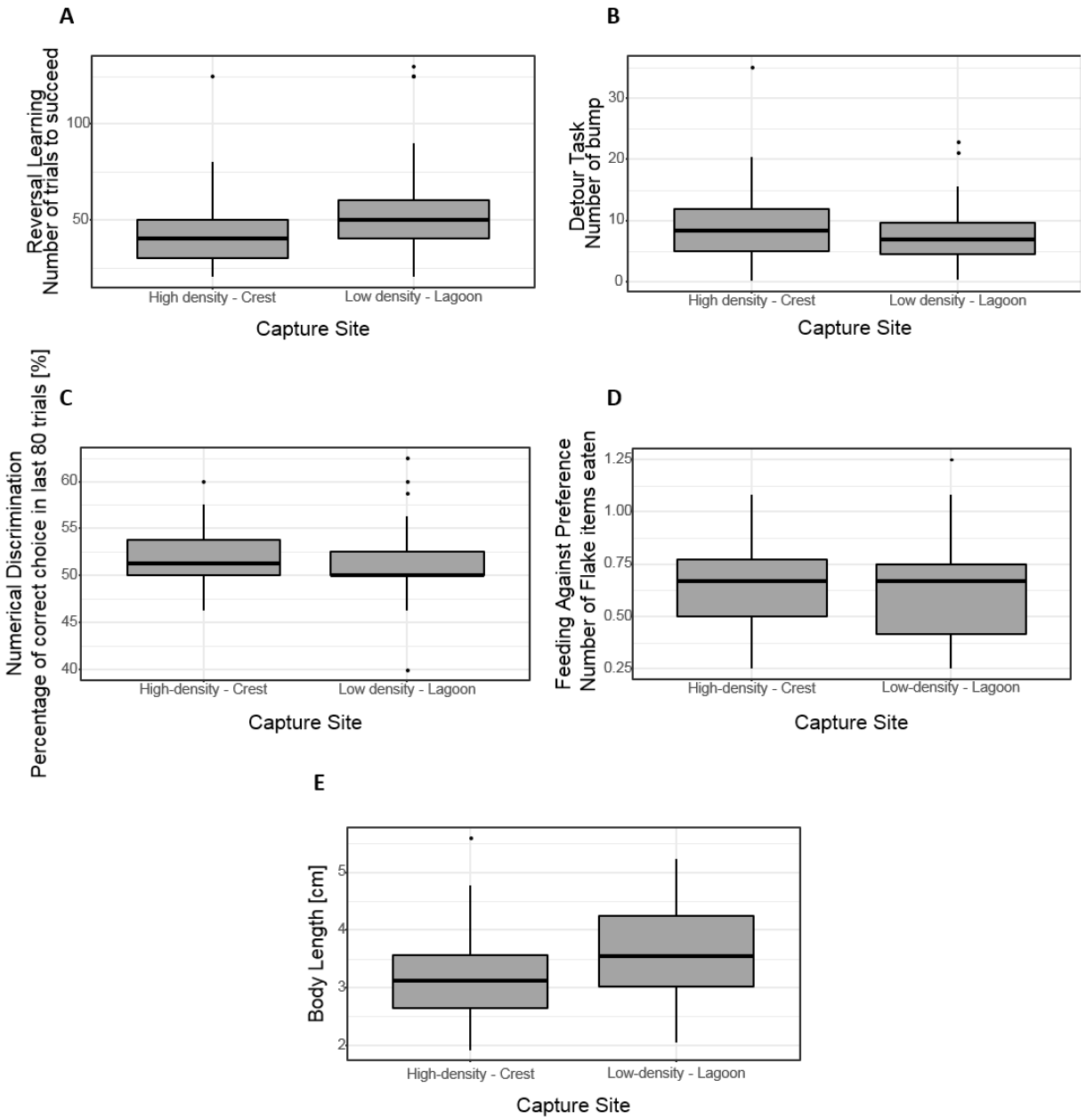

Figure S3: A – D; Performance in each task comparing the capture site (High-density - Crest on the left, and Low-density - Lagoon on the right). No significant difference was found between capture sites and the performance in the four different tasks RL (A), DT (B), ND (C), and FAP (D) (linear model, $p > 0.1$). No difference was found neither between capture sites and body length (E) (Wilcoxon-test, $p = 0.12$).

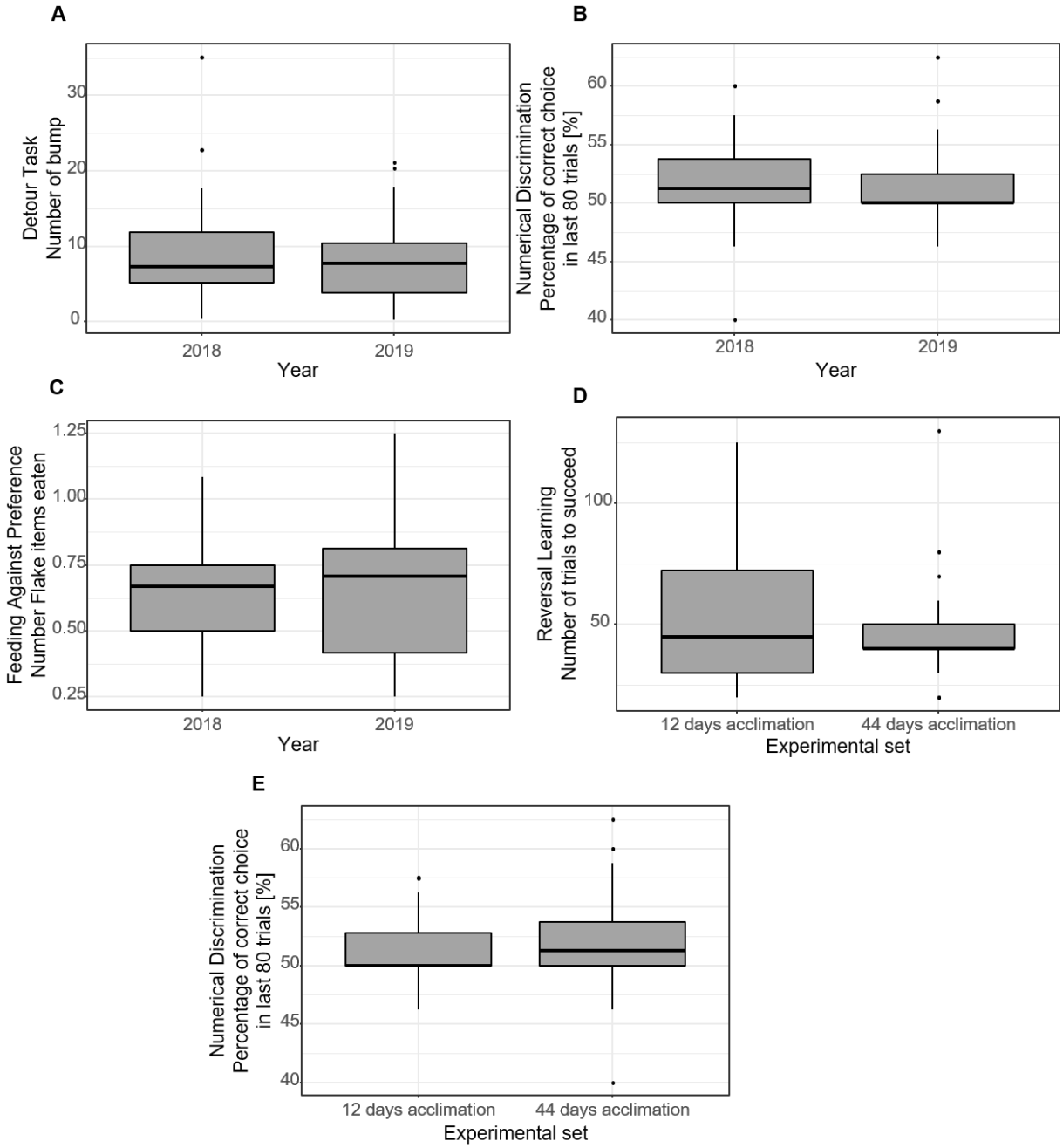

Figure S4: A – C; Performance in Detour Task, Numerical Discrimination, and Feeding Against Preference comparing the two different years (2018 on the left, and 2019 on the right). No significant difference was found between years and the performance in DT, ND, and FAP (A – C) (linear model, $p > 0.3$). D – E; Performance in Reversal Learning and Numerical Discrimination comparing the two different experimental sets (12 days acclimation on the left, and 44 days acclimation on the right). No significant difference was found between the experimental sets and the performance in RL and ND (D – E) (linear model, $p = 0.38$).

Table S1: General intelligence studies since 2017.

Reference	*Species*	N	Experiment in the Field/Lab	Wildcaught/ lab-raised	N° of tasks	Tasks	Result	*g*
Anderson et al. 2017	*Melospiza melodia*	19 males 22 females	lab	wildcaught at 3-6 days, handreared	5	Foraging skill Colour association Colour reversal Spatial learning Detour reaching + song learning	The pairwise correlations between song measures and cognitive measures gave little evidence of any association between song and cognitive ability. By contrast, the generalized linear mixed model analysis uncovered a complex pattern of associations between song and cognition.	yes/no
Ashton et al. 2018	*Gymnorhina tibicen*	47 – 56 Both sex	Outside lab	Ringed, habituated	4	Inhibitory control Associative learning Reversal learning Spatial memory	Strong evidence for a positive manifold. Together, our results point to a major role for the social environment in driving both the development and evolution of cognitive traits.	yes

Damerius et al. 2018	*Pongo abelii* and *Pongo pygmaeus*	13 and 40 respectively (23 females and 30 males)	rehabilitation stations	humans, station, wild, unknown	5	Box task Detour reaching Honey tool trap Reversal learning Tube trap task	The psychometric findings of *g* in orangutans captured the positive correlations among test scores of important problemsolving components, such as the abilities of memorizing, associative learning, spatial flexibility and causal reasoning. The core definition of general intelligence captures exactly these logical problem-solving functions, reasoning- and learning abilities.	yes
Dubois et al. 2018	*Melospiza georgiana*	20 – 49 All males	field	individual marked	5	Novel foraging Colour association Colour reversal Spatial learning Detour reaching + song learning	None of the song features that we measured showed a statistical association with any of the measures of cognitive performance we produced for male swamp sparrows.	no

van Horik et al. 2018	*Phasianus colchicus*	31 chicks Both sex	lab	reared	9	Motor skills Positional ability Positional Learning Positional Memory Discrimination learning Colour Learning Inhibitory control Colour Reversal Detour Reach	Individuals were rarely consistent in their performance across multiple tasks, both within similar and across different cognitive domains. There was little overall consistency in individual performance across tasks.	no

General discussion

D.1 Summary

In my thesis, I focused on the cognitive abilities of *Labroides dimidiatus*, their ability to integrate information and to adjust their behaviour accordingly. The findings from this thesis brought additional information to the field of common vertebrate cognition. Moreover, it emphasised that my particular fish study species, despite their small brain, showed advanced cognitive abilities previously thought to be unique to large-brained species. The ecological relevance of the tasks did not seem to affect cleaners' performance. In the first chapter, I tested whether the ecology of both cleaners and noncleaners would affect their performance in an ecologically relevant task, i.e. delay of gratification paradigm. As the first chapter showed that the specific ecology of cleaners aided their decision making, I was curious whether cleaners could perform as well but in an abstract experimental design, and so the second chapter tested cleaners in matching-to-sample (MTS) and delayed matching-to-sample (DMTS) experiments. In the third chapter, because cleaners did not show any particular difficulties to solve either ecological or non-ecological cognitive tasks, I tested whether cleaners possess general intelligence using a cognitive test battery. The battery test was composed of four non-ecological tasks from four cognitive processes such as learning – flexibility, inhibition – spatial orientation, quantitative reasoning, and working memory. Additionally, an ecological task was tested based on inhibition cognitive processes.

The results found in the first chapter were expected from cleaners but surprising from non-cleaners. Firstly, cleaners' and non-cleaners' ability to control themselves in order to get more food suggest a similar capacity to monkeys (Evans and Beran, 2007; Pelé et al., 2010, 2011; Ramseyer et al., 2006; Stevens et al., 2005). Furthermore, two *L. dimidiatus* individuals showed extreme waiting times, until 4 and 8 minutes, which is comparable to chimpanzees' performance (Beran et al., 1999; Dufour et al., 2007). As non-cleaner wrasses performed as well as cleaners, it seems that only the ecology affects inhibition process, at least not in the tests I used, i.e. with a food reward. Those results demonstrated that self-control relating to food is not linked with brain size, i.e. a large brain does not improve selfcontrol when food is involved. Furthermore, from those results, we can ask if ectotherms, i.e. animals in which body temperature mirrors the environmental temperature (Davenport, 1992; Ohlberger, 2013), do have their own perception of time as non-cleaner wrasses perform similarly to cleaners and monkeys without ecological relevance. Ectotherms have a slow metabolic rate which might affect their performance in this particular task. It has been shown that perception of temporal information is linked to metabolic rate as well as to body size (Healy et al., 2013), i.e. slower metabolic rate and larger body size species are slower in processing high temporal resolution sensory information. We can ask if this can have an impact on the perception of time in ectotherms. On the other hand, temporal perception has been linked to associative learning (Church, 2006) and that learning is related to a temporal map (Balsam and Gallistel, 2009). As we know that fish are capable of associative learning, they might have learned that when the immediate reward plate is introduced while they had not eaten the previously available food item, a bigger/tastier plate reward became available after a few seconds. As events are related in time, this temporal relation could have been used by cleaners in this paradigm and helped them to wait. It was also interesting when looking at the quitting time, i.e. time when an individual stopped waiting and ate the immediate food reward, to see that fish anticipated their decision to wait or not in the trial for a bigger food reward. Again, these results are similar to what was found in Tonkean and long-tailed macaques, brown capuchins, and chimpanzees in quantitative tasks and crows and Goffin cockatoos in qualitative tasks (Auersperg et al., 2013; Dufour et al., 2007, 2012; Pelé et al., 2010, 2011) which are all considered large-brained animal species that have advanced cognitive abilities. However, when analysing the quitting time, but per individual this time, two individuals per cleaner species seemed to anticipate their decision at different time-lapses. These results showed a difference between cleaners and non-cleaners. Overall

non-cleaners tended to wait more before failing compared to cleaners, who took decisions earlier. Apparently, non-cleaners did not seem to understand why they were waiting, whereas cleaners seemed to understand the task and made an active decision on waiting or not. Moreover, the opposite results between cleaners, *L. dimidiatus*, and corvids in the quantitative and qualitative tasks might reflect the specific ecology of each animal group. Overall, results emphasise how ecology is important when studying cognition as ecology drives cognitive abilities. These findings are in line with the ecological hypothesis stipulating that ecological conditions will shape cognitive abilities (Kamil, 1998; Shettleworth, 2001). Besides the ecology, specific physiological mechanisms may also affect cognitive abilities as shown with non-cleaners, i.e. ectotherms.

For the second chapter, the overall results of the six individuals in the MTS task revealed that cleaners are able to learn concepts. This ability has been shown in several species although for many species only few individuals succeeded in these studies (Blough, 1959; Bodily et al., 2008; D'Amato et al., 1985; Finch, 1942; Gierszewski et al., 2013; Giurfa et al., 2001; Goldman and Shapiro, 1979; Herman et al., 1989; Iversen, 1993; Newport et al., 2014; Nissen et al., 1948; Oden et al., 1988; Pack et al., 1991; Truppa et al., 2010; Wright et al., 1988; Zerbolio and Royalty, 1983). In those previous studies the number of trials animals needed to understand the rule in the training phase with between two and forty-two stimuli was high (more than 300 trials). Additionally, the number of trials needed to succeed in the transfer was between 48 and 2000 trials. Depending on the study, the transfer trials were presented with a mix of known and new stimuli in some cases. In the end, the experimental design changed few factors such as the use of known and new stimuli in the transfer trials, and the number of stimuli used in the training phase. The inconsistent experimental design between the different studies may have rendered the experiments more or less easy eventually. In my study, as no training was given to cleaners, their performance cannot be directly compared to previous studies. However, when looking at the high number of training trials needed for other species to reach criterion, cleaners did not seem to perform worse. Even so, again the criterion was not the same in each study. Therefore, other species would have to be tested in our more general way, and we should test cleaners with training and transfer trials as previously done. The second experiment in this chapter, DMTS, which was done with one individual only, was inconclusive. Because of the small sample size (n = 1), current conclusions that cleaners lack working memory can only be preliminary. Since working memory has been shown in another fish species, groupers, that wait in front of the hidden place of a prey fish for twenty-five minutes (Bshary et al., 2006; Vail et al., 2013), further studies are needed to conclude whether cleaners have this ability. From an ecological point of view, it does not seem that cleaners need working memory, i.e. object permanence, in their everyday life as they are getting their food from client fish in the surroundings. If a client fish hides in crevices in the reef, cleaners do not need to wait for it in order to get food as it can simply clean another client fish in the area. That is why we need more studies on that topic to disentangle whether this process is absent in cleaner fish for the reason that it is not useful in their ecological system.

Finally, the third chapter was a pioneer study in general intelligence, *g*, with an ectotherm species. As shown in various reviews (Burkart et al., 2017; Chabris, 2007; Matzel et al., 2011; Searcy and Nowicki, 2019; Shaw and Schmelz, 2017), overall studies in endotherm species, i.e. mammals and birds, concluded similarly that endotherms are endowed with general intelligence. However, bird studies are controversial as *g* is not always found, i.e. absence of positive correlations between tasks (Anderson et al., 2017; Boogert et al., 2011; Farrell et al., 2016; Guillette et al., 2015; Keagy et al., 2011; Nettle et al., 2015). Here, we did not find a psychometric *g* in cleaners as all the tasks were not loading positively in the first factor. This result may suggest that general intelligence might be linked to the cephalisation index that is found to be 10-fold bigger in endo- compared to ectotherms (Jerison, 1973). Several future studies are needed to increase the evidence going in this direction to have robust and consistent results. Furthermore, it was interesting to see that the two tasks, ecological and non-ecological, from the same cognitive process, i.e. inhibition, were not correlated. This allows us to ask

whether ecological and non-ecological tasks stimulate the same part of the brain even when activating the same cognitive process. In contrast to what Ashton et al. found in 2018, I did not find an effect of social environment, i.e. all individuals performed equally in the different tasks regardless of whether fish came from a high- or low-density fish area. As general intelligence is absent in cleaners, there is no apparent reason for social environment to affect cognitive abilities in that context. However, only three cognitive processes were implemented in the analysis, such as learning – flexibility, inhibition, and quantitative reasoning, other cognitive processes such as reasoning by exclusion, planning, visual-spatial processing, problemsolving should be considered for future studies (Table 1). There is no doubt that more studies are needed in this field of research to discover whether general intelligence is something only found in endotherms as related to their 10-fold relative larger brains compared to ectotherms.

Table 1: Presentation of advanced cognitive abilities in cleaners and what still need to be done. Working memory is the only cognitive process in which cleaners were not successful so far (highlighted in grey in the table).

	What it is done with cleaners	**Ability**	**What it needs to be done with cleaners**
Advanced cognitive process – task	Inhibition: - delay of gratification - detour task	Yes	Planning – tool use for future event
	Anticipation – quitting time in delay of gratification	Yes	Problem-solving – tool use
	Concept learning – matchingto-sample (MTS)	Yes	Working memory – DMTS standard version
	Working memory: - delayed MTS - object permanence	No	Working memory – object permanence with more salient object
	Learning – flexibility – reversal learning	Yes	Reasoning by exclusion – oddfrom-sample (OFS)
	Quantitative reasoning – numerical discrimination	~ Yes	Visual-spatial processing - maze
	Theory of mind – seen not seen experiment	Yes	
	Empathy – consolation experiment	Yes	

D.2 Big picture

D.2.1 Why study fish cognition and how does it help us understanding brain-cognition evolution?

Existing studies on brain functions revealed similarities among vertebrates indicating a more conservative brain evolution than previously believed (Broglio et al., 2005; Butler and Hodos, 2005; Nieuwenhuys et al., 1998; Salas et al., 2003; Striedter, 2005). The central nervous system was found to be structured in the same way in vertebrates as well as the telencephalon part where voluntary actions are controlled (Braford, 1995, 2009; Nieuwenhuys, 2009, 2011; Nieuwenhuys et al., 1998; Northcutt, 1995, 2008; Northcutt and Braford, 1980; Northcutt et al., 2009; Wullimann and Mueller, 2004). Furthermore, the hippocampus, amygdala, and neocortex of telencephalon subdivisions in tetrapod have their homologous parts in classes of bony fish: ray-finned fish or Actinopterygii (Braford, 2009; Butler and Hodos, 2005; Mueller and Wullimann, 2009; Northcutt, 2008; Northcutt and Braford, 1980; Wullimann and Mueller, 2004; Yamamoto et al., 2007). It became clear then that fish are as important as other animal taxa to study cognition as they possess the same fundamental brain

structures which are involved in advanced cognitive processes. The question of fish cognition arises as the proper tool to understand the ancestral brain of vertebrate species and what cognitive abilities it was endowed with.

Nowadays, plenty of studies show a panel of highly sophisticated behaviours in a wide range of animal species, fish included (Brown et al., 2011; Kappeler and Anthes, 2010; Shettleworth, 2001). The cleaner fish *L. dimidiatus* being no exception with several studies showing cleaners' performance in sophisticated strategies in ecologically relevant tasks (Bshary, 2001; Bshary and Noë, 2003; Bshary et al., 2014; Hammerstein and Noë, 2016; Noë and Hammerstein, 1994; Noë et al., 2001; Pinto et al., 2011; Prétôt et al., 2016; Salwiczek et al., 2012; Triki et al., 2019; Wismer et al., 2016, 2019). Those highly sophisticated behaviours were even based on supposedly advanced cognitive processes. Most interestingly, cleaners adapt their strategies depending on their environment. If cleaners live in complex areas, i.e. high fish density, they will show more complex strategies than when they are living in less complex areas, i.e. with low fish density (Triki et al., 2018; Wismer et al., 2014). This flexibility is part of advanced cognitive processes. Therefore, my thesis digs down in this direction, i.e. advanced cognitive processes, and it reveals that both ecological (chapter 1) and abstract (chapter 2) tasks were solved by cleaners. Those results add to the emerging picture that fish, i.e. ectotherms, can evolve many different advanced cognitive processes. This provides increased evidence that animals are better than previously thought, even small-brained ones. Thereby, the factor 10 in relative brain size that exists between endo- and ectotherms cannot be explained by advanced cognitive processes. In chapter 3, no general intelligence was found in cleaners which might be a plausible explanation to this factor 10, as general intelligence is found in general in endotherms.

D.2.2 Main findings and implications

D.2.2.1 Ecological intelligence hypothesis: ecology drives cognitive abilities

The ecological hypothesis stipulating that ecology shapes cognitive abilities (Kamil, 1998; Shettleworth, 2001) was partly strengthened with the first chapter of this thesis about impulse self-control. Interestingly, I found that non-cleaner wrasse, lacking ecological relevance in the delay of gratification paradigm, waited as long as cleaners for whom the task was ecologically relevant. This result raises the question if, after all, this paradigm was cognitively demanding or whether it was more about physiological mechanisms (as ectotherms have a lower metabolic rate compared to endotherms). Nonetheless, when looking at the quitting time, only four individuals were found to quit earlier than expected, all of them being cleaners. None of the non-cleaner wrasses were found to be capable of such an anticipation process. So far, anticipation has been found in large brain animals such as primates and birds (Tonkean and long-tailed macaques, brown capuchins, chimpanzees, crows and Goffin cockatoos (Auersperg et al., 2013; Dufour et al., 2007, 2012; Pelé et al., 2010, 2011)). This result is a piece of evidence that cleaner fish show advanced cognitive processes, i.e. anticipation, specifically linked to their specific complex environment. The presence of the cognitive process of anticipation in cleaners and its absence in non-cleaners is of particular interest when knowing that the only largebrained species lacking anticipation are young human children, i.e. around two years of age (Steelandt et al., 2012). This shows that cognitive processes may develop with age and experience, i.e. the cognitive process of anticipation is found in adults but not in human children. In the case of fish, cleaners show anticipatory processes because their ecology drives them to have this ability which permits them to increase their foraging success. Non-cleaners lack this anticipatory process as this ability is not needed in their environment.

D.2.2.2 Also in abstract design cleaners show cognitive abilities

Cleaners showed capacity to concepts learning, i.e. relating stimuli between them via their function, here the concept of same/different, and generalised the concept to new stimuli. Because of the

different experimental design used here, the comparison with previous studies on MTS was not feasible. However, the number of training trials presented in previous studies was quite high (minimum of 300 trials), whereas the number of transfer trials varied greatly between the different animal species (48 – 2000). A comparison can be made with the study of Truppa et al. (2010) with capuchin monkeys. After several training steps (more than 10'000 trials), the last phase consisted of a session of a hundred trials with new stimuli presented at each trial. It is similar to what we did with cleaners except that cleaners did not have any training trials prior to be tested. Truppa et al. (2010) found that the 6 capuchin monkeys made the correct choice on average 61% (74% - 55%) of the time over the 100 trials. Cleaners over 200 trials chose on average the correct stimuli 56% of the time (75% - 49%) with one of the 6 cleaners tested only in 40 trials. With 5 cleaners tested in 200 trials, the average correct choice was of 52.5% (57% - 49%). These close results in the percentage of correct choices between the two studies suggest that using new stimuli at each trial (trial-unique stimuli) might have help the cleaners to understand and generalise the task at a conceptual level more rapidly. Moreover, no proactive interference occurred, i.e. the capacity to retain new information is restrained by old memories, which may have helped cleaners as well to comprehend the task. The second task, DTMS, did not allow us to reveal the cognitive process of working memory in cleaners. However, it is important to highlight that we tested only one individual and that the retention interval was pretty long, around 30s, which may have affected cleaner performance. Indeed, the retention interval was found to decrease performance in such tasks in other studies (Nelson and Wasserman, 1978). Therefore, the presence of such a process in cleaners is not excluded as this process was revealed in groupers waiting up to twenty-five minutes in front of a crevice in order to catch a prey (Bshary et al., 2006; Vail et al., 2013). For this particular fish species, it seems that the specific hunting behaviour of groupers triggers object permanence ability. Additional studies are needed to verify the presence/absence of this process in cleaners, which is distinctly possible after the multiple studies showing other advanced cognitive processes in cleaners. Why this process compared to others would lack in cleaners? Is this ability not at all needed in cleaners' environment? This would be the reason for its absence with the ecological approach point of view. Or as cleaners show already many advanced cognitive processes, does this additional process take too much brain volume? This still needs to be clarified with in-depth studies.

D.2.2.3 General intelligence: a plausible explanation for cephalisation index difference between endo- and ectotherms

The results presented in the third chapter about general intelligence are foremost the first ones about ectotherms. Furthermore, in contrary to general findings in mammals and birds, we did not find any evidence of such general intelligence in cleaner fish. If general intelligence would have been related to brain activities in general, we would have found general intelligence as well in fish, and this was not the case. Thereby, to have a brain does not involve possessing general intelligence innately. This result allowed us to test whether general intelligence might be at least part of the explanation of the cephalisation index difference of 10-fold between endo- and ectotherms (Harvey and Krebs, 1990; Jerison, 1969, 1973). We will need more studies on ectotherms to prove or disprove this hypothesis.

Interestingly, the site where cleaners were caught did not have any effect on their performance in any tasks. The finding of Triki et al. in 2018, i.e. effect of population densities on sophisticated strategies, did not apply here in general intelligence. The main difference between Triki's study and general intelligence remains in that sophisticated strategies rely on decision making which adapt to a given situation and relate to the environment, whereas the cognitive processes tested in general intelligence were independent of any ecological relevance. Therefore, the fact of coming from a more or less complex environment did not affect the performance of cleaners in the general intelligence experiment.

D.3 Work in parallel and in progress

I had the pleasure to collaborate with scientists on side projects and co-supervised four master students while doing my PhD. All master students had Professor Redouan Bshary as the main supervisor. For three of them, their projects were related to my thesis topic. Hereafter, I will present the different projects' main results coming both from my different collaborations and from my master students. All the various results lead to a better understanding of cognition in the cleaner wrasse *L. dimidiatus*.

D.3.1 Strategic sophistication in cleaners

D.3.1.1 Third-party punishment

Strategic sophistication occurs when individuals interact with each other and the exchange of goods is involved, i.e. like in a market situation (Binmore, 2007), and refers to the adjustment of decision making within a given situation (Triki et al., 2019). For example, individuals have to make a suitable decision when reciprocity is appropriate behaviour and when punishment has to be applied and thus depending on the situation. Parallel work to my PhD (Annex 'Third-party punishment in a fish') continued on the 'old' approach of Redouan Bshary's group, i.e. describing games and strategies that have been argued to warrant a big brain without necessarily complex underlying mechanisms.

In cooperative interactions, it happens that individuals cheat instead of reciprocating, which induces punishment by the partner. Punishment is a strategy to maintain cooperation. Indeed, punishing cheating individuals will lead them to avoid defection during the next interaction, re-establishing cooperation (Clutton-Brock and Parker, 1995; Raihani et al., 2012). Two types of punishment exist, direct and third-party punishment. Direct punishment occurs between two individuals interacting together. If an individual A defects in the interaction with individual B, B will punish A. Whereas thirdparty punishment happens when an individual C, which is uninvolved in the interaction between the two individuals, punishes the defector even though not directly implied in the interaction. Third-party punishment has been argued to be extremely important for human cooperation (Fehr and Fischbacher, 2003, 2004), but lacking evidence in non-human species as considering to be a sophisticated strategy. However, a simple form of third-party punishment has been described in the cleaner wrasse (*Labroides dimidiatus*) (Bshary et al., 2008; Raihani et al., 2010). In the situation where cleaners are inspecting in pairs, the cheating behaviour of the female is costly to the client principally. However, males undergo a cost as well as lose a foraging opportunity as a result of the client leaving, so males cannot be considered as uninvolved in this co-inspecting interaction. Therefore, only a simple form of third-party punishment has been shown so far. We, therefore, tested third-party punishment in its strict sense, i.e. without sharing a cost. The experiment consisted of removing the partner's foraging lost by presenting a model client to each partner separately. Data were collected in 2016 (by myself) and in 2019 by Pietro Storelli (a master student). In the first experimental part, cleaner couples were separated with a Plexiglas barrier and forage singly on their client model. In the second experimental part, couples were this time not separated with a barrier but the model clients assigned to each individual were sufficiently apart to avoid any supplementary foraging from both parties. The difference remains in the client's model behaviour of females which either stayed in the aquarium, i.e. mimicking cooperative behaviour from females, or moved out of the aquarium, suggesting client leaving behaviour and therefore cheating behaviour from females. Moreover, during the second experimental set (2019), the experiment was done with two supplemental conditions, couples were tested with their familiar or with an unfamiliar partner. On the whole, the results revealed evidence of proper third-party punishment in cleaners as males punished females when cheating on the client model while males did not suffer personal foraging loss. The punishment rate tended to be higher toward unfamiliar females. This might suggest that males ensure unfamiliar females' cooperative level

(Raihani et al., 2010), and/or it may be a dominant behaviour as cleaners are protogynous hermaphrodite fish (Nakashima et al., 2000; Robertson, 1972; Sakai et al., 2001). Overall, it does not seem that third-party punishment is an ability unique to humans. These results suggest that the evolution of third-party punishment might be triggered by other ecological features than only social learning abilities and between-group competition as previously thought. What matters in cleaners' life is the reputation of their cleaning station, meaning the better a reputation a cleaning station has, the more likely it is that clients come back. In consequence, a better reputation means more clients, i.e. more foraging opportunities. It is therefore clear that males will control females to cooperate with clients instead of cheating as cleaner pairs share common client fish coming to their station. Cleaners revealed once more that their specific ecology drives their sophisticated strategy.

D.3.2 Advanced cognitive processes

D.3.1.2 Visual discrimination and generalization by cleaner wrasse *Labroides dimidiatus*

Discrimination is important for animals in daily life, as they must adapt their behaviour as a function of different stimuli, which can be rewarded and/or unrewarded stimuli (Shettleworth, 2013; Spence, 1936; Sutherland, 1964). Category discrimination is when an individual can discriminate between different stimuli and relate them to different categories (Pearce, 1994). We can distinguish between functional and relational categories. Functional categories are linked to the physical pattern of the stimuli, e.g. round shape vs not round shape, whereas relational categories are related to the relationship between stimuli, e.g. same vs different stimuli. Several studies show the abilities of diverse animal species in discrimination (D'Amato and Salmon, 1982; Hepper, 1988; Newport et al., 2016; Parr et al., 2000; Schluessel et al., 2012; Siebeck et al., 2009; Sutherland, 1957). Discrimination is used in generalisation, i.e. same reaction to different but same stimuli (Ferrari et al., 2007; Harlow et al., 1972; de Mendonça-Furtado and Ottoni, 2008; Mercado et al., 2000; Pack et al., 1991). Generalisation is important as it permits individuals to behave flexibly when encountering new situations, they can rely on what they know and adjust to new conditions (Emery and Clayton, 2004). Thereby, individuals are quicker to adapt to new situations than if they would have to learn each problem independently (known as the learning sets; Harlow, 1949). This ability to generalise was thought to be unique to humans, but simpler rules have been shown in non-human animals (Murphy et al., 2008).

Cleaners were shown to use generalisation in a social tool context, i.e. generalise the rule to swim toward a predatory fish in order to not be punished by a cheated client. The project of Alexia Steiger conducted in Moorea, French Polynesia, at the Gump Research Station between May – June 2017, aimed to investigate cleaner fish *L. dimidiatus* visual discrimination abilities. Always in an ecologically relevant situation but without social context, we aimed to assess cleaners' abilities to distinguish between two client fish species from two distinctive families and their ability to generalize between these two families when presented with different member species. In other words, we investigated if cleaners were able to learn a discrimination rule to form categories and to apply this rule to different fish species in order to categorize them efficiently, and thus generalize the rule. She tested 6 individuals: 3 were taught to receive a reward when choosing a butterflyfish laminated picture, and the 3 others were rewarded when choosing a damselfish laminated picture. Individuals ran trials with a specific pair of two laminated pictures, one picture from each client fish family when they reached criteria, the second pair of two laminated pictures was presented. The process continued until eight pairs were tested. The pairs' presentation order was different for each individual, avoiding sequence effects. After individuals reached criteria with the eighth pair, we used hybrid-fish laminated pictures to determine what features the cleaners focussed on. We used the tail of a common surgeonfish, *Ctenochaetus striatus*, and combined it with the head of either butterflyfish or damselfish. In the prevention of previous learning, we changed the pairs' species combinations. Taken together, the results indicated that cleaners discriminate between damselfish and butterflyfish laminated pictures,

and they generalized client fish family patterns through different species. On average, the number of trials needed to succeed decreased over time and with new client fish pairs. Moreover, even when the overall shape of the species changed (hybrid-fish), cleaners were still able to discriminate between the two family client species significantly above chance. As a result, cleaner fish *L. dimidiatus* are capable of generalisation in an ecological context and apply their knowledge to novel objects. This study was another evidence that cleaners do have advanced cognitive abilities when related to their ecology. It seems that advanced cognitive abilities are not linked to brain size in fish, but special environmental configuration triggers cognitive abilities, i.e. ecological intelligence hypothesis.

D.3.1.3 Theory of mind

Theory of mind (ToM) is defined as the ability of an individual to attribute mental states, specific knowledge states to itself and to others (either to same or to different species), and therefore to understand that others might have a different mental state compared to oneself (Dally et al., 2010; Premack and Woodruff, 1978). This advanced cognitive ability is an aspect of social cognition as it is used in social context to understand other individuals' behaviour and to behave accordingly (Gweon and Saxe, 2013). This notion is therefore applicable to different animal species such as primates, corvids, and ravens where several studies showed the existence of basic ToM in those species (Bräuer et al., 2007; Bugnyar, 2011; Bugnyar and Heinrich, 2005; Bugnyar and Kotrschal, 2002; Bugnyar et al., 2016, 2016; Clayton et al., 2007; Dally et al., 2010; Hare et al., 2000; Krupenye et al., 2016; Schmelz et al., 2011, 2013). It seems that the evidence for key components of ToM is restricted to large-brained mammals and birds. This pattern raises the question of whether large brains are a pre-requisite for ToM components or whether selective pressure for successful deception may shape the evolution of ToM capacities even in smaller brained species, like ectotherms. Therefore, the second Annex 'Do cleaner fish know what others can and cannot see?' was a project in collaboration with Laurie R. Santos, Katherine McAuliffe, and Lindsey A. Drayton on the ability of female cleaners to accordingly behave depending on whether they were seen or not by males. As a result, females used a visual barrier to hide their cheating behaviour from males such as primates do (Flombaum and Santos, 2005; Hare et al., 2000, 2006; Karg et al., 2015). Moreover, females were able to make the distinction between what males can or cannot see, and therefore females decided to eat behind an opaque barrier when males were watching. This distinction between what partners can and cannot see is one important component of ToM abilities in humans which seems to be present in female cleaners' behaviour. These outcomes go in line with the ecological intelligence hypothesis since in this experiment it revealed that ecological pressures can drive cognitive abilities thought to be related only to human first, and large-brained species secondly. Most recently, cleaners were found to succeed in the mirror test (Kohda et al., 2019), i.e. to self-recognise itself in a mirror (Gallup, 1970). This visual self-recognition capacity tends to measure self-awareness in animals. However, the success in this task is not sufficient to demonstrate self-awareness strictly speaking (e.g. ants (Cammaerts and Cammaerts, 2015)). This result adds more weight to the hypothesis that various components of a ToM can be present in ectotherm species, i.e. species with a comparatively small brain.

D.3.1.4 Behavioural responses of cleaner wrasse toward a stressed individual: evidence for consolation?

Theory of mind is defined as cognitive perspective-taking, whereas empathy, which is a related concept of ToM as it is linked to the attribution of mental states to others but particularly on an emotional level, and, therefore, defined as emotional perspective-taking (Hynes et al., 2006). Since the basics form of ToM is described in several animal species, empathy strictly speaking will be hard to be revealed in animals. However, consolation, i.e. comforting behaviour that happens when an individual is stressed and another individual comfort the stressed party via physical contact, is a form of empathy (Zahn-Waxler et al., 1992). This form of empathy has been demonstrated in few animal species so far in aggressive social context such as in canids (Cools et al., 2008; Palagi and Cordoni,

2009), corvids (Fraser and Bugnyar, 2010; Seed et al., 2007), elephants (Plotnik and de Waal, 2014), and great apes (Clay and de Waal, 2013; De Waal and van Roosmalen, 1979). Furthermore, consolation behaviour, i.e. an increase in physical contact from an individual towards a stressed one which has the effect of reassuring and calming the stressed individual (De Waal and van Roosmalen, 1979), has been revealed in prairie voles (rodents; Burkett et al., 2016). The comforting behaviour happened in a non-social context. Consolation was thought to require advanced cognitive abilities (Russon et al., 1998), but this last study on rodents raises the question of whether this capacity requires less cognitive capacities as previously thought, or whether living in a specific social context triggers such behaviour.

In order to investigate the degree to which basic forms of these mechanisms are evolutionarily widespread and what is the relative importance of ecological conditions versus having a large brain, we tested cleaners in consolation experiment. This project was conducted by two of my master students: on the one hand by Léonore Bonin in Moorea, French Polynesia, at the Gump Research Station between July – August 2016, and in May 2017, and on the other hand by Pietro Storelli on Lizard Island Research Station, Australia, between February – April 2019. Their project aimed to investigate whether consolation behaviour exists in couples of cleaner wrasse *L. dimidiatus* and if a familiarity's bond affects it. The general experimental design was composed of an initiation phase where the couple were together, a separation phase which ended with or without stressing event, and the reunion phase. In the different experiments, the stress level of individuals, the aggression rate, and consolation behaviours were recorded at both the initiation and the reunion phases. After analysis of the different experimental design data, we found some evidence for consolation in couples of cleaner wrasses. An increase in voluntary tactile stimulation as well as physical contact was observed and reflected actions modulated by the stress level of the recipient. On the other hand, we found an increase in males' aggressive behaviours toward the stressed female, which could have been interpreted as stimulation from the males towards the females in order to keep them active and available to potential clients and/or predators. The partner's degree of visibility modulated the females' stress level. Females were more stressed when the males were absent or when the visual contact was altered compared to when the two individuals were together or when the visual contact between the couple was clear. Hence, the well-being of females is potentially linked to the presence of their partners who provide comforting behaviours when they are stressed. Overall, consolation behaviour was found to be higher in the reunion phase in the stressing event treatment compared to the control where no stress was induced, and thus invariably if the females were familiar or not. We did find a significant difference between the conditions, as males are more aggressive towards females and thus in general (at both phases). With these results, we can, affirm that cleaner wrasse *L. dimidiatus* showed consolation behaviour when females are exposed to an unsocial stressful event as found in prairie wolves (Burkett et al., 2016). Moreover, males make the distinction between familiar and unfamiliar partners, in that they are more aggressive with an unknown partner. The presence of consolation behaviour in cleaners adds weight to what was previously found by Burkett et al. in 2016, suggesting that consolation might not require advanced cognitive abilities. However, they put forward the link with maternal care which is non-existent in cleaners as they have pelagic larvae stage (Victor, 1986). Consequently, consolation behaviour might appear under certain environmental conditions. As consolation behaviour is considered a component of empathy, and empathy being a related concept to ToM, we can therefore attribute to cleaners an emotional component of the cognitive process of ToM.

D.4 Future research and work

The various results from my PhD thesis demonstrate the advanced cognitive abilities of cleaners *L. dimidiatus* in different cognitive processes. So far, cleaners did not fail in any cognitive process task used to test complex cognition, apart for working memory. In the future, we would not be surprised to find more advanced cognitive abilities from cleaners, such as reasoning by exclusion,

problemsolving, visual-spatial processing, and planning. In Table 1, I propose various tasks that could be used to test cleaners on other cognitive processes. As environmental challenges are claimed to be more important for brain size evolution, it would be interesting to test cleaners in such environmental intelligence, e.g. complex diet, tool use, spatial cognitive map. As some behaviour might be isolated/not frequent, it seems difficult to test. Other ectotherms, such as lizards, might be easier to test for environmental intelligence as we could recreate their environment in captivity, e.g. as scientists do with some birds. Therefore, it would be easier to record data and even change environmental factors to check if it affects cognitive abilities, and if so how.

A follow up on general intelligence performance and brain size is actually held by Yasmin Emery, PhD student in the Behavioural Ecology laboratory. After the second experimental year on general intelligence (2019), I kept all the fish (n=38) and let Yasmin test them in other tasks prior to choosing half of the individuals from each capture site and collected their brain. The brain size was compared between the two sites as well as related to their performance in *g*. The brain size was not found different between the two capture sites and did not affect individual performance either. The absence of difference in brain size between the two sites goes against what was previously found by Triki et al. in 2019a, i.e. forebrain size correlated with population densities. However, the two different capture sites were connected in the sense that cleaners could swim from one site to the others, which might explain the lack of difference in brain size in our study. Moreover, the analysis of brain cell numbers is still under investigation, and secondly, data on fish density from both sites have not been analysed yet. With this in-depth analysis, we will be able to see whether brain size affects the performance of cleaners in a test battery on general intelligence. Furthermore, we will be able to test whether we find the same results as Triki et al. in 2019 (a).

As the results from *g* show no effect of body size on cognitive performance in cleaners, with Zegni Triki, we want to explore how does brain size scale with body size if cognitive abilities remain constant, i.e. cognitive equivalence.

D.4.1 Final conclusion

In conclusion, the various results from this thesis are in line with the ecological intelligence hypothesis which might be the most accurate explanation for brain evolution in the cleaner fish *L. dimidiatus*. To have a small brain does not necessarily imply to lack advanced cognitive abilities as this thesis suggests with cleaner wrasse as model species. Furthermore, the absence of general intelligence in this fish species opens the possibility that the cephalisation index shows a 10-fold difference between endo- and ectotherms might be explained by the presence/absence of *g*. Present and future studies will definitely increase our knowledge and understanding of what selection pressure drives brain-cognition evolution through vertebrates, fish being an exceptional study system model.

D.5 References

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Annex | parallel research

Title: Third-party punishment in a fish

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MA and PS collected the data and ran the analyses. MA, NJR and RB contributed to the writing.

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Abstract

The tendency for an uninvolved bystander to intervene on behalf of a victim ('third-party punishment') is common in humans and has been proposed as a pivotal mechanism driving the large-scale expansion of cooperative human societies. Such behaviour has not been observed in any non-human species, although a prototypical form of third-party punishment has been described in the cleaner fish *Labroides dimidiatus*. Here, males often chase their female partners for cheating a jointly inspected 'client' fish by eating mucus instead of ectoparasites. In this case, the evidence for third-party punishment is suggestive but not conclusive because males suffer the immediate loss of foraging opportunities due to the client leaving. Here, we show experimentally that males also punish a cheating female that cheats a client during solo inspections, providing the first experimental evidence of third-party punishment by an uninvolved bystander in a non-human species. This finding challenges the assumption that third-party punishment is a uniquely human trait.

Keywords: *Labroides dimidiatus*; cooperation; third-party punishment; human trait

Introduction

Punishment has been defined as an action in which the punisher accepts a cost in order to inflict a cost on the target in response to a selfish action by the latter (Clutton-Brock and Parker, 1995; Raihani et al., 2012a). In humans, punishment may be given not only by the victim of a selfish act but also by otherwise uninvolved bystanders whose payoffs were unaffected (Fehr and Fischbacher, 2003, 2004). It has been argued that such 'third-party punishment' relies on a tendency to copy other group members, which promotes within-group cooperation and increases success in inter-group competition (Bowles and Gintis, 2004; Boyd et al., 2003; Fehr and Gächter, 2002). The social learning rules that may facilitate the spread of third-party punishment within groups are a matter of debate (Burton-Chellew et al., 2017; Lehmann et al., 2008). Nevertheless, in line with the rather specific scenario of cultural group selection, that involves advanced cognitive processes along with rare ecological pressures, thirdparty punishment has not yet been described in other species.

A prototypical form of third-party punishment has been described in the cleaner wrasse *Labroides dimidiatus* (Bshary et al., 2008; Raihani et al., 2010). Cleaner fish typically live in harems comprised of one male and several smaller females (Robertson, 1972). While male and female cleaners are breeding partners, females are also potential future competitors to the male since large females can switch sex (Robertson, 1972). Cleaner fish provide a service to reef-fish 'clients' by removing ectoparasites but prefer to eat client mucus (Grutter and Bshary, 2003), leading to a conflict of interest with clients since mucus feeding harms clients. Male cleaners sometimes co-inspect large clients with a female partner. If either cleaner cheats (eats mucus), the client flees with a 50% probability (Bshary and Schäffer, 2002). As one cleaner obtains the benefits of mucus feeding while both cleaners share the cost of client departure, the resulting payoff matrix between cleaners is equivalent to a prisoner's dilemma (Bshary et al., 2008). Male cleaners are larger than and dominant to their female partners. When clients flee the male often punishes the female, especially when the latter cheated (Bshary et al., 2008). Females subsequently behave more cooperatively in the next pairwise interaction with a client (Bshary and Grutter, 2002a, 2002b, 2005; Bshary et al., 2008; Raihani et al., 2010, 2012b, 2012c). Male punishment of cheating females is superficially similar to third-party punishment since the client, rather than the male, is the primary victim of female cheating. Nevertheless, males also suffer the immediate loss of foraging opportunities due to the client leaving and cannot therefore be described as truly uninvolved. Here, we asked whether males would punish their female partners for cheating a client even if the male was an uninvolved observer of the interaction rather than co-foraging. If so, punishment would not be a response to personal immediate losses.

Material and methods

(a) Study site and study animals

We collected data in two phases (Study 1a & 2a: 2016; Study 1b & 2b: 2019). In 2016, we collected data on 12 cleaner fish couples at the Gump Research Station on Moorea (French Polynesia). In 2019, we collected data on 18 cleaner fish couples at Lizard Island Research Station (Australia). The couples were caught with a barrier net (2m long, 1.5m high, mesh size 0.5cm) on nearby reefs. For Studies 1a and 2a (2016), blue round tanks (212 cm in diameter x 86.5 cm high) were divided with white Plexiglas sheets such that four compartments were constructed of roughly 100 x 30 cm in size with a water level of 35 cm (Figure 1C). Each compartment harboured one couple. It could be separated in two parts of roughly equal size for Experiment 1 (Figure 1C). Cleaner fish were acclimatized for at least 21 days before taking part in experiments. For Studies 1b and 2b (2019), couples were placed in glass aquaria. Six aquaria were of a dimension of 90 x 38 x 38 cm and three aquaria of 80 x 38 x 38 cm. Each aquarium was divided into two compartments with an opaque separation in order to have 18 home aquaria (Figure 2C). Cleaners were acclimatized for at least two weeks prior to start the experiment. At that

point, cleaners were already accustomed to feeding off Plexiglas plates. Cleaners foraged on plates that offered small items of preferred prawn and less-preferred fish-flake mixed with prawn (hereafter 'flake'). All cleaners were trained that feeding against preference (eating prawn) resulted in the removal of the Plexiglas plate (via a lever attached to the plate). All cleaners ate some flake items during six learning trials, i.e. they were exposed to the contingencies that eating flake has no negative consequences while eating prawn does. Cleaners from Studies 1a& 2a (2016) subsequently took part in experiments other than reported here in which eating against preference was a major component. Those experiments lasted 25 days. As a consequence, subjects were well trained for the current study in that they were used to discriminate between prawn and flake items, and they had learned that eating prawn led to the fast removal of the plate while eating flake had no negative consequences. This prior experience formed the basis for our first experiment in which females were exposed to a) either flake items only or to a mixture of flake and prawn items, and b) fast, slowly or non-fleeing plates. If males paid attention to those details they could have been able to interpret their observations based on own experience.

(b) Experimental design

i. Training

Prior to Study 1a, cleaners were trained once more to feed against their preference, using plates offering twelve flake and two prawn items. Plates were removed as soon as a first prawn item was eaten and re-introduced after 30s until the second prawn item was eaten. The next trial took place around 35 min later. We first conducted six trials with males and females exposed separately with the opaque divider in place. Then, we conducted another six trials with males and females foraging together, again removing the plate as soon as a first prawn item was eaten. Except for couples B and K, where the respective females never cheated during the joint inspection of the plate, the schedule ensured that all females gained the experience that eating a prawn item leads to being chased by their male partner.

Every morning, fish were fed ad libitum flake for 5 minutes 15 minutes before starting the trials. In Studies 1a & 2a (2016), a camera was placed inside the compartment to record the feeding and most importantly any male aggression during the 30s following the removal of the barrier. Studies 1b and 2b (2019) were video recorded with a CANON camera fixed on top of a tripod and positioned in front of the glass aquarium.

ii. Study 1a (Figure 1A)

During each trial, a barrier was first inserted into the aquarium, before introducing the male and female plates. Males and females were separated while foraging and the barrier was removed immediately afterward. Cleaners were exposed to five different treatments that differed with respect to three aspects: barrier type (opaque/transparent), number and type (flake and prawn) of food items, and client response to female foraging. We used three different model client plates. Client 'A' was a 'normal' client, which offered three flake and one prawn items (3 flake; 1 prawn), where eating the prawn item lead to the removal of the plate; Client 'B' was a 'low value' client, (3 flake; 0 prawn) that stayed inside the aquarium even after the female had finished eating all items; and Client 'C' was a 'high value' client (12 flake; 0 prawn) that also remained after the female had eaten all items. In all trials, males interacted only with Client B (3 flake; 0 prawn). Regarding the client response, we introduced three options: fast removal, slow removal and no removal. Removal was invariably a response to the female eating the prawn item off client 'A', while clients 'B' and 'C' invariably remained in the tank after the female had finished eating. Fast removal occurred within 1s of prawn eating so that the female could not eat any other food item, while slow removal took 3-5s and the female was hence able to eat all remaining items before the plate was extracted. Male plates always contained

three flake items and were never removed from the aquarium. More specifically, we tested the following five treatments:

1. Opaque barrier / Client A (3 flake; 1 prawn) / fast removal: In this condition, the plate is removed fast when female eats the prawn item but the male does see neither the cheating nor the plate removal. Therefore, we expected males to show baseline levels of aggression in this treatment.
2. Transparent barrier / Client A (3 flake; 1 prawn) / fast removal: Here, the plate is quickly removed when the female cheats and males can observe both the cheating and plate removal. If males use either observed prawn eating or fast removal of a plate as a cue for the female cheating, they should be more aggressive than in treatment 1.
3. Transparent barrier / Client A (3 flake; 1 prawn) / slow removal: If males use fast removal of a plate as a cue for the female cheating, they should only show baseline aggression in this condition. In contrast, if they use client leaving during interaction and/or females eating a prawn item as a cue then they should be as aggressive as in treatment 2.
4. Transparent barrier / Client B (3 flake; 0 prawn) / no removal: Control clients have no prawn items and thus cheating is not possible. If males use plate removal (fast/slow) and/or eating a prawn item as a cue for the female cheating, they should only show baseline aggression in this condition (i.e. equivalent to treatment 1).
5. Transparent barrier / Client C (12 flake; 0 prawn) / no removal: High-value clients contain 12 flake items and thus no cheating is possible. If males use any form of removal of a plate and/or eating a prawn item as a cue for the female cheating, they should only show baseline aggression in this condition. In contrast, if they see females as competitors, aggression would serve as punishment for depleting a resource rather than punishment for cheating a client. In that case, males should be most aggressive in this condition. In contrast, if males care about positive evidence for female partners behaving cooperatively, males should be least aggressive in this treatment. This is because treatment 5 is the only condition in which males properly see females foraging on the plate and the plate remaining: females continue foraging beyond their male partner terminating with his own plate because of the extra items.

Each couple performed 12 trials per treatment, i.e. 60 trials in total. Trials were grouped into units of five trials in which each treatment occurred once. The order within a unit was determined by a lottery. The experimenter exposed each couple to one unit before moving on to the next couple. A single unit consisted of the following actions. First, the camera was placed in the compartment to record the feeding and most importantly any male aggression during the 30s following the removal of the barrier. Then, the fish were separated with the appropriate barrier (opaque or transparent). Habituated cleaners can easily be guided with hand movements in the water to achieve the separation. 30s after the separation had been put in place, the plates corresponding to the pre-determined first treatment were introduced in both compartments. Depending on the treatment the female plate was removed fast or slowly as soon as the female ate the prawn item, or not at all when only flake items were offered. Once the female either stopped eating or had finished the flake items on her plate, the separation was lifted so that individuals could interact. After 40s the couple was again separated with the introduction of a barrier and the next trial could begin. This procedure was repeated until the unit was completed.

Once each couple had been exposed to a unit the next round of units began. One round of units took about 2.5 h. Therefore, three units, i.e. 15 trials per couple could be completed within a day, starting at 8:00 and finishing at 16:30. A lunch break of 1 hour took place after two rounds; the third round took place in the afternoon. Thus, experiment 1 lasted four days. Only trials where neither male nor female foraged on the other's plate were used in the analysis to avoid the possibility that male/female foraging interfered with males being aggressive. All conditions combined, we removed between 1-3 trials in 8 couples out of 12.

iii. Study 1b (Figure 1B)

Two plates containing six flake items each were introduced simultaneously at opposite ends of one compartment sidewall. Fish were hence free to choose where to go. As a consequence, either male or female ate off both plates during several trials (1-5, depending on couple identity). We discarded such trials for the analyses, kept the pre-scheduled order of trials and only repeated failed trials at the end. Whenever the fish chose to inspect different plates, we collected data. The male plate remained at the spot where it was introduced into the compartment. The female plate remained at the site of introduction in 50% of trials while it was moved after the female ate a first item towards the male end of the compartment before being removed in the other 50% of trials. The speed of plate movement was adjusted to the female speed of following in such a way that she remained close and could potentially eat items off the plate. The process took about 8-10s (based on the video analysis of 20 randomly chosen trials), during which the plates covered a distance of about 30cm. Males invariably finished their own plate and most of the time joined the female at her plate. Only trials where males had nothing left to forage on the females' plate were included in the analysis (see criterion above). As soon as all foraging had ceased and the plate removed or not we quantified male aggression towards the female during the following 30s.

Couples were tested in units of four trials with the aim to obtain 12 successful control and 12 successful experimental trials. Within a unit, two plates were introduced simultaneously about every 60s. For each couple the order of presentation of control (female plate remains) and experiment (female plate moves) was obtained with a semi-random lottery: the same treatment could not occur more often than three times in a row, and the entire sequence was produced prior to experiments. We repeated initially failed trials in a random order. Importantly, we kept a balance between the experimental and control conditions. This meant that certain couples exceeded the initially planned 12 trials per condition. Furthermore, we had to discard few trials after thorough video-analysis because males or females turned out to have eaten on both plates. Final sample sizes, therefore, ranged between 11 – 14 trials in the experimental treatment and between 10 – 14 trials in the control treatment.

iv. Study 2a (Figure 2A)

We proceeded the same way as in Study 1a with the difference that here only two treatments were tested. This time the treatments differed with respect to two aspects: number and type (flake and prawn) of food items, and client response to female foraging. We used two different model client plates. Client 'A' was a 'normal' client, which offered three flake and one prawn items (3 flake; 1 prawn), where eating the prawn item lead to the removal of the plate; Client 'B' was a 'low value' client, (3 flake; 0 prawn) that stayed inside the aquarium even after the female had finished eating all items. In all trials, males interacted only with Client B (3 flake; 0 prawn). Regarding the client response, we introduced two options: fast removal, and no removal. Removal was invariably a response to the female eating the prawn item off client 'A', while client 'B' invariably remained in the tank after the female had finished eating. Fast removal occurred within 1s of prawn eating so that the female could not eat any other food item. Male plates always contained three flake items and were never removed from the aquarium. More specifically, we tested the following two treatments:

1. Transparent barrier / Client A (3 flake; 1 prawn) / fast removal: In this condition, the plate is quickly removed when the female cheats and males can observe both the cheating and plate removal. If males use either observed prawn eating or fast removal of a plate as a cue for the female cheating, they should be more aggressive than in treatment 2.
2. Transparent barrier / Client B (3 flake; 0 prawn) / no removal: Control clients have no prawn items and thus cheating is not possible. If males use plate removal and/or eating a prawn item as a cue for the female cheating, they should only show baseline aggression in this condition.

Afterward, we proceeded similarly as during Study 1a.

Here, we added the condition familiar and unfamiliar partner. We started with 10 couples in the familiar condition and the other 8 with the unfamiliar condition. At the end of the experiment, we reversed the condition for each couple and ran the experiment one more time. In the unfamiliar condition, the individuals were reunited 5 minutes prior to the start of the experiment. Every couple was tested for 6 rounds in each condition. A round was defined as 4 trials for couple. In this case, we had 2 treatments (1 trial each), 1 round was composed of both treatments tested twice (4 trials in total). For each couple, we randomized the treatments to avoid that a couple started every trial with the same treatment. In one day, we tested 18 couples for 1 round. Therefore, we needed 12 days to complete the experiment with the 2 conditions. Only trials where neither male nor female foraged on the other's plate were used in the analysis to avoid the possibility that male/female foraging interfered with males being aggressive. All conditions combined, only the unfamiliar couple M – male, N – female had 10 trials available for the analysis. All other couples had the 12 trials available for the statistics. v. Study 2b (Figure 2B)

We proceeded similarly to Study 2a. The only differences remain in the number of flake items displayed on the plate: five instead of six, as well as an additional treatment: the males' plate moving.

As in the Study 1b, we added the condition of familiarity between the partners. Therefore, every couple was tested for 12 rounds in each condition. To complete one round the couple was tested for each treatment, they completed 3 trials (1 trial per treatment). The treatments were randomized to avoid that the same couple was tested with the same treatment sequence in consecutive rounds. In one day, we were able to run 2 rounds with 17 familiar couples and 16 unfamiliars. We needed 12 days to complete this experiment in the 2 conditions. Final sample sizes, after the removal of failed trials, except for the couple I – male, J – female where 6 trials were available in treatment 'males' plate move', ranged between 10 – 15 trials in the experimental treatments and between 11 – 12 trials in the control treatment by couple.

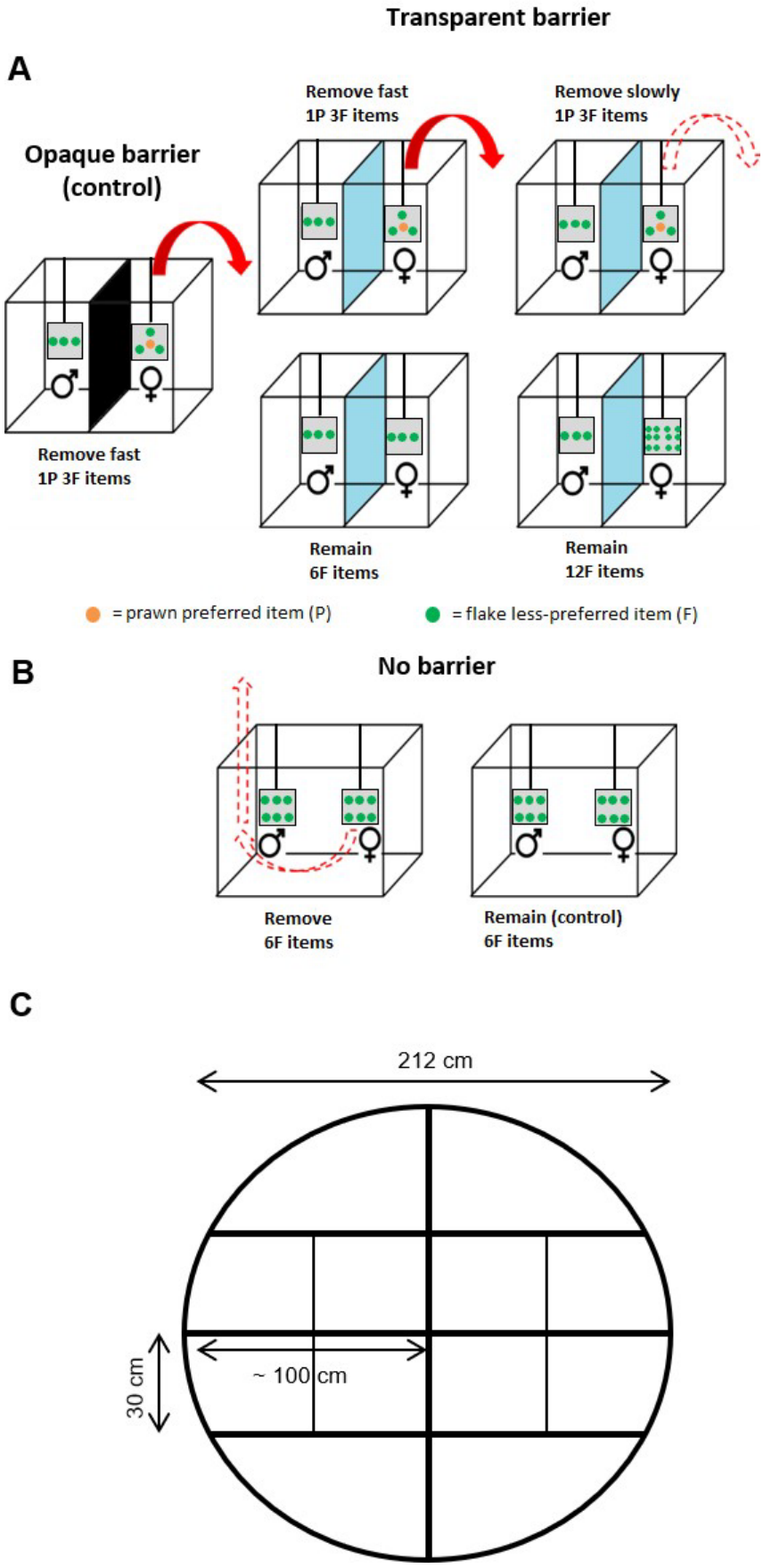

Figure 1: Studies 1a & 2a (2016). (A) Experimental design of the first experiment showing the 5 different treatments offered to females. (B) Experimental design of the second experiment showing the two different treatments offered to females. (C) Schematic view of the four compartments of roughly 100 x 30 cm in size with a water level of 35 cm constructed with white Plexiglas sheets in the blue round tanks (212 cm in diameter x 86.5 cm high). Each compartment harboured one couple. It could be separated in two parts of roughly equal size for experiment 1.

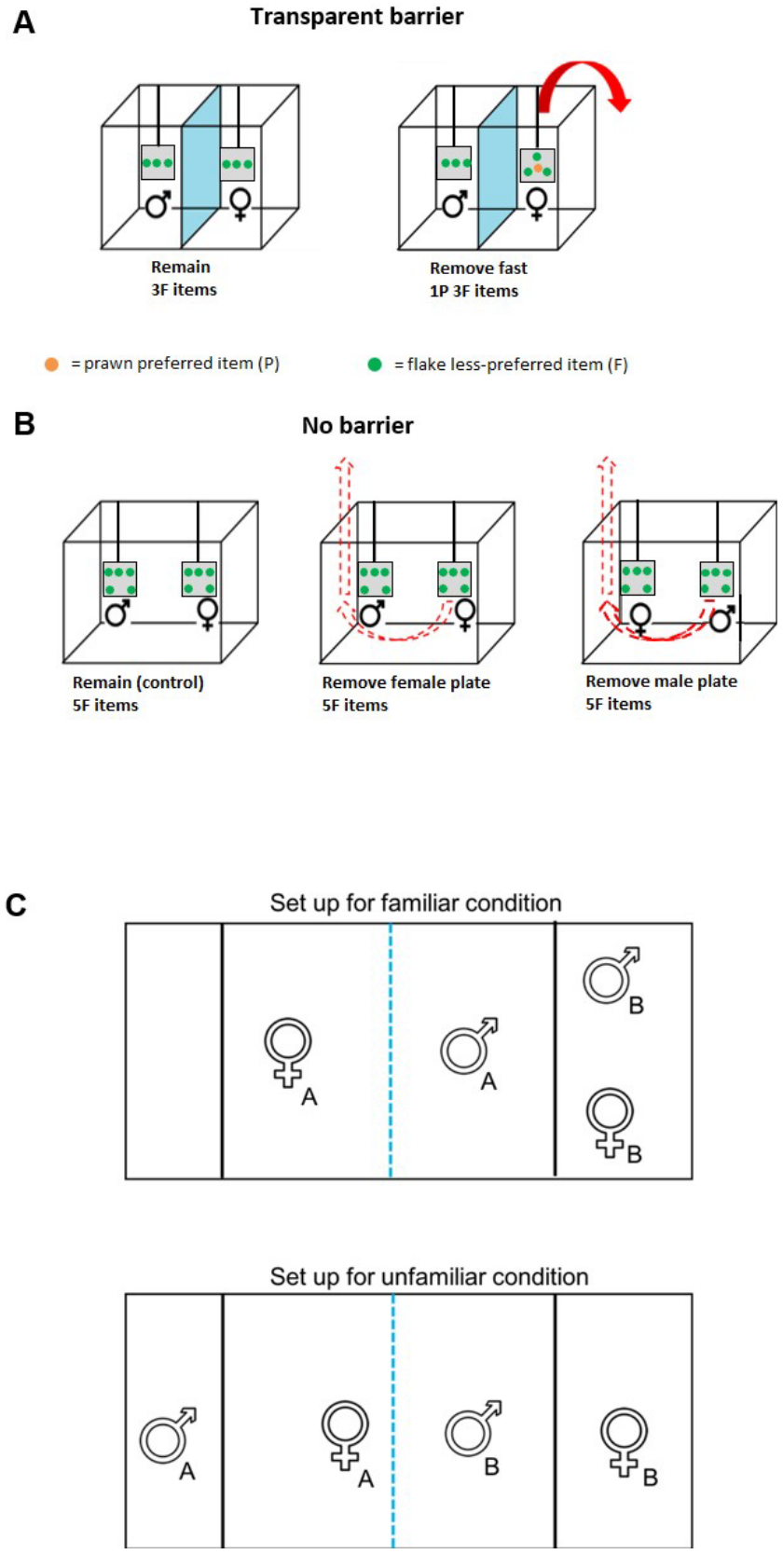

Figure 2: Studies 2a & 2b (2019). (A) Experimental design of the first experiment showing the 2 different treatments offered to individuals. (B) Experimental design of the second experiment showing the three different treatments offered to individuals. (C) Schematic view of the two conditions: familiar and unfamiliar. The part of the aquarium where the experiment was carried was of a dimension 60 x 38 x 38 cm in size with a water level of 20 cm constructed with white Plexiglas sheets in the glass aquaria. The blue dashed line corresponds to the different separations that are used during the diverse experiments. Those separations can be transparent, opaque or absent depending on the treatment of the experiment. The separation between the couple which is doing the experiment and the untested individuals is always opaque.

(c) Data analysis and statistics

All statistics were implemented in R version 1.2.5019, © 2009-2019 RStudio, Inc.) (TEAM, R. Core., 2016).

Given that we could not predict how cleaners would respond to the two different experimental designs, and given that we would interpret a single positive result as meaningful, we lowered the alpha to 0.025 (Holm, 1979). In Studies 1a & 1b, we conducted 12 trials per treatment and noted for each trial whether or not the male chased the female during the 30s following the removal of the barrier. The effect of our treatment on the binary outcome 'aggression' (yes/no) and on the condition for the second experimental set was assessed using a generalized linear mixed model (GLMM) using the package 'lme4' (Bates et al., 2015). Couples and units (nested within couples) were the random effects in the model accounting for the pseudo-replication in our data, according to the design explained above.

For Study 1, we used the Tukey method to perform post-hoc multiple comparisons. For Study 2, the model contained the interaction term between treatment and condition in addition. The 'emmeans' package was used (Searle et al., 1980) permitting to show the effect of a given treatment on a specific condition and vice versa.

For Studies 3 & 4, we compared male aggression towards females in 12 trials where the female's plate was removed (30s observation after removal) and in 12 trials where the male's plate was removed to 12 trials where her plate was not removed (30s observation after female finished foraging). As above, the effect of our treatment on the binary outcome 'aggression' (yes/no) and on the condition for the second year (2019) was assessed using a GLMM using the package 'lme4' (Bates et al., 2015). Couples and units (nested within couples) were the random effects in the model accounting for the pseudoreplication in our data, according to the design explained above. For Study 4, the model contained the interaction term between treatment and condition in addition. The 'emmeans' package was used (Searle et al., 1989) permitting to show the effect of a given treatment on a specific condition and vice versa.

Results

i. Study 1a

The hypothesis that male aggression is a form of competition, rather than punishment, predicts that males would be most likely to chase females when females interacted with a 'high value' client (since in this treatment, females eat the most food items). In contrast to this prediction, post hoc test following an overall significant result ($\chi^2 = 16.488$, df = 4, p = 0.002) revealed that males chased females in significantly fewer trials when the female interacted with the high value plate (36%) compared to both the control (63%; SE = 0.2711, z value = -1.042, p = 0.001) or to the fast-fleeing plate (57%; SE = 0.2842, z value = -3.048, p = 0.02), while aggression for the low-value (53%; SE = 0.2834, z value = 2.465, p = 0.098) or slow-leaving plate (50%; SE = 0.2827, z value = -2.066, p = 0.24) were intermediate and not significantly different from other treatments (Figure 3A). Reduced aggression with the highvalue client is not an artefact of the male being able to co-forage on the high-value plate since we ensured that females had eaten all food items before removing the barrier. This finding, therefore, suggests that male aggression is not simply an expression of competitive behaviour.

ii. Study 2b

Males punished females significantly more when the female's plate was removed compared to when the plate remained in the tank (SE = 0.3552, z value = -2.334, p = 0.02; mean aggression level in removal

condition (25%), and control condition (remain) (12%) respectively, Figure 3B). Males that chased their female partner typically finished eating all items off their plate before chasing the female. Thus, male aggression cannot be explained as a response to personal foraging losses.

iii. Study 1b

A significant difference in the punishment rate was found between treatments ($\chi^2 = 11.1979$, df = 1, p = 0.0008). Only a tendency was revealed between familiar and unfamiliar partner, males being more aggressive toward the unfamiliar partner ($\chi^2 = 3.6499$, df = 1, p = 0.056). No interaction term between the two variables was significant ($\chi^2 = 0.0274$, df = 1, p = 0.9). The analysis of the contrast between treatment 1 and treatment 2 in each condition showed significant results (familiar: SE = 0.2732663, z value = -2.179, p = 0.03; unfamiliar: SE = 0.2577318, z value = -2.552, p = 0.01; Figure 4A).

iv. Study 2a

No significant difference was found between the different treatments ($\chi^2 = 1.4743$, df = 2, p = 0.478), as well as no interaction term between treatments and condition ($\chi^2 = 0.2088$, df = 2, p = 0.9; Figure 4B). While the condition showed a weak tendency that males punished more unfamiliar females than familiar ones ($\chi^2 = 3.1566$, df = 1, p = 0.08).

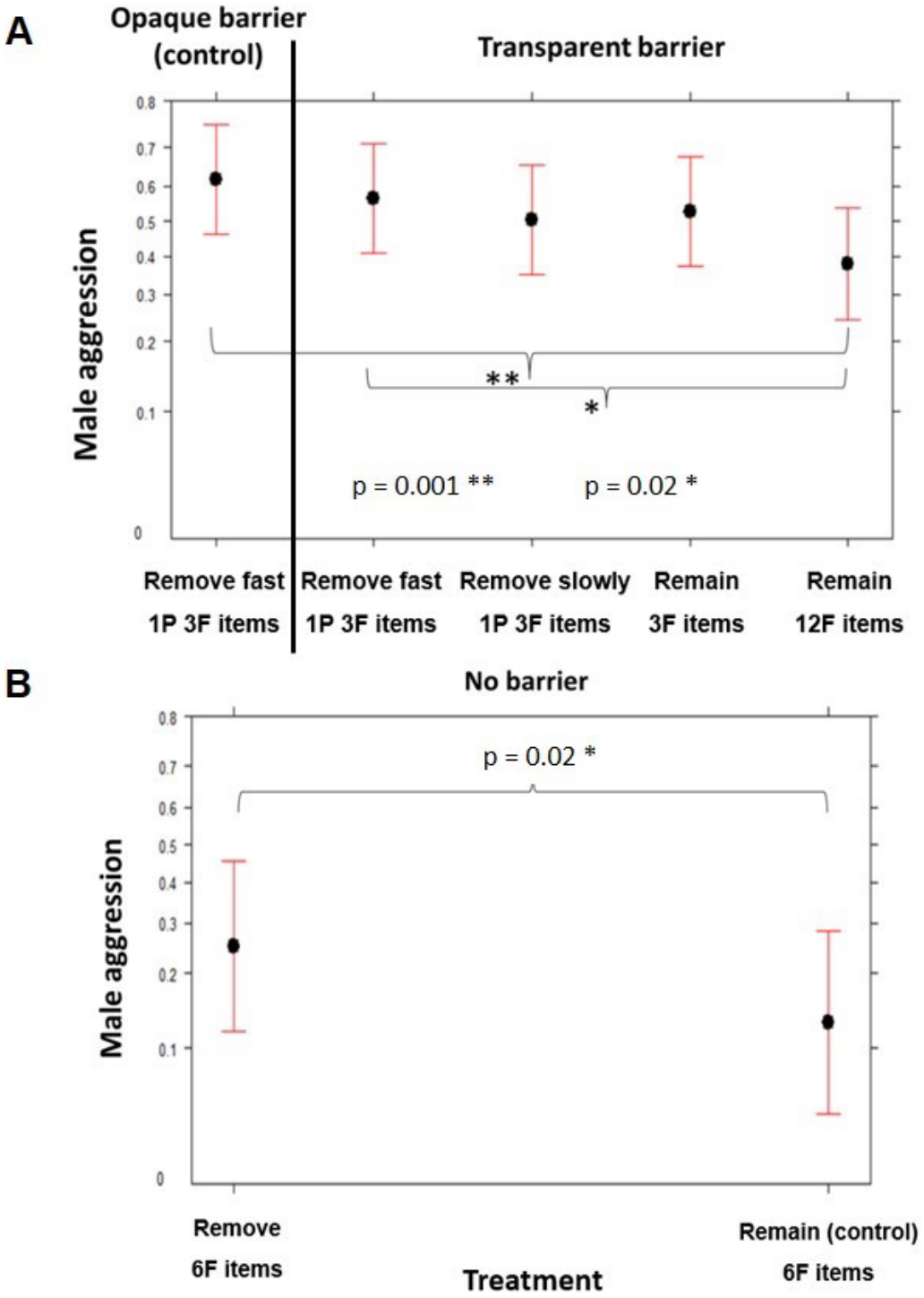

Figure 3: First experimental set (2016). Effect of the different treatments on the probability that males show aggression towards female partners. Twelve couples of *L. dimidiatus* performed twelve trials in each treatment. Shown are model mean probabilities of aggression and 95% confidence interval. (A) Male and female were separated with a Plexiglas barrier (transparent or opaque depending on the treatment). Males invariably received a permanent plate with 3 flake items. Females were treated in 5 different ways that are characterised by the female being visible or not ('transparent barrier' versus 'opaque barrier'), the number of prawn items ('P') and flake items ('F') and how the plate was moved by the experimenter in response to female foraging ('Remove fast', 'Remove slowly' or 'Remain). In removal treatments, the experimenter started removal of the plate in response to females eating the preferred prawn item. Fast removal meant that no further items could be eaten. Slow removal meant that the female could still eat any remaining flake items before the plate was out of the water. The barrier was only removed once the female stopped foraging and male aggression (present or absent) was scored for the following 30s. (B) Couples were not separated and split voluntarily between two plates (containing six flake items each) presented simultaneously at opposite corners of the same aquarium side. Females were treated in two different ways: the plate either remained stationary as for males, or it was moved away from the female through the aquarium, with adjusted speed in such a way that she remained close and could potentially eat items off the plate, around in the aquarium, past the male and out of the aquarium.

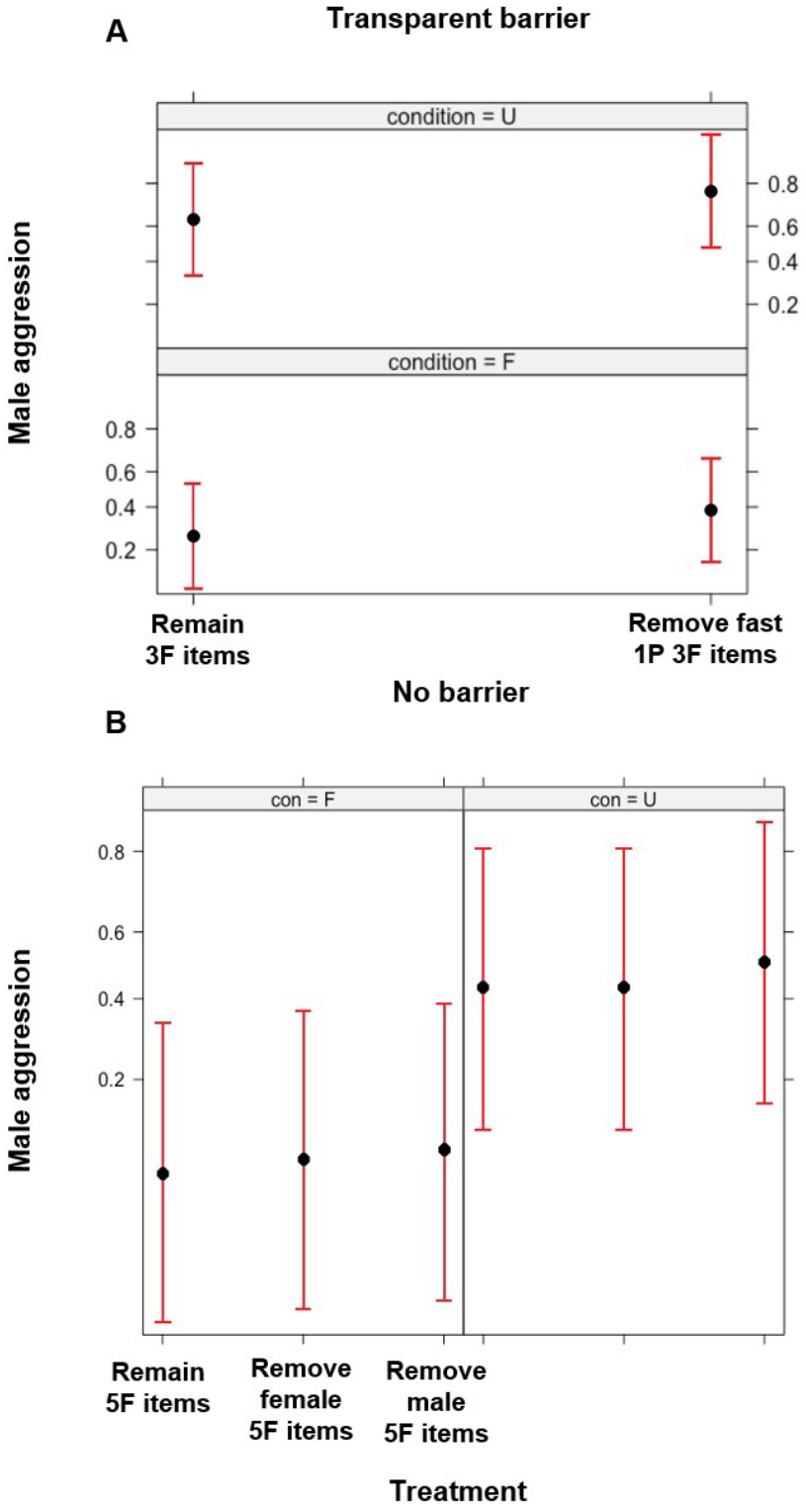

Figure 4: Second experimental set (2019). Effect of the different treatments on the probability that males show aggression towards female partners. Eighteen couples of *L. dimidiatus* performed twelve trials in each treatment. Shown are model mean probabilities of aggression and 95% confidence interval. (A) Male and female were separated with a transparent Plexiglas barrier. Males invariably received a permanent plate with 3 flake items. Females were treated in 2 different ways that are characterised by the number of prawn items ('P') and flake items ('F') provided on the plate. In the removal treatment, the experimenter started removal of the plate in response to females eating the preferred prawn item. Fast removal meant that no further items could be eaten. The barrier was only removed once the female stopped foraging and male aggression (present or absent) was scored for the following 30s. (B) Couples were not separated and split voluntarily between two plates (containing five flake items each) presented simultaneously at opposite corners of the same aquarium side. The plate either remained stationary for both individuals, or it was either moved away from the female through the aquarium or from the male, with adjusted speed in such a way that the individual remained close and could potentially eat items off the plate, around in the aquarium, past the partner and out of the aquarium.

Discussion

Our results provide several lines of evidence that personal immediate losses are not needed for male cleaners to engage in aggression towards females in a foraging context. In Study 1b, males were more aggressive to females when the latter apparently caused a food plate to leave. This aggression occurred despite the fact that males never gained access to food on the female's plate in this experiment. Similarly, in Study 2a, males chased females more frequently if the female's plate moved and left the aquarium, despite males not losing any items off their own plate. Thus, males were uninvolved bystanders in both experiments. Furthermore, there are good explanations why the other two experiments did not yield significant results (see below). Taken together, the results provide the first evidence for third-party punishment in a non-human species.

The precise cues that males may use to decide whether or not to punish warrant further investigation. As punishment was once triggered by fast removal of the plate (Study 1b, 2019) and once by slow removal of the plate (Study 2a, 2016), a food plate disappearing appears to be an important cue. However, when we included the treatment 'male plate moves slowly before being removed' in Study 2b (2019), males were less likely to chase a female partner when the female's plate was removed. One possible interpretation of this latter result is that males learn through personal experience that some plates leave even when they are not cheated, and that this learning affects subsequent punishment decisions. Nevertheless, this hypothesis cannot be addressed with the data we present here.

In Study 1a (2016), male aggression was highest in the control condition when the male was visually isolated from the female. This result was unexpected as it is not linked in any way to punishment for cheating clients or competition over food. By way of explanation, we suggest that male aggression could have been an artefact of visual isolation and linked to social control. Sex change by female *L. dimidiatus* has been shown to be largely induced by the combination 'male absence' and 'lack of being the target of aggression' (Robertson, 1972), and hence our male subjects may have asserted their dominance after reunification. Aggression as a means of social control has also been documented in other group-living species without sex change (Mulder and Langmore, 1993; Nakashima et al., 2000; Sakai et al., 2001). Further experiments would be needed to test this idea.

Male cleaner fish did not seem to adjust third-party punishment tendencies based on partner familiarity. Previous work has found that males are more aggressive towards unfamiliar females (Raihani et al. 2012). The effects we observed here are in the same direction, though not significant at conventional levels. We found no evidence to suggest that males adjust punishment frequency according to partner familiarity. This implies that male cleaners exhibited general behavioural rules with respect to third-party punishment. On a phenotypic level, this general rule ressembles human norms that were postulated to underlie third-party punishment in humans (Fehr and Fischbacher, 2004). Apparently, different mechanisms in humans and fish can cause similar decision rules.

In cases of third-party punishment in humans, the ways in which punishers benefit are still debated (Lehmann et al., 2007). A recent study showed that people are more likely to invest in third-party punishment when they believe that the cheat would also harm them, emphasizing the role of repeated encounters (Krasnow et al., 2016). As male and female cleaners interact repeatedly, it appears clear how male cleaner fish benefit from punishing as currently uninvolved bystanders. Previous research showed that punished females will behave more cooperatively during future interactions (Raihani et al., 2010). Such increased cooperation by females will benefit males directly in two ways. First, when they co-inspect a client the male experiences reduced risk that the client leaves because of female cheating. Second, even if the female inspects a client alone, increased levels of cooperation will increase the probability that this client will return for future inspections, due to the repeated nature of cleaning interactions (Bshary and Schäffer, 2002), and hence be accessible to the male. In conclusion, the rather uniquely human combination of social learning abilities and between-group

competition is not a pre-requisite for the evolution of third-party punishment. Instead, we suggest that other ecological features might also favour the evolution of third-party punishment in different systems. In the cleaner fish case, third-party punishment is facilitated due to the dominance asymmetry between male and female cleaners but also because cleaner pairs share a network of clients, where clients might sometimes return to the cleaning station but also have the choice between alternative service providers.

Ackowledgements

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Author contributions

R. B. and N. J. R. conceived the project. All the authors designed the experiments. M. A. collected the data in 2016 and P. S. in 2019. M. A. and P. S. performed the analyses and all authors analysed the results. M.A, N. J. R. and R. B. wrote the manuscript and revised it.

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Do cleaner fish know what others can and cannot see?

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Abstract

Much of human experience is informed by our ability to attribute mental states to others, a capacity known as theory of mind (ToM). Comparative research has shown that non-human primates possess some key components of ToM, like the ability to represent what others perceive and know. While evidence for ToM in animals to date has largely been restricted to primates and other large-brained species, the use of ecologically-valid competitive contexts hints that ecological pressures for strategic deception may give rise to ToM abilities in distantly-related taxonomic groups. In line with this hypothesis, we show that cleaner wrasse (*Labroides dimidiatus*) exhibit ToM capacities akin to those observed in primates in the context of their cooperative cleaning mutualism. These results suggest that ecological pressures for strategic deception can drive human-like cognitive abilities even in very distantly related species.

Keywords: Theory of mind; cleaner fish; audience effects; knowledge state attribution; perspective taking