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Causes and consequences of social phenotypic variation in the Caribbean facultative cleaning goby *Elacatinus prochilos*

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Résumé

Les animaux présentent des comportements sociaux d'une diversité fascinante qui ont longtemps attiré l'attention de chercheurs de différentes disciplines. Malgré un intérêt commun, certaines disciplines se sont plus concentrées sur les explications ultimes (fonctionnelles) des interactions sociales alors que d'autres se sont concentrées sur les explications proximales (mécaniques) de ces comportements. Cependant, afin de comprendre comment la sélection naturelle façonne les mécanismes sous-jacents, il est nécessaire d'utiliser une approche intégrative qui examine à la fois les explications mécaniques et fonctionnelles. Le but de mon projet de thèse était de comprendre les causes proximales et ultimes des variations sociales du gobie des Caraïbes *Elacatinus prochilos*. J'ai d'abord utilisé une approche intégrative combinant les données écologiques, comportementales, cognitives et d'anatomie cérébrale pour expliquer les potentiels mécanismes qui expliquent les variations phénotypiques comportementales observées. J'ai ensuite utilisé une approche comparative intra- et inter-espèce pour étudier comment les mesures cérébrales varient entre des espèces apparentées mais aux phénotypes habitat-alimentation différents. Les individus de l'espèce *Elacatinus prochilos* adoptent un des deux phénotypes suivants : nettoyeur ou habitant dans les éponges. Les gobies nettoyeurs sont flexibles dans l'utilisation de leur habitat et se nourrissent principalement d'ectoparasites d'autres espèces de poissons du récif. Au contraire, les gobies habitant dans les éponges vivent en groupes allant jusqu'à 70 individus et expriment une hiérarchie claire basée sur la taille des individus. Dans l'introduction générale, je présente des données de base qui révèlent les différences d'utilisation de l'habitat, de comportement social et de structure du groupe entre les deux phénotypes. Dans le premier chapitre, j'ai exposé des individus des deux phénotypes à des conditions sociales standardisées en laboratoire et j'ai étudié si les différences d'environnements sociaux et écologiques naturels influencent la flexibilité comportementale des adultes. Dans le second chapitre j'ai testé si les différences de phénotype habitat-alimentation permettent de prédire les performances d'apprentissage lors de deux épreuves de discrimination à deux choix où la réponse correcte était indiquée par des indices différents. Dans le troisième et dernier chapitre, j'ai comparé la structure cérébrale des deux phénotypes d'*E.prochilos* à celle de deux autres espèce du même genre avec des phénotypes habitat-alimentation diffèrent : le nettoyeur obligatoire *Elacatinus evelynae* et l'habitant des éponges obligatoire *Elacatinus chancei*. De façon surprenante, je n'ai pas trouvé de preuve que les différences de phénotype chez *E.prochilos* sont liées à des différences de préférence d'habitat, de règles de décisions sociales, de capacités d'apprentissage associatif et de structure cérébrale : nous ne savons pas comment les différences phénotypiques fonctionnent. Comme je n'ai trouvé de différence ni dans les mécanismes, ni dans la structure cérébrale, il est actuellement impossible d'expliquer quels mécanismes ont conduit à l'apparition d'un clade habitant les éponges et d'un clade nettoyeuse habitant les coraux. Cependant, j'ai trouvé des différences dans les aires cérébrales liées à l'axe sensoriel visuel/de la ligne latérale entre les deux clades, ce qui suggère des changements indépendants dans des aires cérébrales fonctionnellement corrélées qui peuvent être écologiquement adaptatifs. En conclusion, les résultats de mon étude challengent les concepts qui lient l'expérience individuelle à la flexibilité comportementale. Comprendre pourquoi les gobies sont une exception est un enjeu majeur pour les recherches futures.

Mots-clés: gobies nettoyeurs, comportement social, cognition, morphologie cérébrale

General Summary

The fascinating diversity of social behavior displayed by animals has long attracted the attention of researchers from different disciplines. Despite the common interest in the topic, some disciplines have focused more on the ultimate (functional) explanations for social interactions while others have mainly focused on the proximate (mechanistic) explanations for these behaviors. However, in order to understand how natural selection shapes the mechanisms underlying social behavior, it is necessary to use an integrative approach examining both mechanistic and functional explanations for behavior. The aim of my Ph.D. project was to understand the proximate and ultimate causes of social behavior variation in the Caribbean cleaning goby *Elacatinus prochilos*. First, I used an integrative approach that combined ecological, behavioral, cognitive and brain morphology data in order to unveil the potential mechanisms underlying the behavioral phenotypic variation observed in the system. Second, I used a within and between species comparative approach for investigating how brain measurements vary across closely related species with different habitat-feeding phenotypes. Individuals in the species *Elacatinus prochilos* may adopt two habitat-feeding phenotypes: cleaning or sponge-dwelling. Cleaning gobies, in general, are flexible in their habitat use and obtain most of their food by eating ectoparasites off other reef fish species. In contrast, sponge-dwelling gobies live in groups of up to 70 individuals and do express a clear size-based hierarchy. In the general introduction, I provide some background data that revealed the differences in habitat use, social behavior and group structure between the two phenotypes. In the first chapter, I exposed individuals from both phenotypes to standardized group conditions in the laboratory and asked whether the differences in their natural social and ecological environment impose constraints on adult behavioral flexibility. In the second chapter, I tested whether the habitat-feeding phenotype differences predicted learning performance in two discriminatory two-choice tasks that differed with respect to the relevant cues available to identify the correct choice. In the third and final chapter, I compared the brain structure of the two *E. prochilos* phenotypes to that of two other species in the genera that also differ in the habitat-feeding mode: the obligatory cleaner *Elacatinus evelynae* and the obligatory sponge-dwelling *Elacatinus chancei*. Surprisingly, I did not find any strong evidence that the differences between *E. prochilos* phenotypes are related to differences in habitat preference, social decision rules, associative learning skills, and brain structure. This means that at this moment, I cannot answer the question of how the differences between phenotypes work. Since I could not find differences in the mechanisms, or in brain structure, it is also currently impossible to answer what differentiation in mechanisms drove the evolution of a sponge-dwelling clade versus a coral-dwelling cleaning clade. However, I found differences in brain areas related to the visual/lateral line sensory axis between the obligatory cleaning versus the obligatory sponge-dwelling species, which revealed independent changes in functionally correlated brain areas that might be ecologically adaptive. In conclusion, the results of my study provide a challenge for various concepts that link individual experience to constraints in behavioral flexibility. Understanding why the gobies are an apparent exception will be the major challenge for future research.

Key words: cleaning gobies; social behavior; cognition; brain morphology.

Table of contents

General Introduction

I.1. Integrative approaches for the study of social behavior

In nature, we can observe a diversity of strategies employed by organisms for growth, survival, and reproduction. This diversity is generated through the combination of different morphological, behavioral, and physiological traits. Among these traits, behaviour is considered the most flexible. The vast majority of animal species adjust their behavior in response to environmental changes or to the behavior of other individuals. In the last few years, there is an increased interest in studying the causes and consequences of behavioral variation among individuals of the same population (Bergmüller and Taborsky, 2010). The main question is whether these behavioral changes represent unavoidable deviations from an optimal phenotype, or if the variations are a result of natural selection in environments where more than one strategy would lead to the same positive results in terms of fitness (Bergmüller, 2010). In order to answer this question, it is necessary to investigate which underlying mechanisms relate to behavioral variation if this variation is heritable and what the fitness consequences are for individuals expressing different behavioral phenotypes (Bergmüller, 2010).

Various researchers proposed that an integrative approach that combines mechanism and function is necessary for the study of social behavior (Bshary and Oliveira, 2015; Hofmann et al., 2014; McNamara and Houston, 2009; O'Connell and Hofmann, 2011a; Taborsky and Oliveira, 2012). Social behavior is a good candidate for integrative approaches because the complexity of the social environmental generates a high degree of unpredictability that individuals have to flexibly adjust to in order to optimize their response to the social stimuli (Taborsky and Oliveira, 2012). Most of the integrative and comparative approaches for the study of social behavior highlight the importance of simultaneously collecting behavioral, life history, ecological, and neurobiological data for as many species as possible (Hofmann et al., 2014; Rubenstein and Abbot, 2017; Taborsky et al., 2015). Alternatively, other integrative frameworks highlight the need to understand the mechanisms causing individual phenotypic plasticity and their consequences for the evolution of social behavior (Taborsky and Oliveira, 2012; West-Eberhard, 2003). Such an approach should be more appropriate for the study of the causes and consequences of intraspecific variation in social behavior.

The social competence approach proposes that behavioral flexibility should be studied in the same way as other types of phenotypic plasticity: focusing on the mechanisms that allow individuals to adapt to a variety of complex social environments (Taborsky and Oliveira, 2012). This suggestion is based on the fact that behavioral flexibility is a powerful way to adjust to environmental conditions, but might or might not be genetically determined and transferred across generations (Taborsky and Oliveira, 2012; West-Eberhard, 2003). Behavioral flexibility has been connected to many different underlying mechanisms (e.g. learning, neural circuits, neuroendocrine regulation, gene expression, epigenetic regulation) that link the social and ecological habitat to the expression of the appropriate social behaviour (Rubenstein and Hofmann, 2015; Taborsky and Oliveira, 2012). By identifying the mechanisms involved in behavioral flexibility we should gain insights into its evolutionary dynamics (Taborsky and Oliveira, 2012).

Although integrative approaches for the study of behavior are gaining prominence, there are still some challenges that prevent integrating proximate and ultimate questions to the same model species (Taborsky et al., 2015). This happens partially due to the long-standing differences in terminology and conceptual framework between mechanistic and functional research areas (McNamara and Houston, 2009; O'Connell and Hofmann, 2011a) but also because the species traditionally used by mechanistic research are not the same used in behavioral and ecological research (Taborsky et al., 2015). In this context, fish species are good candidate models for an integrative study of social behavior. This group shows a great diversity of social behaviors and social organizations (Bshary et al., 2014; Taborsky and Wong, 2017) and are a more accepted model for neuroendocrine manipulations than mammals or birds. Importantly, all vertebrates share genetic, developmental and physiologic mechanisms due to common ancestry, allowing scientists to generalize across clades (O'Connell and Hofmann, 2011b; Woolfe et al., 2004).

I.2. Social behavior, cognition, and brains

Many disciplines try to understand the relationships between social behavior, cognition, and brains. What evolutionary forces select for larger/more complex brains? How do social behavior and social complexity correlate with cognitive skills? Which brain characteristics best correlate with complex social interactions and cognitive abilities? Studies across different taxa have tried to answer these questions and have documented a diversity of positive correlations. For example, studies with humans, primates and other mammals have found that brain size is correlated to a number of life history, ecological, social, cognitive and behavioral traits (Aiello and Wheeler, 1995; Armstrong, 1983; Deaner et al., 2007; Dunbar and Shultz, 2017; Harvey and Bennet, 1983; Isler and van Schaik, 2009; van Schaik et al., 2012). Social learning, innovation, and tool use are correlated in primates (Deaner et al., 2006) and corvids can use social information for developing new tools (Kenward et al., 2006). Social complexity is correlated to better performance in transitive inference in corvids (Bond et al., 2003) and fishes (Grosenick et al., 2007). Social complexity and cognitive abilities are correlated to differences in brain architecture in primates, birds, and fishes (Burish et al., 2004; Dunbar and Shultz, 2007; Gonzalez-Voyer et al., 2009; Pollen et al., 2007). Given the many correlations found across different taxa, it may not be surprising that some studies have found inconsistent results. For example, one study with primates did not find the usual correlation between brain size and sociality (DeCasien et al., 2017) and studies with fish have found inconsistent correlations between brain size and habitat complexity or social organization (Ahmed et al., 2017; Pollen et al., 2007; Reddon et al., 2016).

The comparative approach has brought valuable insights into the relationship among social complexity, cognition, and brains across contemporary species. However, with the increase of the number of comparative studies increases the concerns about how to interpret results obtained with varied approaches and methods, and about how to choose the appropriate methods in the first place (Dunbar and Shultz, 2017; Logan et al., 2018; Pollen and Hofmann, 2008; Shettleworth, 1993). For example, researchers trying to understand why particular cognitive abilities evolve usually compare closely related species that face different cognitive challenges while researchers interested in general cognitive processes compare distantly related species in similar neutral tasks (Shettleworth, 1993). Inferring cognitive abilities by testing individuals in neutral tasks might be problematic because we cannot differentiate whether low performance is due to lack of cognitive abilities or because the task has no ecological relevance for the individuals (Gingins and Bshary, 2016; Prétôt et al., 2016a, 2016b; Salwiczek et al., 2012). Finally, comparative studies trying to understand how sociality and cognition relate to brain evolution current lack information on how natural selection actually modifies the mechanisms underlying behavior and cognition (Healy and Rowe, 2007; Herculano-Houzel, 2011; Logan et al., 2018). This is particular important for studies using brain size as the only neuro correlate in comparative studies as we currently lack the knowledge on about how brain size scales with body size, about how brain regions scale with brain size and finally, about how brain size is associated with cognition for many species (Logan et al., 2018).

Recently, Logan and collaborators (Logan et al., 2018) summarized all the concerns regarding comparative studies that try to understand the relationship between behavior, cognition and brain evolution, and proposed two alternative frameworks for reducing heterogeneity in comparative studies. First, a top-down approach focusing on cross-species correlations between brain measures and a trait of interest should be used for generating brain evolution hypotheses. Second, these hypotheses should be tested with a bottom-up approach that focus on directly testing behavior and cognition in individuals to determine how they relate to brain measures in these particular individuals of a particular species. The main argument of a bottom-up approach is similar to the argument of using a mechanistic approach for the study of social behavior in the sense that they both encourage researchers to have a better understanding of the causal links between the variables measured. In that context, a bottom-up approach focused on individuals within species with high behavioral divergence should be particularly useful in providing important insights into the ecological correlates and fitness consequences of variation in particular brain measures (Logan et al., 2018).

I.3. The Ph.D. thesis approach and objectives

The aim of my Ph.D. project was to understand the proximate and ultimate causes of social behavior variation in the Caribbean cleaning goby *Elacatinus prochilos*. I first used a bottom-up approach that integrated ecological, behavioral, cognitive and brain morphology data obtained from the same individuals while exposing them to ecologically relevant contexts. This approach aimed to unveil the potential mechanisms underlying the behavioral phenotypic variation observed in the system. Second, I used a top-down comparative approach for investigating how brain measurements vary across closely related species with different behavioral phenotypes. This approach aimed to compare the relative importance of ecological and phylogenetic factors in explaining the observed variation in brain structure and getting potential insights into how this trait evolved.

I.4. Study species and study site

The Caribbean cleaning gobies are an ideal model system for using an integrative approach for investigating the causes and consequences of intraspecific behavioral variation. The thesis focused on the social behavior variation found in the species *Elacatinus prochilos* but in one of the chapters and in some parallel projects I included two species of the same genus for comparative analyses: *Elacatinus evelynae* and *Elacatinus chancei*. Therefore, I will first give a short description of the *Elacatinus* group and then give more details about the main model species. The *Elacatinus* genus includes around 31 species and is separated in two main distinct phylogenetic clades, one comprising mostly cleaning species, and the other comprising sponge-dwelling (Colin, 2010; Ruber et al., 2003; Taylor and Hellberg, 2006). The cleaning species live on corals or substrata other than sponges, where they maintain cleaning stations and feed mostly on ectoparasites removed cooperatively from larger fish known as clients (Côté and Soares, 2011). In contrast, sponge-dwellers live in close association with barrel sponges or tubular sponges and feed mainly on microinvertebrates buried in the sponges' tissues (Colin, 1975). Interestingly, the habitat-feeding mode separation can occur within some species (Whiteman and Côté, 2004a, 2002). The species that present habitat-feeding mode separation are known as facultative and the ones that do not have such a separation as obligatory cleaning gobies (Côté and Soares, 2011). The reasons driving this intraspecific variation in habitat use are still poorly understood, but it is clear that it has great consequences with respect to the social organization of the individuals. The phenotypes inhabiting cleaning stations are usually found in socially monogamous pairs (Harding et al., 2003; Whiteman and Côté, 2003), the ones inhabiting tubular sponges are found in pairs or small groups (Colin, 1975) and the ones inhabiting large barrel sponges are found in large groups of up to 70 individuals (Personal observation, (Whiteman and Côté, 2004b)). Here and after we will refer to the habitat-feeding phenotypes (individuals living in similar ecological and social characteristic) or species living in cleaning stations as cleaning gobies and the ones living in sponges as sponge-dwelling gobies or sponge-dwellers.

The study was conducted in Barbados, at the Bellairs Research Institute. In Barbados, three species of the *Elacatinus* genus co-occur: *E. evelynae*, *E. prochilos* and *E. chancei*. The two former species belong to the cleaning clade and the latter to the sponge-dwelling clade. *E. evelynae* is an obligatory cleaning species (In Barbados this species is mostly found in cleaning stations, but it has been registered as being facultative on another Caribbean island, St. Croix (White et al., 2007)), *E. prochilos* is a facultative species and *E. chancei* is an obligatory sponge-dwelling species found in tubular sponges. The main core of my work was conducted on the North and South Bellairs fringing reefs on the west coast of Barbados (**Fig. 1**). The fringing reefs of the west coast are characterized by four reef zones: the back reef, reef crest, spurs and grooves and fragmented spur zones (**Fig. 1**) (Lewis, 1960). After the fragmented spur zones, a sand stretch separates the fringing reefs from the offshore reefs, with patchily distributed reef islands occurring in some areas (hereafter called patch reefs). The barrel and tubular sponges are more abundant in the patch reefs and almost absent in the spurs and grooves zones (personal observation). The two zones are separated by 5 to 15 meters of sand.

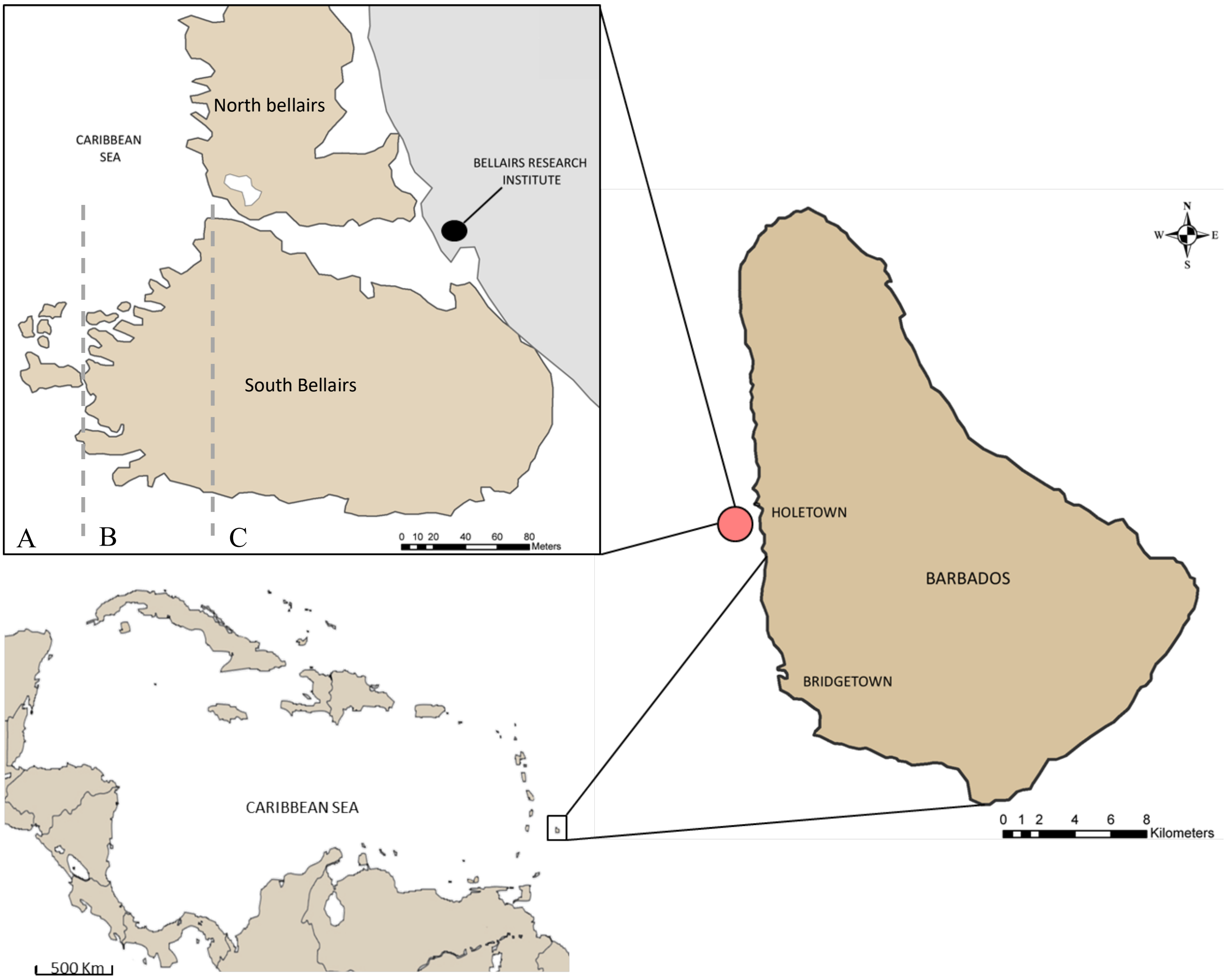

Figure 1: Study area map giving details of the location of Barbados in the Caribbean, the location of the research stations and the location of the main site of the study in the west coast of Barbados (red dot - North and South Bellairs reefs). The detail of the reefs show the three characteristic zones of the reef (A – patch reefs, B – spurs and grooves, and C – reef crest).

I.5. Thesis structure

In the background data section, I first present some important descriptive data about the phenotypic behavioral variation observed in *E. prochilos*. I considered that it was important to confirm the results obtained in studies conducted more than 10 years ago in order to make sure that the differences observed then were consistent with the situation during my research project. For that purpose, I conducted field observations and registered all intra- and interspecific interactions for both phenotypes. I also conducted visual transects to register the habitat use patterns and number of individuals occupying cleaning stations and barrel sponges. Finally, I present in this section some preliminary results of genetic analysis and initial attempts of conducting translocations and habitat choice experiments. This data can give us an indication of potential mechanisms involved in the observed differences in habitat use.

In the first chapter, I tested whether the social environment experienced in the wild affects adult behavioral response in standardized laboratory experiments in the contexts of group formation and food sharing. The experiment consisted of creating groups composed either of cleaning or sponge-dwelling individuals, in a way that simulated the social hierarchies observed in the wild and introducing a food context. With this experiment, I aimed to understand whether the decision rules on how to interact towards individuals of different sexes and status would follow what is observed in the wild or would be flexibly adjusted according to the context.

In the second chapter, I tested whether individuals from each phenotype use different cues for associative learning in a food context. For that purpose, I tested the same cleaning and sponge-dwelling gobies that were tested in the group manipulations in two discriminatory two-choice tasks that differed with respect to the relevant cues available to identify the correct choice. In the pattern-cued task, individuals had to identify the specific pattern of a plate that consistently provided food, while in the spatial task they had to identify the location of a plate where food was consistently provided. We predicted that the tasks would have different ecological relevance to the phenotypes and by comparing their performance in the two tasks I expected to understand how general individual cognitive performance is.

For the third chapter, I removed the brains of the gobies tested in the previous chapters and compared the brain structure of the two phenotypes to other two *Elacatinus* species found in Barbados: the closely related and obligatory cleaning goby *Elacatinus evelynae* and the distantly related and obligatory sponge-dwelling goby *Elacatinus chancei.* We specifically measured the relative volume of different brain areas and nuclei and used different types of analyses for comparing how the brain structure differs across species and how the different brain areas change in relation to each other and to the total brain size. By comparing the brains of the two phenotypes with the two species we aimed to unveil whether variations in the relative volume of brain parts are better predicted by ecological or phylogenetic factors.

I.6. Background data

The data that I will present here is the product of exploratory or pilot studies that provided important background information about the system. This data helped me to establish the research questions for the thesis' chapters and to obtain extra information about the system. For each study, I will shortly present the main motivation for collecting the data, the methods, data analysis, and main results. All the data analysis were conducted using R version 3.4 (R Core Team, 2017). Then I discuss the main conclusions and implications of these results.

I.6.1. Survey of gobies natural distribution

This data was collected in order to explore the distribution of the gobies at the main study site. We conducted underwater visual surveys in August of 2015 on the patch reefs of the North and South Bellairs reefs and the spurs and grooves zone of the South Bellairs reef. The patch reefs are usually on the seaward edge of the fringing reef (7 to 10 meters deep) and are separated from the spurs and grooves zone (3 to 7 meters deep) by a sandy stretch of 5 to 15 meters. We performed in total 14 strip transects perpendicular to the shore (20 x 2 m each; 7 on patch reefs and 7 on spurs and grooves) for the surveys of the gobies abundance. We registered the size and habitat use of all *Elacatinus prochilos* observed in each transect. Observers visually estimated the gobies size by placing a measuring tape on the exact place the cleaner was occupying on the substrate (Sazima et al., 2000). The surveys were conducted from 9:00 to 12:00h on four different days. We analyzed the data by applying a logit transformation to the gobies density and including it as dependent variable in a linear model (function lm from package stats (R Core Team, 2017)) that included substrate (barrel sponges x other substrates), reef zone (patch reefs x spurs and grooves) and their interaction as fixed factors. The model was validated with graphical inspection of the distribution of the residuals and the significance of the main factors and interactions tested with the function Anova from package car (Fox and Weisberg, 2011). The density of gobies significantly varied across substrates ($F = 17.5$, $p < 0.001$) and reef zones ($F = 13.9$, $p < 0.001$). The model indicated that there was a significant interaction between substrates and reef zones ($F = 44.3$, $p < 0.0001$). There were significantly more gobies in the sponges in the patch reefs zone and significantly more gobies in other substrates in the spurs and grooves zones (**Fig.2**).

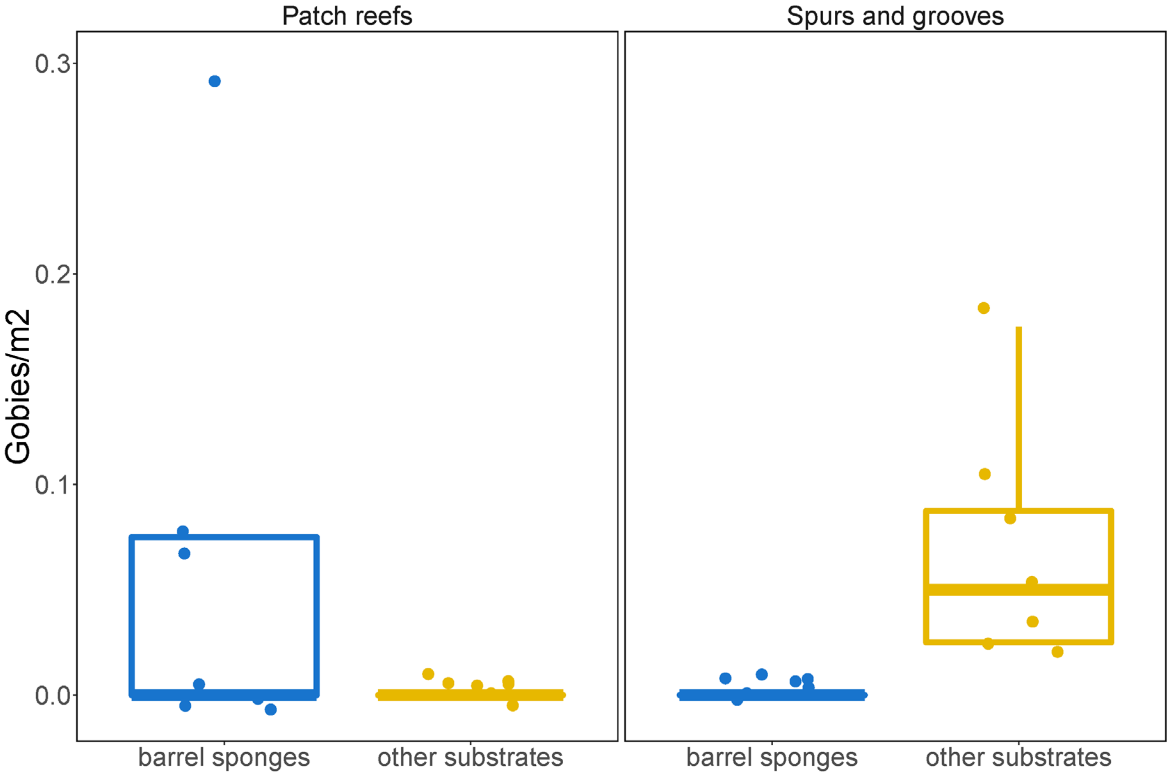

Figure 2: Density of *E. prochilos* gobies (gobies / m2) on barrel sponges from the species *Xestospongia muta* (barrel sponges) or other substrates found on two different reef areas within the North and South Bellairs reef: patch reefs and spurs and grooves. The central line in boxes, the boxes, and the whiskers indicate the median, Q1, Q3, and 1.5*QR.

I.6.2. Behavioral observations

Different ecological and behavioral studies have highlighted the differences in cleaning and social behavior between cleaning gobies and sponge-dwellers (White et al., 2007; Whiteman and Côté, 2002, 2004b). Here we conducted field observations to confirm the patterns previously described. Behavioral observations were conducted from March to June of 2016, on SCUBA, between 08:30 and 17:30h, from a distance of 2 m, at the South Bellairs reef (13.191542° N, 59.641882° W) on the west coast of Barbados. We conducted approximately 20 hours of underwater observations in total, with individual observations on 27 cleaning gobies (10h) and 25 sponge-dwellers (9h14min) distributed in 23 cleaning stations (3.5 to 8.7 m deep) and 18 sponges (4.5 to 9.4 m deep). Observation duration for each individual varied from 15 to 30 minutes. We registered the frequency, duration, and species involved for all cleaning interactions that occurred and we also noted all aggressive interactions (chases) toward conspecifics. We used the Welch Two Sample t-tests for testing whether cleaning and sponge-dwelling gobies differed significantly with respect to the following variables: number of individuals occupying the same cleaning station or sponge, frequency and average duration of cleaning interactions, proportion of time spent in cleaning interactions and number of chases given (frequency in which gobies chased away conspecifics or heterospecifics from their territories). We ran the t-tests by using the function t.test from package stats (R Core Team, 2017), after which we used Holm's p-values adjustment of multiple comparisons (Holm, 1979) with the function p.adjust from package stats (R Core Team, 2017). We found that cleaning stations were inhabited by significantly fewer gobies than barrel sponges (**Fig. 3a**, t = -4.69, corrected p-value < 0.001). In terms of cleaning activity, cleaning gobies spent more time on cleaning interactions than sponge-dwellers (**Fig. 3b**, t = 2.48, corrected p-value= 0.039). We registered a total of 149 cleaning interactions with cleaning gobies and only 13 interactions with sponge-dwellers. Cleaning gobies engaged in more cleaning interactions per minute than sponge-dwellers (**Fig. 3c**, t = 3.48, corrected p-value = 0.004), although the average duration of these interactions did not significantly differ between phenotypes (**Fig. 3d**, t = 2.12, corrected p-value=0.063). Clients initiated the interactions with cleaning gobies 94.8% of the time (129 out of 136 interactions for which initiators were registered) and in 84.6% of the cases with sponge-dwellers (11 out of 13 interactions). Finally, sponge-dwelling gobies significantly chased conspecifics more often than cleaning gobies did (**Fig. 3e**, t = -4.64, corrected p-value < 0.001).

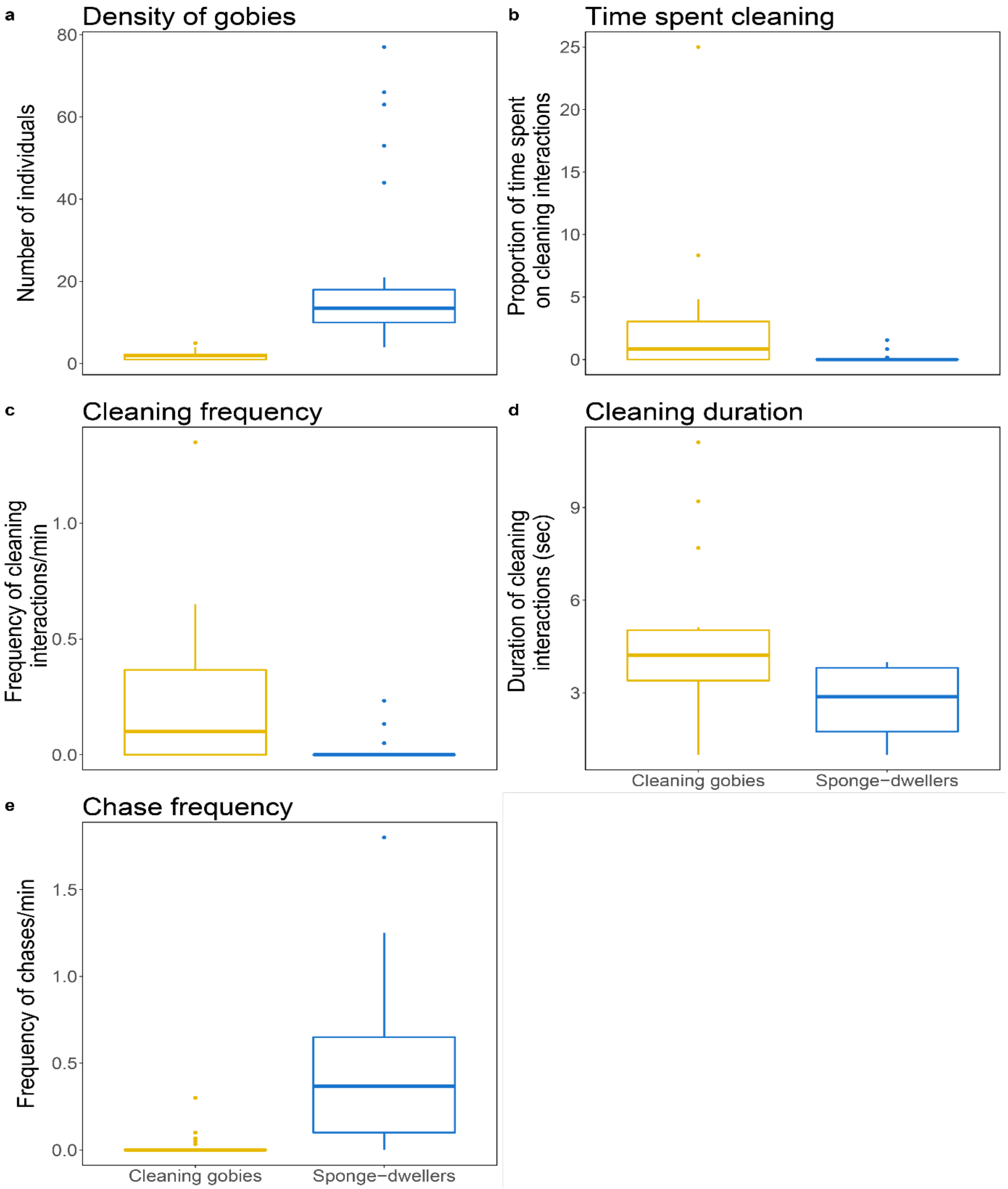

Figure 3: Natural abundance and behavior of cleaning gobies and sponge-dwellers registered in the field: (a) abundance of cleaning gobies at cleaning stations (n=23) and of sponge-dwellers at barrel sponges (n=18); (b) Frequency of cleaning interactions per minute of observation of individual cleaning gobies (n=27) and sponge-dwellers (n=25); Average duration (sec) of cleaning interactions registered for cleaning gobies (n=149) and sponge-dwellers (n=13); (d) Proportion of time cleaning gobies (n=27) and sponge-dwellers (n=25) spent on cleaning interactions (e) Frequency of chases per minute of observation of individual cleaning gobies (n=27) and sponge-dwellers (n=25). The central line in boxes, the boxes, and the whiskers indicate the median, Q1, Q3, and 1.5*QR, consequently. Dots represent outlier values (>median+1.5*QR).

I.6.3. Experimental test of habitat preference in the field

The only study that investigated microhabitat selection in *E. prochilos* found inconsistent results with respect to which habitat is preferred by the gobies (Whiteman and Côté, 2004a). Resource selection indices suggested a preference for sponges, and occupancy of sponges was higher than of corals. However, after experimental removal of gobies, adult population increase was higher on corals than in sponges (Whiteman and Côté, 2004a). Here we aimed to unveil the habitat preference of cleaning and sponge-dwelling individuals by directly testing their habitat preference in the field. These tests were conducted in May of 2018. A total of 10 sponge-dwelling gobies and 9 cleaning gobies *E. prochilos* were collected from both corals and barrel sponges on a patch reef in the Carlisle Bay (13.085908° N, -59.612192°W), on the southwest coast of Barbados. It should be noted that this site is located in a patch reef zone (where the barrel sponges are particularly abundant), and it is very likely that the gobies captured from corals were just temporarily on this substrate but actually establish their territories in the sponges (we have seen this happens many times when tagging individuals for a parallel project). Therefore, this test was more valid for testing whether sponge-dwelling gobies prefer sponges or not. The individuals were tested in two different sandy areas adjacent to the patch reef, where we placed one brain coral head and one barrel sponge, separated by 1.5 to 2.0 meters and isolated from the surrounding reef by at least 5 meters. In the first area, we used one brain coral head of the species *Pseudodiploria strigosa* and a barrel sponge of the species *Xestospongia muta*. in the second area, we used a brain coral head of the species *Colpophyllia natans* and another *Xestospongia muta* barrel sponge. The corals and sponges had similar diameters and heights. The North/South or East/West orientation of the substrates were changed between testing days, whenever possible, to avoid that the predominant currents affected the gobies choice. The gobies were captured with hand nets after being sprayed with an anesthetizing mixture of clove oil and ethanol (1:4). All gobies were larger than 2 cm and smaller than 3 cm. Immediately after being captured, they were placed in sealed plastic bags and left inside it to recover from the anesthetic for 15 to 30 minutes. After that, we transported the gobies to the sandy areas and individually tested them in a nearly counterbalanced order that depended on the number of individuals from each group collected in each testing day. Before the trial, we transferred each goby to a transparent cup and placed it in the middle distance between the coral and the sponge. The cup would be positioned with its opening facing down and slightly dug into the sand. The goby would be left to acclimate in the cup for one minute, during which it would be able to have visual contact with both habitats. The experimenter would then lift the cup with a string that was attached to its bottom. The experimenter was at least 3 m vertically above the goby before lifting the cup. The first habitat to be touched by the goby was then registered. Finally, we recovered the gobies from the testing habitats immediately after they made a choice and returned them to the adjacencies of their original territories. The trials were conducted from 10:00 to 16:00h and took 4 days to be completed. We tested for differences in preference between the two phenotypes by running a logistic regression (family=binomial, link=logit) using the function glm from package stats (R Core Team, 2017). The habitat of choice entered in the model as a categorical response (coral or sponge) and the habitat of origin, where the gobies were captured, as a categorical explanatory variable. The model was validated with graphical inspection of the distribution of the residuals and the significance of the main factors and interactions tested with the function Anova from package car (Fox and Weisberg, 2011). We found no significant differences between gobies captured form corals and gobies captured from sponges in the proportion of individuals that chose each habitat (**Fig. 4**, LR $\chi2$ = 0.006, p = 0.9372).

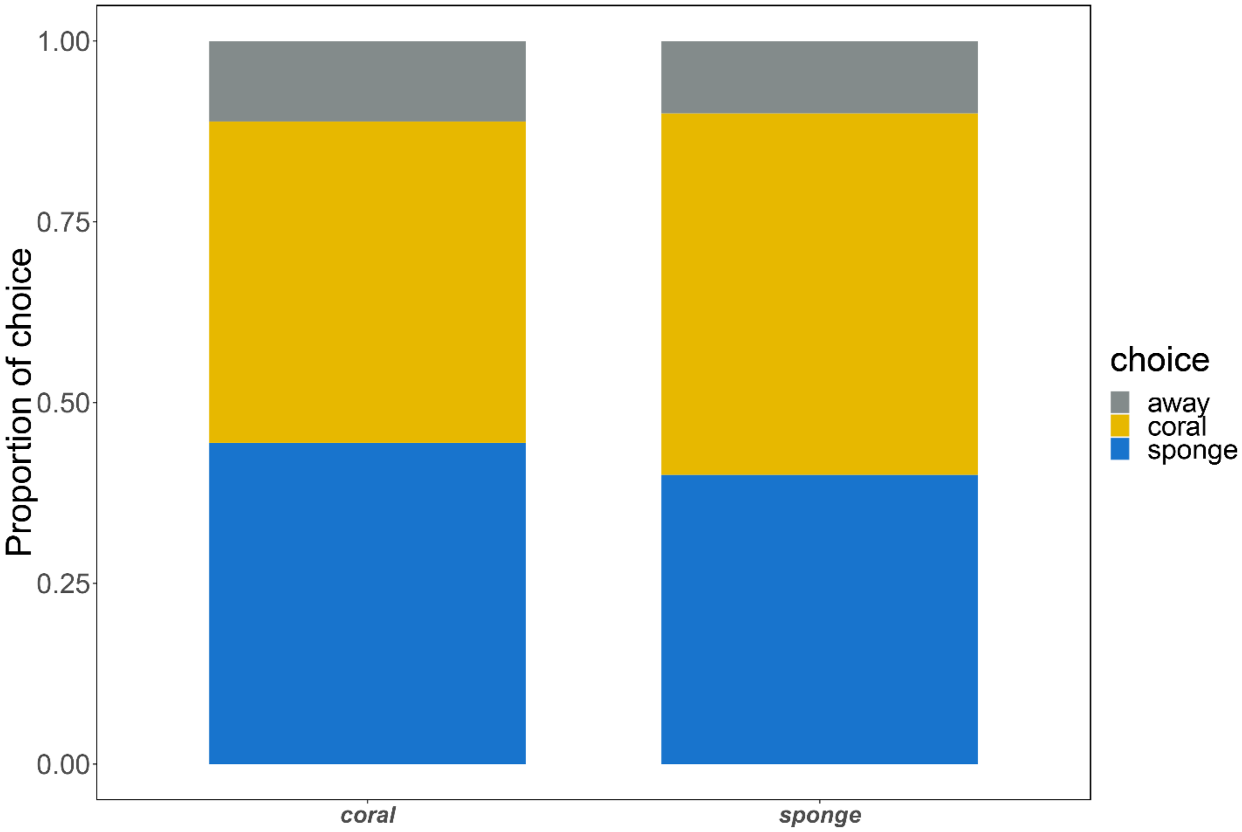

Figure 4: Proportion of *E. prochilos* collected from either corals or sponges (x-axis), which chose coral (yellow) or sponge (blue) within the experimental area or that swam away without choosing one of the two available habitats (grey).

I.6.4. Translocation trials

We aimed to investigate how flexible cleaning and sponge-dwelling gobies were with respect to habitat use by assessing their permanence and behavior after translocations between habitats. The translocations were conducted in the South Bellairs reef, from March to July 2016. We captured 15 cleaning gobies and 15 sponge dwellers, each from different cleaning stations and sponges, except for two cleaners that were captured at the same cleaning station but after a 25 days' interval. Cleaners and sponge-dwellers were captured by spraying a mixture of clove oil and ethanol (1:4) and using hand nets or plastic bags. Individuals in the plastic bags were then transferred to the hand nets, where fish were injected with elastomers implants. With a few exceptions when we couldn't capture the dominant individual after observation, the captured individuals were the largest dominant ones from both cleaning stations and sponges. After injection, individuals were returned to plastic bags and assigned to one of the three following treatments: (i) translocation (n=13): individuals from cleaning stations were transferred to sponges and individuals from sponges transferred to cleaning stations (separated by at least 10 meters); (ii) tag control (n=13): in order to assess the influence of elastomers injections, we swam with individuals inside the plastic bags for 2 minutes but returned them to the site of capture. (iii) habitat change control (n=4): individuals from cleaning stations were transferred to a different cleaning station and individuals from sponges to a different sponge. In all treatments, individuals were captured in the evening and placed inside cages that were positioned at close proximity from the destination sponge or cleaning station (transparent plastic bottles that permitted visualization of the surroundings, covered at the opening with a mesh net to allow water circulation and with a shelter (PVC pipe) inside). Fish were left overnight in the cage and released the next morning. Behavioral observations took place before capture, after cage release, the evening of the release day and the next morning. Due to logistical problems, we couldn't monitor all individuals with the same frequency after translocation experiments, so I will present the results for two time periods: the next day and next week. It is important to note that some individuals were monitored only after a week of the release, others only at the next day and some at both periods. From the 2 sponge-dwellers assigned to habitat control treatment, one was found dead on the next day and the other disappeared on the next day. From the 2 cleaners, one stayed until the next day but disappeared before a week and the other disappeared the next day. Data for tag control and translocations are presented in **Figure 5**. As can be seen, the frequency of disappearance was very high, independently of the treatment. Thus, it appears likely that marking with elastomer causes increased mortality under field conditions, or at least to a loss of dominance status leading to migration/expulsion and hence disappearance. Nevertheless, the data indicate that at least cleaning gobies can adjust to living in sponges. Such flexibility provides some evidence that the two eco-types belong to the same population, i.e. that there is gene flow between eco-types.

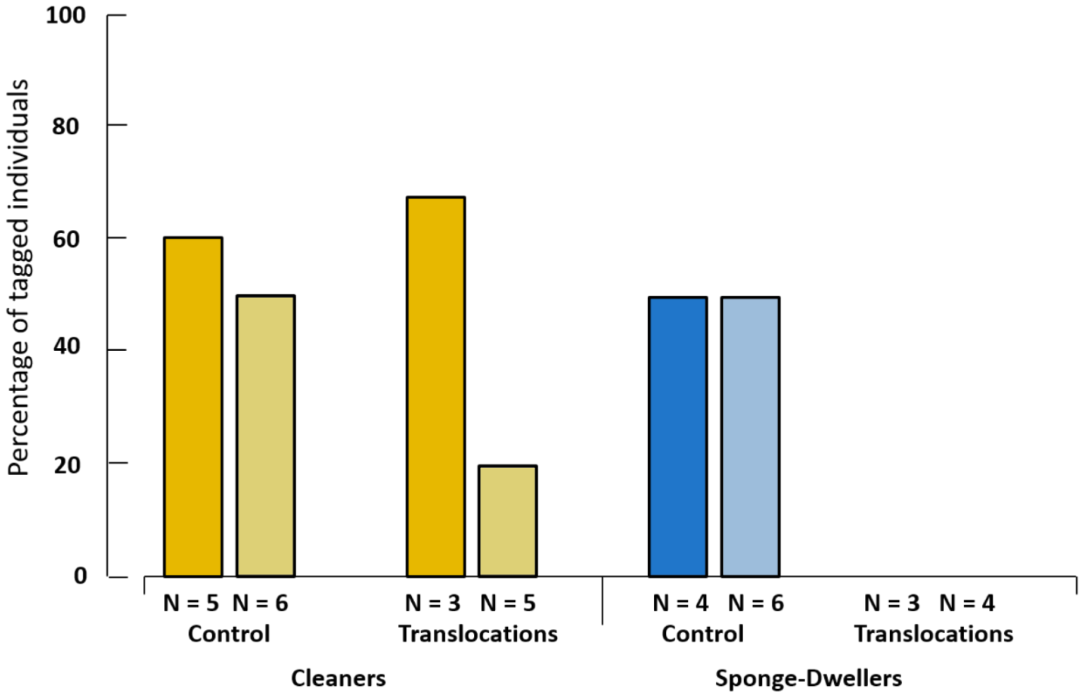

Figure 5: Percentage of cleaners (yellow) and sponge-dwellers (blue) that remained in the same place after a day (dark colors) and/or a week (light colors) of being tagged and released in the same original habitat (control) or translocated to a different habitat (translocations).

I.6.5. Genetic analysis

In order to investigate whether differences between cleaners and sponge-dwellers have a genetic component, we captured some individuals for genetic analysis. We captured 13 cleaners and 13 sponge-dwellers from South Bellairs (20) and North Bellairs (6) reefs. Fish were captured with a mixture of clove oil and alcohol, sacrificed with a clove oil overdose and preserved in 95% alcohol until analysis. Total genomic DNA was extracted from pectoral fins tissue and 444 bp from the mitochondrial cytochrome b (mtcyb) gene were amplified through polymerase chain reaction (PCR). The analyses results provide evidence that individuals from both habitats belong to the same species (nucleotide diversity= 0.0022, haplotype diversity= 0.67, FST=0.015, P>0.05).

I.7. Background data conclusions

This preliminary assessment of the *E. prochilos* gobies gave us some important insights about the differences and similarities between phenotypes. Concerning the habitat use differences, the occurrence of the cleaning goby phenotype seems to be a consequence of the distribution of sponges in the reefs of Barbados (**Fig. 2**). Our data showed that in the patch reefs, where the sponges are more abundant (personal observation) the gobies are exclusively found in the barrel sponges. In contrast, in the spurs and grooves, where only a few sponges are present (personal observation) most of the gobies are found on other substrates. This indicates a potential preference for sponges since substrates suitable for cleaning stations seem to be largely available in the patch reefs (personal observation). However, our preference tests conducted at another patch reef site with even more sponges available did not confirm that sponge-dwellers or gobies found on corals (but not necessarily establishing territories on corals) had a spontaneous preference for sponges (**Fig. 4**). However, the sample size for this particular experiment was quite low and there are at least two additional potential problems. First, the gobies might have been too stressed by the handling to make a proper choice. Second, maybe gobies only have a preference for sponges that are already occupied by other gobies. Therefore, more tests need to be conducted in order to obtain a better picture of habitat preferences. In the general discussion, I will give more information about a parallel project under my co-supervision that aims to better understand the mechanisms causing habitat use variation in *E. prochilos* gobies.

Concerning the social organization and behavioral differences, the results from the field observations conducted in this study confirmed the patterns observed in previous studies with the same species and in the same reefs (Whiteman and Côté, 2004a, 2004b). Cleaning stations are usually occupied by 1 to 5 individuals and sponges by groups of 3 to 77 individuals, cleaning gobies do engage more often in cleaning interactions and sponge-dwelling gobies do engage in more aggressive interactions per minute than cleaning gobies. Concerning flexibility, the translocation experiments revealed that 3 cleaners stayed for at least a few days at the sponges while no sponge-dwellers stayed at the chosen cleaning stations. However, we have to be very cautious in interpreting these initial data. Besides the very small sample size, we couldn’t control for the sex of the individuals being translocated, so it could be possible that only females were translocated from cleaning stations to sponges and that females are more flexible than males. Second, while sponges are well delimited and easy to monitor, the location and borders of the cleaning stations are more difficult to delimit. We tried to translocate the sponge-dwellers to cleaning territories that we had observed for a couple of days and that had always been inhabited by cleaning gobies, but it remains unknown whether the habitat quality of the chosen cleaning stations and sponges were similar or not. At least for the period of our monitoring, we never saw a translocated sponge-dweller returning to a sponge. Therefore, it could also be possible that the sponge-dwellers did not stay at the selected cleaning station, but moved to another one. Finally, the genetic analyses indicate that the two phenotypes belong to the same species, but this was based on only one genetic marker and therefore genetic causes of behavioral variation cannot be excluded at this stage. For the studies presented in the following chapters, I always tried to maximize the differences between cleaning gobies and sponge dwellers and for that purpose, I always collected cleaning gobies from sponges-free zones. Furthermore, I always tried to collect cleaning gobies adult couples and adult sponge-dwelling gobies from groups larger than five individuals.

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Chapter 1 | Social experience does not affect how the facultative Caribbean cleaning goby *Elacatinus prochilos* behave towards conspecifics in a group manipulation experiment.

Abstract

Animals living in groups often have to decide how to behave and respond to other members of the group. The decision-making process can be influenced by a variety of pre-determined or plastic mechanisms, which can be expressed in different stages of an individual's life. The social competence approach for the study of social behavior provides a very useful framework for investigating how individuals adjust their social behavior in order to optimize their social relationships. This framework has been very successful in explaining how the early social environment predicts social competence in the context of size-based dominance hierarchies. Nevertheless, most, if not all, of these studies manipulated the early social environment in captivity. Here we aimed to investigate how social experience affects an individual's behavior in a group with experiments on wild-caught individuals that have experienced naturally occurring variation in their social environment. The model system for this study was the facultative cleaning goby species *Elacatinus prochilos*. In this species, individuals may adopt two habitat-feeding strategies: cleaning or sponge-dwelling. The habitat-feeding phenotype has strong consequences for the social organization and social behavior of the individuals: Cleaning gobies live in social monogamy and are very aggressive towards large same-sex intruders while sponge-dwelling gobies live in large groups and express a clear size-based hierarchy that includes both sexes. We exposed individuals from both phenotypes to standardized group conditions in laboratory and asked whether the differences in their natural social and ecological environment impose constraints on adult behavioral flexibility. Surprisingly, we found that both phenotypes seem to be able to make monogamous pairs and, at the same time, keep a dominance hierarchy in the group. We hypothesize that in smaller/larger groups, the decision mechanism of cleaning and sponge-dwelling gobies on how to behave towards individuals of the opposite sex might change. Moreover, the possibility of adjusting the decision mechanisms should remain to the adult stage if group sizes in the wild for both phenotypes are changeable. Further studies should test these hypotheses in order to investigate why keeping a variation/plasticity in the decision rules at an adult stage is important for this species.

1.1. Introduction

Animals living in groups are often exposed to multiple social possibilities that require decision-making. For example, they have to decide whether to cooperate, defect, compete or mate with another member of the group. The decision-making process depends on the individual's ability to perceive and evaluate the social and ecological environment and determine the appropriate behavior (Taborsky & Oliveira, 2012). Such an ability to regulate the expression of social behavior in order to optimize social relationships is referred to as social competence (Taborsky & Oliveira, 2012). Socially competent behavior may sometimes be quite straightforward, for instance, when dominants are aggressive and subordinates, submissive. The more stable the environment is, the less advantageous it should be to keep collecting further information (Fawcett and Frankenhuis, 2015; Frankenhuis and Panchanathan, 2011; Stamps and Krishnan, 2014; and empirical examples in Taborsky, 2017). Flexible decision rules of adults, including many "if, then…" rules, may be under positive selection only if the social environment is highly variable, but at the same time, such flexibility might be constrained by the fact that social decision-making is produced by a single neural circuit (O'Connell & Hofmann 2011; Bshary & Oliveira, 2015).

There are other reasons why behavioral flexibility may be constrained, especially in an adult stage. Behaviors might have a genetic component and/or the genotype might interact with the environment during development to allow an individual to adjust to the current local conditions, as long as they are predictive of the individual's future conditions (developmental plasticity; West-Eberhard, 2003). Often, organizing effects of hormones are involved in preparing an individual for the overall life history trajectory. Behavioral endocrinologists have studied the organizing effects of hormones during ontogeny as an approach to explaining consistent individual phenotypic variation (e.g.: maternal effects like yolk testosterone levels in birds (Schwabl, 1996); the effects of the sex of neighbour embryos on adult behaviour (Ryan and Vandenbergh, 2002); early life conditions in lizards influencing adult territoriality (Moore et al., 1998)). Furthermore, learning plays such an important part in the development of behavior that early experimental psychologists assumed that all behaviors are learned (Wynne, 2001). Different reinforcement regimes and/or different exposures during early life stages may lead to consistent behavioral differences (e.g. superstitions in pigeons (Skinner, 1948); various mechanisms of imprinting in birds (Bolhuis, 1991)).

Behavioral flexibility is particularly advantageous when living in stable groups with size-based dominance hierarchies. As individuals grow older and larger, with the largest group members dying off, they should show increasing dominance and less submissive behavior. The group structure of social hierarchies often results in asymmetric access to food and reproductive partners by the individuals, which imposes a strong selection force toward getting a dominant position (reviewed in Clutton-Brock and Huchard, 2013). The accurate representation of the dominance relationships can help individuals to scale up in the hierarchy, with or without forming effective social alliances (Cheney and Seyfarth, 2005). Information about social status can be obtained by physical conflict, visual, chemical, olfactory and auditory cues or a combination of all that signals (Fernald, 2014). Most importantly for the current study, the social competence of individuals in social hierarchies can be affected by previous experience (Bastian et al., 2003; Branchi et al., 2013a, 2013b, 2006; D'Andrea et al., 2007; Fischer et al., 2015). Nevertheless, all these studies manipulated the early social environment in captivity. Therefore, it would be useful to complement the data with experiments on wild-caught individuals that have experienced naturally occurring variation in their social environment.

This study focused on the Caribbean goby species *Elacatinus prochilos* as a suitable model for testing whether social experience affects the way individuals interact with conspecifics. *Elacatinus prochilos* individuals may adopt two habitat-feeding strategies: cleaning or sponge-dwelling. Cleaning gobies, in general, are flexible in their habitat use and obtain most of their food by eating ectoparasites off other reef fish species (Côté and Soares, 2011). They live in social monogamy and benefit from guarding a large mate (Whiteman and Côté, 2003). Mate fidelity is maintained by strong aggression toward large same-sex intruders (Harding et al., 2003; Whiteman and Côté, 2003). Moreover, cleaning gobies females and males share the same territory and can even share the same client, in a cooperative manner (Soares et al., 2009). In contrast, sponge-dwelling gobies live in groups of 3 to 70 individuals (background data, (Whiteman and Côté, 2004)) and express a clear size-based hierarchy that includes both sexes (Whiteman and Côté, 2004). They rarely clean but mainly feed on polychaetes inside the barrel sponges, where dominance yields access to the best foraging spots (Whiteman and Côté, 2004). Dominant individuals maintain exclusive territories, monopolizing areas with high food abundance, while subordinate fish occupy broader home ranges (Whiteman and Côté, 2004).

Given these major differences in social organization between cleaning gobies and sponge dwellers, our aim was to determine whether experience affects adult behavioral response in standardized laboratory experiments in the following contexts: group formation and food sharing. We predicted that, when introduced into a new group, cleaning gobies would display a higher frequency of positive behaviors toward a size-matching goby of the opposite sex, show stronger aggression toward same-sex individuals and be more willing to share the territory and food with a partner. On the other hand, we predicted that sponge-dwellers would show stronger aggression toward smaller members, independently of the sex, keep separate territories and compete for food. In summary, we predicted that, when introduced to a new group of conspecifics, the priority for cleaners should be to find a mate, and for sponge-dwellers, to establish dominance. This study is an initial attempt to understand whether decision-making rules have a role in the differentiation of the two phenotypes by affecting their social performance in social hierarchies and monogamous pair bonding.

1.2. Methods

1.2.1. Capture and housing

Gobies were caught from around 17 barrel sponges (sponge-dwellers, N=75) and 23 cleaning stations (cleaning gobies, N=68) from three fringing reefs on the west coast of Barbados (North and South Bellairs 13.191542° N, 59.641882° W and Greensleeves 13.225882° N, 59.644153° W). Sponge-dwellers were caught from giant barrel sponges (*Xestospongia muta*) in the patch reef zone (6 to 10 meters) and group sizes of sponges varied from 5 to 70 individuals. In contrast, cleaning gobies were mostly in pairs, mainly associated with live coral, but also with other substrates like coralline algae and dead coral in the spurs and grooves zone (3 to 8 meters). The gobies were captured using plastic bags and hand nets after sedating them with a spray mixture of 50 ml of clove oil and 200 ml of ethanol 70% (1:4) diluted in approximately 700 ml of seawater. Fish were then transported to the lab inside zip lock bags. Individuals belonging to the same sponge or the same cleaning station were transported together in the same bag and were housed together either in a glass aquarium (60 x 40 x 39 cm) or mesh bags (made of mosquito screens) placed inside an aquarium (15cm diameter), depending on the number of individuals captured. Within two days after capture, each goby was sexed, sized, weighted and individually housed in a mesh bag. To determine the sex, we checked the gobies' shape of the urogenital papilla under a binocular microscope. The papilla is conical and long in males and short and truncate in females (Thresher, 1984). Because the individuals had to be flipped over for the papilla observation, we sedated each goby before the procedure in a petri dish containing 100 ml of seawater and around 5 ml of clove oil mixture. Together, sexing, sizing and weighting (scale with three digits precision) took less than 2 minutes per goby. The gobies were left to recover in another petri dish with seawater before being returned to their mesh bags. Another 2 to 3 days after this procedure, the gobies were injected with an elastomer implant. The implants were used for identification of each individual within the groups. Within 2 days after the implant injections, the gobies were assigned into the experimental groups. The gobies were fed with mashed prawn spread in a rectangular white Plexiglas plate (5x8cm) starting from the second day of arrival in the lab.

1.2.2. Experimental set-up and procedure

This experiment was conducted in July 2017 and from April to May 2018. We composed 13 groups of cleaners and 15 groups of sponge-dwellers, making sure that all individuals within groups were unfamiliar with each other. Each group contained two male-female pairs and 1 to 2 juveniles whose sex could not be determined. Individuals' size and weight ranged from 20 mm to 33 mm. In the wild, cleaning gobies male-female pair's sizes are strongly positively correlated (Harding et al., 2003) and sponge-dweller groups dominance hierarchies are strongly based on size differences (Whiteman and Côté, 2004). Therefore, we combined the pairs in each group according to their body sizes. We kept the difference in size within pairs as small as possible (average difference of 2.5 mm within larger pairs and 0.3 mm within smaller pairs) and the differences between same-sex individuals as large as possible (average difference of 4.5 mm between males and 2.3 mm between females). The experimental set-up consisted of a larger compartment (30 x 40 cm) and a smaller compartment (15 x 40 cm) separated by an opaque Plexiglas partition containing a small door (5x5cm). Two small PVC pipes (diameter: 2.0 cm; length: 10 cm) were provided as a shelter in the larger compartment.

The experimental procedure started with group formation (Day 0). The gobies belonging to the same group would be transferred one by one, in descending order of size. The first member was transferred from the mesh bags to the larger compartment and left there to acclimate for 5 minutes. At the same time, the second member would be let to acclimate in the smaller compartment, also for five minutes. After the five minutes, the small door separating the two compartments was lifted and the goby from the smaller compartment would be gently directed to the larger compartment. The same procedure was applied to the next members until the whole group was together at the larger compartment. Each group member was observed during the 5 minutes immediately after they entered the larger compartment. All interactions among the new individuals entering and the others already in the group were recorded. At the end of the group formation, the door was opened and the gobies allowed exploring the smaller compartment. In the consecutive four (N=8) or five days (N=10) after group formation, each group member (except the juveniles) was observed for 5 minutes, during which all interactions involving that member were registered (20 min block observations per group). The observations were conducted from 10:00 to 17:00 and the group observation hour changed every day. At the end of each group observation session, the food plate was inserted in the large compartment and the gobies allowed to eat freely for 20 to 60 minutes. On experimental days 3, 4 and 5, the feeding plate was video recorded with a GoPro Hero3+ camera, from above, for 10 minutes.

1.2.3. Data analysis

We tested for differences in the within-group interactions between phenotypes by running generalized linear mixed models (GLMM) using the function glmer from package lme4 (Bates et al., 2015). We categorized as dominants the larger female-male pair and as subordinates the smaller female-male pair. We aimed to test whether the behaviors performed by each individual in a group depended on the sex and status of who performed the behavior (hereafter referred as actor, four categories = dominant male, dominant female, subordinate male, subordinate female) toward whom the behavior was directed (hereafter referred as recipient, same four categories), the social experience of the actor (phenotype) and the familiarity with the other group members (day of observation). Because the size difference between males and females and dominants and subordinates were not always the same across groups, we included the size difference between the actor and the recipient of the behavior as a fixed factor. Because some behaviors were performed only by some categories of individuals in the group and not always directed to all categories of receivers (for example, subordinate females almost never chased dominant males) we analyzed the behaviors separately for each actor/receiver category. We made that choice to avoid problems with parameters estimation caused by complete separation in random effects (Sauter and Held, 2016).

We first analyzed whether each category approaches towards other categories differed between cleaning gobies and sponge-dwellers. For these analyses, we included frequency of approaches as the response variable, phenotype, towards whom category, size difference and day as fixed factors and towards whom category within group identity as random factor. The results of this model were compared to binomial models in which the frequency of approach was analyzed as 1 or 0. Whenever differences in the significance of the factors were found between the two models, we confirmed the robustness of the results with bootstrapping using the function ctree from package party (Hothorn et al., 2006). Second, we analyzed whether the responses to approaches varied across the category of receivers. For that, we categorized different types of responses to approaches into three categories: submissive responses, positive responses, and dominance responses (see the descriptions of the behaviors included in each category in **Table 1**). We ran one binomial GLMM for each behavior category and receiver category combination, using the proportion of responses in each category as response variables, phenotype, the category of who approached, size difference and day as fixed factors and group identity as the random factor. In both the approaches and responses modeling, initial models included all interactions between phenotype, towards whom category and size differences and the interaction between observation day and phenotype. Third, we categorized all other behaviors performed (except approaches) into three categories: negative behaviors, positive behaviors and dominance behaviors (descriptions in **Table 1**). We ran one negative binomial GLMM for each behavior category and actor category combination, using the frequency of behaviors as response variables, phenotype, towards whom category, size difference and day as fixed factors, and the interactions between phenotype and the other fixed factors and goby identity as random factors. For the dominance behaviors performed by dominant males and subordinate males, we used the function glmmadmb from package glmmADMB in order to meet the assumptions of the data distribution (Fournier et al., 2012).

We also tested for differences between cleaning gobies and sponge-dwellers in the proportion of time individuals spent sharing the food plate by running a linear model with the function lm from package stats (R Core Team, 2017). The proportion of time on the plate was arcsine transformed and used as the response variable. Phenotype, number of individuals sharing the plate (1, 2 or >3), observation day (day 3 to 5) and the interaction between phenotype and number of individuals sharing a plate were included as fixed factors. Moreover, we tested for differences between phenotypes in the general proportion of chases per feeding attempt using a binomial GLMM that included phenotype, observation day and their interaction as fixed factors and group identity as random factor. Finally we tested for differences between phenotypes in the proportion of time the four categories (dominant males, dominant females, subordinate males and subordinate females) spent in the larger versus the smaller compartment by running a LMM that included phenotype, observation day, the individual category and their interaction as fixed factors and group identity as random factor.

All models were validated by visual inspection of residuals and confirmed with bootstrapping when residuals distribution was not ideal. Non-significant interactions were excluded from the final models. The significance of each factor and interactions in the final models were obtained with the function Anova from package car (Fox and Weisberg, 2011). We did not perform pairwise comparisons due to the large number of tests required. Rather, we interpreted pairwise differences between phenotypes by looking at the model effects in effect plots produced with the function effects from package effects (Fox and Weisberg, 2018). The linearity between response variables and levels of factors was checked with the function gam from package mgcv (Wood, 2011). Day 0 was excluded from all analysis except in the analysis of dominance behavior because individuals were observed in a slightly different way in the first day (see methods section) but most of the dominance behaviors happened in the first day. Juveniles were excluded from the approach, response and behavioral analysis because of the restricted number of behaviors towards juveniles. We corrected the p-values of multiple tests for a same response variable with the function p.adjust, using the Holm method (Holm, 1979).

Table 1: Ethogram table with the descriptions and categorizations of the behaviors observed during the experiment.

CONTEXT	BEHAVIOR	DESCRIPTION
Neutral	approach	the focal fish approaches another fish by less than 5 cm
Dominance	circle	focal fish swims around another fish while vibrating the caudal fin
	jumps/strong jumps	focal fish does some threatening body contortions
	display	focal fish has all fins erected and the dorsal part of the body is curved
	mount	focal fish places the head and pectoral fins over the dorse of another fish
	parallel	focal fish stays side by side with another fish, head to tail.
	tail up	focal fish erects the caudal fin
Submission	avoid	the focal fish avoids another approaching fish by taking a distance of more than 5cm
	leave	the focal fish abandons its positions after another fish approaches and swim to another place
	escape	the focal fish swims very fast while escaping from a chase
Negative	bite	focal fish bites another fish
	chase	focal fish quickly darts towards another fish
	fight	two fish try to bite each other at the same time
Positive	follow	focal fish keeps swimming after another fish
	share shelter	two fish stay inside the same shelter without fighting
	swim together	two fish swim side by side
	tail shake	the focal fish moves the caudal fin horizontally (slower than the vibrations)
	touch	the focal fish touches another fish with the mouth or head without opening the jaws

1.3. Results

1.3.1. General social interaction patterns

Contrary to expectations, sponge-dwellers and coral dwellers did not show any significant differences in any recorded social behavior (Statistics and p-values for all response variables and all factors tested can be found in **Table S1** of the supplementary materials). Instead, the frequency and valence of the interactions between members within a group depended exclusively on the sex and social status of the individuals interacting. Dominant males and females showed most affiliative social behavior towards each other: they approached each other significantly more often than they approached subordinates (**Fig. 1a**; recipient factor: DM: $\chi 2 = 40.2$, corrected p. value < 0.00001; DF: $\chi 2 = 53.0$, corrected p. value < 0.00001; effects plots (a,b) on supplementary material). In addition, dominant males did not show the dominance response they frequently express when approached by subordinates towards the dominant females (**Fig. 1d**; actor factor: $\chi 2 = 17.8$, corrected p. value < 0.01; effects plot (c) on supplementary material). The proportion of submissive responses did not significantly differ according to the actor category for any receiver category (Tab. S1, **Fig. 1b**).

Positive behaviors and responses were only observed between dominant males and dominant females (Tab. S1, **Fig. 1c,** and **Fig. 2b**). In contrast, dominant males displayed significantly more negative behaviors towards subordinates than towards dominant females (**Fig. 2a**; DM; recipient factor: $\chi 2 = 57.0$, corrected p. value < 0.00001; effect plot (d) on supplementary material). Also, subordinate males were selective in the targets of their negative behaviors, displaying more towards the subordinate females than towards the dominant females (**Fig. 2a**; SM; recipient factor: $\chi 2 = 21.9$, corrected p. value < 0.00001; effect plot (e) on supplementary material). Independently of the subjects' previous social experience, dominance behaviors significantly decreased with habituation to the group (observation day; DM: $\chi 2 = 14.1$, corrected p. value < 0.01; DF: $\chi 2 = 14.6$, corrected p. value < 0.01; SM: $\chi 2 = 18.0$, corrected p. value < 0.001; effect plots (f,g) on supplementary material). Furthermore, we found a significant interaction between recipient category and size difference in the model for dominance behaviors performed by dominant males ($\chi 2 = 7.4$, corrected p. value = 0.05). Dominant male dominance behaviors decreased with the increase of size difference for dominant females (less frequent toward smaller dominant females) and increased with the increase of size difference for subordinate males (more frequent toward larger subordinate males) (effect plot (h) on supplementary material).

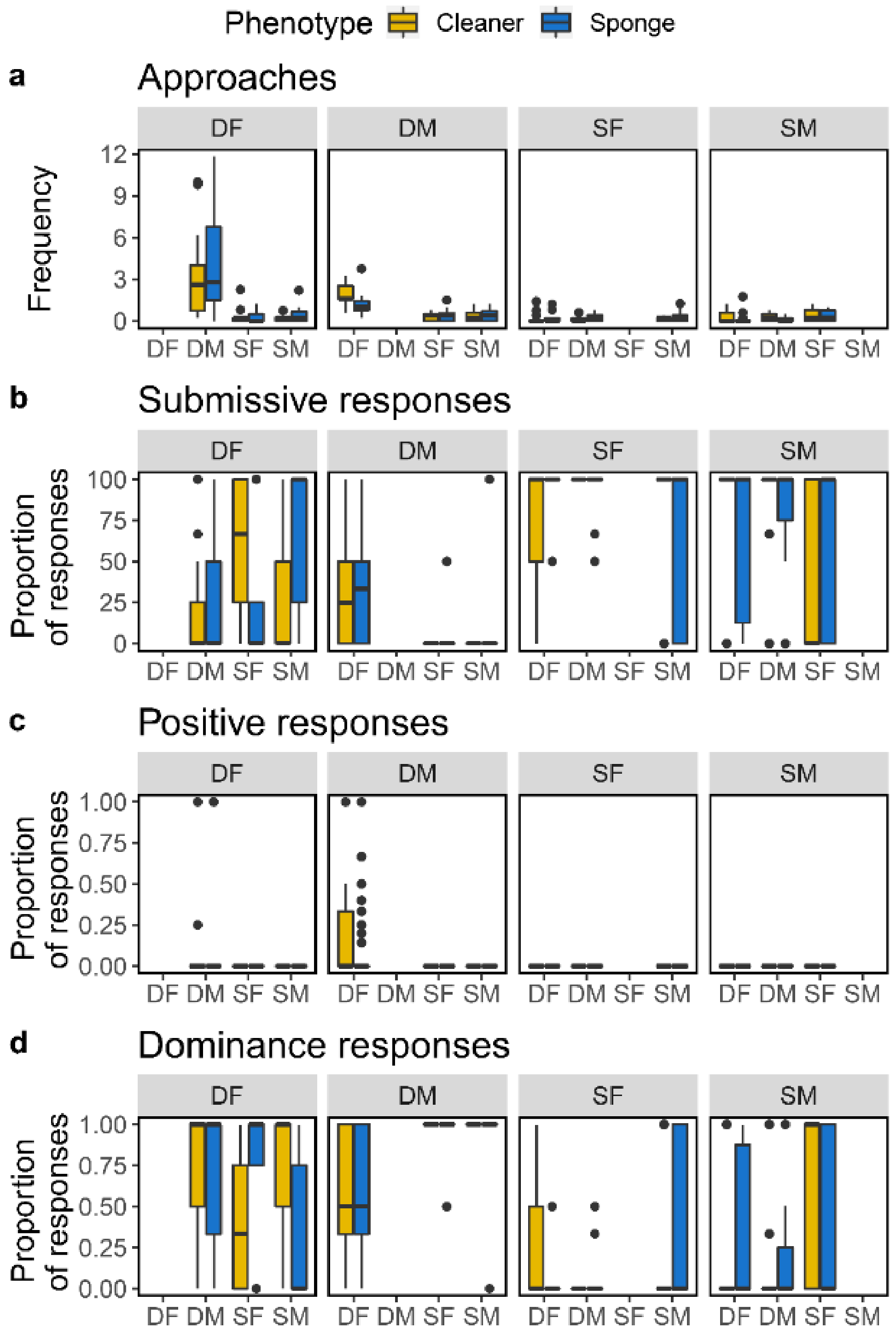

Figure 1: (a) average frequency of approaches for each combination of actor (plots) and recipient (x-axis) and (b-d) proportion of responses to approaches for each category that responded (plots) to each category of actor approaching (x-axis), per observation day (5 min per actor) for cleaning gobies (yellow) and sponge-dwellers (blue). DF = dominant female, DM = dominant male, SF = subordinate female and SM = subordinate male. The types of behaviors included in each category of response (Submissive (b), Positive (c) and Dominance (d)) are described in **Table 1**. The central line in boxes, the boxes, and the whiskers indicate the median, Q1, Q3, and 1.5*QR, consequently. Dots represent outlier values (>median+1.5*QR).

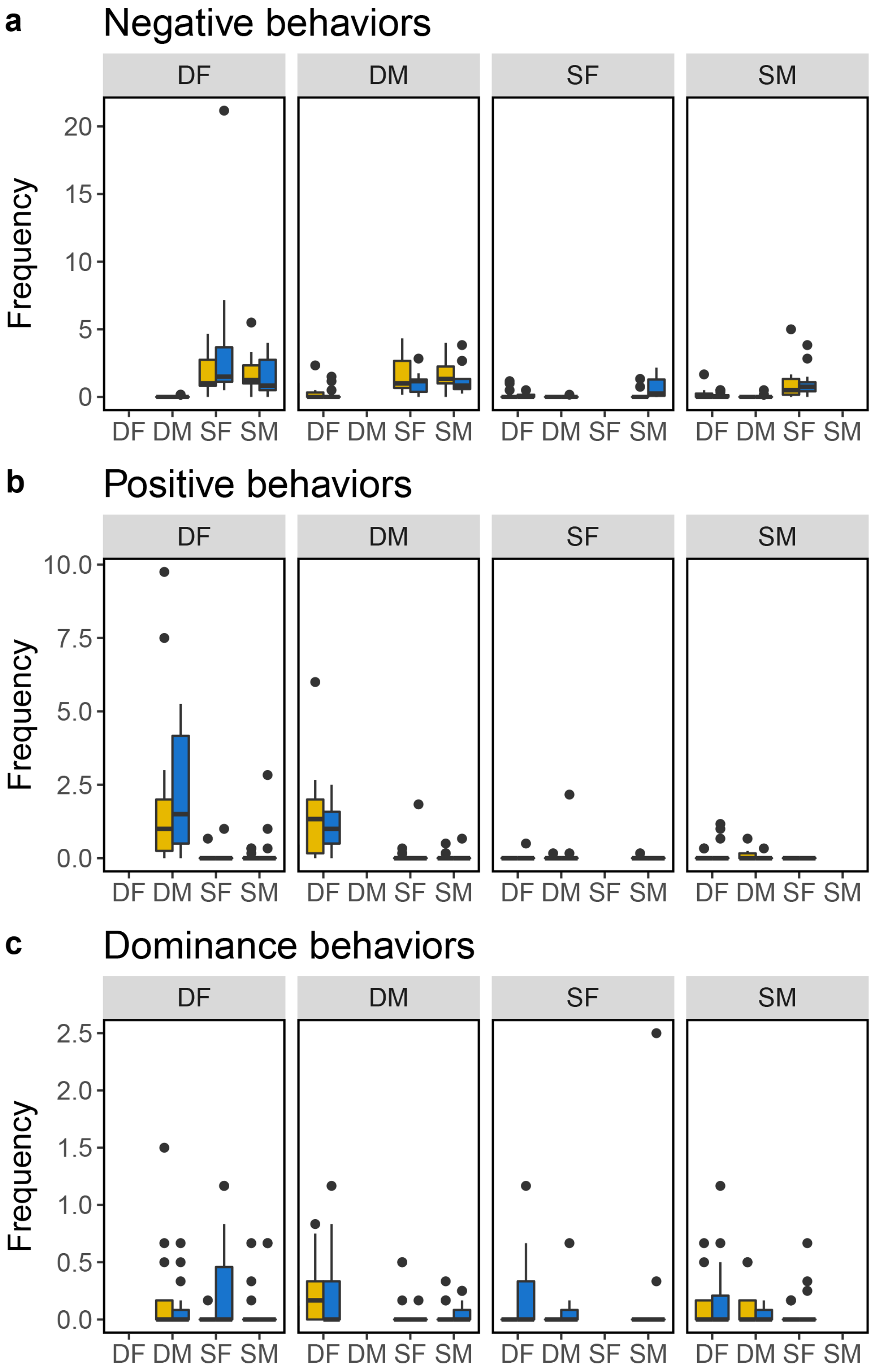

Figure 2: Average frequency of (a) negative, (b) positive and (c) dominance behaviors for each combination of actor (plots) and recipient (x-axis) per observation day (5 min per actor) for cleaning gobies (yellow) and sponge-dwellers (blue). DF = dominant female, DM = dominant male, SF = subordinate female and SM = subordinate male. The types of behaviors included in each category are described in **Table 1**. The central line in boxes, the boxes, and the whiskers indicate the median, Q1, Q3, and 1.5*QR, consequently. Dots represent outlier values (>median+1.5*QR).

1.3.2. Co-feeding and tolerance near food-providing plates

Similarly to the general interaction patterns, most results were not affected by the subjects' previous social experience. Gobies from both corals and sponges spent significantly more time feeding alone on the food plates than they did in pairs or groups (> 3 individuals) (**Fig. 3**; F = 963.44, $p < 0.00001$). The proportion of time feeding was not significantly affected by the day of observation (**Fig. 3**; F = 0.01, p = 0.99), the phenotype (**Fig. 3**; F = 0.01, p = 0.92) or the interaction between phenotype and the number of gobies in the plate (**Fig. 3**; F = 1.37, p = 0.25). Subordinates consistently spent significantly more time in the smaller compartment than dominants (**Fig. 4**; $\chi2 = 63.3$, $p < 0.00001$), independently of the observation day ($\chi2 = 3.49$, p = 0.47), the phenotype ($\chi2 = 0.15$, p = 0.69) or any of the tested interactions (Phenotype:day: $\chi2 = 1.35$, p = 0.85; Phenotype:who.category: $\chi2 = 2.27$, p = 0.51; Day:who.category: $\chi2 = 5.85$, p = 0.92; Phenotype:day:who.category: $\chi2 = 15.5$, p = 0.21). Finally, there was a significant effect of previous social experience on the number of chases per feeding attempts, with sponge-dwelling groups showing higher frequencies that cleaning groups on days 4 and 5 (**Fig. 5**; observation day: $\chi2 = 10.5$, p = 0.005; interaction phenotype: observation day: $\chi2 = 16.5$, p = 0.0002).

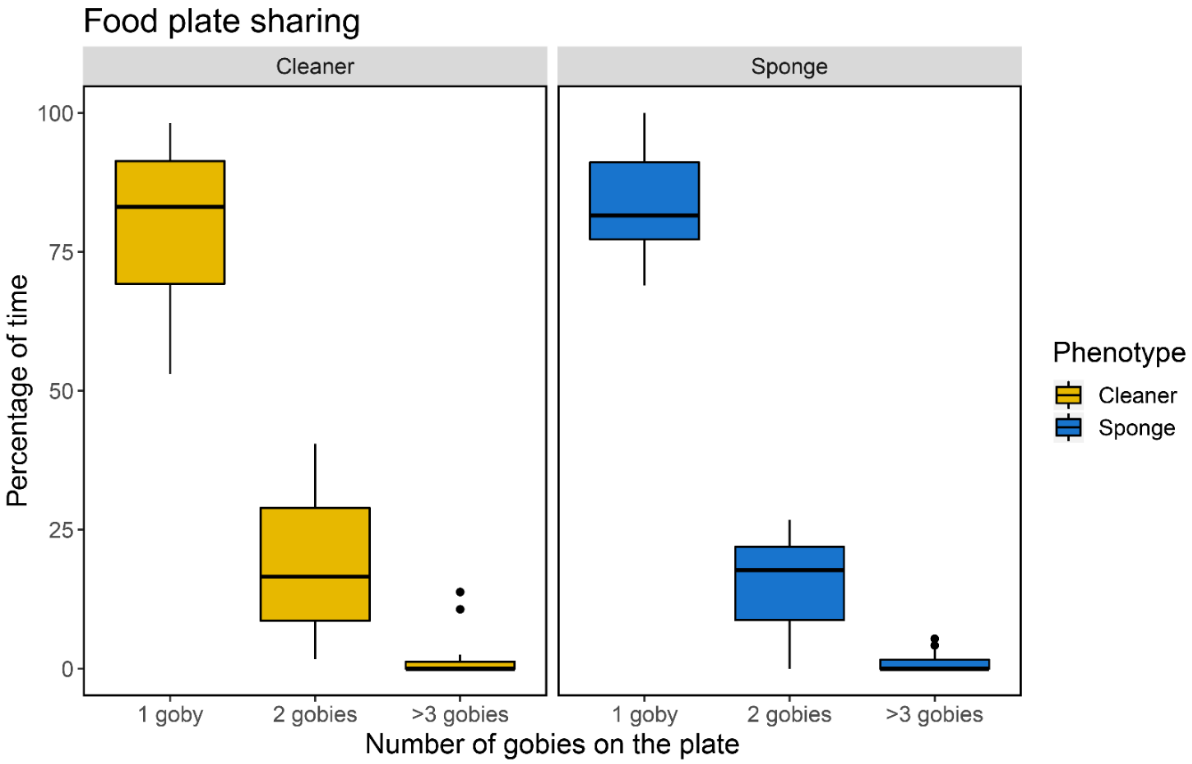

Figure 3: Percentage of time that cleaning (yellow) and sponge-dwelling (blue) gobies spent on the feeding plates alone (1 goby), in pairs (2 gobies) or in groups (>3 gobies). The central line in boxes, the boxes, and the whiskers indicate the median, Q1, Q3, and 1.5*QR, consequently. Dots represent outlier values (>median+1.5*QR).

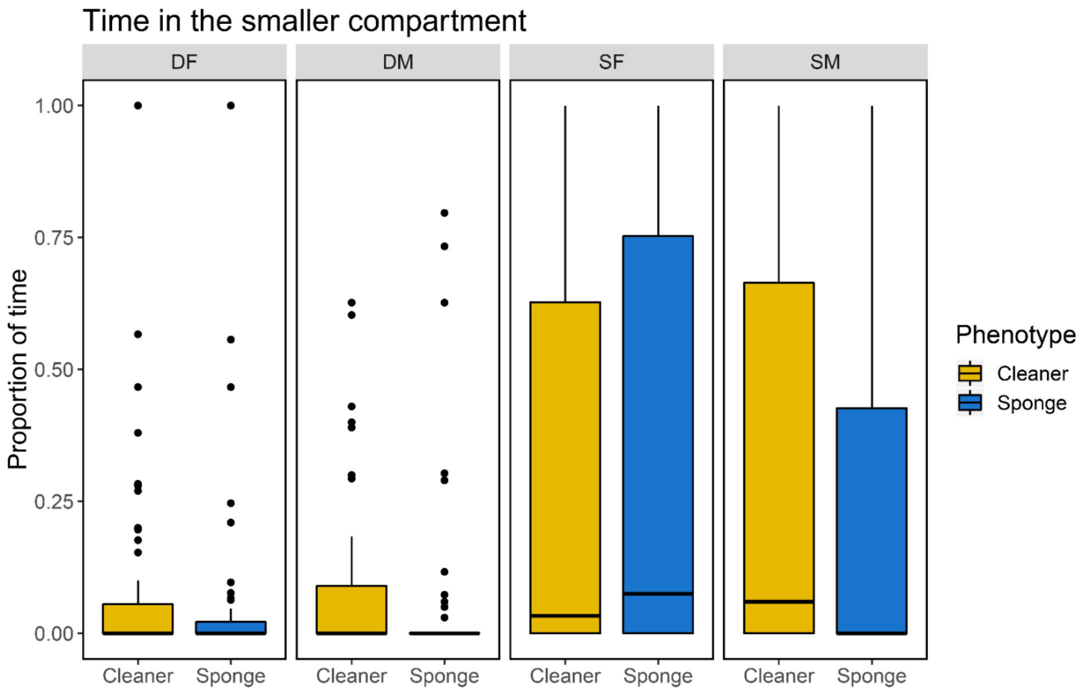

Figure 4: Proportion of time that cleaning (yellow) and sponge-dwelling (blue) gobies spent in the smaller compartment for all observation days summed. The central line in boxes, the boxes, and the whiskers indicate the median, Q1, Q3, and 1.5*QR, consequently. Dots represent outlier values (>median+1.5*QR).

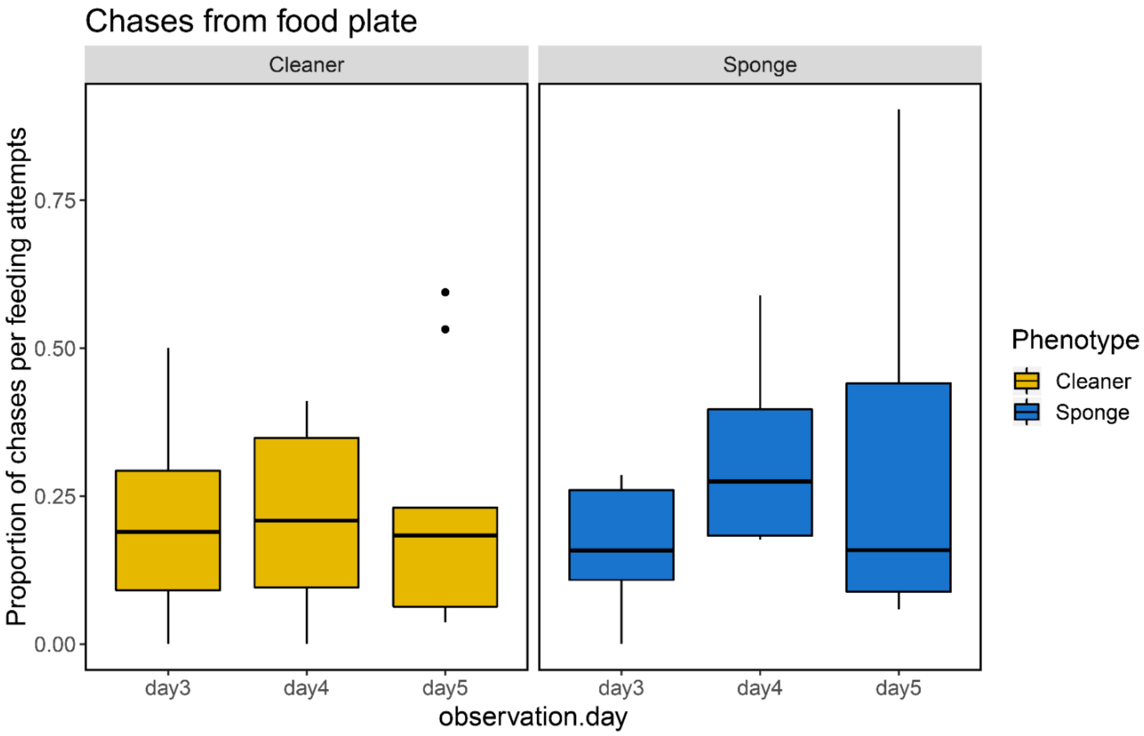

Figure 5: Proportion of chases per feeding attempts in cleaning (yellow) and sponge-dwelling (blue) groups for each day of observation in which feeding behavior was recorded (days 3 to 5). The central line in boxes, the boxes, and the whiskers indicate the median, Q1, Q3, and 1.5*QR, consequently. Dots represent outlier values (>median+1.5*QR).

1.4. Discussion

The aim of this study was to investigate the hypothesis that differences in the previous social experience of cleaning gobies versus sponge-dwellers affect the level of social competence they display in laboratory-based experiments. Based on previous studies and the field observations, we had predicted that pair-living cleaning gobies would be more apt at developing positive relationship between the largest male and female but also less apt to establish stable relationships (characterized by low aggression) between other group members when compared to sponge-dwellers, that are used to live in size-based hierarchies. We also expected sponge-dwellers to monopolize food more efficiently. Despite the standardized group composition in the experiments, we expected differences in social behavior because the mechanism(s) underlying alternative life histories are predicted to impose constraints on adult behavioral flexibility. In contrast to our predictions, we found that both phenotypes behaved very similarly. More specifically, we had predicted that cleaning gobies would display a higher frequency of positive behaviors toward a size-matching goby of the opposite sex, show stronger aggression toward same-sex individuals and be more willing to share the territory and food with a partner. Second, we predicted that sponge-dwellers would show stronger aggression toward smaller members (at least, initially), independently of the sex, keep separate territories and compete for food. Nevertheless, the frequency of approaches, positive, negative and dominant behaviors, as well as the proportion of positive, submissive and dominant responses toward the different categories (dominant males, dominant females, subordinate males, and subordinate females), were not significantly different between the two phenotypes. Moreover, they seem to share food equally often and divide their territory in a similar way. The only difference that we found was that sponge-dweller groups were, in general, more competitive over food than cleaning groups.

Both dominant cleaning-gobies and sponge-dwellers behaved and responded more positively toward a size-matching partner of the opposite sex and more negatively and dominantly toward smaller individuals, irrespectively of the sex and size differences. They also shared the most valuable territory (larger compartment) with their partners and kept subordinates away from it. Regarding the cleaning gobies, this result is similar to what is found in the wild and is appropriate considering that cleaning gobies are monogamous, share a territory and benefit from guarding a large mate (Whiteman and Côté, 2003). However, we expected that cleaning gobies would behave more negatively toward subordinates of the same sex, especially to the ones that were more similar in size. It is possible that cleaning gobies only feel more threatened by reproductive competitors that are larger than they are and smaller individuals are always seen as food competitors, irrespectively of the sex. On the other hand, sponge-dwellers behaved as expected towards subordinates, but not towards a size-matching partner. In the wild, sponge-dwellers have never been seen behaving positively towards the opposite sex when on the barrel sponges, especially when the partner has a similar size and enters its feeding territory (Personal observation, Whiteman & Côté, 2004). The fact that sponge-dwellers were forming "couples" and sharing their territory with a partner in a similar way as cleaning gobies indicate that sponge-dwellers adjust their behavior to different contexts. In the wild, sponge-dwellers seem to mate and find crevices for laying eggs outside of the sponges (Personal observation; Whiteman & Côté, 2004). Therefore, their feeding and reproductive environments are separated. In our experiment, the shelters and food were provided in the same compartment (larger compartment) and most of the positive behaviors occurred inside these shelters. We hypothesize that in such a small scale it is difficult for the individuals to separate the two contexts completely and therefore sponge-dwellers behave more positively toward a size-matching partner and allow them to share their territory and food with them. However, the higher levels of chases per feeding attempts in the sponge dweller groups indicate that they keep a higher competitive state. Unfortunately, we could not see who directed chases towards whom in this experiment because the tagging colors were not large or bright enough to be observed in the videos.

Such persisting behavioral flexibility might be favored by natural selection if changes in social conditions are frequent and rather unpredictable. In favor of this hypothesis, we note that gobies have a rather short maximum life expectancy of about 12 months (Colin, 1975), and may frequently change their status, especially in sponges due to large old individuals dying and new young recruits joining. Life-long flexibility in intra- and interspecific social behavior could be important if individuals readily switch between habitats at some point of their lives, but we currently lack evidence that they do so. First, there is no evidence for an ontogenetic shift in habitat type and feeding strategy, as the mean ages of cleaning and sponge-dwelling gobies are not significantly different (Unpublished personal data, White, Grigsby, & Warner, 2007). Second, none of the 64 *E. evelynae* tagged by White et al (2007) were seen to move less than 2 meters from the initial location or to switch between habitat types. Moreover, results of a pilot study with *E. prochilos* go in the same direction (unpublished data). Sponge-dwelling *E. prochilos* can be found siting in corals located around the sponges from time to time, but we have never seen those completely leaving the sponges and establishing cleaning stations in monogamous pairs (personal observation).

In conclusion, the results of our study do not provide evidence that social experience and/or underlying genetic factors modulate the behavior of the two *E. prochilos* phenotypes in a way that would prevent individuals from converging in their social behavior when exposed to the same social environment. Both phenotypes seem to be able to make monogamous pairs and, at the same time, keep a dominance hierarchy in the group. Further studies should investigate why keeping this plasticity is important at an adult stage. Additionally, we should investigate how social experience affect the two phenotypes in other aspects of their social environment. For example, the higher frequency of cleaning interactions observed for cleaning gobies could potentially affect their ability of distinguishing across different types of clients and choosing the most appropriate ones. Finally, we should better understand what determinants, costs, and benefits are involved in adopting a cleaning and sponge-dwelling strategy in terms of food acquisition, mating and reproductive success, and survival. For instance, we should investigate whether there is a trade-off between foraging and reproductive success for sponge-dwellers. This would help us to understand the real costs of behaving flexibly as an adult in *E. prochilos*.

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1.6. Supplementary Information

Table S1: Analysis of covariance table: Generalized linear models for testing behavioral differences. The frequency or proportion of the different types of behaviors (Type) in the different categories (Cat) were included as response variables. Phenotype (cleaning x sponge-dwelling), actor/recipient category (DF, DM, SF, SM), the size difference between the actor the recipient (size differences) and day of observation (day) were included as main factors. Only significant interactions are presented. DF = dominant female, DM = dominant male, SF = subordinate female and SM = subordinate male. Significant p values and corrected p values (p cor.) are in bold.

			DM				**DF**			
Cat	**Type**	**Predictors**	**Chisq**	**Df**	**Pr(>Chisq)**	**p cor.**	**Chisq**	**Df**	**Pr(>Chisq)**	**p cor.**
Approach		Phenotype	1,133	1	0,287	1	0,414	1	0,520	1
		recipient	40,258	2	**<0,001**	**<0,001**	53,046	2	**<0,001**	**<0,001**
		Size difference	0,028	1	0,868	1	2,422	1	0,120	1
		Day	0,045	1	0,833	1	0,786	1	0,375	1
Response	Submissive	Phenotype	0,532	1	0,466	1	0,499	1	0,480	1
		actor	na	na	na		0,726	2	0,696	1
			*responses towards DF only							
		Size difference	0,759	1	0,384	1	2,132	1	0,144	1
		Day	0,025	1	0,874	1	5,024	1	**0,025**	0,375
	Dominance	Phenotype	0,055	1	0,814	1	0,621	1	0,431	1
		actor	17,853	2	**<0,001**	**0,002**	0,625	2	0,731	1
		Size difference	3,940	1	**0,047**	0,660	2,576	1	0,109	1
		Day	0,163	1	0,686	1	7,373	**1**	**0,007**	0,099
	Positive	Phenotype	1,220	1	0,269	1	0,028	1	0,867	1
		actor	na	na	na		na	na	na	
			*responses towards DF only				*responses towards DM only			
		Size difference	4,078	1	**0,043**	0,261	0,411	1	0,522	1
		Day	0,106	1	0,745	1	1,394	1	0,238	1
Behaviors	Negative	Phenotype	0,378	1	0,539	1	3,333	1	0,068	0,747
		recipient	57,010	2	**<0,001**	**<0,001**	4,077	1	**0,043**	0,522
							*actions towards SM and SF only			
		Size difference	1,785	1	0,182	1	1,014	1	0,314	1
		Day	4,558	1	**0,033**	0,426	3,189	1	0,074	0,747
	Dominance	Phenotype	0,661	1	0,416		0,001	1	0,981	1
		recipient	6,370	1	**0,012**	0,093	0,509	1	0,475	1
							*actions towards DM and SM only			
		Size difference	9,396	1	**0,002**	**0,022**	0,024	1		
		Day	14,138	1	**<0,001**	**0,002**	14,685	1		
		recipient: size	7,406	1	**0,006**	**0,058**	na	na		
	Positive	Phenotype	0,210	1	0,647	1	0,624	1		
		recipient	na	na	na		na	na		
			*actions towards DF only				*actions towards DM only			
		Size difference	0,292	1	0,589	1	1,777	1		
		Day	3,907	1	0,048	0,282	4,206	1		

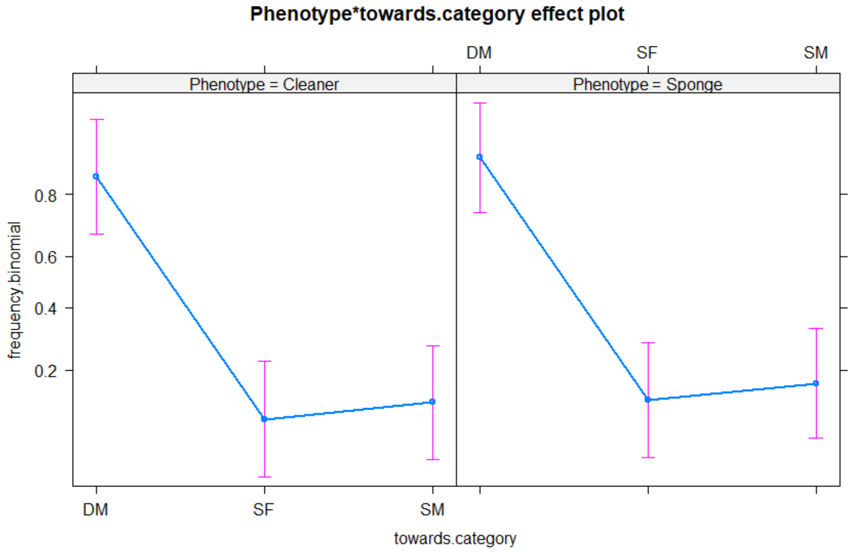
		Phenotype: Size	3,984	1	0,046	0,282	na	na		

Table S1: continuation

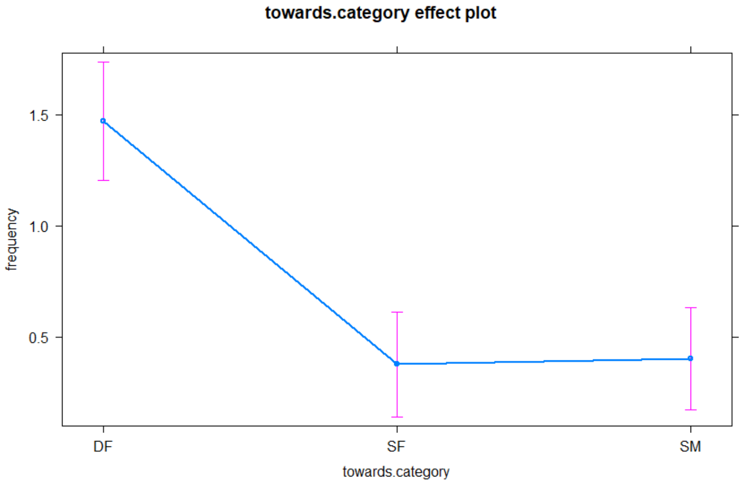
			SM				**SF**			
Cat	**Type**	**Predictors**	**Chisq**	**Df**	**Pr(>Chisq)**	**p cor.**	**Chisq**	**Df**	**Pr(>Chisq)**	**p cor.**
Approach		Phenotype	0,840	1	0,360	1	1,639	1	0,201	1
		recipient	2,555	2	0,279	1	0,333	2	0,847	1
		Size difference	0,003	1	0,957	1	0,938	1	0,333	1
		Day	0,231	1	0,631	1	0,058	1	0,811	1
Response	Submissive	Phenotype	1,248	1	0,264	1	0,664	1	0,415	1
		actor	0,649	2	0,723	1	2,829	2	0,243	1
		Size difference	2,292	1	0,130	1	0,044	1	0,834	1
		Day	2,226	1	0,136	1	0,109	1	0,742	1
	Dominance	Phenotype	1,248	1	0,264	1	0,664	1	0,415	1
		actor	0,649	2	0,723	1	2,829	2	0,243	1
		Size difference	2,291	1	0,130	1	0,044	1	0,834	1
		Day	2,226	1	0,136	1	0,109	1	0,742	1
	Positive	Phenotype	na	na	na		na	na	na	
		actor	na	na	na		na	na	na	
			*no positive responses				*no positive responses			
		Size difference	na	na	na		na	na	na	
		Day	na	na	na		na	na	na	
Behaviors	Negative	Phenotype	0,039	1	0,844	1	3,013	1	0,083	0,747
		recipient	21,933	1	**<0,001**	**<0,001**	0,014	1	0,905	1
			*actions towards DF and SF only				*actions towards DF and SM only			
		Size difference	0,337	1	0,562	1	2,882	1	0,090	0,747
		Day	5,094	1	**0,024**	0,336	0,122	1	0,727	1
	Dominance	Phenotype	0,159	1	0,690	1	na	na	na	
		recipient	4,655	2	0,098	0,683	na	na	na	
							*not enough registers			
		Size difference	1,405	1	0,236	1	na	na	na	
		Day	18,068	1	**<0,001**	**<0,001**	na	na	na	
		recipient: size								
	Positive	Phenotype	na	na	na		na	na	na	
		recipient	na	na	na		na	na	na	
			*no positive behaviors				*no positive behaviors			
		Size difference	na	na	na		na	na	na	
		Day	na	na	na		na	na	na	
		Phenotype: Size	na	na	na		na	na	na	

Effects plot: Effects plot of glmm model predictions (parameter estimate and 95% CI) of the models that found significant effects in the actor (who.category)/recipient (towards.category) factors and interactions.

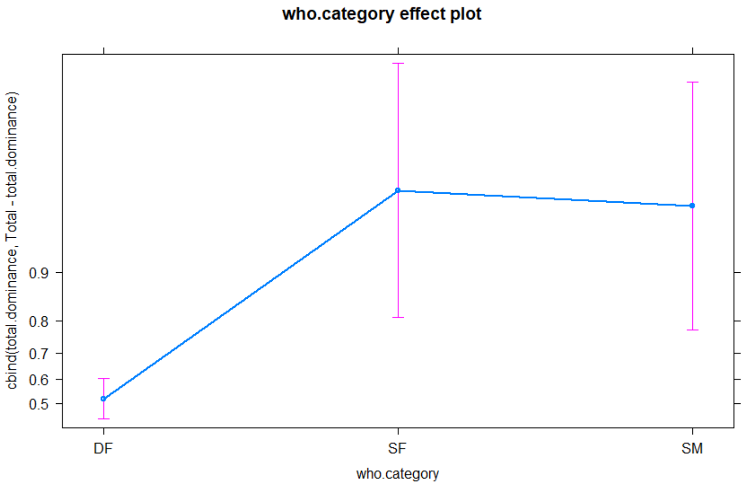
(a) Approaches by dominant females



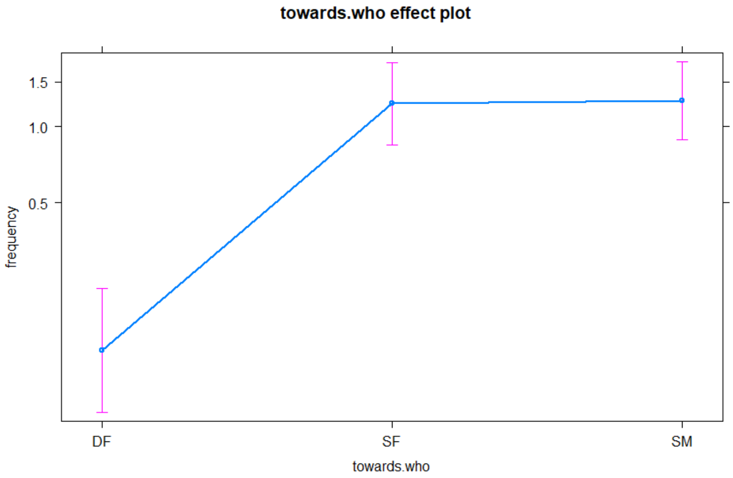
(b) Approaches by dominant males



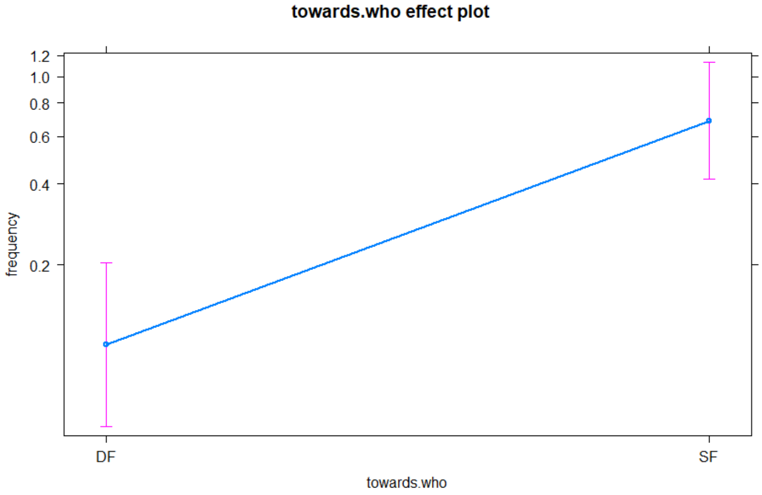
(c) dominance responses by dominant male



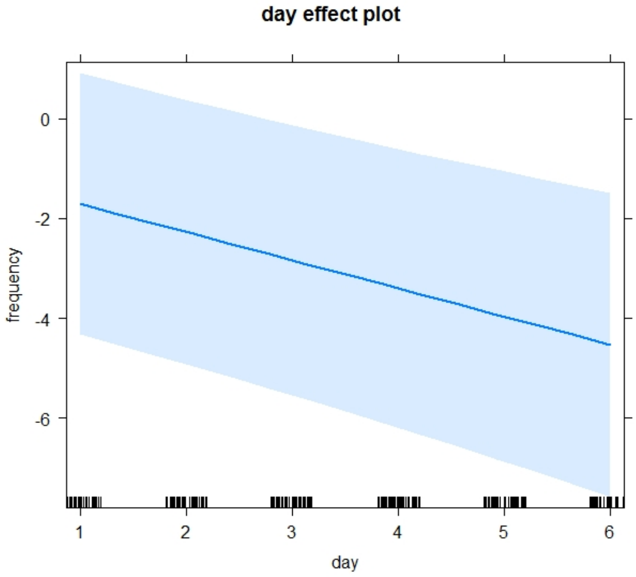
(d) Negative behaviors by dominant male



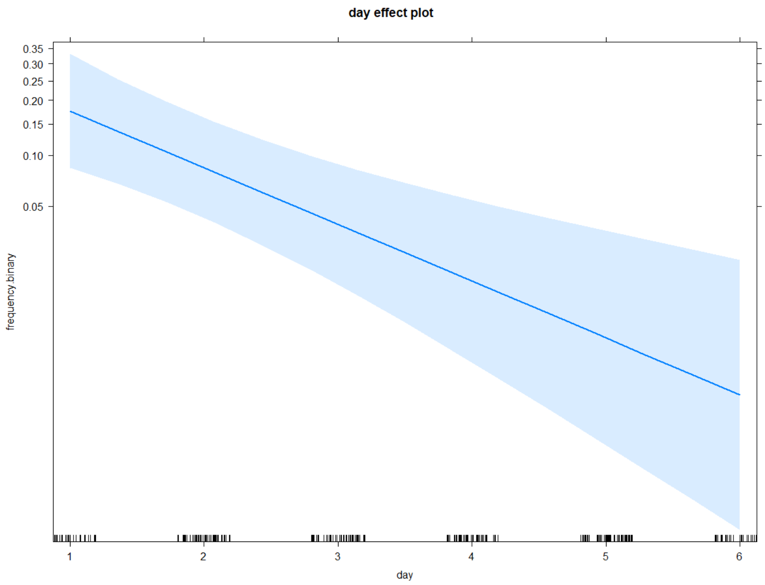
(e) Negative behaviors by subordinate male



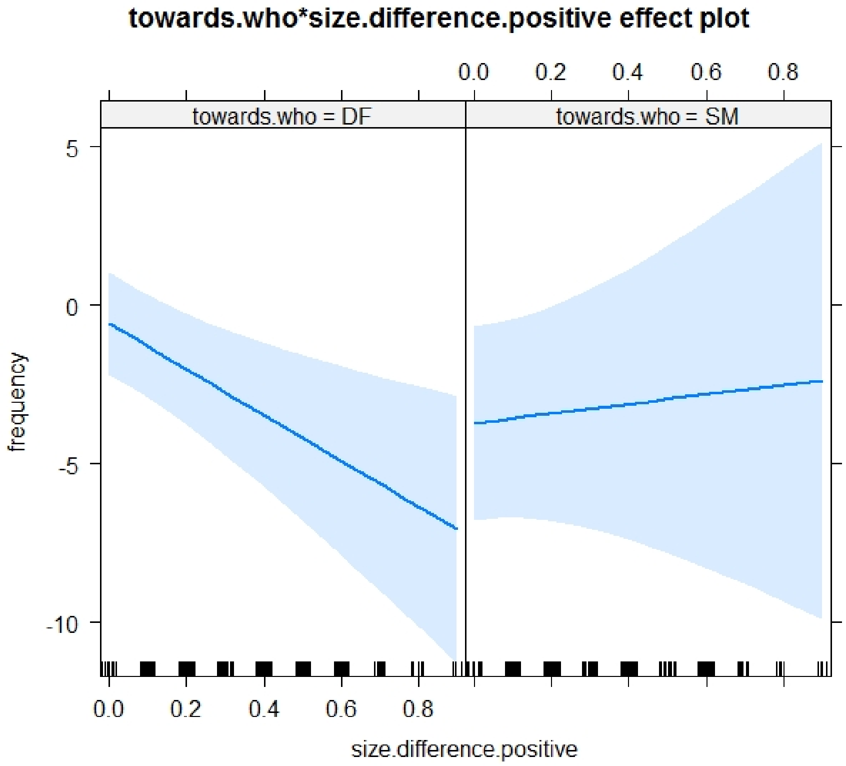
(f) Dominance behaviors by dominant male



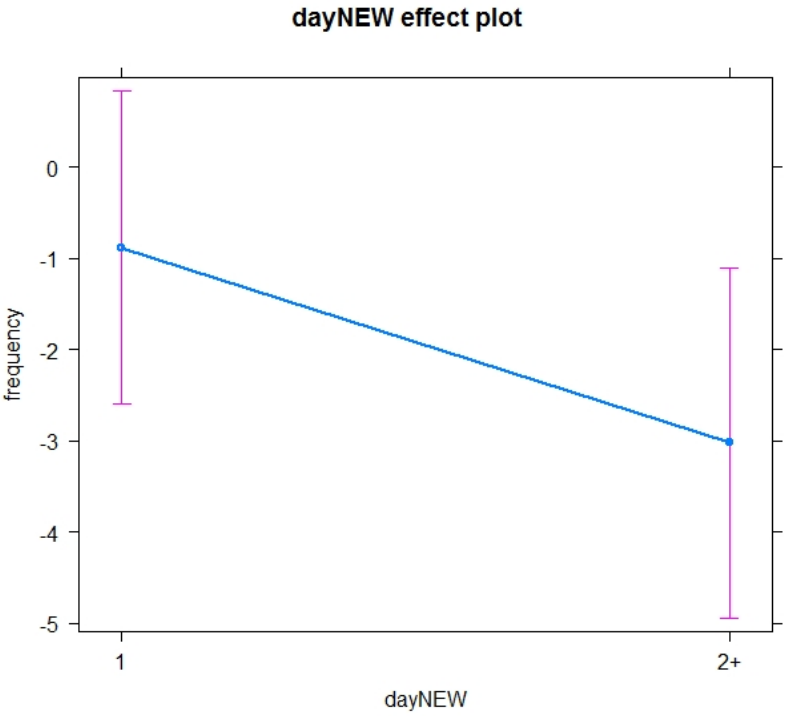
(g) Dominance behaviors by dominant females



(h) Dominance behaviors by dominant male



(i) Dominance behaviors by subordinate males



Chapter 2 | Ecological differences in the facultative Caribbean cleaning goby *Elacatinus prochilos* do not predict learning performance in discriminatory two-choice tasks

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Abstract

The ecological approach to comparative cognition predicts in which ecological and social contexts cognitive abilities should emerge. We used this approach to test whether differences in habitat use and social behavior in the facultative Caribbean cleaning goby *Elacatinus prochilos* predict learning performance in two discriminatory two-choice tasks. This species has two behavioral phenotypes: one that frequently engages in cleaning interactions and inhabits corals in male-female pairs (cleaning gobies) and another that rarely engages in cleaning interactions and inhabits barrel sponges in large groups (sponge-dwellers). We predicted that cleaning gobies would outperform sponge-dwellers in a pattern-cued task, which consisted of identifying the pattern on a plate that consistently provided food, while sponge-dwellers would outperform cleaning gobies in a spatial task, which consisted of identifying the location of the plate. Contrary to our predictions, there was no difference in performance between the two phenotypes. Most of the gobies performed poorly in the pattern-cued task and well in the spatial task. The most likely explanation for these results is that the association of a pattern with positive and negative reinforcement may not be a pre-requisite for engaging in cleaning interactions, while spatial skills might be equally required in both phenotypes. Therefore, the ecological differences within the species *E. prochilos* do not seem to cause significant differences in individual performance in the two learning tasks. Nevertheless, further research should investigate which aspects of cleaning and sponge-dwelling gobies ecological and social environment may require spatial learning.

2.1. Introduction

The ecological approach to comparative cognition emphasizes that ecology may be a better predictor of cognitive performance than phylogenetic relatedness (Kamil, 1988; Shettleworth, 2010, 1993). It is based on the idea that individuals behaviors and brains are largely shaped by the challenges associated with the environment in which they live (Dukas, 1998; Healy and Braithwaite, 2000). This approach successfully explains why closely related species that face different ecological pressures diverge in cognitive performance as well as why distantly related species show similar cognitive performances when exposed to similar ecological challenges (Shettleworth, 2010). For example, food-storing species of birds are better at processing and remembering spatial information than non-storing species (Balda and Kamil, 1989; Olson et al., 1995). Similarly, rock-pool gobies learn to find a food reward faster in a spatial task than sand-gobies from more homogeneous environments (White and Brown, 2014a, 2014b). On the other hand, species of distantly related groups like corvids and apes use similar reasoning abilities to solve social and physical problems (Emery, 2004; Emery and Clayton, 2004a, 2004b; cf. Seed et al., 2009). Most importantly, high performance often appears to be tightly linked to specific ecological needs. For instance, seed-caching birds excel in spatial memory tasks but do not in other forms of memory testing (Olson et al., 1995), and cleaner wrasses outperform other wrasses in tasks related to their cleaning interactions with client reef fishes but do not in a non-ecologically relevant spatial discrimination task (Gingins and Bshary, 2016). Likewise, black-capped chickadees from harsh environments outperform individuals from more stable environments only in ecologically relevant spatial tasks (Pravosudov and Clayton, 2002). Finally, cleaner wrasse may match or even outperform mammals and birds in tasks that capture the demands of their cleaning ecology (Pepperberg and Hartsfield, 2014; Salwiczek et al., 2012; Zentall et al., 2018, 2016; Prétôt et al., 2016a, 2016b). Therefore, the ecological approach encourages the comparison of closely related and distantly related species in a variety of tasks so that each species is confronted with both ecologically relevant and irrelevant problems (Shettleworth, 2010).

The logic of the ecological comparative approach has also been used to study intraspecific variation of performance in cognitive tasks. For example, early exposure to environmental enrichment enhances later cognitive performance in Lake Tanganyika cichlids (Kotrschal and Taborsky, 2010). Moreover, a study on the cognitive performance of Australian magpies recently provided the first evidence that living in a larger group correlates with general intelligence parameters (Ashton et al., 2018), although the precise ecological pressures driving that correlation remain unknown. Another example is how strategic sophistication in the cleaner wrasse *Labroides dimidiatus* is specifically linked to its ecological needs

(Triki et al., 2018; Wismer et al., 2014). Cleaner fish remove ectoparasites from the body surface and cavities of other larger marine organisms referred to as clients (Côté, 2000). Conflicts of interest between cleaners and clients can arise for various reasons. For instance, cleaner wrasses prefer to eat client mucus, which constitutes cheating (Grutter and Bshary, 2003). In addition, because cleaners interact with about 2000 clients per day (Grutter, 1996), clients may seek services simultaneously and thus compete over priority of access. Consequently, both clients and cleaners show sophisticated strategic adaptations to these conflicts, like image scoring, audience effects and partner switching (Bshary and Grutter, 2006; Bshary and Schäffer, 2002; Pinto et al., 2011). As an example, cleaners can prioritize client species that can switch partners over clients that cannot (Bshary and Grutter, 2002). However, cleaner wrasses living in a socially simpler environment (characterised by low cleaner and client densities) failed to solve the same cognitive tests on audience effects and prioritising of ephemeral food sources that are solved by cleaners living in a more complex social environment (Triki et al., 2018; Wismer et al., 2014). Apparently, cleaners in socially simple environments do not use these abilities because clients do not exert choice (Triki et al., 2018), possibly because partner switching is too costly when cleaner density is low.

While ecological variation in the cleaner wrasse system is rather subtle, the facultative Caribbean cleaning goby *Elacatinus prochilos* provides an ideal model for a comparative study aimed at investigating how social environment and habitat structure link to specific intraspecific differences in cognitive abilities in the context of cleaning interactions. In this species, habitat use is related to differences in social and feeding behavior (Whiteman and Côté, 2004a, 2002). *Elacatinus prochilos* gobies living on corals and other substrates are usually found in pairs or small groups that depend mostly on cleaning interactions for feeding (Whiteman and Côté, 2004a, 2003, 2002). In contrast, *E. prochilos* gobies inhabiting barrel sponges are organized into larger groups, with dominance hierarchies, that feed primarily on micro-organisms living inside the sponges' tissue and only rarely engage in cleaning interactions (Whiteman and Côté, 2004b). We refer to the former as cleaning gobies, and to the latter as sponge-dwelling gobies. Like cleaner wrasses, cleaning gobies are able to categorize different clients (Soares et al., 2007), which suggests that they are able to recognize individual clients, just as wrasses are (Tebbich et al., 2002). However, gobies engage in fewer cleaning interactions (Whiteman and Côté, 2002) and do not exhibit the same complex behaviors used for partner control as cleaner wrasses (Soares et al., 2008). The intraspecific differences between cleaning and sponge-dwelling gobies provide an ideal system to investigate how ecological and social factors link to intraspecific variation in cognitive performance. Finally, comparing the performance of cleaning gobies to previous findings for cleaner wrasses will give us the opportunity to evaluate whether the degree of complexity of cleaning interactions correlates with performance in cleaning-related cognitive tasks.

We tested the gobies in two discriminatory two-choice tasks that differed with respect to the relevant cues available to identify the correct choice. In the pattern-cued task, individuals had to identify the specific pattern of a plate that consistently provided food, while in the spatial task they had to identify the location of a plate where food was consistently provided. We predicted that the pattern-cued task would be more ecologically relevant for the cleaning gobies because it mimics a situation in which the cleaner has to choose between two clients arriving simultaneously at the cleaning station. In this situation, the cleaner needs to recognize the most valuable client because the ignored client might leave the station (Bshary and Schäffer, 2002). Since cleaning gobies are able to discriminate between clients, we expected that cleaning gobies would outperform sponge-dwellers in the pattern-cued task. On the other hand, we predicted that the spatial discrimination task would be more relevant to sponge-dwellers because the food in barrel sponges is patchily distributed and sponge-dwellers are known to be able to locate their home sponge from over 40 meters away (Whiteman and Côté, 2004b). By testing cleaning and sponge-dwelling gobies in the two tasks, we expect to understand how general individual cognitive performance is. If cognition is tightly linked to the ability to solve an ecologically relevant challenge, cleaning gobies and sponge-dwellers should outperform each other only in the ecologically relevant tasks.

2.2. Methods

2.2.1. Capture and housing

Laboratory experiments were conducted from March to July of 2017. Gobies were caught from both barrel sponges (sponge-dwellers) and cleaning stations (cleaning gobies) from three fringing reefs on the west coast of Barbados (North and South Bellairs 13.191542° N, 59.641882° W; Heron Bay 13.196817° N, 59.643521° W and Greensleeves 13.225882° N, 59.644153° W). Sponge-dwellers formed groups of 5 up to 80 individuals and were associated with giant barrel sponges (*Xestospongia muta*) in the patch reef zone (6 to 10 meters). In contrast, cleaning gobies were found either solitary or in pairs, mainly associated with live coral, but also with other substrates like coralline algae and dead coral in the spurs and grooves zone (3 to 8 meters). The gobies were captured using plastic bags and hand nets after sedating them with a spray mixture of 50 ml of clove oil and 200 ml of ethanol (1:4) diluted in approximately 700 ml of seawater. Fish were then transported to the lab inside zip lock bags. Individuals belonging to the same sponge or the same cleaning station were transported together in the same bag and were housed together either in a glass aquarium (60 x 40 x 39 cm) or mesh bags placed inside an aquarium (15cm diameter), depending on the group size. Within two days after capture, each goby was sexed, sized, weighted and individually housed in a mesh bag. Individuals' size and weight ranged from 2.2 to 3.4 cm and 0.07 to 0.39 g. To determine the sex, we observed the shape of the urogenital papilla under a binocular microscope. The papilla is conical and long in males and short and truncated in females (Thresher, 1984). Because the individuals had to be flipped over for the papilla observation, we sedated each goby before the procedure in a petri dish containing 100 ml of seawater and around 5 ml of clove oil mixture. Together, sexing, sizing and weighting took less than 2 minutes per goby. The gobies were left to recover in another petri dish with seawater before being returned to their mesh bags. Another 2 to 3 days after this procedure, part of the gobies (48 out of 64 individuals) were injected with an elastomer implant. The implants were used for individual identification in a social group manipulation that occurred prior to the learning tasks. The social group manipulation consisted of forming groups of cleaners or sponge-dwellers, each containing 6 individuals (one pair of dominants, one pair of subordinates and one pair of juveniles), and registering all interactions among individuals. After the group manipulations, cleaning gobies and sponge-dwellers were assigned to one of the two different learning tasks, based on their sizes and sex, in a counterbalanced manner. Individuals stayed in the lab for a maximum of 3 to 7 weeks before being sacrificed for a separate project on brain anatomy.

2.2.2. Experimental set-up and acclimation

Associative learning processes in fish can be investigated by using Plexiglas plates offering food as proxies of real clients. The Caribbean cleaning gobies can learn to eat items off Plexiglas plates (Soares et al., 2010) and this experimental paradigm has been used extensively with cleaner wrasses (Bshary and Grutter, 2006, 2002; Gingins et al., 2018; Gingins and Bshary, 2016; Grutter and Bshary, 2003; Triki et al., 2018; Wismer et al., 2014). This paradigm captures the essence of cleaning interactions as demonstrations of key results have been reproduced in experiments with real cleaner–client interactions (Pinto et al., 2011). The experimental set-up (**Fig.1**) consisted of a holding compartment (15 x 40cm) and an experimental compartment (45x40cm), separated by an opaque Plexiglas partition containing a small door (5x5cm). Fish were given 1-2 days to get used to the experimental set-up and to the feeding location. They were fed with mashed prawn spread on a rectangular white Plexiglas plate (5x8cm) starting from the second day of arrival in the lab. After testing, fish were kept in the experimental compartment, with the door closed and a PVC pipe provided as shelter. Every morning the gobies were directed to the holding compartment and kept there for acclimation with the door closed for 20 minutes. Meanwhile, the experimental compartment was cleaned. Fish that ate little during the experimental trials (because of many wrong decisions or because some fish would not consume items after a correct choice in the beginning) were fed with some mashed prawn by the end of the day to reduce any potential variance in individual performance caused by differences in satiation levels.

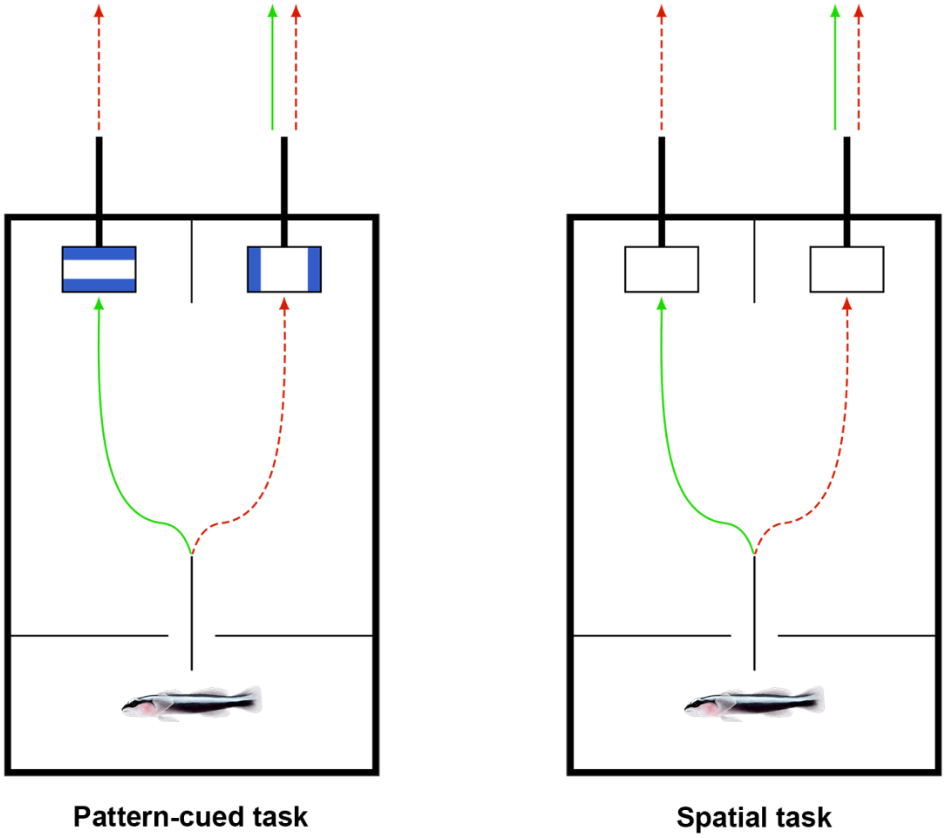

Figure 1: Diagram of the (a) pattern-cued task and (b) spatial task procedure as viewed from above. The goby is illustrated in the holding compartment and the plates are positioned in the experimental compartment. At the beginning of each trial, a small opaque barrier separating the two compartments was lifted. In both tasks, the gobies were presented with two plates separated by a small opaque partition. In the pattern-cued task (a), the plates had different patterns, whereas the plates were identical in the spatial task (b). Both plates contained a small piece of food reward located at the back of the plate. The solid and dashed lines illustrate what happened to the plates when a goby made a choice. When the goby made a correct choice (solid arrows), the plate with the incorrect pattern (a) or at the incorrect side (b) was removed and the goby is allowed to access the reward behind the correct plate. When a goby made an incorrect choice (dashed lines), both plates were removed and the goby had no access to a reward. The correct/incorrect pattern or side was determined according to the initial preference of each subject during the acclimation phase. Figure modified with the permission of S. Gingins (Gingins et al., 2018).

2.2.3. Learning and reversal learning tasks

In total, 64 gobies were assigned to one of the two different tasks: (i) pattern-cued task, in which the fish had to learn to eat on a plate with a pre-established correct pattern and (ii) spatial task, where the fish had to learn to eat on a plate positioned in a pre-established correct side of the aquarium (**Fig. 1**). In both tasks, two Plexiglas plates (5x8cm) were placed on the side of the aquarium opposite to the door. In the pattern-cued task, the plates had two different patterns (two horizontal blue stripes or two vertical blue bands) while in the spatial task both plates were white. We chose to use the blue color because it is one of the most ubiquitous colorations in reef fish due to its conspicuousness against the reef background (Marshall, 2000). We first conducted 10 familiarization trials with each goby, during which the gobies were free to eat from both plates. The opposite of the most frequent choice made during the familiarization trials (horizontal x vertical or right x left) was determined as the correct pattern for the pattern-cued task and the correct side for the spatial task. This was done to ensure that successful performance was based on learning and not preference-bias. In case a goby showed no preference, we would determine the correct pattern/side based on the preferences of other individuals from the same habitat, in order to counterbalance the correct options within subjects from the same habitat as much as possible. The subject was considered to choose a plate when it touched it or passed behind it. Whenever the subject made the correct choice, the fish would be given a maximum of 1 minute to eat on the plate. In case it chose the wrong plate, both plates were removed simultaneously and no food reward was available. Both plates had equal amounts of food on their backside, to ensure that choices were not based on olfactory cues. The left–right position of the plates was switched every trial. As soon as a trial ended, fish were gently directed back to the holding compartment with the handle of a hand net. After some trials, most of the fish had learned to swim back to the holding compartment by themselves at the end of the trial. If a fish took more than 5 minutes to choose a plate, this was considered to be an incorrect choice. In the event that this occurred for a fish that was close to reaching the learning criteria, the trial would be repeated at the end of the session. One session consisted of ten trials. The fish was considered to learn a task if: (i) it solved 9/10 or 10/10 trials in a row, (ii) it solved 8/10 trials twice in a row, (iii) it solved 7/10 trials three times in a row. We provided a maximum of 100 trials for each fish to solve the task. The successful individuals would immediately start the reversal trials in the next session, in which the correct pattern/side would be reversed and the individuals would be given another 100 trials to solve the task using the same procedure and learning criteria. Experiments were performed from 8 am to 7 pm.

Each individual was tested for a maximum of 20 trials per day with a minimum interval of 15 minutes between trials.

2.2.4. Data analysis

In order to compare the performance of phenotypes and sexes in each task we performed a survival analysis using the function Surv from package survival (Therneau, 2015). This analysis was chosen because the data is right censored, meaning that we do not know whether individuals that failed to solve the task within 100 trials could have learned if given more trials. The number of trials to solve the task was used as the response variable and a status variable was created to inform the function whether the observations were censored (individuals that did not solve the task after a 100 trials) or not (individuals that solved the task). The Surv function creates a survival object that compiles the response variable and censoring information. One survival object was created for each phase of the spatial test (learning and reversal) and used as dependent variable in the survival regression models. Cox's proportional hazards models were fitted using the function coxph from package survival (Therneau, 2015). Phenotype, sex and the interaction between them were included as fixed factors in each model. In order to investigate the role of contextual variables in the learning performance of individuals, we further included the following variables as fixed factors in both models: preference ratio, which refers to the number of trials in which an individual chose a certain pattern/side during the preference assessment; average time to enter the experimental compartment, which refers to the average time individuals took to pass through the door and enter the experimental area; average time to choose a plate, which refers to the average time individuals took to choose a plate after entering the experimental area and proportion of feeding, which refers to the proportion of correct trials in which individuals ate the food reward. We used the function Anova (type II test) from package car (Fox and Weisberg, 2011) to assess the significance of each particular factor in the models. Finally, we used the function lm from package stats (R Core Team, 2017) to test whether accuracy (percentage of correct trials) depended on the average decision making time (average time to choose a plate). Phenotype, sex, and their interactions were also included as fixed factors in this model. The significance of factors was obtained with the function Anova from package car (Fox and Weisberg, 2011). Finally, we assessed the models' validity by visual inspection of residuals homogeneity and normality.

2.3. Results

Two male sponge-dwellers solved the pattern-cued task in 10 and 60 trials whereas no cleaning gobies were able to solve it before 100 trials (**Fig. 2a**). On top of that, only one of the two sponge-dwellers solved the reversal task, after 70 trials (**Fig. 2b**). In contrast, most of the cleaning gobies and sponge-dwellers managed to succeed in the spatial task. Because only two individuals completed the pattern-cued task, we compared the performance of phenotypes and sexes exclusively for the spatial task. There were no significant differences between the two phenotypes in performance, both in the learning (**Fig. 2c**, $\chi2 = 0.50$, $p = 0.48$) and reversal trials (**Fig. 2d**, $\chi2 = 0.49$, $p = 0.48$). Additionally, the performance of males and females did not differ in the spatial learning ($\chi2=0.54$, $p=0.46$) and spatial reversal ($\chi2 = 0.01$, $p=0.94$) trials. We also found no interactions between phenotype and sex in either of the models (spatial learning: $\chi2=0.40$, $p=0.53$; spatial reversal learning: $\chi2=3.8$, $p=0.051$). Finally, the contextual variables had no effect on the gobies' performance in the learning or reversal learning of the spatial task (statistics (**Tab. S1** and **Tab. S2**) and figures (**Fig. S1**) in the electronic supplementary material) and there was no significant correlation between accuracy and decision making time during the learning and reversal learning trials of the spatial task statistics (**Tab. S3** and **Tab. S4**) and figure (**Fig. S2**) in the electronic supplementary material). However, we did find a significant crossover interaction between decision-making time and phenotype for the learning trials, meaning that the relationship direction depended on the phenotype (**Tab. S3** and **Fig. S2** in the electronic supplementary material).

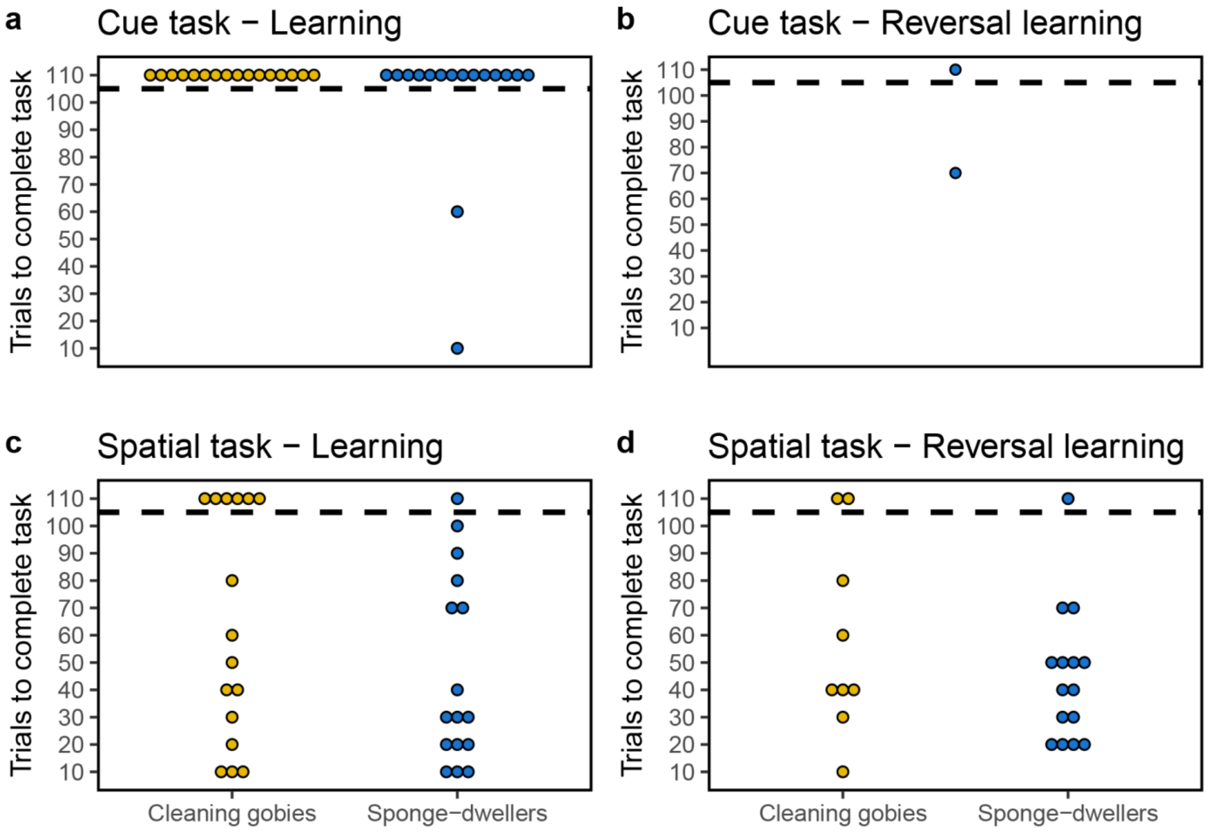

Figure 2: The performance of cleaning gobies and sponge-dwellers on the two discriminatory two-choice tasks. The plots show the number of trials (individual dots) that the gobies needed to complete the learning (a, c) and reversal learning (b, d) of the pattern-cued and spatial learning tasks. The dots above the dashed line represent fish that failed to complete the task in the allotted number of trials.

2.4. Discussion

The aim of this study was to investigate how two different forms of sociality – pair living while largely relying on cleaning interactions versus living in larger groups with little to no cleaning interactions – may predict performance of *E. prochilos* in two different learning tasks. We predicted that cleaning gobies would outperform sponge-dwellers in the pattern-cued task and that the opposite would be true in the spatial task. Contrary to our predictions, there was no difference in performance between the two types of gobies. Furthermore, the pattern-cued task was apparently very difficult as only two individuals solved it within 100 trials, which is in stark contrast to the performance of cleaner wrasses (12 individuals solved the task, Cardoso et al., 2015). Below, we further discuss which factors could have influenced the performance of the gobies in the pattern-cued and spatial tasks and what factors are potentially associated with individual variation in performance within tasks.

2.4.1. Pattern-cued task

When animals cannot solve a certain cognitive task, it is always challenging to determine if the underlying causes are related to the species' ecology, social environment, cognitive abilities, the experimental procedure or a combination of these factors (Prétôt et al., 2016b; Shettleworth, 2010). It was surprising that only two of the gobies could solve the pattern-cued task. We expected that the cleaning individuals would find this task relatively easy to solve, as they feed off a variety of client species that differ in pattern and parasite load. Indeed, cleaner wrasses can easily solve a similar dichotomous choice task in less than 40 trials (Cardoso et al., 2015). However, our study only used plates with different patterns while the cleaner wrasse study used plates with different patterns as well as with different colors (Cardoso et al., 2015). It is possible that fish perceive color cues as more salient than pattern cues, i.e. that they can more readily form associations between color cues and a reward (Ingle, 1965). In the specific case of cleaning gobies, size variation might even be more relevant. Cleaning gobies preferentially clean client species that carry more ectoparasites (Soares et al., 2007) and ectoparasite infestation is positively related to body size (Coile and Sikkel, 2013; Grutter, 1999a; Grutter and Poulin, 1998; Poulin, 2000). Nonetheless, discriminating among patterns should not be such a difficult task for fishes. The Ambon damselfish *Pomacentrus amboinensis* are able to discriminate between different printed shapes (Siebeck et al., 2009) and between plates with distinct 2D images of the same color (Binning et al., 2018). Importantly, the fact that gobies were able to solve the spatial task, which had a similar experimental procedure, indicates that the low performance in the pattern-cued task was not related to the task procedure.

Differences in cleaning interaction patterns between *E. prochilos* gobies and *L. dimidiatus* wrasses may partially explain the differences in learning performance in the pattern-cued task between the two species. We observed that *E. prochilos* gobies spent much less time in cleaning interactions (2.47% for cleaners and 0.10% for sponge-dwellers) than cleaner wrasses usually do (26-35%, Grutter, 1999b, 1996). They also interact with fewer client species (background data, Bansemer et al., 2002; Luiz et al., 2016), and are largely reactive while cleaner wrasses are proactive (7% initiation of interactions versus 35%; (Oates et al., 2010). These differences alone indicate that *E. prochilos* gobies have fewer opportunities to learn than cleaner wrasses. In addition, there is no punishment, partner switching or tactile stimulation involved in the cleaning goby mutualism (Soares et al., 2008). Contrary to cleaner wrasses that prefer to eat mucus (Grutter and Bshary, 2003), which protects client skin surface (Eckes et al., 2008; Shephard, 1994) cleaning gobies do prefer to eat ectoparasites (Soares et al., 2010), meaning that there is no conflict of interests between the gobies and their clients. The absence of conflicts between the cleaning gobies and their clients may result in a lower selective pressure on cleaning gobies for associating the pattern with positive and negative reinforcements.

2.4.2. Spatial task

Despite the differences in habitat and food acquisition between *E. prochilos* cleaning gobies and sponge-dwellers, the two phenotypes performed similarly in the spatial task. Previous studies with intertidal gobies have shown that species from rocky platforms have better spatial memory abilities than species from sandy beaches (White and Brown, 2014a, 2014b). Here we predicted that sponge-dwellers would outperform cleaning gobies in the spatial task because the food is patchily distributed in sponges, with specific locations consistently offering more food (Whiteman and Côté, 2004b). The average performance of *E. prochilos* in the spatial task (~40 trials until reaching learning criterion) was similar to that of cleaner and non-cleaner wrasse species that were tested in the same paradigm (Cardoso et al., 2015; Gingins et al., 2018, 2013). There are two possible non-exclusive explanations for why we did not find differences between the two phenotypes: either sponge-dwellers do not need improved spatial skills for finding their territory/food or cleaning gobies do match the sponge-dwellers' spatial skills for other purposes. For example, although cleaning gobies do not need to locate their main source of food (clients carrying ectoparasites), they might still use spatial learning for finding shelters and potential mates. Further research should investigate how the different components of *E. prochilos'* social and ecological environment might impose challenges that require spatial cognition.

2.4.3. Factors associated with individual performance

Although we found no differences in performance between *E. prochilos* phenotypes in the spatial task, there was large individual variation in performance within-phenotype in this task. Some studies with fish have found that individual differences in learning performance correlate with measures of boldness and sociality (Kareklas et al., 2017; Mamuneas et al., 2015; Trompf and Brown, 2014). There is currently a debate on whether there is a connection between behavioral types (e.g. in respect to boldness, aggressiveness, exploration tendency) and cognitive speed-accuracy trade-offs (Sih and Del Giudice, 2012). Some studies have found that individuals making faster decisions were less accurate (Burns and Rodd, 2008; Wang et al., 2015) whereas others have found that some aspects of decision-making and learning are related to boldness and sociality, but independent of speed-accuracy trade-offs (Kareklas et al., 2017; Mamuneas et al., 2015; Trompf and Brown, 2014). In our study, we found no evidence for the decision speed and decision accuracy trade-off because the average time for making a choice and choice accuracy were not significantly correlated. Moreover, contextual variables like the latency to enter the experimental compartment and the average time taken to make a choice did not explain the observed differences in learning performance in the spatial task. Therefore, our additional analyses failed to explain any part of the variance in goby learning performance within spatial tasks.

In conclusion, the ecological differences between cleaning and sponge-dwelling populations of *E. prochilos* do not seem to cause differences in individual performance in the two learning tasks. While we lack information on whether habitat choices are linked to any innate preferences in a genetically diverging population or whether choice is based on individual decisions linked to the current local distribution of conspecifics, the two social and environmental conditions appear to be so different that we expected genetic or ontogenetic divergence to have taken place. With this in mind, we chose two learning tasks that we expected to capture relevant ecological differences. However, it seems that associating the choice of a pattern with positive or negative reinforcement is not important for engaging in cleaning interactions in gobies. Furthermore, it appears that spatial memory skills are equally present in a foraging context in both phenotypes. We hypothesize that the benefits of associative pattern learning may be reduced for *E. prochilos* cleaning gobies because they spent less time in cleaning interactions, interact with a lower diversity of client species and have no conflict of interests with their clients compared to cleaner wrasses. Further research should investigate which aspects of cleaning and sponge-dwelling gobies' ecological and social environment may require spatial learning. Of particular interest could be to explicitly test the intraspecific social skills of gobies from the two habitats. The social competence approach (Bshary and Oliveira, 2015; Taborsky and Oliveira, 2012) predicts that individual social decision rules should be adjusted to the specifics of the social environment. Typically living in pairs, as cleaning gobies do, should require different social skills than living in large groups with a hierarchy, as sponge-dwelling gobies do. This intraspecific variation in social organization that can be observed within a single location offers great potential to study the ontogeny, physiology, cognition and brain organization underlying social behavior.

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2.6. Supplementary Information

Table S1. Analysis of covariance table: **Survival model for learning performance in the spatial task**. The number of trials needed to learn was included as the response variable. Average time to enter the experimental compartment (average time individuals took to pass through the door and enter the experimental area), proportion of feeding (proportion of correct trials in which individuals ate the food reward), average time to choose a plate (average time individuals took to choose a plate after they entered the experimental area), preference ratio (number of trials (1 to 10) in which an individual chose a certain pattern/side during the preference assessment), phenotype (cleaning goby or sponge-dwellers) and sex (male or female) were included as main effects. All interactions included in the model are presented.

SOURCE OF VARIATION	LR CHISQ (X^2)	PR (>CHISQ) (P VALUE)
Main Effects		
Average Time To Enter The Experimental Compartment	3.1965	0.07380
Proportion Of Feeding	2.4881	0.11471
Average Time To Choose A Plate	3.3305	0.06801
Preference Ratio	0.3620	0.54738
Phenotype	0.5060	0.47687
Sex	0.5438	0.46086
Two-Way-Interactions		
Phenotype X Sex	0.3964	0.52895

Table S2. Analysis of covariance table: **Survival model for reversal learning performance in the spatial task**. The number of trials needed to learn was included as the response variable. Average time to enter the experimental compartment (average time individuals took to pass through the door and enter the experimental area), proportion of feeding (proportion of correct trials in which individuals ate the food reward), average time to choose a plate (average time individuals took to choose a plate after entering the experimental area), preference ratio (number of trials (1 to 10) in which an individual chose a certain pattern/side during the preference assessment), phenotype (cleaning goby or sponge-dwellers) and sex (male or female) were included as main effects. All interactions included in the model are presented.

SOURCE OF VARIATION	**LR CHISQ (X^2)**	**PR (>CHISQ) (P VALUE)**
Main Effects		
Average Time To Enter The Experimental Compartment	2.8187	0.09317
Proportion Of Feeding	0.3303	0.56548
Average Time To Choose A Plate	0.5816	0.44567
Preference Ratio	1.6820	0.19465
Phenotype	0.4956	0.48143
Sex	0.0054	0.94143
Two-Way-Interaction		
Phenotype X Sex	3.8117	0.05089

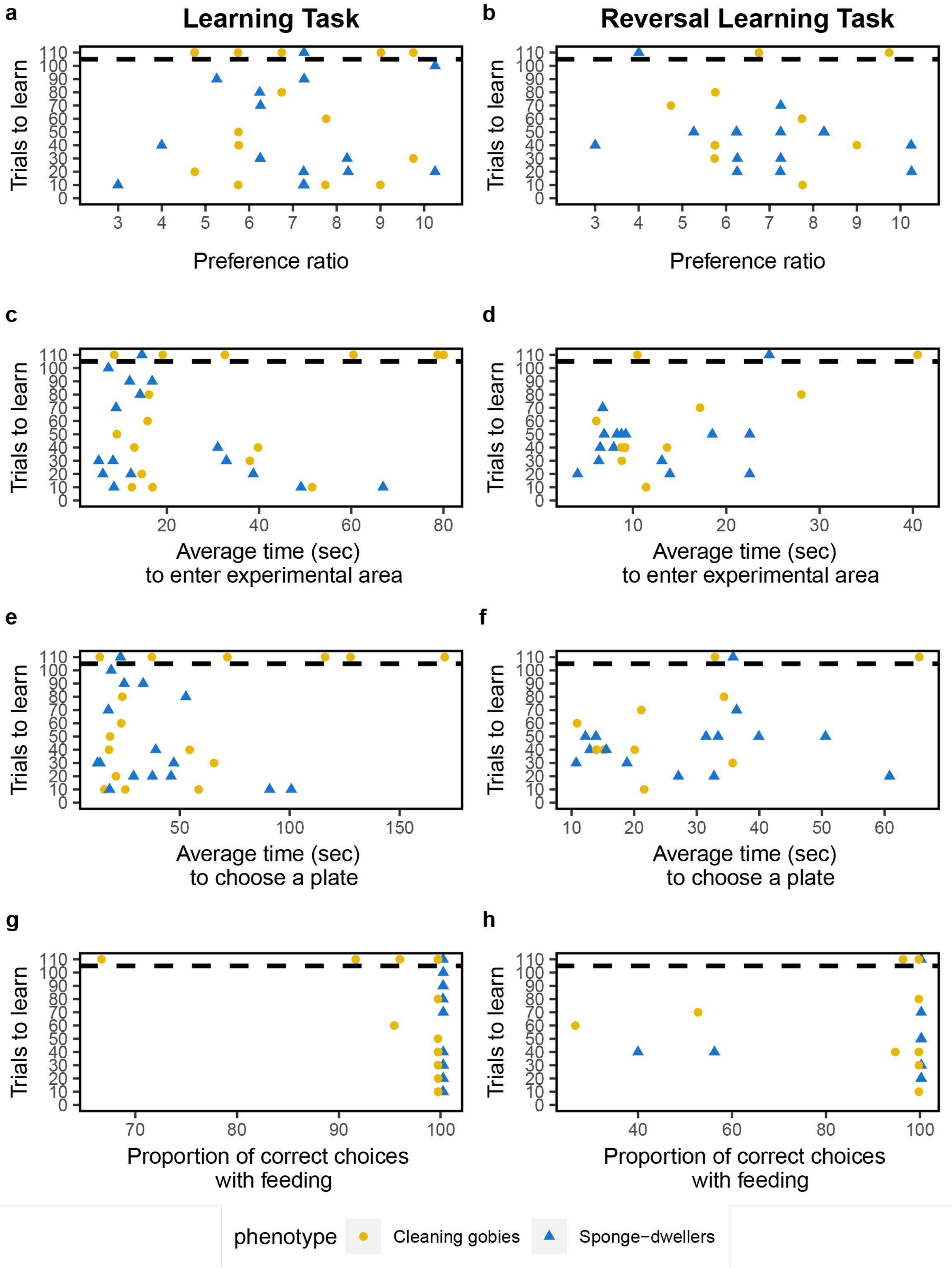


Figure S1: Relationship between learning (left column) and reversal learning (right column) performance in the spatial task and the following contextual variables: (a, b) preference ratio: number of trials in which an individual chose a certain pattern/side during the preference assessment; (c, d) average time individuals took to pass through the door and enter the experimental area; (e, f) average time individuals took to choose a plate after entering the experimental area; (g, h) proportion of correct trials in which individuals ate the food reward. Dots represent the performance of cleaning gobies and triangles the performance of sponge-dwellers. The dots and triangles above the dashed line represent fish that failed to complete the task in the allotted number of trials.

Table S3. Analysis of covariance table: **Linear model for learning accuracy in the spatial task**. The percentage of correct trials was included as response variable. The average time to choose a plate, phenotype (cleaning goby or sponge-dweller) and sex (male or female) were included as main effects. All interactions included in the model are presented. Asterisks depict significant effects (α = 0.05).

SOURCE OF VARIATION	SUM SQ	F VALUE	PR (>F)
Main Effects			
Average Time To Choose A Plate	601.1	1.4989	0.232717
Phenotype	242.6	0.6050	0.444289
Sex	1407.1	3.5090	0.073265
Two-Way-Interactions			
Average Time To Choose A Plate X Phenotype	4347.1	10.8407	0.003067*
Average Time To Choose A Plate X Sex	394.1	0.9829	0.331371
Phenotype X Sex	146.6	0.3657	0.551034
Three-Way-Interaction			
Average Time To Choose A Plate X Phenotype X Sex	661.7	1.6501	0.211208

Table S4. Analysis of covariance table: Linear model for **reversal learning accuracy in the spatial task**. The percentage of correct trials was included as the response variable. The average time to choose a plate, phenotype (cleaning goby or sponge-dweller) and sex (male or female) were included as main effects. All interactions included in the model are presented.

SOURCE OF VARIATION	SUM SQ	F VALUE	PR (>F)
Main Effects			
Average Time To Choose A Plate	222.9	0.6139	0.4441
Phenotype	70.1	0.1930	0.6660
Sex	0.3	0.0007	0.9793
Two-Way-Interactions			
Average Time To Choose A Plate X Phenotype	0.2	0.0005	0.9829
Average Time To Choose A Plate X Sex	275.9	0.7600	0.3955
Phenotype X Sex	90.9	0.2504	0.6232
Three-Way-Interaction			
Average Time To Choose A Plate X Phenotype X Sex	16.3	0.0448	0.8348

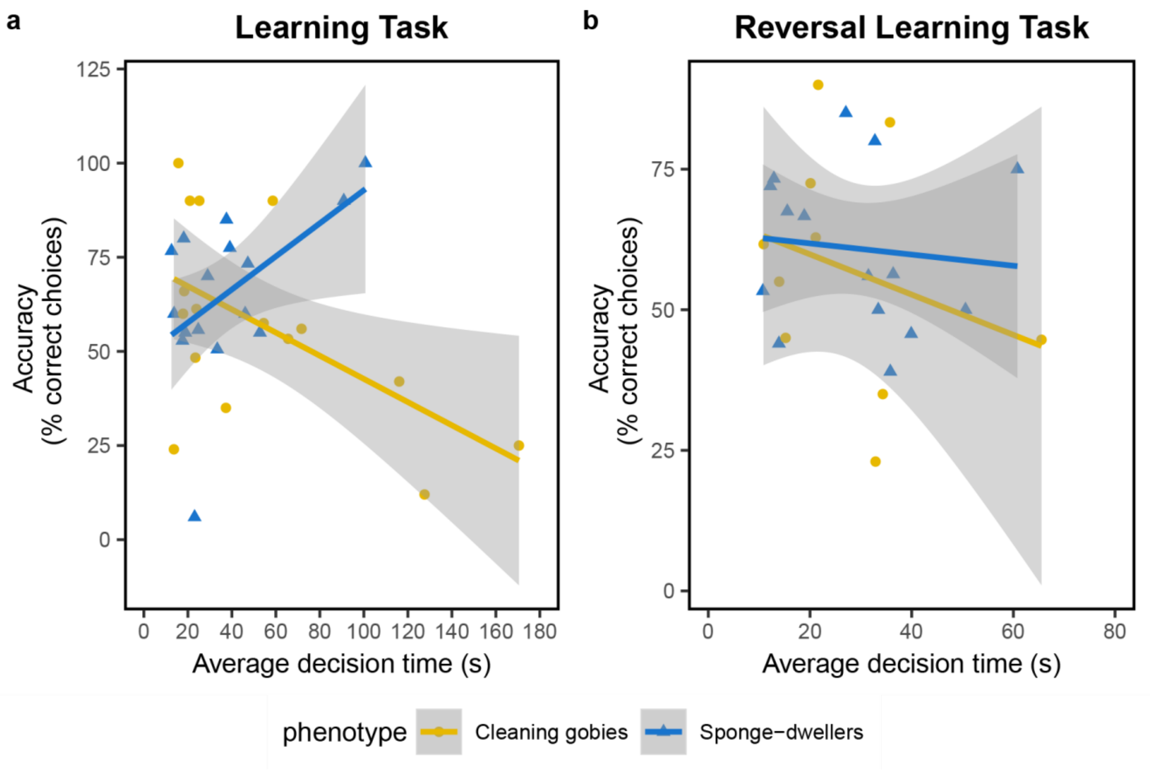


Figure S2: Relationship between learning (a) and reversal learning (b) accuracy in the spatial task and the average time to choose a plate for cleaning gobies (dots) and sponge-dwellers (triangles). The solid lines indicate the linear model prediction and the grey areas the 95% confidence interval.

Chapter 3 | Brain structure differences in cleaning and sponge-dwelling Caribbean *Elacatinus* gobies

Abstract

Comparative studies of brain anatomy between closely related species have been very useful in demonstrating selective changes in brain structure. They have identified mosaic patterns of brain evolution, i.e. when variation in brain structure across species is the result of specific selection pressure to certain brain regions. Within-species comparisons are also useful for identifying changes in brain structure caused by contrasting environmental selection pressures. In the present study, we aimed to understand how differences within and between species in habitat use and feeding behavior influence brain morphology on both ecological and evolutionary time scales. We used as model system three species of the *Elacatinus* genus that differ in their habitat-feeding mode. The obligatory cleaning goby *Elacatinus evelynae* inhabits mainly corals and feed mostly on ectoparasites removed from larger fish during cleaning interactions. In contrast, the obligatory sponge-dwelling goby *Elacatinus chancei* inhabits tubular sponges and feed on microinvertebrates buried in the sponges' tissues. Finally, the facultative cleaning goby *Elacatinus prochilos* individuals can adopt either phenotype, the cleaning or the sponge-dwelling habitat-feeding mode. By comparing the brains of the facultative goby phenotypes to the brains of the obligatory species we can test whether brain morphology is better predicted by phylogenetic relatedness or the habitat-feeding modes (cleaning x sponge-dwelling). We specifically compared the relative volume of different brain areas and nuclei across species and found that *E. prochilos* brains from both types (cleaning and sponge-dwelling) were highly similar to each other, and in general more similar to the brains of the most closely related species, *E. evelynae* (obligatory cleaning species), than to the brains of *E. chancei* (sponge-dwelling species). In contrast, we found significant brain structure differences between the cleaning species (*E. evelynae* and *E. prochilos*) and the sponge-dwelling species (*E. chancei*) that revealed independent changes in functionally correlated brain areas that might be ecologically adaptive. *E. evelynae* and *E. prochilos* had a relatively larger visual input processing axis and a relatively smaller lateral line input processing axis than *E. chancei*. These results indicate that the use of tubular sponges in the non-cleaning clade might play a more important role in selecting for brain structure differences than other ecological variables related to their feeding modes.

3.1. Introduction

Comparative studies of brain anatomy have tried to understand vertebrate brain evolution by exploring the association of relative brain size to a number of life history, ecological, cognitive and behavioral traits (Aiello and Wheeler, 1995; Armstrong, 1983; Deaner et al., 2007; Dunbar and Shultz, 2017; Harvey and Bennet, 1983; Isler and van Schaik, 2009; van Schaik et al., 2012). Although this approach has brought valuable insights into the factors shaping variation in brain structure across contemporary species, it has yielded some inconsistent results. For example, some studies with primates did not find the usual correlation between brain size and sociality (DeCasien et al., 2017) or brain size and longevity (Allman et al., 1993). Studies with fish have also found inconsistent correlations between brain size and habitat complexity or social organization (Ahmed et al., 2017; Pollen et al., 2007; Reddon et al., 2016). The main problem with using brain size or relative brain size as a correlate for comparative studies is that it requires many assumptions about the methods used for measuring the brain, on how brain size scales with body size, on how brain regions scale with brain size and finally, on how brain size is associated with cognition (Logan et al., 2018). In other words, the current comparative approach lacks information on how natural selection actually modifies the mechanisms underlying behavior and cognition (Healy and Rowe, 2007; Herculano-Houzel, 2011; Logan et al., 2018). By focusing exclusively on brain size and/or the size of major brain areas like telencephalon or diencephalon, we ignore the changes in specific brain areas or nuclei that cause behavioral, cognitive and brain size differences (Logan et al., 2018).

The comparative detailed analysis of the relative size of different brain areas or nuclei can help reveal how selection operates within the brain. Two non-exclusive models have been proposed for explaining how brains evolve. The mosaic evolution model suggests that variation in brain structure across species are the result of specific selection pressure to certain brain regions (Barton and Harvey, 2000; Smaers and Soligo, 2013). In contrast, the developmental constraints model recognizes that genetic pleiotropic effects and constraints of the developmental process may coordinate brain development in a way that variation in brain structure is followed by the adjustment of the proportions of the different brain areas (Finlay et al., 2001; Finlay and Darlington, 1995; Jerison, 1991). Because the two models are non-exclusive, the real question here is to which extent developmental constraints can prevent selection from acting on specific brain components. Selection on specific brain components is likely to be happening when (i) partial correlations among individual components correspond to functional connections, (ii) individual components deviate from general patterns of correlated evolution and (iii) variation in component size is more correlated with ecology than with overall brain size (Montgomery et al., 2016).

Comparisons of brain structure within species have been particularly useful for demonstrating selective changes in the brain. Individuals from a same species are more likely to have similar developmental rules for brain scaling and therefore, differences in the relative size of brain areas are more likely to be caused by contrasting environmental selection pressures. This hypothesis has found empirical support in artificial selection studies. For example, artificial selection of brain sizes in guppies revealed that brain regions respond similarly to strong directional selection on relative brain size (Kotrschal et al., 2017). This indicates that there are no scaling rules that selective cause changes in certain areas with brain size increase. Instead, brain anatomy variation in contemporary species is most likely due to direct selection on key regions (Kotrschal et al., 2017). Similarly, artificial selection in mice for motor performance caused a selective increase in the midbrain that was independent of the size of other brain areas (Kolb et al., 2013). Finally, studies with recently diverged wild populations also identified mosaic patterns of brain evolution at a micro-evolutionary scale. Environmental or behavioral variation has been found to be associated with variation of brain architecture independent of overall brain size in nine-spine sticklebacks, three-spine sticklebacks and brown trout (Gonda et al., 2011; Kolm et al., 2009; Park and Bell, 2010). Despite these recent advances in the understanding of brain morphology in respect to micro-evolutionary changes, more studies are needed for a proper comparative analysis.

Here we aim to fill this gap by conducting between and within species comparisons of brain structure in the Caribbean *Elacatinus* gobies. The *Elacatinus* species are separated in two distinct phylogenetic clades, one comprising mostly cleaning species, and the other comprising sponge-dwelling species (Colin, 2010; Ruber et al., 2003; Taylor and Hellberg, 2006). The cleaning species live on corals or substrata other than sponge, where they maintain cleaning stations and feed mostly on ectoparasites removed from larger fish (Côté and Soares, 2011). In contrast, sponge dwellers live in close association with barrel sponges or tubular sponges and feed mainly on micro invertebrates buried in the sponges' tissues (Colin, 1975). Interestingly, the habitat-feeding mode separation can occur within some species (Whiteman and Côté, 2004, 2002). The clade hence provides a unique opportunity for analyzing how differences in habitat use and feeding behavior influence brain morphology on both ecological and evolutionary time scales.

We specifically compared the relative volume of different brain areas and nuclei among three species of the *Elacatinus* genus that co-occur in the reefs of Barbados: *Elacatinus evelynae*, *Elacatinus prochilos* and *Elacatinus chancei*. The two former species belong to the cleaning clade and the latter to the sponge-dwelling clade. Despite being more closely related to the cleaning species, *E. prochilos* is actually a facultative species that exhibit intraspecific variation in the habitat-feeding mode. Therefore, *E. prochilos* individuals either adopt the cleaning or the sponge-dwelling habitat-feeding mode. First, we asked whether the brain structure of the two individual phenotypes of *E. prochilos*, in terms of the relative volume of different brain areas and nuclei, is rather conserved and hence invariably more similar to *E. evelynae* than to *E. chancei*. Alternatively, if there is a major effect of habitat selection, cleaner *E. prochilos* individuals might have brain morphology that is more similar to *E. evelynae*, while sponge-dwelling *E. prochilos* individuals might have a brain morphology that is more similar to *E. chancei*. Second, we asked whether variations in the relative volume of brain areas across species and phenotypes indicate a concerted or mosaic pattern of brain evolution. A concerted pattern would be characterized by all species/phenotypes having similar residual brain area volumes, while a mosaic pattern would be characterized by residual differences in some particular components between species/eco-types.

3.2. Methods

3.2.1. Capture and housing

The *Elacatinus prochilos* gobies were caught in 2017 from both barrel sponges (sponge-dwellers, N=32) and cleaning stations (cleaning gobies, N=32) from three fringing reefs on the west coast of Barbados (North and South Bellairs 13.191542° N, 59.641882° W; Heron Bay 13.196817° N, 59.643521° W and Greensleeves 13.225882° N, 59.644153° W). Sponge-dwellers formed groups of 5 up to 80 individuals and were associated with giant barrel sponges (*Xestospongia muta*) in the patch reef zone (6 to 10 meters). In contrast, cleaning gobies were found either solitary or in pairs, mainly associated with live coral, but also with other substrates like coralline algae and dead coral in the spurs and grooves zone (3 to 8 meters). The gobies were captured using plastic bags and hand nets after sedating them with a spray mixture of 50 ml of clove oil and 200 ml of ethanol (1:4) diluted in approximately 700 ml of seawater. Fish were then transported to the lab inside zip lock bags. Individuals belonging to the same sponge or the same cleaning station were transported together in the same bag and were housed together either in a glass aquarium (60 x 40 x 39 cm) or mesh bags placed inside an aquarium (15cm diameter), depending on the group size. Within two days after capture, each goby was sexed, sized, weighted and individually housed in a mesh bag. Individuals' size and weight ranged from 2.2 to 3.4 cm and 0.07 to 0.39 g. To determine the sex, we observed the shape of the urogenital papilla under a binocular microscope. The papilla is conical and long in males and short and truncated in females (Thresher, 1984). Because the individuals had to be flipped over for the papilla observation, we sedated each goby before the procedure in a petri dish containing 100 ml of seawater and around 5 ml of clove oil mixture. Together, sexing, sizing and weighting took less than 2 minutes per goby. The gobies were left to recover in another petri dish with seawater before being returned to their mesh bags.

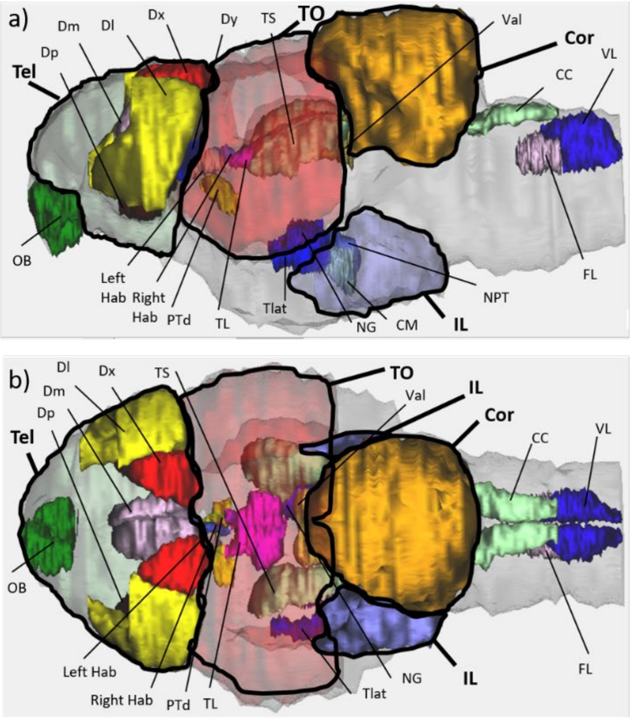
Another 2 to 3 days after this procedure, part of the gobies (48 out of 64 individuals) was injected with an elastomer implant. The implants were used for individual identification in a social group manipulation that occurred prior to the learning tasks. The social group manipulation consisted of forming groups of cleaning and sponge-dwelling gobies, each containing 6 individuals (one pair designated to a dominant position, one pair designated to a subordinate role and one to two juveniles), and registering all interactions among individuals (see details on Chapter 1). After the group manipulations, all the 64 gobies were assigned to one of the two different tasks: (i) pattern-cued task, in which the fish had to learn to eat on a plate with a pre-established correct pattern and (ii) spatial task, where the fish had to learn to eat on a plate positioned in a pre-established correct side of the aquarium (see details on Chapter 2). The sex and size of individuals within a task was counterbalanced for each phenotype. Individuals stayed in the lab for a maximum of 3 to 7 weeks before being sacrificed for brain anatomy analysis. The laboratory experiments with *E. prochilos* were conducted from March to July of 2017. The *Elacatinus evelynae* (N=16) and *Elacatinus chancei* (N=15) gobies were collected from South Bellairs and Carlisle Bay (13.085908° N, -59.612192°W) reefs in May of 2018. *E. evelynae* were mostly found in pairs and associated with coral heads while *E. chancei* were found mostly alone inside the tubes of the tubular sponge *Aplysina fistularis*. The capturing procedure was the same described for *E. prochilos*. No behavioral or cognition experiments were conducted with these species, therefore individuals were sacrificed within 2 days after capture.

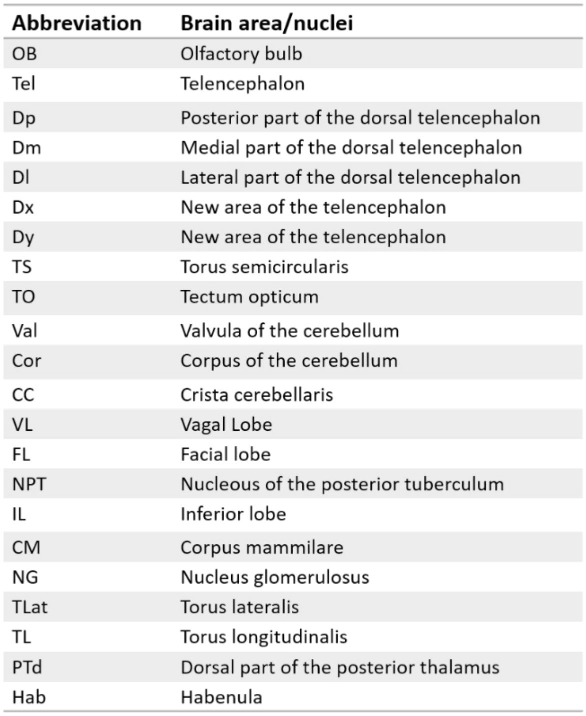
3.2.2. Preparation of samples and brain processing

All gobies were anesthetized and euthanized with a clove oil mix overdose (50 ml of clove oil and 200 ml of ethanol (1:4) diluted in approximately 100ml of seawater). The individuals were left in the clove oil solution for a minimum of 5 minutes or until the heartbeat could not be noticed anymore under a binocular microscope. The sex, size, and weight of individuals were reassessed and the dorsal portion of the cranium was removed. Individuals were fixed in 4% unbuffered formalin and remained preserved until brain removal (3 to 5 months later). The brains were carefully removed from the skull with tweezers and scissors and transferred to the same fixative. After at least a day in fixative, the brains were watered for 24 h, placed in 70% ethanol for three days and subsequently stained with 0.5% cresyl violet solution + 0.1% Triton X-100 for 3h. The Cresyl violet is commonly used in the Nissl staining method, which is a classical histological method for staining neural tissue. In this method, the basic dye binds to negatively charged structures like RNA and DNA. After staining, the samples were washed several times with distilled water and stored overnight. The day after, the brains were dehydrated through immersion in a series of alcohol solutions of ascending concentration (50%, 70%, 80%, 90% and 96% Ethanol). Each immersion took 30 min, including the replacement of the Ethanol after 15 min. At the end of the alcohol series, the brains were incubated for 3 h in a pre-infiltration solution (1:1 solution of 96% ethanol and Technovit 7100 methacrylate) and then let overnight in an infiltration solution (mixture of 100 ml Technovit 7100 methacrylate + one package of hardener 1). The following day, the brains were embedded in Teflon or silicon molds with 15ml of the infiltration solution mixed with 1ml of hardener II. During all steps of the staining and embedding process, the brains were placed on a shaker table in order to facilitate the infiltration of the substances and the staining process. After the embedding process, the last step of brain preparation consisted of removing the hardened brains from the molds and gluing them to a bolt using the two-component resin Technovit 3040 (2:1 ratio powder and liquid). The Methacrylate blocks were then cut in caudal-frontal orientation on an automatic microtome equipped with a fluorescence microscope (rhodamine filter set). The cresyl violet fluorescence of the block face was imaged after every section (5 µm thickness) and the images stored on a computer as a stack.

3.2.3. Volume measurements

The brain sections images were analyzed in the software Animalexplorer 2.0 (designed by Hofmann, M.; unpublished information). Individual brain part volumes are obtained by manually segmenting the different brain sections. Due to the extraordinary number of sections produced with the microtome, not all sections were segmented. For a given brain part, the first and final section were identified and a skipping factor determined how many sections could be skipped according to the size of each area. For the gobies brains, at least 30 to 50 sections of each brain area were segmented. The final absolute volume of each brain part is calculated based on the segments made for each brain area. The brain areas investigated for this study are listed and illustrated in **Figure 1**. Hereafter, we use the term brain areas for the major brain divisions (Telencephalon, Tectum opticum, Inferior lobe, Cerebellum) and brain nuclei for all the other areas listed in **Figure 1**. The brain areas and nuclei were chosen based on previous histological analysis that confirmed the similarities and borderlines of cell groups found in other gobies species (Gebhardt and Hofmann, unpublished data).Two brains could not be segmented due to methodological problems during the cutting or staining process





Abbreviation	Brain area/nuclei
OB	Olfactory bulb
Tel	Telencephalon
Dp	Posterior part of the dorsal telencephalon
Dm	Medial part of the dorsal telencephalon
Dl	Lateral part of the dorsal telencephalon
Dx	New area of the telencephalon
Dy	New area of the telencephalon
TS	Torus semicircularis
TO	Tectum opticum
Val	Valvula of the cerebellum
Cor	Corpus of the cerebellum
CC	Crista cerebellaris
VL	Vagal Lobe
FL	Facial lobe
NPT	Nucleous of the posterior tuberculum
IL	Inferior lobe
CM	Corpus mammilare
NG	Nucleus glomerulosus
TLat	Torus lateralis
TL	Torus longitudinalis
PTd	Dorsal part of the posterior thalamus
Hab	Habenula

Figure 1: Illustrative indication in (a) lateral view an (b) dorsal view of brain areas/nuclei measured in this study and listed on the table. The black thick lines are just for illustration of major brain divisions and do

not represent real measurements. The colored areas represent real reconstructions of brain nuclei volumes based on segmentations made on a female cleaning *Elacatinus prochilos* brain sections.

3.2.4. Data analysis

In order to test whether the volume of different brain areas and nuclei are better predicted by phylogenetic relatedness or ecological factors, we ran univariate and multivariate linear models (LM) using the function lm from package stats (R Core Team, 2017). The univariate linear model included total brain volume as dependent variable and the multivariate model included the volumes of all brain areas and nuclei indicated in **Figure 1** (using the cbind function), except olfactory bulb, which could only be accurately measured in a few individuals. Both models included the two species and the two *E. prochilos* phenotypes (the four groups will be hereafter referred to as phenotypes) and sex as fixed factors. The univariate model for brain volume included body length as covariate and the multivariate model for brain areas included brain volume as covariate. To test for differences in the allometric relationships across the different phenotypes and sex, we first ran the models including all interaction terms between the factors phenotype, sex and the covariates (body length or total brain volume) (Engqvist, 2005). We tested the significance of the main factors and interactions using the function Anova from package car (Fox and Weisberg, 2018) and conducted post-hoc pairwise comparisons between the phenotypic groups using the function lsmeans from package lsmeans (Lenth, 2016). For the multivariate model, we conducted pairwise comparisons separated for each brain area/nuclei in order to establish which brain areas are causing the observed differences between phenotypes in the multivariate analyses. The lsmeans function automatically adjusts the p value levels for multiple tests within groups of comparisons (in this case, 4). Two individuals for which the body length was missing were excluded from the univariate model. For both models, we checked if the results were consistent when setting the dependent variables as absolute volumes, the log of the absolute volumes or residual volumes (residuals obtained from the regression of total brain volume/brain areas volume and body length/brain volume). We decided to keep the results of both models because there is no consensus in the literature on which is the best way of correcting brain volumes data (Deaner et al., 2007; Engqvist, 2005) and therefore we should evaluate whether different models can lead us to different interpretations. All models were validated with graphical inspection of the distribution of the residuals. In addition, we constructed a multidimensional scaling plot (MDS) for better visualizing how similar the brains of the different phenotypes were. For constructing this plot, we first calculated the distance matrix using the dist function and then used the cmdscale function, from package stats (R Core Team, 2017). Finally, in order to control for potential effects of our cognitive/social competence tests in the brain volumes of the *E. prochilos* phenotypes, we ran separate LMs to test whether the residual total brain volume differed between individuals subjected to different cognitive tests (pattern-cued task or spatial task) or social status (dominant or subordinate). In addition, we checked whether the residual brain size correlated with performance (number of trials to solve the task) in the spatial task.

In order to test whether the variations in the relative volume of brain areas across species and phenotypes indicate a concerted or mosaic pattern of brain evolution, we checked for positive or negative correlations between the residual volumes (i.e. variation in the volume of brain areas that is independent of total brain volume) across all the measured areas. The residual volumes were obtained from the regression of the multivariate dependent variable and brain volume using the function lm from package stats (R Core Team, 2017). We visualized the correlations using the pairs function from package lattice (Deepayan, 2008). In order to look into the differences between species and phenotypes in more detail, we used the Volume Ratio Transformation (VRT) method (Hofmann, in review). This method allows comparisons between the phenotypes by using matrices of the ratios of each brain part divided by another. The ratios comparison do not need any scaling correction and are particularly indicated when one cannot measure all the areas of the brain. In these cases, using total brain size as correcting factor can generate misleading comparisons of a particular area between groups, because the changes in the relative volume of the area might actually be caused by the decrease/increase of other areas. With the VRT method, we are able to identify the brain areas that consistently take a higher percentage of other brain areas in a certain group, and therefore are likely to be enlarged in that particular group. The advantage of using this method for analyzing the patterns of change in the brain is that it filters out all scaling differences common to both groups and only shows allocation differences between brain areas.

3.3. Results

Concerning the differences between species/phenotypes, we found significant differences in the total brain volume across phenotypes, a significant positive correlation between brain volume and body length, but no significant differences in brain volume between sexes (**Fig. 2**, **Table 1a**). No significant interactions were found in the complete model (Phenotype:Length, $F = 1.33$, $p = 0.269$;

Phenotype:Sex, F = 0.24, p = 0.865; Length:Sex, F = 1.2298, p = 0.2709904; Phenotype:Length:Sex, F = 2.17, p = 0.098). *Elacatinus prochilos* cleaning gobies' brains were significantly larger than the brains of all other phenotypes (**Fig. 2**, **Table 1b**). Even after correcting for body length differences, there were still significant differences across phenotypes (F = 6.40, p < 0.001) and no differences differences across sexes (F = 1.50, p = 0.223) nor significant interactions (Phenotype:Sex, F = 0.51, p = 0.67). Finally, the separate analysis for *E. prochilos* phenotypes revealed that there were no significant differences in the corrected total brain volume between individuals subjected to the different cognitive tasks (F = 0.62, p = 0.433) or subjected to different social status (F = 1.25, p = 0.292), as well as no significant interactions between phenotype and task (F = 0.06, p = 0.803) or phenotype and social status (F = 0.7023, p = 0.500). We also did not find a significant correlation between corrected brain size and number of trials to learn the spatial task (F = 1.64, p = 0.213) nor a significant interactions with the phenotype (F = 0.34, p = 0.565).

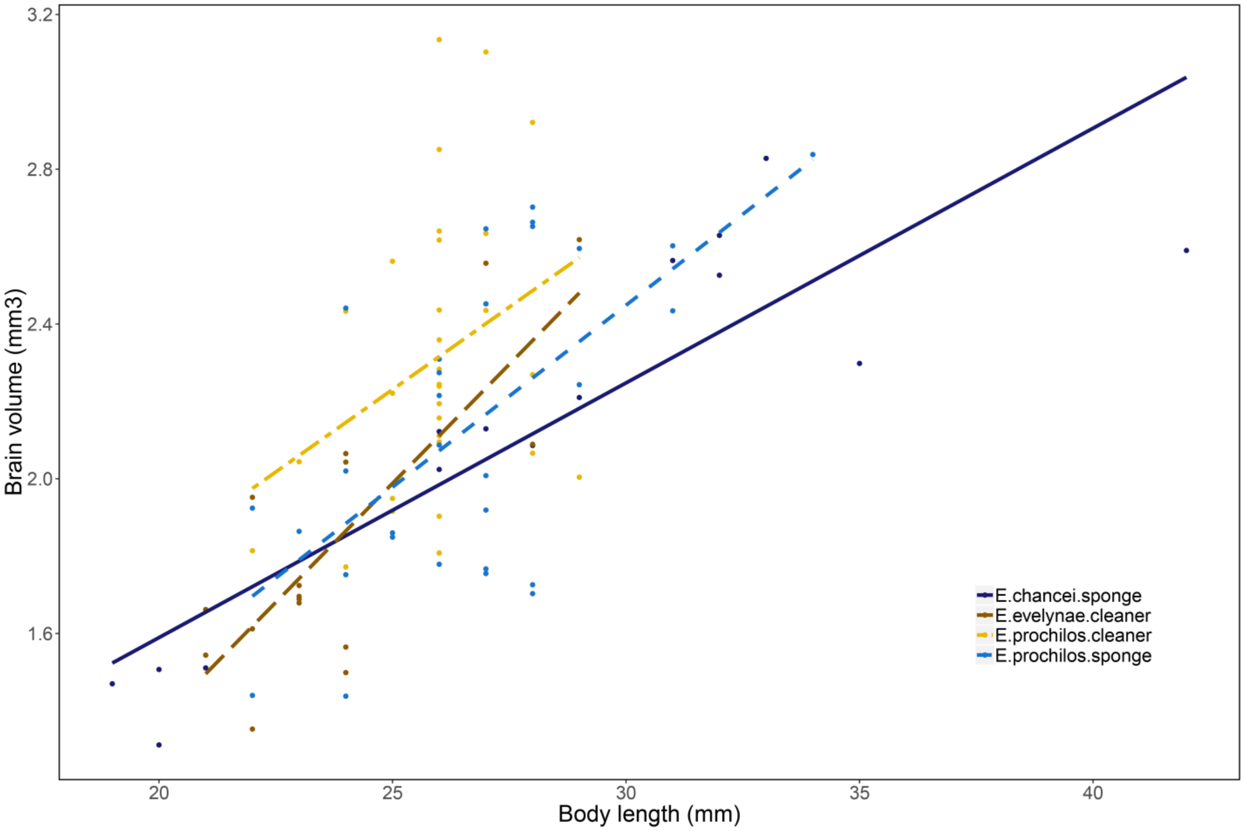


Figure 2: Relationship between body length (mm) and total brain volume (mm3) for the four phenotypes: sponge-dwelling gobies from the species *E. chancei* and *E. prochilos* (sponge – dark blue/light blue lines) and cleaning gobies from the species *E. evelynae* and *E. prochilos* (cleaner – dark yellow/ yellow lines).

Table 1a: Analysis of covariance table for lm model. Total brain size was included as response variable and the phenotypes (*E. chancei*, *E.evelynae*, *E. prochilos* cleaning and *E. prochilos* sponge-dwelling), individuals size (length.mm) and sex (male x female) were included as main effects. Asterisks depict significant effects (α = 0.05).

PREDICTORS	SUM SQ	DF	F VALUE	PR(>F)	
phenotypes	1,8198	3	6,4725	0,0005346	*
Length.mm.	5,7636	1	61,4987	1,18E-11	*
Sex	0,144	1	1,5365	0,2185476	
Residuals	7,9661	85			

Table 1b: Pairwise contrasts between phenotypes.

CONTRASTS	ESTIMATE	SE	DF	T.RATIO	P.VALUE
E.chancei.sponge - E.evelynae.cleaner	-0,0779	0,12	85	-0,649	0,9158
E.chancei.sponge - E.prochilos.cleaner	-0,3738	0,1002	85	-3,732	0,0019*
E.chancei.sponge - E.prochilos.sponge	-0,1353	0,0983	85	-1,377	0,5173
E.evelynae.cleaner - E.prochilos.cleaner	-0,296	0,0974	85	-3,038	0,0164*
E.evelynae.cleaner - E.prochilos.sponge	-0,0575	0,0997	85	-0,576	0,9389
E.prochilos.cleaner - E.prochilos.sponge	0,2385	0,0795	85	2,999	0,0183*

The multivariate analysis of the volume of different brain areas also revealed significant differences across phenotypes, a significant positive correlation with brain volume, and no significant differences between sexes (**Table 2a**). Again, no significant interactions were found in the complete model (Phenotype:Length, approx. F = 0.95, p = 0.565; Phenotype:Sex, approx. F = 0.87, p = 0.689; Length:Sex, approx. F = 1.28, p = 0.309; Phenotype:Length:Sex, approx. F = 0.83, p = 0.753). The brain areas that significantly differed between species are presented in **Table 2b**. The multivariate model using the residuals of brain area volumes as dependent variable gave slightly different results (**Table 2c**) concerning the brain areas that were different between groups.

Both models consistently found that *E. chancei* had significantly smaller Inferior lobe (**Fig. 3b**) and Nucleus glomerulosus (**Fig. 4h**) than all other groups, significantly smaller Tectum opticum than both phenotypes of *E. prochilos* (**Fig. 4c**), significantly larger Valvula of the cerebellum than *E. prochilos* sponge-dwelling phenotypes (**Fig. 4o**) and significantly higher Torus semicircularis (**Fig. 4k**), Torus longitudinalis (**Fig. 4l**), Crista cerebellaris (**Fig. 4m**) and Habenula (**Fig. 4f**) than all other groups. The model using the corrected brain areas size also indicated that *E. prochilos* cleaning phenotypes have larger Inferior lobes than the sponge-dwelling phenotypes (**Fig. 3b**) and larger Dy (**Fig. 4e**, new area of the telencephalon) than *E. evelynae*. The corrected model also found that *E. chancei* have larger Dl (**Fig. 4b**) and Dx (**Fig. 4d**) (dorso lateral and another new dorsal area of the Telencephalon) than the *E. prochilos* cleaning phenotypes.

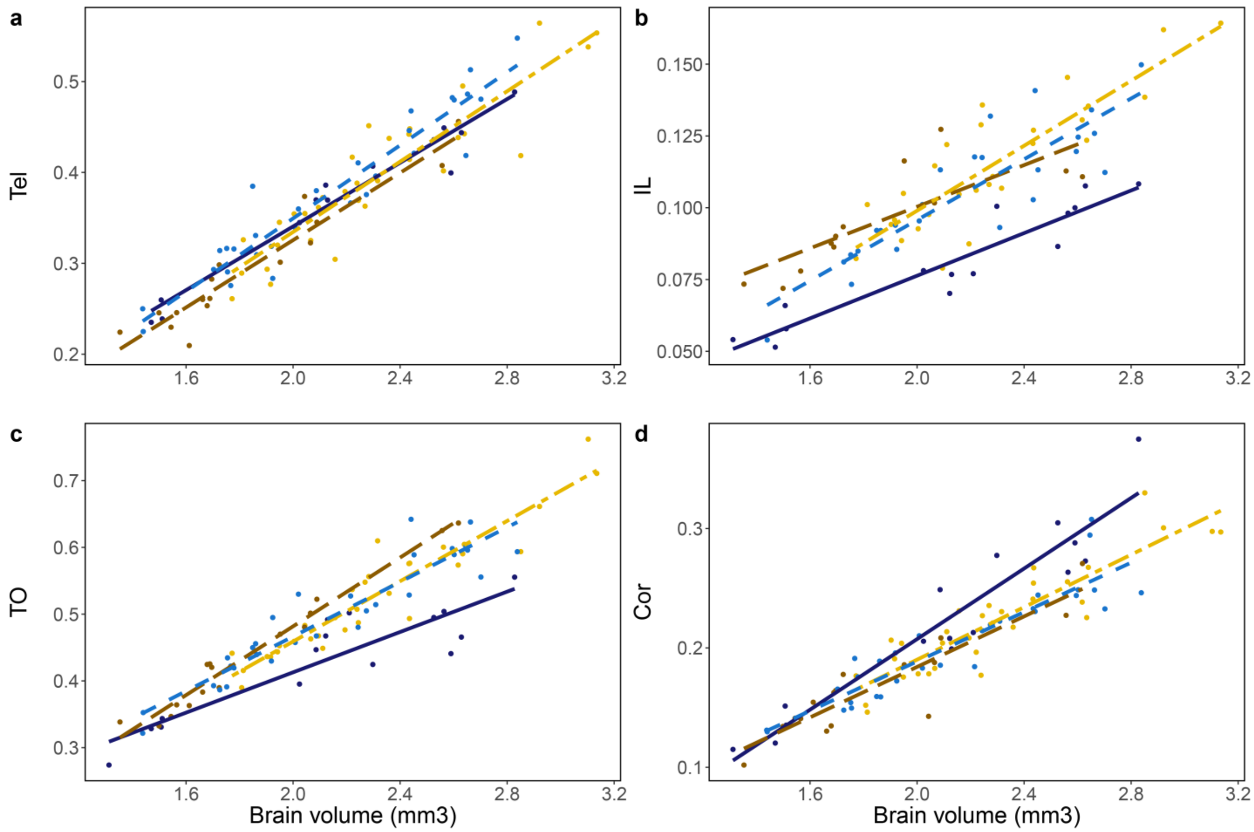


Figure 3: Relationships between total brain volume (mm3) and brain areas volume (mm3) for the four phenotypes: sponge-dwelling gobies from the species *E. chancei* and *E. prochilos* (sponge – dark blue/light blue lines) and cleaning gobies from the species *E. evelynae* and *E. prochilos* (cleaner – dark yellow/ yellow lines). (a) Tel = Telencephalon, (b) IL = Inferior lobe, (c) TO = Tectum opticum, (d) Cor = Corpus of the Cerebellum.

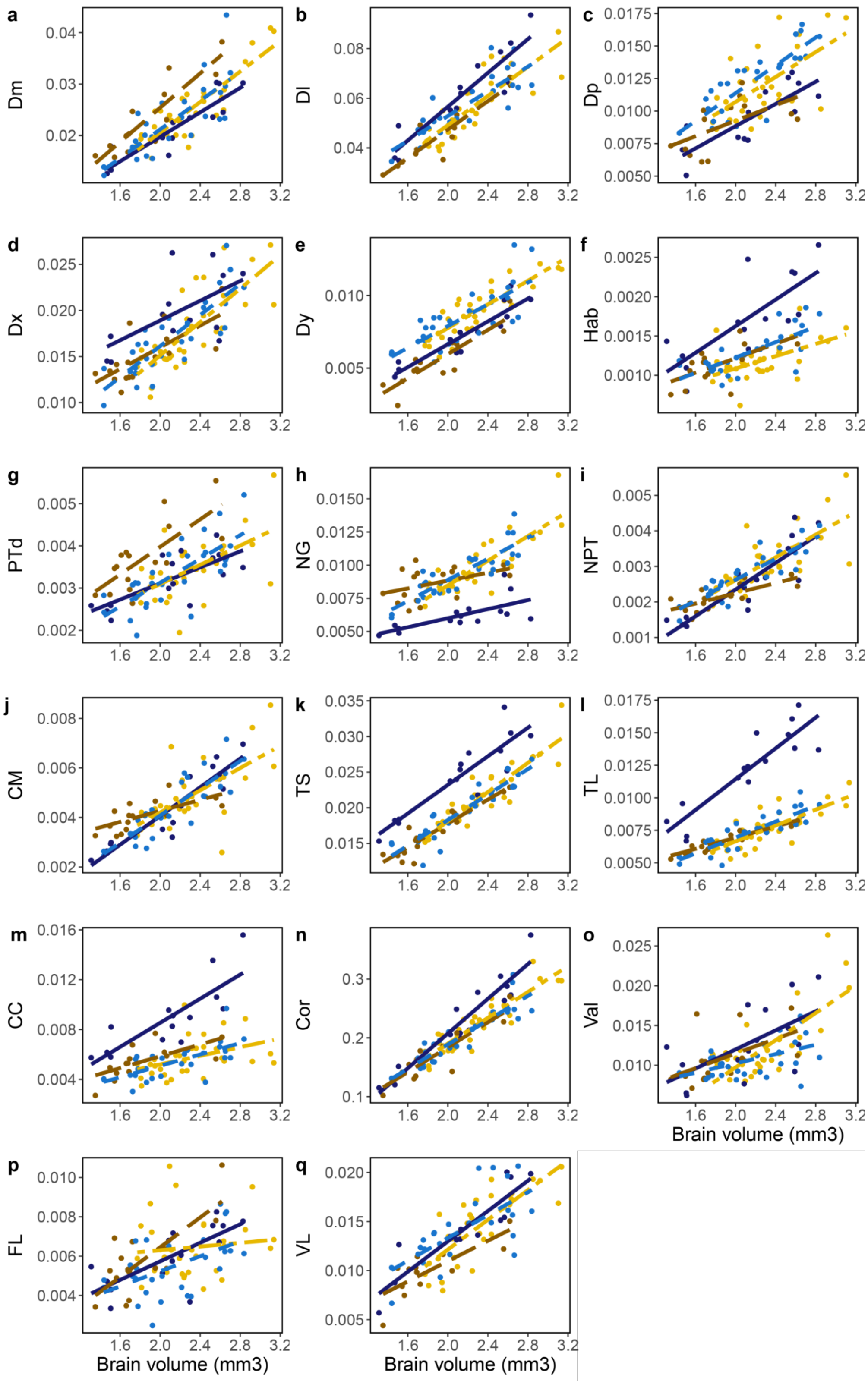


Figure 4: Relationships between total brain volume (mm3) and brain nuclei volume (mm3) for the four phenotypes: sponge-dwelling gobies from the species *E. chancei* and *E. prochilos* (sponge – dark blue/light

blue lines) and cleaning gobies from the species *E. evelynae* and *E. prochilos* (cleaner – dark yellow/ yellow lines). (a) Dm = Medial part of the dorsal telencephalon, (b) Dl = Lateral part of the dorsal telencephalon, (c) Dp = Posterior part of the dorsal telencephalon, (d) Dx = New area of the telencephalon, (e) Dy = New area of the telencephalon, (f) Hab = Habenula, (g) PTd = Dorsal part of the posterior thalamus, (h) NG = Nucleus glomerulosus, (i) NPT = Nucleous of the posterior tuberculum, (j) CM = Corpus mammilare, (k) TS = Torus semicircularis, (l) TL = Torus longitudinalis, (m) CC = Crista cerebellaris, (n) Cor = Corpus of the cerebellum, (o) Val = Valvula of the cerebellum, (p) FL = Facial lobe, (q) VL = Vagal lobe.

Table 2a: Analysis of covariance table for multivariate model. Brain size off all brain areas and nuclei were included as response variable and the phenotypes (*E. chancei*, *E.evelynae*, *E. prochilos* cleaning and *E. prochilos* sponge-dwelling), individuals size (length.mm) and sex (male x female) were included as main effects. Asterisks depict significant effects (α = 0.05).

	DF	TEST STAT	APPROX F	NUM DF	DEN DF	PR(>F)	
phenotype	3	2,11005	3,2742	63	87	1,89E-07	*
Length.mm.	1	0,79739	5,06	21	27	5,98E-05	*
Sex	1	0.51891	0.8218	21	16	0.6683	

Table 2b: Pairwise contrasts between phenotypes for the multivariate analysis using total volume as response variable. Significant values are depicted in bold.

CONTRASTS	TEL	DM	DL	DP	DX	DY	HAB
	p.value	p.value	p.value	p.value	p.value	p.value	p.value
E.chancei.sponge - E.evelynae.cleaner	0,9693	0,2401	0,3488	0,9994	0,482	0,8829	**0,0269**
E.chancei.sponge - E.prochilos.cleaner	0,3327	0,125	0,9961	**0,0247**	0,5222	**0,007**	**0,0036**
E.chancei.sponge - E.prochilos.sponge	0,3122	0,1625	0,9941	0,0064	0,8621	0,0524	0,0385
E.evelynae.cleaner - E.prochilos.cleaner	0,1713	0,9934	0,2751	0,0343	0,9596	0,0013	0,9962
E.evelynae.cleaner - E.prochilos.sponge	0,2157	0,9909	0,3807	0,0194	0,798	0,0198	0,8312
E.prochilos.cleaner - E.prochilos.sponge	0,9996	0,9998	1	0,9002	0,9155	0,8567	0,8015
	PTD	**NG**	**NPT**	**CM**	**IL**	**TO**	**TS**
	p.value	p.value	p.value	p.value	p.value	p.value	p.value
E.chancei.sponge - e.evelynae.cleaner	0,7783	**0,0074**	0,9995	0,9	**0,0386**	0,7088	**0,0047**
E.chancei.sponge - e.prochilos.cleaner	0,3217	**<.0001**	**0,0125**	0,3528	**<.0001**	**0,0051**	0,1021

E.chancei.sponge - E.prochilos.sponge	0,6362	<.0001	0,165	0,8712	0,0008	0,0027	0,0146
E.evelynae.cleaner - E.prochilos.cleaner	0,9754	0,4519	0,0385	0,9063	0,0681	0,2422	0,1448
E.evelynae.cleaner - E.prochilos.sponge	1	0,4413	0,3573	0,9995	0,9652	0,2059	0,6096
E.prochilos.cleaner - E.prochilos.sponge	0,9397	0,9959	0,6402	0,7455	0,0674	0,9806	0,7356
	TL	**TLAT**	**CC**	**COR**	**VAL**	**FL**	**VL**
	p.value	p.value	p.value	p.value	p.value	p.value	p.value
E.chancei.sponge - E.evelynae.cleaner	**<.0001**	0,9965	**0,0321**	0,8971	0,1355	0,6603	0,513
E.chancei.sponge - E.prochilos.cleaner	**<.0001**	0,1282	**0,0002**	0,8983	0,5829	0,6999	0,8153
E.chancei.sponge - E.prochilos.sponge	<.0001	0,985	<.0001	0,9926	0,0434	0,9959	0,7821
E.evelynae.cleaner - E.prochilos.cleaner	0,9981	0,3002	0,8937	0,46	0,4356	0,9768	0,0769
E.evelynae.cleaner - E.prochilos.sponge	0,9982	0,9999	0,6713	0,9511	0,9994	0,4762	0,1095
E.prochilos.cleaner - E.prochilos.sponge	0,9676	0,154	0,9007	0,679	0,3105	0,4414	0,9996

Table 2c: Pairwise contrasts between phenotypes for the multivariate analysis using the corrected volume as response variable. Significant values are depicted in bold and significant values in red represent values that were consistently significant in the comparisons using the corrected and non-corrected (**Table 2b**) brain volumes as response variables.

CONTRASTS	**TEL**	**DM**	**DL**	**DP**	**DX**	**DY**	**HAB**
	p.value	p.value	p.value	p.value	p.value	p.value	p.value
E.chancei.sponge - E.evelynae.cleaner	0,975	0,1195	0,1368	0,9984	0,6387	0,9932	0,0414
E.chancei.sponge - E.prochilos.cleaner	0,8473	0,9584	0,0154	0,316	0,0259	0,1003	0,0001
E.chancei.sponge - E.prochilos.sponge	0,9772	0,7041	0,1428	0,0877	0,2728	0,2674	0,0141
E.evelynae.cleaner - E.prochilos.cleaner	0,9895	0,1034	0,9699	0,3995	0,4317	0,0405	0,5101
E.evelynae.cleaner - E.prochilos.sponge	0,8003	0,4016	0,9831	0,1167	0,9642	0,1386	1
E.prochilos.cleaner - E.prochilos.sponge	0,3696	0,8316	0,7095	0,6953	0,5661	0,9525	0,299
	PTD	**NG**	**NPT**	**CM**	**IL**	**TO**	**TS**

	p.value	p.value	p.value	p.value	p.value	p.value	p.value
E.chancei.sponge - E.evelynae.cleaner	0,25	0,0001	0,8657	0,791	0,0001	0,0477	<.0001
E.chancei.sponge - E.prochilos.cleaner	0,5474	<.0001	0,1149	0,9192	<.0001	0,0087	<.0001
E.chancei.sponge - E.prochilos.sponge	0,846	<.0001	0,3809	0,9894	0,0004	0,0019	<.0001
E.evelynae.cleaner - E.prochilos.cleaner	0,7518	0,9997	0,5172	0,9565	0,6856	0,9999	0,9963
E.evelynae.cleaner - E.prochilos.sponge	0,5164	0,9936	0,8804	0,8658	0,5159	0,8573	0,9557
E.prochilos.cleaner - E.prochilos.sponge	0,9284	0,9632	0,8725	0,9775	0,0097	0,7459	0,7629

	TL	**TLAT**	**CC**	**COR**	**VAL**	**FL**	**VL**
	p.value	p.value	p.value	p.value	p.value	p.value	p.value
E.chancei.sponge - E.evelynae.cleaner	<.0001	0,9271	0,0254	0,9399	0,1778	0,9867	0,7402
E.chancei.sponge - E.prochilos.cleaner	<.0001	0,5307	<.0001	0,3893	0,1899	0,9445	0,9757
E.chancei.sponge - E.prochilos.sponge	<.0001	0,9472	<.0001	0,2241	0,0322	0,6445	1
E.evelynae.cleaner - E.prochilos.cleaner	0,482	0,9237	0,1833	0,7925	0,9557	0,7539	0,8287
E.evelynae.cleaner - E.prochilos.sponge	0,781	0,5785	0,1564	0,5572	0,9646	0,3883	0,6172
E.prochilos.cleaner - E.prochilos.sponge	0,9451	0,066	0,9917	0,927	0,5672	0,7985	0,9396

The MDS plot indicates that *E. chancei* have the most different brains when comparing across groups (**Fig. 5**). Concerning the patterns of changes in the brains, it is clear that changes in some areas are positively or negatively correlated to changes in some areas but not to changes in others, independently of brain volume changes (more details on **Figure 6**). Finally, the VRT method also found more significant differences between *E. chancei* and the other phenotypes and indicate similar correlations between brain areas that some areas change across species, but not others (**Fig. 7**).

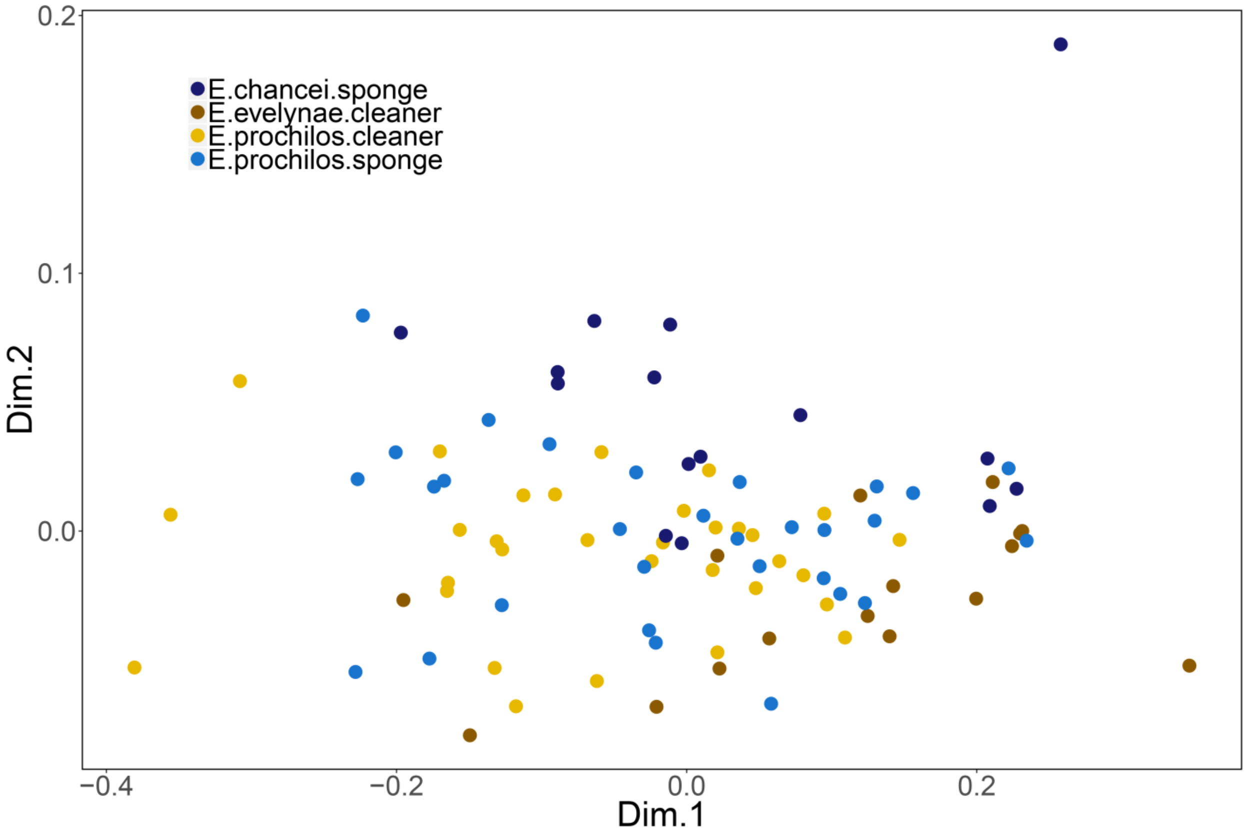
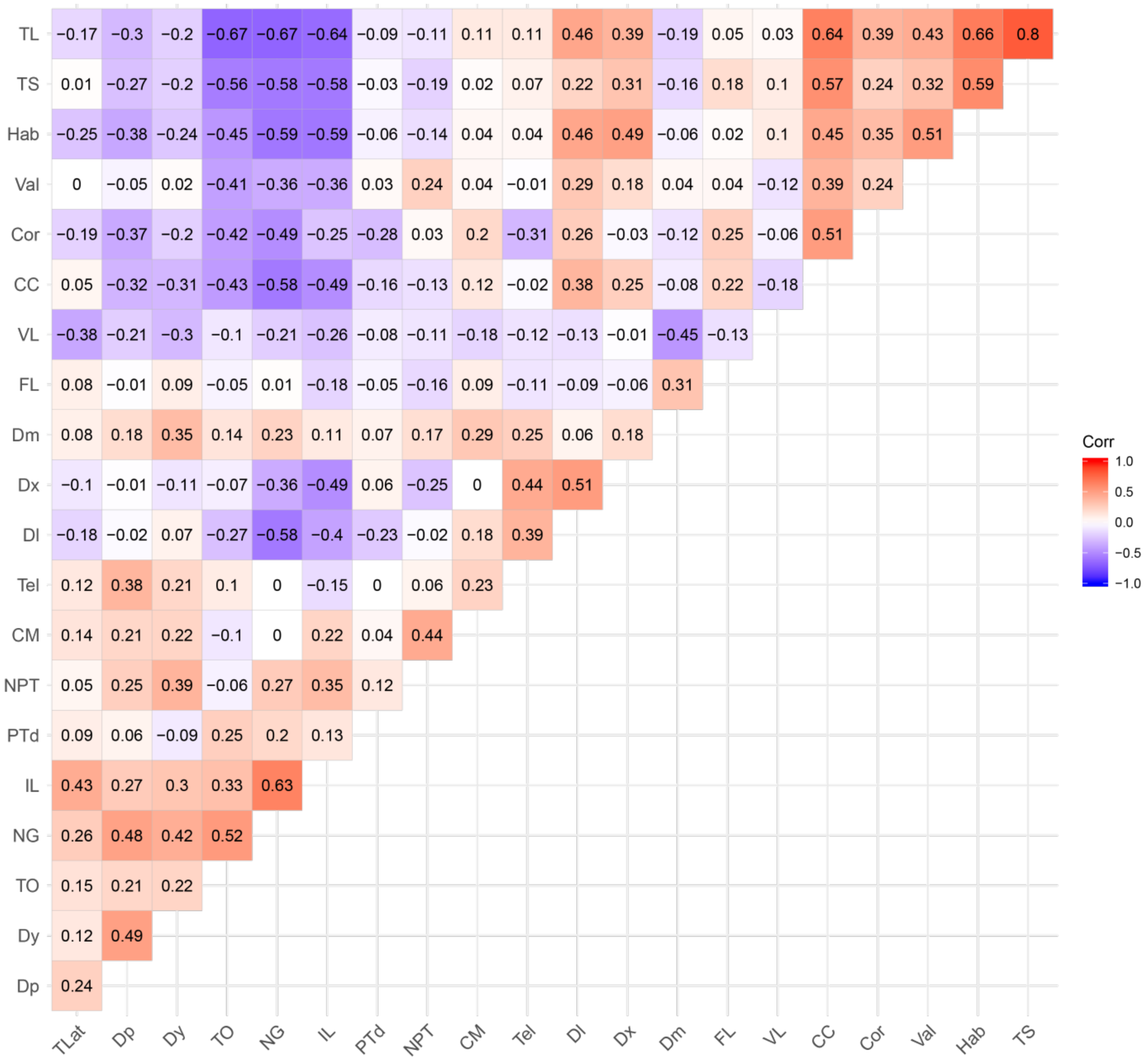


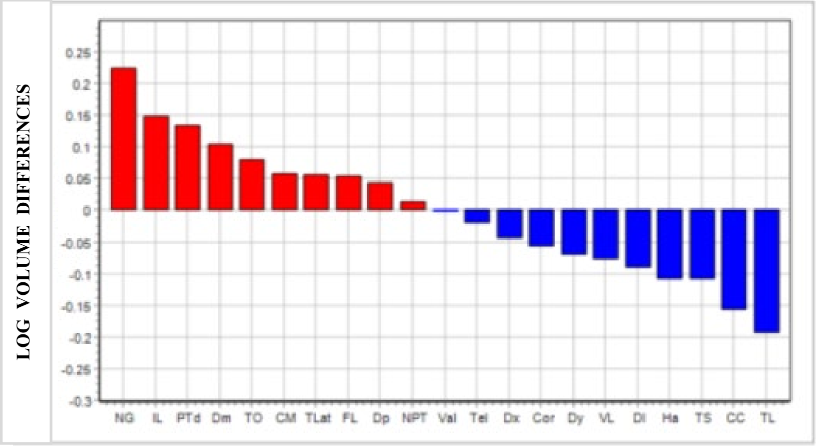
Figure 5: Multi-dimensional scaling (MDS) analysis showing similarities between individuals from the different phenotypes (dark blue - *E. chancei*, dark yellow - *E.evelynae*, light yellow - *E. prochilos* cleaning and light blue - *E. prochilos* sponge-dwelling) in respect to the volumes of the different brain areas and nuclei.



	TLat	Dp	Dy	TO	NG	IL	PTd	NPT	CM	Tel	Dl	Dx	Dm	FL	VL	CC	Cor	Val	Hab	TS
TL	−0.17	−0.3	−0.2	−0.67	−0.67	−0.64	−0.09	−0.11	0.11	0.11	0.46	0.39	−0.19	0.05	0.03	0.64	0.39	0.43	0.66	0.8
TS	0.01	−0.27	−0.2	−0.56	−0.58	−0.58	−0.03	−0.19	0.02	0.07	0.22	0.31	−0.16	0.18	0.1	0.57	0.24	0.32	0.59	
Hab	−0.25	−0.38	−0.24	−0.45	−0.59	−0.59	−0.06	−0.14	0.04	0.04	0.46	0.49	−0.06	0.02	0.1	0.45	0.35	0.51		
Val	0	−0.05	0.02	−0.41	−0.36	−0.36	0.03	0.24	0.04	−0.01	0.29	0.18	0.04	0.04	−0.12	0.39	0.24			
Cor	−0.19	−0.37	−0.2	−0.42	−0.49	−0.25	−0.28	0.03	0.2	−0.31	0.26	−0.03	−0.12	0.25	−0.06	0.51				
CC	0.05	−0.32	−0.31	−0.43	−0.58	−0.49	−0.16	−0.13	0.12	−0.02	0.38	0.25	−0.08	0.22	−0.18					
VL	−0.38	−0.21	−0.3	−0.1	−0.21	−0.26	−0.08	−0.11	−0.18	−0.12	−0.13	−0.01	−0.45	−0.13						
FL	0.08	−0.01	0.09	−0.05	0.01	−0.18	−0.05	−0.16	0.09	−0.11	−0.09	−0.06	0.31							
Dm	0.08	0.18	0.35	0.14	0.23	0.11	0.07	0.17	0.29	0.25	0.06	0.18								
Dx	−0.1	−0.01	−0.11	−0.07	−0.36	−0.49	0.06	−0.25	0	0.44	0.51									
Dl	−0.18	−0.02	0.07	−0.27	−0.58	−0.4	−0.23	−0.02	0.18	0.39										
Tel	0.12	0.38	0.21	0.1	0	−0.15	0	0.06	0.23											
CM	0.14	0.21	0.22	−0.1	0	0.22	0.04	0.44												
NPT	0.05	0.25	0.39	−0.06	0.27	0.35	0.12													
PTd	0.09	0.06	−0.09	0.25	0.2	0.13														
IL	0.43	0.27	0.3	0.33	0.63															
NG	0.26	0.48	0.42	0.52																
TO	0.15	0.21	0.22																	
Dy	0.12	0.49																		
Dp	0.24																			

Figure 6: Correlation matrix plot presenting the correlation coefficients for the regressions of the residual volumes of each brain area and nuclei, for all phenotypes, against each other (produced with the function pairs from package lattice (Deepayan, 2008)). The gradient from blue to red shows the strength of the negative (blue squares) and positive (red squares) correlations. Non-significant correlations are not colored (white squares). In this figure, one should notice that some areas show strong positive/negative correlations in the residual volume variation with some areas but not others, forming strong red/blue clusters.

a. ***E. evelynae*** **(cleaner) /** ***E. chancei*** **(sponge)**

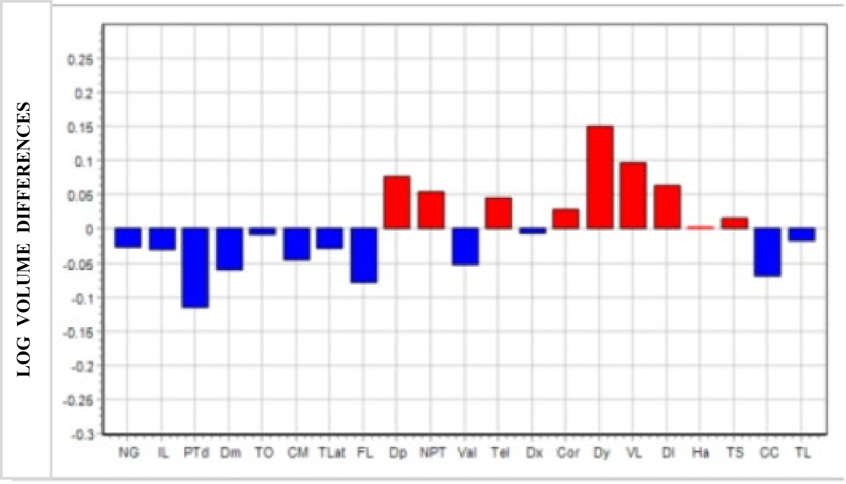


b. **Cleaner / sponge** ***E. prochilos***

c. ***E. prochilos*** **cleaner /** ***E. evelynae***

d. ***E. prochilos*** **cleaner /** ***E. chancei***

e. ***E. prochilos*** **sponge /** ***E. evelyane***



f. ***E. prochilos*** **sponge /** ***E. chancei***

Figure 7: Comparison of the relative volumes of the different brain areas/nuclei across phenotypes using the VRT analysis (methods). The bars values correspond to the average difference between phenotypes in the ratio of a particular brain area/nuclei in comparison to all other areas. The plot shows the brain areas that consistently take a higher (red) or lower (blue) ratios of other brain areas in one phenotype compared to the other, and therefore are likely to be enlarged in that particular group. The plots show the reference values for the species showed first in the title. For example, (a) shows that the areas in red are on average relatively larger in *E. evelynae* compared to *E. chancei*. The same is true for all the other comparisons (b-f).

3.4. Discussion

We had first asked whether the brain structure of the two individual types of *E. prochilos*, in terms of the relative volume of different brain areas and nuclei, is rather conserved and hence invariably more similar to *E. evelynae* than to *E. chancei*, or whether habitat choice and/or diet (cleaning versus non-cleaning) may have a major effect on brain structure. We used different approaches for answering this question and all approaches indicated that *E. prochilos* brains from both types (cleaning and sponge-dwelling) were more similar to the brains of the most closely related species, *E. evelynae* (obligatory cleaning species), than to the brains of *E. chancei* (sponge-dwelling species). Furthermore, some results did not fit either of our two hypotheses: that the total brain volume of the *E. prochilos* cleaning phenotypes was significantly larger than the brains of the other groups, with some brain areas volumes of *E. prochilos* cleaning individuals differing from both the sponge-dwelling individuals and *E. evelynae*. Secondly, we asked whether the variations in the relative volume of brain areas across species and phenotypes indicate a concerted or mosaic pattern of brain evolution. The fact that some areas changed across species while others remained the same, independently of brain size changes, indicates a mosaic pattern of brain evolution. The functional relationship of these areas will be discussed below.

3.4.1. Habitat use and brain structure

Despite the great differences in habitat use and feeding behavior between *E. prochilos* cleaning gobies and sponge dwellers, we did not find great differences in the brain structure between the two groups. However, the *E. prochilos* cleaning phenotypes brains were larger than the brains of the other groups and a few differences were found in the volume of the brain areas in this species: *E. prochilos* cleaning phenotypes had significantly larger Inferior lobe than the sponge-dwelling phenotypes and one part of the telencephalon (Dy) is significantly larger in the cleaning phenotypes if compared to *E. evelynae*. The function of the Dy area of the telencephalon is still unknown but the Inferior lobe includes parts of the diencephalon (**Fig. 1**). The diencephalon has been connected to the modulation of social behavior in different vertebrates as it harbors most nuclei of the mesolimbic reward system (O'Connell and Hofmann, 2011). The diencephalon had previously been found to be relatively large in a cleaner fish species that present complex social interactions, the cleaner wrasse *Labroides dimidiatus,* compared to other perciform species (Chojnacka et al., 2015). However, if the increased size of the inferior lobe were related to the complexity of engaging in cleaning interactions, we would have expected that the obligatory cleaner, *E. evelynae*, should also have significantly larger Inferior lobes than *E. prochilos* sponge-dwelling phenotypes, which was not the case in our study. It therefore appears that the relative enlargement of the Inferior lobe in *E. prochilos* cleaners is not related to cleaning activities. Furthermore, as both *E. prochilos* cleaning gobies and *E. evelynae* typically live in pairs, social structure and resulting emotional aspects like pair bonding apparently cannot explain this result either. Unfortunately, not all areas of the diencephalon could be measured in this study, which opens the possibility that other diencephalon part sizes may be adjusted as a function of cleaning and/or pair living.

The differences in relative volume of brain areas found between the two species of the cleaning clade, *E. prochilos* and *E. evelynae* on the one hand and the species of the sponge-dwelling clade, *E. chancei* on the other hand seem to be linked to the presence of a relatively larger visual input processing axis and a relatively smaller lateral line input processing axis in the cleaning species. The visual axis would be composed by the Nucleus glomerulosus, the Tectum opticum and the Inferior lobe. The Nucleus glomerulosus receives tectal input from the Tectum opticum and projects to the Inferior lobe. The Tectum opticum is the primary visual center in fishes (Butler and Hodos, 2005) and some fish species that do rely more on vision do have larger Tectum opticum (Keagy et al., 2018). On the other hand, the lateral line axis would be composed by the Torus semicircularis, Crista cerebellaris and the Torus longitudinalis. Acoustic and mechanosensory input from the lateral line system is processed in the Torus semicircularis (Meek and Nieuwenhuys, 1998; Wulliman et al., 1996). The Crista cerebellaris also receives input from the lateral line and although the function of the Torus longitudinalis is unknown, it is often larger in species with larger Crista cerebellaris (Hofmann, unpublished data). These differences in the visual/lateral line processing axis may be related to the differences in habitat use between the species. The sponge-dwelling *E. chancei* spend most of their time inside the tubular sponges (Mazzei, personal observation) and therefore, they might rely less on vision for navigating in the reef habitat and finding food. On the other hand, the cleaning species, in the same way as other coral reef fishes, might largely rely on vision for navigating in the reef and finding food. This should be especially true for the cleaning phenotypes, that have to find ectoparasites on the bodies of larger client fishes, but could also be true for the sponge-dwelling phenotypes, which inhabit the larger and more exposed barrel sponges.

Recent studies comparing fish phenotypes within the same species have successfully provided evidence for the mosaic brain hypothesis. Studies with nine-spined sticklebacks (*Pungitius pungitius*) documented that brain size relative to body size was larger in pond than in marine phenotypes, while the telencephalon tended to be larger in marine than in pond phenotypes (Gonda et al., 2011). Moreover, by subjecting individuals from wild phenotypes to reproduction in the laboratory for a common garden experiment, they found evidence that the brain changes are caused by environmentally induced phenotypic plasticity (Gonda et al., 2009). The same phenotypic plasticity was found in another tropical fish (*Poecilia mexicana*), for which changes in the brain were shown to be related to local adjustment to perpetual darkness (Eifert et al., 2015). Similarly, studies with freshwater sticklebacks (*Gasterosteus aculeatus*) have found that phenotypes from structurally complex habitats have a different telencephalon morphology than phenotypes that feed more in open habitats on plankton (Park and Bell, 2010). Finally, wild brown trout (*Salmo trutta*) individuals with different mating strategies differ in brain size and brain structure (Kolm et al., 2009): migratory fish have a larger cerebellum but overall smaller brains than non-migratory fish (Kolm et al., 2009). All these examples of apparent phenotypic plasticity suggest that the fish brain can readily be subjected to fine-scale adaptive evolutionary changes in response to changes in life histories, social systems and/or habitat use. In our study, the comparison between species from the two clades (cleaning x sponge-dwelling) indicates that changes in some brain areas reflect adaptations to the ecological needs of the species, like the potential reduction of the visual axis in the sponge-dwelling species. On the other hand, we only found few differences in the cleaning goby clade, despite the supposedly large ecological differences between *E. evelynae* and *E. prochilos* cleaners versus sponge-dwelling *E. prochilos*. We propose the following non-exclusive hypotheses for why we found these results: (i) the habitat structure differences between barrel and tubular sponges might provide different ecological conditions for sponge-dwelling gobies (for example, there might be more reliance on visual input in barrel sponges than in tubular sponges),

(ii) there are developmental constraints that impede the *E. prochilos* sponge-dwelling population brains to change and/or (iii) reducing visual acuity might not be adaptive if habitat use is not pre-determined and instead established during recruitment (especially if visual acuity is important for finding ectoparasites).

A puzzling result that needs further investigation is that *E. prochilos* cleaners had significantly larger brains than the other groups sampled. Previous research failed to find important differences between cleaning and sponge-dwelling *E. prochilos* individuals with respect to cognitive performance (**chapter 3**), social strategies in groups with standardized composition (**chapter 2**) and interaction patterns with predatory and harmless client reef fish in laboratory experiments (Emery et al. unpublished data). Therefore, we have currently no indication what ecological factor(s) may cause such increase in brain volume within species. The comparison with *E. evelynae* does not allow us to make any predictions either as our current knowledge emphasizes the similarities: cleaning and pair-living. Therefore, according to current assessment, we would have predicted similar brain sizes in *E. evelynae* and cleaning *E. prochilos* individuals.

In summary, we did not find a signal of habitat-feeding mode in predicting brain structure in the two *E. prochilos* phenotypes, since their brains were similar to each other and to the more closely related species, *E. evelynae*, irrespectively of the important differences in habitat and feeding behavior. However, differences between the cleaning species (*E. evelynae* and *E. prochilos*) and the sponge-dwelling species (*E. chancei*) revealed independent changes in functionally correlated brain areas that might be ecologically adaptive. Although we only found small differences in the brain structure between the cleaning and sponge-dwelling phenotypes, further studies should go beyond neuroanatomical measurements and look for potential differences in cellular composition, gene expression or hormonal modulation in this species. These approaches can give more detailed information about the constraints involved in changing the brain structure and reveal brain plasticity mechanisms that are not related to brain structure.

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General discussion

D.1. Summary

The framework proposed by Niko Tinbergen in 1963 for the study of the proximate and ultimate explanations of behavior provides the basis for an integrative understanding of why individuals behave in a certain way (Bateson and Laland, 2013; Tinbergen, 1963). In my thesis, I tried to understand why *Elacatinus prochilos* adopt different habitat-feeding modes by asking two of the four of Tinbergen's questions: how does it work (mechanism) and how does it evolve (phylogeny). I started by testing the role of different mechanisms in producing the observed differences in habitat use, social behavior, and feeding mode. In the background data, presented in the general introduction section, I tested whether differences in habitat use were caused by differences in habitat preference between individuals. In the first chapter, I tested whether differences in the social behavior were caused by different decision rules on how to interact toward conspecifics. In the second chapter, I asked whether adopting different feeding strategies involved learning different types of cues. In the third chapter, I then focused on the main organ controlling and coordinating all these mechanisms, the brain, and tried to get an insight on how it evolved in the *Elacatinus* group by comparing the brain between three species that differ in the habitat feeding mode.

As the Background data confirmed that there are key differences in group size/structure and feeding behavior between cleaning and sponge-dwelling gobies, a cascade of predictions were made for the main results chapters. The most striking mismatch between predictions and results were described in chapter 1: the lack of differences with respect to social behavior between the two goby phenotypes. I consider this result very surprising due to great evidence in the literature that small differences in the early social environment lead to significant differences in the social competence of individuals (Desjardins et al., 2012; Fischer et al., 2015; Hesse et al., 2016; Kasper et al., 2017; Lovic et al., 2001; Nyman et al., 2017; Ruploh et al., 2014, 2013; Taborsky et al., 2012; Taborsky and Oliveira, 2012). How to explain the fact that both the cleaning gobies and sponge-dwellers behavior deviated from what I expected, considering their previous social experience, and converging on highly similar social interaction patterns? No matter whether one considers genetic differentiation, different gene-environment interactions, or different learning opportunities between the two habitats, all these mechanisms would predict that social interaction patterns should differ. Furthermore, in another recent study led by Yasmin Emery for her Master thesis, we found no differences in the time cleaning gobies and coral dwellers spent interacting with predatory or non-predatory clients, when exposed to those in the lab (Emery et al. unpublished data). These results reinforce that the early social environment does not impose constraints on how the individuals can adjust their behavior. One potential explanation is that gobies possess a rather extensive innate/plastic decision-rule with many "if, then…", which allows them to flexibly adjust to groups of different sizes, to their own role within the group, and to foraging opportunities. Such persisting flexibility might be favored by natural selection if changes in social conditions are frequent and rather unpredictable. In favor of this hypothesis, I note that gobies have a rather short maximum life expectancy of about 12 months (Colin, 1975), and may frequently change their status, especially in sponges due to large old individuals dying and new young recruits joining.

Once the lack of differentiation in both intra- and interspecific social behavior is documented, the lack of differences in the learning tasks (chapter 2) as well as the only minor differences in brain morphology (chapter 3) was to be expected. Nevertheless, I still found surprising that almost none of the gobies could solve the pattern-cued task, as this type of task is usually solved by fish (Binning et al., 2018; Siebeck et al., 2009; White and Brown, 2015) and much more complex tasks are solved by the cleaner wrasse *Labroides dimidiatus* (Bshary et al., 2014; Gingins and Bshary, 2016; Salwiczek et al., 2012). Despite that, I suggest that more cognitive tasks should be used in future studies, manipulating different types of cues and/or focusing on the cognitive skills used in the context of social hierarchies, like transitive inference (Fernald, 2014). These cognitive experiments could give us better insights on whether the overall slightly larger brains found in *E. prochilos* cleaning gobies have any relationship with cognitive abilities.

D.2. The big picture

D.2.1. Why study the mechanisms of phenotypic variation?

Adaptive evolution is usually visualized as beginning with a genetic mutation that directly affects the phenotype in a way that increases its fitness and therefore is selected and passed to the next generations. However, more commonly, adaptive evolution starts with plastic phenotypes induced by environmental factors and causing phenotypic variation. The plastic phenotypes can be selected due to its positive fitness effects, and when gaining a genetic component, cause cross-generational changes in phenotypic and genotypic frequencies (West-Eberhard, 2003). This gives a fundamental role to the underlying mechanisms of phenotypic plasticity as they can directly affect the evolution of phenotypic variation. This might be the main raw material for producing adaptive phenotypic plasticity and evolution (Taborsky, 2016; West-Eberhard, 2003).

Concerning the *Elacatinus prochilos* system, we still do not know whether phenotypic variation in *E. prochilos* is induced by genetic or environmental factors. We only tested for differences between populations in one genetic marker, the cytochrome B, and although we did not find significant differences in this marker, we cannot exclude other genetic sources of variation. We expected that by looking at potential mechanisms related to phenotypic variation we would provide the raw material for further studies aimed at finding if these mechanisms were genetically or environmentally determined. However, none of the mechanisms investigated in our study (behavioral, cognitive, habitat preference) seem to be directly related to the behavioral differences observed in the wild.

On the other hand, the group manipulations (chapter 1) can still give us some insights on which should be the future directions. The fact that sponge-dwelling *E. prochilos* behaved differently towards females in the laboratory conditions indicate that putative neurohormonal regulation might be involved in the decision making-process, as it happens for other vertebrates (Godwin and Thompson, 2012; Goodson, 2005; O'Connell and Hofmann, 2011). Therefore, further studies should focus on the role of neurohormonal mechanisms in regulating the decision-making process in *E. prochilos* gobies by conducting experiments that manipulate hormonal levels, group size and sex of the individuals from both phenotypes.

D.2.2. Open questions

My PhD thesis yielded an overall puzzling result: the apparently full flexibility of adult phenotypes to adjust to novel social environments independently of their previous experience. This convergence to the specifics of the social environment also made it difficult to apply the concept of social competence to the system. Finally, it was beyond the scope of the thesis to assess fitness consequences of different social environments and diets. It therefore remains unclear whether the social behavior of subjects in the experimental groups represented optimal strategies under natural conditions. Research on *E evelynae* suggests that sponge-dwellers survive and grow better (White et al., 2007). However, these studies do not provide data on lifetime reproductive success and should hence be taken with care. More generally, we should investigate what is the current function of adopting each habitat-feeding mode in *Elacatinus prochilos.* Do the two strategies yield similar fitness benefits for the individuals? Do they represent alternative life history strategies or is one option making the best out of a bad job?

Second, we should investigate how these differences come about. Is the habitat-feeding mode genetically determined or do individuals adopt one strategy only after settling to the different substrates? Whether the strategies represent alternative life history strategies or not and whether they are genetically determined or not should have a strong influence on the plasticity/generality of the mechanisms causing behavioral variation in the two phenotypes. For example, if the strategy is only adopted after settlement, and if there is large variation in the ecological conditions to be encountered by the settler (e.g. availability of sponges versus other substrates, competitors, ectoparasites) it should be beneficial to keep mechanisms that serve both functions, either by being plastic or by including decision rules that serve both phenotypes. Despite the clear evidence in the literature that the two phenotypes largely differ in habitat use, diet, frequency of cleaning interactions, group size, number of interactions with conspecifics and level of aggression towards conspecifics of the same sex (Arnal, 2000; Arnal et al., 2000; Soares et al., 2009; Whiteman and Côté, 2004a, 2004b, 2002), there is no evidence that the two phenotypes represent two diverging populations. No morphological differences are described, and a study using cytochrome B as genetic marker failed to find significant differences between phenotypes (background data). My preliminary translocation experiments indicate that at least the transfer from a coral to a sponge is quite feasible (background data). Nevertheless, a more detailed genomics study as well as a large scale translocation experiment of both juveniles and adults would be necessary to draw strong conclusions.

With regard to the apparent life-long phenotypic plasticity observed in my thesis, it is important to note that most studies focused on cleaning gobies living in pairs and sponge-dwelling gobies living in groups of more than 10 individuals, despite having reported that both cleaning gobies and sponge-dwelling can live in larger/smaller groups of around five individuals, for example. This variation was also clear in my study site. Cleaning stations were occupied by one to five cleaning gobies and barrel sponges by three to 77 sponge-dwelling gobies. As long as such fluctuations may readily occur within a location within the lifespan of individuals, then natural selection may promote the evolution of a social decision rule that includes prescriptions on how to behave in small groups. The decision rule would hence cover the group composition used in our experiment.

Furthermore, not a lot is known about the behavior of the recruits and of juvenile gobies. Maybe both group size and feeding behavior is more variable during these life stages, independently of habitat, which can promote selection on plasticity or yield learning opportunities that can still be used as adults. Long-term effects of early exposure have been documented for example in experiments manipulating the presence/absence of parents or predator cues (Taborsky, 2016; Taborsky et al., 2012). Finally, lacking information on the precise mating systems in the sponges habitats, we may have missed the possibility that sponge-dwelling males and females may also form bonded pairs during reproduction, and that such bonding was hence triggered during the lab experiment.

D.2.3. Work in parallel and in progress

During my Ph.D., I co-supervised three master students that worked on projects related to the topic of my thesis. One of them is still in progress. Below I summarize their main objectives and the results implications for the general understanding of the system.

D.2.4. Determinants of habitat use and fitness consequences in Caribbean cleaning gobies

This project is being conducted as part of the master thesis of Jeniece Germain, under the main supervision of Dr. Henri Valles and Prof. Redouan Bshary. The main objective of this project was to understand the mechanisms that cause habitat use variation in cleaning gobies and identify the fitness consequences for individuals. Jeniece monitored the habitat use, population abundance, settlement events, individual movements and survival of the species *Elacatinus prochilos* and *Elacatinus evelynae*, in a patch reef in Barbados, for one year. The first important result that she found was that in the particular studied site, *E. prochilos* are mainly occupying sponges and *E. evelynae*, coral heads. Tagged *E. prochilos* individuals found in corals eventually return to the sponges and never establish cleaning stations. Second, she found that micro-habitat use seems to be size-dependent for both species. For *E.evelynae*, bigger individuals were more likely to be found on corals than smaller ones, while for *E. prochilos*, bigger individuals were more likely to be found on sponges than smaller ones. It is still unclear whether these size-dependent changes in microhabitat use are driven by small-scale ontogenetic migration, by size-dependent mortality, by differences in growth patterns, or by a combination of these factors. Finally, there seem to be no differences in the survival rates of both species in the different habitats. In conclusion, Jeniece's work first indicates that there might be some important ontogenetic changes that were not considered in my study. Moreover, the fact that we did not find significant differences in survival between *E. evelynae* using the cleaning strategy and *E. prochilos* using the sponge-dwelling strategy indicate that survival should also not be strikingly different between the two eco-types of *E. prochilos* adopting the two strategies. The next step on this project is to conduct removal experiments in order to investigate whether competition plays a role in the habitat use differences of the two species.

D.2.5. Social Competence in cleaning interactions

This project was conducted as part of the master thesis of Yasmin Emery, under the main supervision of Prof. Redouan Bshary. The main aim of this project was to investigate whether the two phenotypes would adjust cleaning behavior during a lab experiments in which they were exposed to two different types of clients: a predatory and a non-predatory species. Previous studies with the species *E. evelynae and E. prochilos* revealed that the cleaning gobies give priority of service to predatory clients in the wild and take less time to approach predatory clients in the wild (Soares et al., 2012, 2007). They also observed that *E. evelynae* gobies exposed to predatory clients in the laboratory have higher holding water cortisol levels than gobies exposed to non-predatory clients and control treatment (Soares et al., 2012). Yasmin and I repeated this experiment and predicted that the cleaning phenotype of *E. prochilos* would behave in the same way as the cleaning species *E. evelynae* while sponge-dwelling individuals would instead avoid the predatory client and potentially even the non-predatory client. Surprisingly, she found no significant differences in the frequency of interactions, time of interactions and latency to interact with both clients between cleaning and sponge-dwelling *E. prochilos*, nor any differences between the two phenotypes. Moreover, there were no differences in the holding water cortisol levels between treatments or phenotypes. These results reveal three important insights about the phenotypic variation in this system: First, cortisol does not seem to be an important modulator of cleaning interactions as it is for *E. evelynae*. Second, sponge-dwelling gobies can flexibly adjust to the cleaning context staged in the lab and interact with clients. Third, *E. prochilos* do no adjust their behavior to the different types of clients, at least not under laboratory conditions. Overall, the results fit my own data in that they demonstrate the ability of both eco-types to converge in their behavior as a function of the current environment, in Yasmin's case the opportunity to obtain food by cleaning client reef fish.

D.2.6. Escape response in Caribbean cleaning gobies

This project was conducted as part of the master thesis of Michelle Lampe, under the main supervision of Dr. Dominique Roche and Dr. Henk Siepel. The main question of this project was whether potentially reduced predation pressure on Caribbean cleaning gobies that are involved in mutualistic cleaning interactions with predatory clients has led to a decreased escape performance through compensated trait loss (Ellers et al., 2012). Cleaner wrasses have been shown to have higher escape performance than facultative or non-cleaner species (Gingins et al., 2017). It was hypothesized that the maintenance of high escape performance by cleaner wrasses was either to avoid predators or punishing clients that chase the wrasses for eating mucus instead of ectoparasites (Bshary and Grutter, 2005). One way to differentiate between the two hypothesis was to ask whether cleaning gobies also had higher or lower escape performance compared to other species in the group. Cleaning gobies do not prefer to cheat (eat mucus instead of ectoparasites, (Soares et al., 2010)), which has been used as explanation why clients do not chase them in response to jolting (Soares et al., 2008). Based on that, Michelle compared the escape performance between the obligate cleaner *E. evelynae*, the facultative cleaner *E. prochilos,* and the non-cleaner *E. chancei*. She found that the obligate cleaner *E. evelynae* outperformed the other species in almost all measures of escape response, while the non-cleaner *E. chancei* was the slowest and travelled the shortest distance in general. Once again, she found no significant differences between the cleaning and sponge-dwelling *E. prochilos* phenotypes. This result is consistent with the results found by Yasmin and indicate that the two phenotypes do not suffer differential levels of predation risk and that predation risk seems to be more important for *E. evelynae* than for *E. prochilos*. Furthermore, the fact that we found that obligatory cleaning gobies do maintain high escape performance, like cleaner wrasses, indicates that the risk imposed by predators to cleaner species should be reassessed. Important findings in the case of the cleaning gobies were that these gobies do not seem to taste well (Tuttle et al., 2015) and that the abundance of the invasive predator *Pterois volitans* does not affect cleaning gobies abundance (Tuttle, 2017). These results indicate that we need alternative explanations for the high scape response performance in cleaning gobies.

D.3. Future work

In the future, I would like to extend my Phd research to other Caribbean goby species, but this time gathering information on life history traits (like longevity, fecundity, dispersal patterns, and body size) as well as characteristics of group social organization (such as genetic relatedness, breeding structure, and sex ratio) for a comprehensive synthesis aimed at explaining the main drivers of the evolution of sociality in this group.

D.4. Final conclusions

In conclusion, we did not find evidence that the differences between *E. prochilos* phenotypes are related to differences in habitat preference, social decision rules, associative learning skills, and brain structure. This means that at this moment, we cannot answer the question of how the differences between phenotypes work. Since we could not find differences in the mechanisms, or in brain structure, it is also currently impossible to answer what differentiation in mechanisms drove the evolution of a sponge-dwelling clade versus a coral dwelling cleaning clade. We would only have had an indication of how the differences evolved if we had found clear differences in mechanisms, which were correlated to brain structure differences, which in turn should be present/absent in related species with similar/different ecological needs. This would have indicated the importance of the habitat-feeding mode relative to phylogeny in explaining the behavioral differences between phenotypes. On the other hand, we found differences in the visual/lateral line sensory axis between species in the cleaning versus sponge-dwelling clade, which revealed independent changes in functionally correlated brain areas that might be ecologically adaptive, therefore supporting the mosaic pattern of brain evolution.

D.5. References

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