Population density affects whole brain and brain region volumes in a wrasse species *Coris batuensis*

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Abstract:

The factors shaping brain evolution and cognition are broadly categorized as being either social or environmental. However, the relative importance of these factors is heavily debated, partly due to the limitations from the standard interspecific evolutionary comparative approach. Here, we adopt a complementary strategy leveraging the high degree of brain plasticity that evolved in fishes to ask how variation in social and environmental factors affect individual brain development. We investigated how overall brain size and brain part sizes varied between demes of the same population in the coral reef associated batu coris *Coris batuensis*. This wrasse species is ideal for our approach because its local population densities (a correlate of intraspecific social complexity) are dissociated from both densities of other species (reflecting interspecific complexity, including competitors and predators) and habitat complexity (represented by the three-dimensional structure of the coral reefs and adjacent areas). We found that individuals from demes with higher intraspecific population density possess larger overall brain volumes than those from lower population density environment, caused by an enlargement of all five main brain regions. Brain anatomical measures did not correlate with measures of interspecific or habitat complexity. Our results suggest that brain plasticity in the batu coris evolved to allow individuals to adapt specifically to variation in intraspecific social challenges. The results support a broader version of the social brain hypothesis, emphasizing the entire brain over specific regions like the neocortex in mammals or the telencephalon in fishes.

Introduction:

The brain is the center of the nervous system and as such, regulates both physiological and cognitive abilities crucial to an animal's fitness. Although there is an ongoing debate about what brain measure most accurately reflects cognition, absolute and relative size remain widely used proxies (Deaner et al., 2007; Herculano-Houzel et al., 2015; Logan et al., 2018). According to the Social Brain Hypothesis (SBH), the factors driving brain size variation are social in nature, and correlate with measures such as group size, mating systems, and social bonds (Byrne and Whiten, 1988; Dunbar, 1998; Humphrey, 1976; reviewed in Whiten, 2018). The environmental intelligence hypothesis, on the other hand, credits brain size evolution to diet-related physical and visual challenges linked to habitat complexity, which include interspecific competition and predation (Clutton-Brock and Harvey, 1977; Iwaniuk and Nelson, 2001; DeCasien et al., 2017; Rosati, 2017), though these later factors could also be considered as social (Oliveria & Bshary 2021). However, despite the advantage of increased brain size on cognition, the high costs associated with creating and maintaining brain tissue mean that its enlargement should directly enhance an organism's energy balance (Aiello and Wheeler, 1995; Isler and Van Schaik, 2006; Heldstab et al., 2022).

Most research investigating the relationship between ecology and brain morphology have focused on interspecific comparisons (reviewed in Healy and Rowe, 2007). While between-species comparisons are a powerful method by which to address evolutionary questions, they remain correlative in nature. In order to isolate the effects of environmental factors on brain morphology, an intraspecific approach can be more informative (Gonda et al., 2013; Hall and Tropepe, 2020). In this respect, fishes are a valuable model clade as they experience neuro-morphological plasticity throughout their lifetime due to their capacity for neurogenesis into adulthood (reviewed in Zupanc and Horschke, 1995; Zupanc, 2006). Plastic responses in brain morphology to current ecological conditions have been demonstrated in fishes through rearing experiments whereby individuals of the same population are reared in different ecological settings. For instance, salmon *Oncorhynchus mykiss* (Kihslinger et al., 2006), guppies *Poecilia reticulata* (Burns et al., 2009), and nine-spined sticklebacks *Pungitius pungitius* (Gonda et al., 2011) reared in the wild exhibit larger overall brain sizes or brain part sizes compared to those reared under laboratory conditions (typically considered less environmentally complex), emphasising the need to study wild animals for ecological relevance (Bshary & Triki 2022). These changes in brain size can be rapid as is the case in guppies that develop enlarged optic tecta and relative overall brain size when exposed to enhanced environments after merely 14 days (Fong et al., 2019). Another means of exploring the effects of ecological factors on brain morphology are intrapopulation studies. For example, bluegill sunfish *Lepomis macrochirus* (Axelrod et al., 2018) and guppies *Poecilia reticulata* (Reyes et al., 2022) collected from environments of varying structural complexity exhibit larger overall brains than individuals from simpler habitats. However, through such studies we cannot completely rule out potential genetic differences between individuals of separate habitats (Ishikawa et al., 1999). In this regard, experiments within populations from different demes exclude potential interpopulation genetic variation and isolate the ecological components influencing brain morphology.

Recent studies on the bluestreak cleaner wrasse *Labroides dimidiatus* (hereafter ‘cleaners’) have documented about 14-19% variation in forebrain size on a very small spatial scale, i.e. within demes (areas of coral reef separated from each other by sandy areas) of the same population (Triki et al. 2019b, 2020). The forebrain, comprised of the telencephalon and diencephalon in teleosts, is an area of the brain thought to encompass the so-called social decision-making network (SDMN), a highly conserved group of interconnected nodes involved in encoding for social behaviours (Newman, 1999; Goodson, 2005; O’Connell and Hofmann 2012). As eggs and larvae are pelagic, no genetic population sub-structure is to be expected (Jones et al., 2010; Gardner et al., 2015; Knutsen et al., 2022), which means that any variation between demes in mean brain morphology can be attributed to individuals adjusting brain development to local cognitive demands. Variation in such cognitive demands could be due to the cleaners’ interactions with their so-called ‘client’ fish (Côté, 2000). Clients visit cleaning stations to have ectoparasites removed, but various conflicts of interest exist, primarily because cleaners prefer to eat the clients’ layer of protective mucus (Grutter & Bshary 2003), because some piscivorous clients may try to eat the cleaners (Grutter 2004), and clients may compete with each other over priority of access (Bshary, 2001). Cleaners have been shown to use sophisticated strategies in their average of 2000 cleaning interactions per day (Grutter, 1995) that include reputation management (Bshary and Grutter, 2006), tactical deception (Binning et al., 2017), and partner manipulation (Bshary and Würth, 2001). The expression of such strategies, as well as performance in ecologically relevant laboratory experiments, varies between demes (Wismer et al., 2014, Triki et al., 2019a; Binning et al. 2017).

Demes characterized by high client density and diversity harbor cleaners that are likely to show high strategic sophistication and increased forebrain sizes (Wismer et al., 2014; Wismer et al. 2019; Binning et al., 2017; Triki et al., 2019a,b; Triki et al., 2020). Some of the strategic components cannot be explained by basic associative learning mechanisms but instead require the ability to chain information from subsequent events and detection of configurations (Quiñones et al., 2020, Prat et al., 2021). Furthermore, cleaners show generalized rule learning and social learning of strategies in the context of cleaner-client interactions (Wismer et al., 2016; Truskanov et al., 2020). These results are in line with the hypothesis that cleaners adjust brain development as a function of the complexity of their cleaning interactions. However, client density and diversity positively correlate with two factors – cleaner fish density and habitat structural complexity – that could provide alternative explanations for variation in forebrain size. Cleaners are protogynous hermaphrodites, where terminal males defend larger territories in which typically 1-7 smaller females have their separate cleaning stations but nevertheless interactions are governed by a size-based hierarchy (Robertson 1972; Nakashima et al. 2000). The reproductive system yields opportunities for strategic decision-making in interactions with conspecifics and life history strategies (Leimar and Bshary., 2022). Females may switch between harems and can spawn with two males in parallel (Sakai et al., 2001). Males inhibit sex change of female harem members through aggression (Robertson, 1972; Kuwamura, 1984), and adjust levels of aggression towards females cheating a jointly inspected client that leads to a client's departure, based on the size of the female and the value of the loss (Raihani et al., 2012 a,b). In return, females are responsive to male aggression (Raihani et al., 2010) and show basic understanding of what a male partner can or cannot see (McAuliffe et al., 2021). Therefore, such intraspecific social complexity, that may well be affected by population density, could in principle also cause variation in forebrain size between demes. Finally, fish density and diversity depend on habitat complexity (Graham and Nash, 2013; Darling et al., 2017). This means that an increase in three-dimensional complexity may increase the challenge of navigating within a territory and remembering the location of conspecifics, clients, potential sit-and-wait predators, as well as hiding spots, opening the possibility that spatial complexity contributes to variation in forebrain size.

To pull apart the effects of intraspecific and interspecific interactions and physical environment on brain morphology, we took advantage of the fact that while the sophistication of the cleaning behaviour is unique to the genus Labroides, their social organization is very similar to various other wrasse species. Wrasse are generally protogynous hermaphrodites, and several species live in rather stable harems. We screened transect data to find a suitable study species that not only lives in harems but differs from cleaners with respect to habitat preferences and hence shows population densities that do not correlate with cleaner and overall fish densities. The batu coris *Coris batuensis* fulfilled our requirement, sharing the same social organization as cleaners but preferring to live on sandy flats or the reef base where it feeds off crustaceans and gastropods in coral rubble and sand (Randall, 1999; Randall et al., 1997; Kramer et al., 2016). While being abundant in the general study area (Green, 1996), batu coris abundance correlates negatively with cleaner abundance on our demes (r= -0.35; Triki and Bshary 2019). As a consequence, their intraspecific social complexity is neither positively correlated with general fish abundance as a correlate of interspecific social complexity, nor with habitat structural complexity. By collecting individuals from demes that differ in batu coris population density and overall fish population density and diversity, we could investigate brain morphology in a species for which different hypotheses for forebrain size variation yield different predictions. To partition the variance in brain morphology to the effects of all three potential explanations, we picked demes such that we could expect a low correlation between interspecific complexity and habitat complexity. As a final point, we note that an absence of systematic variation in brain morphology across habitats would be difficult to interpret: it could be that variation in cleaning interaction complexity (absent in coris, present in L. dimidiatus) is the only factor driving variation in gross brain morphology development, or that all three factors investigated contribute to variation in brain morphology but cancel each other out in batu coris.

Materials and Methods:

The study was conducted in June 2022 at the Lizard Island Research Station at the Great Barrier Reef, Australia. 24 *Coris batuensis* were captured from four separate locations using barrier nets measuring 1 m x 1.2 m. Individuals were guided into the barrier nets using hand nets, with a sample size of 6 individuals per site; Clam Gardens, Corner Beach, Horseshoe and North Horseshoe (Figure 1). Transects were run for each site in order to determine both overall fish density as well as *C. batuensis* densities in each habitat. In Horseshoe and Northern Horseshoe we ran five 30-meter transects that were repeated three times. We used three width measurements to observe fish, starting with a 5-meter width to identify large fish, then 3-meters for medium-sized fish and finally 2-meter width for small fish. For Clam Gardens transects were run as described above 10 times. Because Corner Beach is a sparse and patchy habitat, we ran seven transects in that habitat, one per patch, as this was sufficient to count all fish. Habitat complexity was ranked in order of one to four based on the amount of coral cover and structural diversity observed in each environment (aerial view of habitats shown in supplementary materials Fig. S7).

We collected six fish from each site (n=24, TL: mean ± SD, 8.83 ± 0.32 cm) and transported them to the research station within one hour of capture in individual Ziplock bags filled with seawater. We placed the fish in individual aquaria, each of which was connected to a flow-through water system pumping fresh seawater and containing a shelter for refuge. We euthanized the fish the same day with an overdose of Aqui-S solution. We then weighed (to the nearest 0.1g) and measured (to the nearest mm) each individual before we cut the head behind the opercula and fixed the heads in 4% PFA for a period of 5 days. After the 5 days, we washed the heads two times for 10 minutes in 1X PBS before we dissected the brain out of the skull and took pictures of the brain from the dorsal, ventral and from both lateral sides using the AxioCam Erc 5s camera attached to the Zeiss Stereomicroscope Discovery V8. We then used the software ImageJ to measure the length (L), height (H) and width (W) of each brain region (telencephalon, hypothalamus, optic tectum, cerebellum, and medulla, Fig. S8). Finally, we used the ellipsoid model (equation below) to calculate the volume in mm^3 of each of these brain region (Pollen et al., 2007):

$$V = (L \; x \; W \; x \; H)\frac{\pi}{6}$$

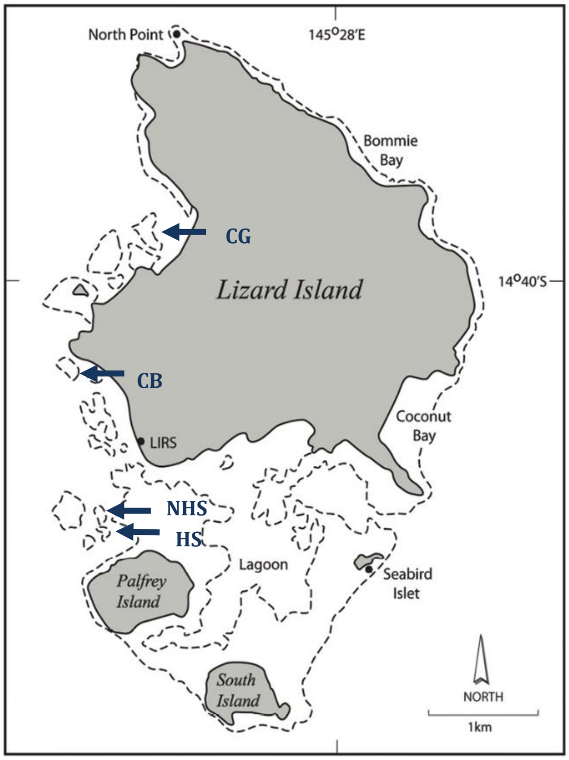

Figure 1. Map of Lizard Island Group Arrows and acronyms represent Clam Gardens (CG); Corner Beach (CB); Northern Horseshoe (NHS) and Horseshoe (HS). Image from DOI: https://doi.org/10.3897/zookeys.491.4932

Statistical analysis

All data were analyzed using the open-sourced software R v. 4.0.3.Prior to statistical analyses, we checked the dataset for outliers using the grubbs.test function in the *outliers* package and found none. To compare overall fish density and *Coris batuensis* densities per 100m^2 between habitats we performed a Kruskal-Wallis test, using each transect as a unit. We then used the Dunn's test to compare differences between groups post-hoc. We chose a non-parametric option because the assumption of homogeneity of variance was violated. To compare variation in brain measurements, we log-transformed the morphological variables to correct for the allometric relationship between body size and brain size, accounting for the multiplicative nature of the data (Huxley, 1924; Pélabon, 2018; Kerkhoff and Enquist, 2009). We then used the transformed values in all the analyses. We used linear models (LM; lm function in R using the package *stats*) to explore the effect of habitat on the size of each brain region as well as the size of the forebrain and total size of the brain. To determine which predictor variable was responsible for explaining brain size variation, it would have been desirable to create a model that included all three variables: intraspecific density, interspecific density, and habitat complexity. However, due to the significant collinearity between interspecific density and habitat complexity, as indicated by Kendall's rank correlation tau ($\tau = 0.67$, $p < 0.001$), these variables were found to be not independent and therefore could not be included in the same model. We thus set brain part sizes as the dependent variable and ran two separate models: first intraspecific density and interspecific density, second intraspecific density and habitat complexity as the fixed factors, with fish length as a covariate to correct for body size. Finally, to determine whether there was selective enlargement of any brain region, we performed linear models as described above, using each region size as the independent variable and habitat and the size of the rest of the brain as the dependent variables. To account for multiple comparisons, we applied a Bonferroni correction to the p-values of each model output. We visually inspected the diagnostic plots of each model to check for normality of residuals and homoscedasticity of variance. The residuals in all models were normal, with no deviations in the q-q plots. Finally, we assessed the proportion of variance explained by the fixed factors in the model by inspecting the multiple and adjusted R^2 of our models.

Results:

We recorded the following intraspecific densities per 100m^2: Clam Gardens (1.56 ± 2.44), Corner Beach (2.04 ± 1.73), Horseshoe (6 ±1.27), and North Horseshoe (11.33 ± 8.55) (Figure 2). A Kruskal-Wallis test revealed a significant difference in interspecific densities based on habitat (H = 9.62, df = 3, p = 0.02). Post-hoc analysis showed that this difference was driven by significantly higher interspecific fish densities in Horseshoe compared to Corner Beach (Z=-2.94, p=0.01, Figure 2a). Intraspecific density comparisons also showed significant differences between habitats (H = 14.43, df = 3, p = 0.002). Specifically, Northern Horseshoe had significantly higher Batu Coris densities than both Clam Gardens (Z=-3.098, p=0.006) and Corner Beach (Z=-2.46, p=0.04, Figure 2b). We see a similar trend between Horseshoe and both Clam Gardens (Z=-2.31, p=0.06) and Corner Beach (Z=-2.61, p=0.08) although those results are only marginally significant (Figure 2b).

We first ran two models, one specifying intra- and interspecific densities as predictors and one with intraspecific and habitat complexity as predictors. Since only intraspecific density showed significant correlation with brain and forebrain volume variation, we chose to use this variable alone as a predictor when comparing each brain part volume (Table 1, and Figure 3). This allowed us to focus on a more meaningful source of variation and simplified our analysis. We found a significant increase in the volumes of each of the five brain regions; telencephalon, hypothalamus, optic tectum, cerebellum, and dorsal medulla, as well as forebrain and total brain (all regions $p < 0.02$, Table 2, Figure 4).

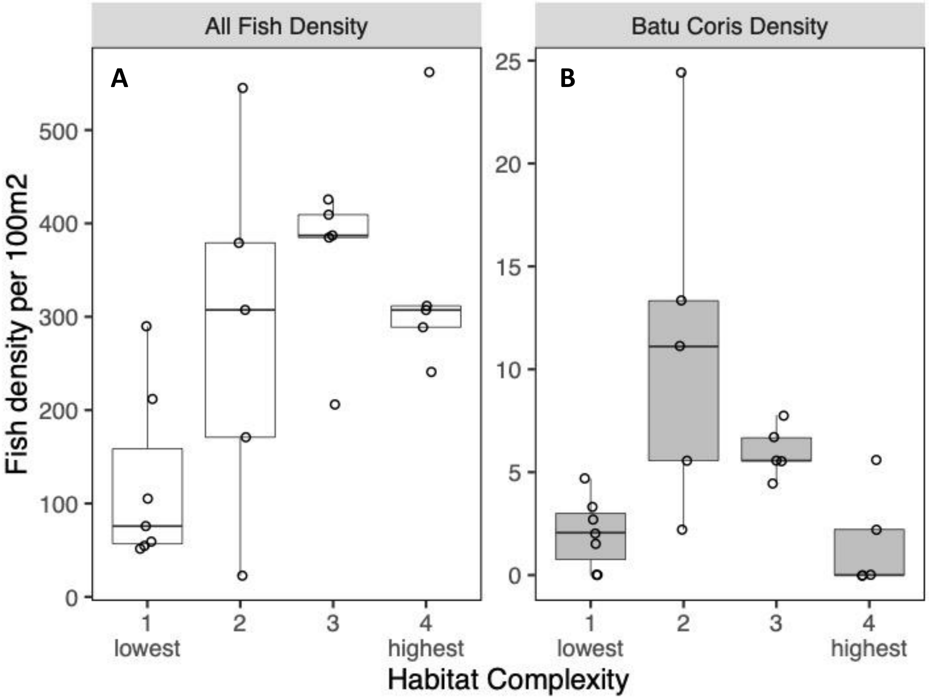

Figure 2. Fish density per 100 square meters by habitat. A) density of all fish species excepting the batu coris and B) batu coris density found in each habitat per 100m^2. Habitats 1 to 4 are shown in order of

increasing complexity which is the following order: Corner Beach, Northern Horseshoe, Horseshoe and Clam Gardens. The length of the boxplot rectangle shows the spread of variability of the data within the interquartile range (IQR) of the middle 50% of the data around the median, shown as a horizontal line. Whiskers represent the range of the data within 1.5 time the IQR.

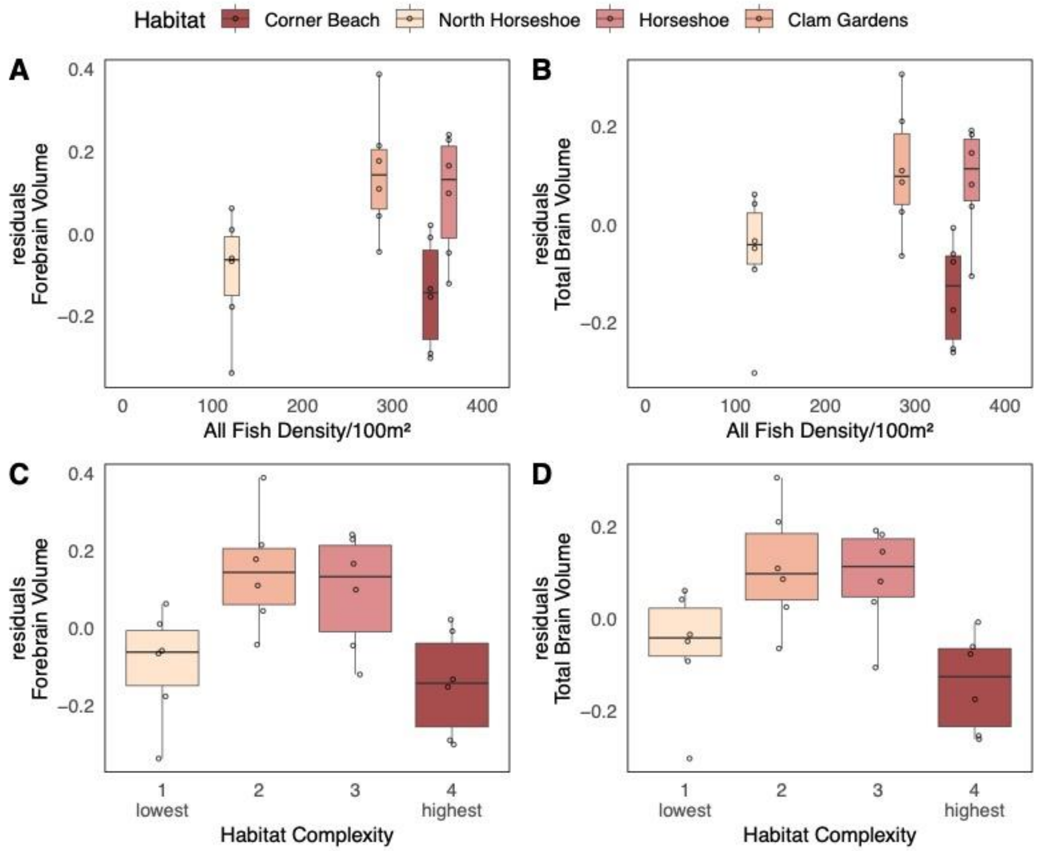

Figure 3. Relationship between residuals of forebrain volume and total brain volume to body length and A-B) interspecific density and C-D) habitat complexity. Residuals are used for visualization purposes only. Boxplots display the interquartile range (IQR) as rectangles with whiskers extending to 1.5 time the IQR.

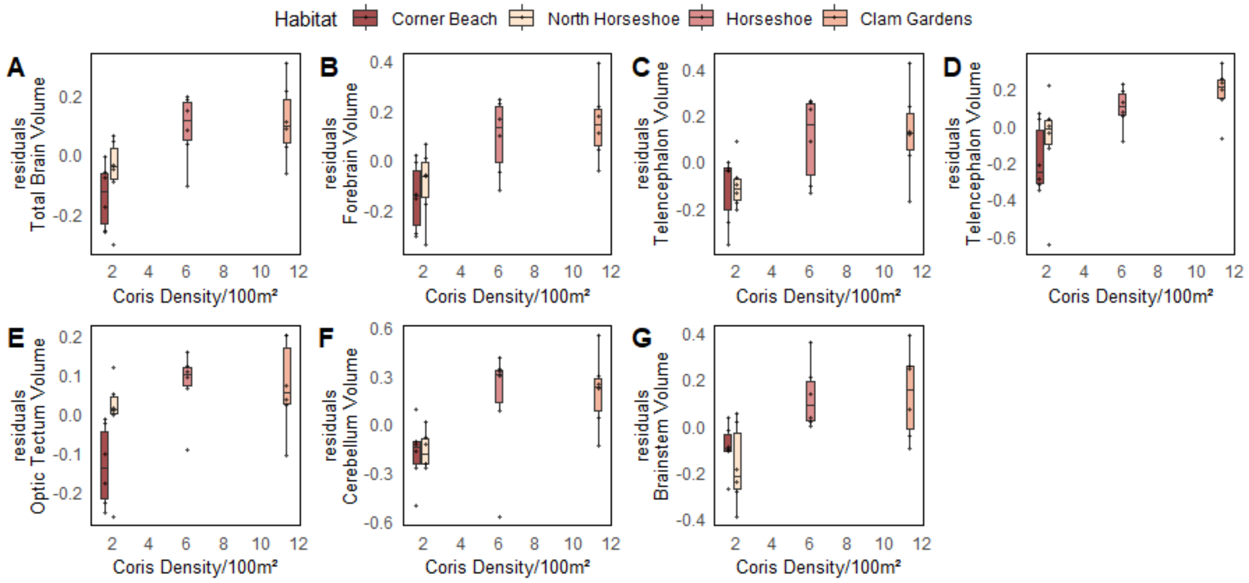

Figure 4. Relationship between residuals of brain region volume on body size and population density. Each of the main brain regions are shown separately (C-G) as well as A) whole brain and B) forebrain volume. Boxplots display the interquartile range (IQR) as rectangles with whiskers extending to 1.5 time the IQR.

response variable	predictor	Estimates	F-value	P-value	Bonferroni corrected p-value	Adjusted R-squared	Figure
total brain	habitat complexity	-0.005	0.05	0.82	1.00	0.78	3B
	coris density	**0.133**	**16.97**	**<0.001**	**0.008**		
forebrain	habitat complexity	0.005	0.03	0.86	1.00	0.76	3A
	coris density	**0.16**	**18.7**	**<0.001**	**0.005**		
total brain	all fish density	-0.02	0.03	0.86	1.00	0.78	3D
	coris density	**0.13**	**16.92**	**<0.001**	**0.008**		
forebrain	all fish density	0.01	0.03	0.84	1.00	0.76	3C
	coris density	**0.16**	**16.9**	**<0.001**	**0.008**		

Table 1. Summary of main statistical effects for total brain and forebrain volume in models including either batu coris density and habitat complexity or batu coris and all fish densities. Each model includes n=24, significance threshold set at alpha ≤ 0.05 *CI* and goodness of fit measures were estimated using Adjusted R^2. Significant results are shown in bold. Bonferroni adjusted p-values were calculated using all 11 models run using brain metrics and include the results from the models in Tables 1 and 2.

predictor	response variable	Estimates	95 % CI		P-value	Bonferroni corrected p-value	Adjusted R-squared
			upper	lower			
coris average density/ 100m2	**total brain**	**0.13**	**0.07**	**0.20**	**<0.001**	**0.004**	**0.79**
	telencephalon	**0.15**	**0.06**	**0.23**	**0.002**	**0.004**	**0.71**
	hypothalamus	**0.19**	**0.09**	**0.29**	**0.001**	**0.01**	**0.66**
	forebrain	**0.16**	**0.09**	**0.24**	**<0.001**	**0.002**	**0.77**
	optic tectum	**0.1**	**0.04**	**0.16**	**0.004**	**0.05**	**0.76**
	cerebellum	**0.23**	**0.1**	**0.36**	**0.002**	**0.02**	**0.59**
	medulla	**0.16**	**0.08**	**0.25**	**0.001**	**0.01**	**0.65**

Table 2. Summary of main statistical effects of relationship between Batu Coris and each brain region. Each model includes n=24, significance threshold set at alpha ≤ 0.05 *CI* at 95% and goodness of fit measure estimated by Adjusted R^2. Significant results are shown in bold. Results are depicted in Figure 4.

Discussion:

We investigated brain size variation in *C. batuensis* with the aim of disentangling the ecological factors responsible for forebrain size variation in a wrasse species. We found that overall brain volume and volume of each brain region varied as a function of batu coris population densities, and we did not detect any effects of either interspecific fish density or habitat structural complexity.

Studies that use the comparative approach face the challenge of identifying the social and environmental factors contributing to variation across species and tend to rely largely on intuitive assumptions (group size as a proxy for social complexity; Dunbar 1992) or post hoc interpretations of significant parameters (pair bonding in birds; Emery et al. 2007). Our study is uncommon in that we study within-species variation in social and environmental challenges, taking advantage of the evolved developmental plasticity causing important variation in individual fish brain sizes. We chose a species for which the available information indicated that intraspecific densities would not correlate positively with interspecific fish densities or habitat structural complexity, allowing us to potentially distinguish between the three possible pressures that could select for differences in brain development. The results uniquely favour the conclusion that batu coris invest in brain development in response to intraspecific social challenges. The results thus clearly support the general ideas of the social brain hypothesis but not any version of environmental hypotheses. In this context, we note that interspecific interactions do not seem to be well classified as either social or environmental in the literature, though a social interpretation seems to be more appropriate in our view (Zuberbühler & Byrne 2006; Oliveira & Bshary 2021).

Our results fit Dunbar's (1992) original assumption that group size, or in our case, population density, is a good proxy for social complexity. However, while Dunbar credited neocortex ratio (neocortex volume divided by the volume of the rest of the brain) as the best measure of social cognitive abilities, the brain size increases we observed were not driven by a selective enlargement of the forebrain. This was unexpected because the social decision-making network, which comprises key brain regions responsible for regulating social behaviour, is mainly located in the forebrain (telencephalon and diencephalon; Goodson 2005; O'Connell & Hofmann 2011, 2012). Given that i) brain parts can evolve in mosaic ways to meet specific demands (see 'mosaic brain hypothesis' by Barton and Harvey, 2000), ii) the telencephalon can be selected for size in fish and up-selection leads to increased cognitive performance in key executive functions (Triki et al. 2022, 2023), and iii) cleaner fish individuals selectively increase forebrain size in response to both intra- and interspecific increased population densities (Triki et al. 2019a, 2020), the enlargement of all brain areas warrants explanation.

We propose three non-exclusive explanations for the variation in overall brain size across sites. First, batu coris – in contrast to cleaners – may face constraints on selective brain region growth. We consider this unlikely, however, as there is little evidence to support the concerted brain hypothesis, especially in fishes (Park and Bell, 2010; Gonda et al., 2013; Fong et al., 2021; Reyes et al., 2022). Secondly, the social cognitive demands modulating brain size differences manifest more broadly in the brain rather than in the forebrain alone. While this concept is supported by a growing body of literature (discussed in Félix and Oliveira, 2021 and Kelly, 2022), we note that it cannot explain the differences between batu coris and cleaners. Thirdly, it has been pointed out in interspecies comparisons that brain size often correlates best with the amount of sensory information processed and the precision of motor control that a species possesses (van Schaik et al. 2023). Examples include electro-sensing in mormyroid fishes (Sukhum et al. 2018), stereoscopic vision in primates (Barton 2004), hand manipulation capacities in primates (Heldstab et al. 2016) or even just the number of legs in lizards (de Meester et al. 2019). Such sensorimotor functions may affect the size of the mesencephalon (optic tectum) and cerebellum without causing an improvement of cognitive processes (Barton 2012; van Schaik et al. 2023).

Batu coris are protogynous hermaphrodites, and as such, their social challenges are most likely centered around managing a size-based hierarchy, i.e. decisions about aggression vs fleeing and key life history decisions regarding growth strategies and sex change. Optimal decision rules should thus be sensitive to shifts in social dynamics dependant on population densities (Fricke 1980; Lutnesky, 1994; van Rooij et al., 1995). Changes in population densities can result in altered group structure, which in turn can influence the degree of site attachment, territory defence, and territory overlap. These changes can subsequently impact various behaviours, including rates of interactions and levels of aggression (Mumby and Wabnitz, 2002). Such effects have been observed in spotlight parrotfish (*Sparisoma viride*), moon wrasses (*Thalasoma bifisciatum*) and circle-cheek wrasse (*Halichoeres miniatus*) where individuals from low density habitats are both more territorial than individuals from high density environments and protect more aggressively those territories than those from high population density environments (van Rooij et al., 1996; Warner & Hoffman, 1980; Ryen, 2007 master's thesis). Overall, high population densities in batu coris should not only select for improved social competence (defined as the use of social information to make appropriate fitness-relevant decisions; Taborsky & Oliveira 2012), but also for improved visual processing and maneuverability, and hence to an overall increase in brain size.

Below we consider two alternative explanations for the observed correlation between batu coris densities and overall brain size and briefly discuss why we consider them unlikely. First, an increase in brain size may be due to increased energy availability in the higher population density environments. However, batu coris feed off crustaceans and gastropods found in sandy areas (Randall, 1999; Randall et al., 1997; Kramer et al., 2016), so if diet related components were responsible for brain size variation, we would expect brain size to correlate negatively with habitat structural complexity but this was not the case. Furthermore, if energy availability caused increased brain size in high density areas, we would have expected a corresponding increase in body condition (mass adjusted for length) but this was not the case (Fig. S3). Finally, an increase in energetic input only leads to a larger brain if the resulting increase leads to fitness-improving functioning of the brain. Thus, irrespective of whether population density correlates with increased energy availability or not, an increase in brain size should necessarily lead to a cognitive advantage counterbalancing the costs of brain enlargement (Aiello and Wheeler, 1995, reviewed in Isler and van Schaik, 2009 and Heldstab et al., 2022), which would be linked to the improved management of social complexity in our case. A second alternative explanation would be that more open sandy habitats cause an increase in predation risk, and that a larger brain yields both the cognitive and sensory-motor capacities necessary to cope with this challenge (see also Zuberbühler & Byrne 2006). However, as with the hypothesis about energy availability, we would have expected brain size to correlate negatively with habitat structural complexity, but this was not the case.

One of the objectives of our study was to shed light on which factors influence brain size variation in cleaner wrasse, whose inter- and intraspecific densities and habitat complexity covary, making it impossible to disentangle how much explanatory power each variable has over brain size variation. Cleaners are able to solve tasks related to their interspecific ecology that other wrasse species, that have otherwise similar social lives, cannot (Gingin and Bshary, 2016), and even much larger-brained species, including primates, cannot (Salwiczek et al. 2012; Zentall et al. 2016).

Furthermore, studies on cleaner wrasses show that population density correlates with both cognitive performance in tasks related to cleaning interactions as well as brain size variation (Wismer et al., 2014; Wismer et al. 2019; Triki et al., 2019a; Triki et al., 2020). High performance in these tasks correlate positively with population density (Wismer et al. 2014; Wismer et al. 2019; Triki et al. 2019a) while forebrain size predicts optimal decision rules in nature as a function intra- and interspecific population densities (Triki et al., 2020). Taken together those results suggest that the cognitive requirements of cleaner wrasses in high density populations are linked to their interspecific social interactions. Here, however, we documented that intraspecific social complexity correlates with overall brain size development in another member of the wrasse family. We recorded a similar effect size of about 14% for involved brain tissues, despite the variation in cleaner densities being much smaller than the variation in batu coris densities (0.47 – 1.6 in Triki et al., 2019b versus 1.56 – 11.33 in the current study). The current results thus offer plenty of opportunities for future research. First, there is a need to study intraspecific interaction and to develop hypotheses about what kind of cognition may have to be enhanced to deal with increased population densities. Second, there is a need to quantify food availability at different sites harbouring different population densities. In cleaner fish, densities correlate tightly with large client densities (Triki et al. 2019a), suggesting that cleaner fish densities are a function of food availability and hence very similar across sites. We do not know for batu coris or other species. Third, there is a need to measure growth curves to determine if increased brain size can result from higher food availability alleviating constraints on brain development, or if it is a result of shifts in energy tradeoffs resulting in more gradual growth patterns. Forth, testing more species may reveal whether any variables predict an overall increase in brain size as in batu coris or a selective increase in forebrain size as in cleaners. These studies will help to assess whether interspecific social competence may contribute to brain size variation in cleaners (Oliveria & Bshary 2021).

In summary, our study provides evidence that intraspecific social complexity drives brain size development in a species with evolved developmental brain plasticity, generally supporting a broad version of the social brain hypothesis. Indeed, the results challenge the conventional focus on the forebrain as the primary site regulating social behaviors, suggesting that improved senso-motoric skills contribute to the expression of appropriate behaviour under high population densities. More generally, ectotherms may provide many opportunities to test experimentally how social and environmental factors affect overall brain and brain parts development and how variation in brain anatomy and physiology affect behaviour and cognition as a means of adaptation to ecological conditions within a population.

Authors' contribution

Y.E and R.B designed study; R.B and L.P collected field data; Y.E collected laboratory data, analysed data and generated figures. Y.E and R.B wrote and finalized the paper.

Competing interests

All authors declare no competing interests

Ethics

The study described herein was reviewed and granted by the Animal Ethics Committee of Queensland, Australia (DAFF) under permit number CA 2022/04/1609.

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Supplementary Materials

Figure S1. Length and Body condition of fish per habitat. A) length in centimetres of fish per habitat and B) body condition calculated as body mass (g) adjusted for body length (cm) per habitat. Boxplots display the interquartile range (IQR) as rectangles with whiskers extending to 1.5 time the IQR.

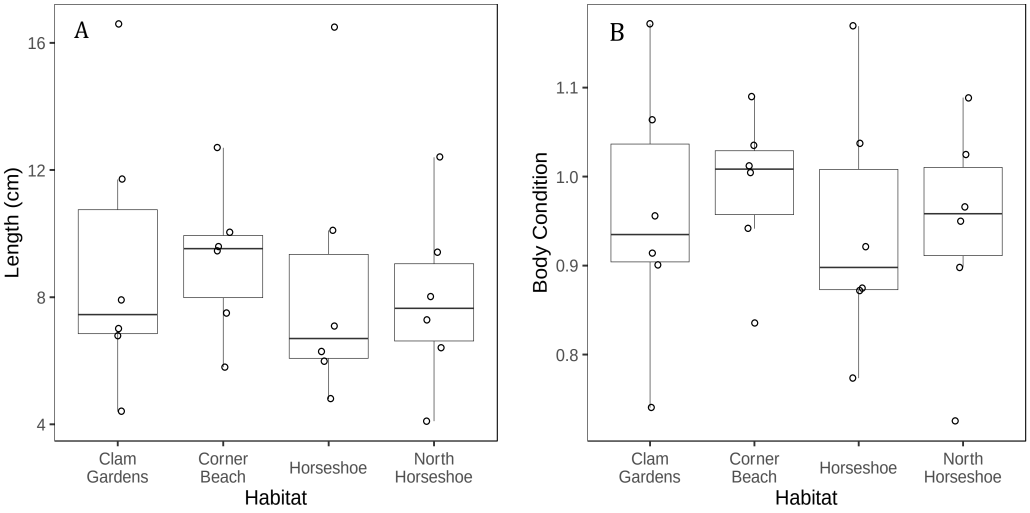


Fig S2. Brain photos for ellipsoid calculation. *Coris batuensis* brains were photographed from A) dorsal B) lateral and C) dorsal angles. W, L and H refer to the width, length, and height of each structure with numbers representing each of the brain regions as follows: 1) telencephalon, 2) optic tectum, 3) cerebellum, 4) dorsal medulla and 5) hypothalamus.

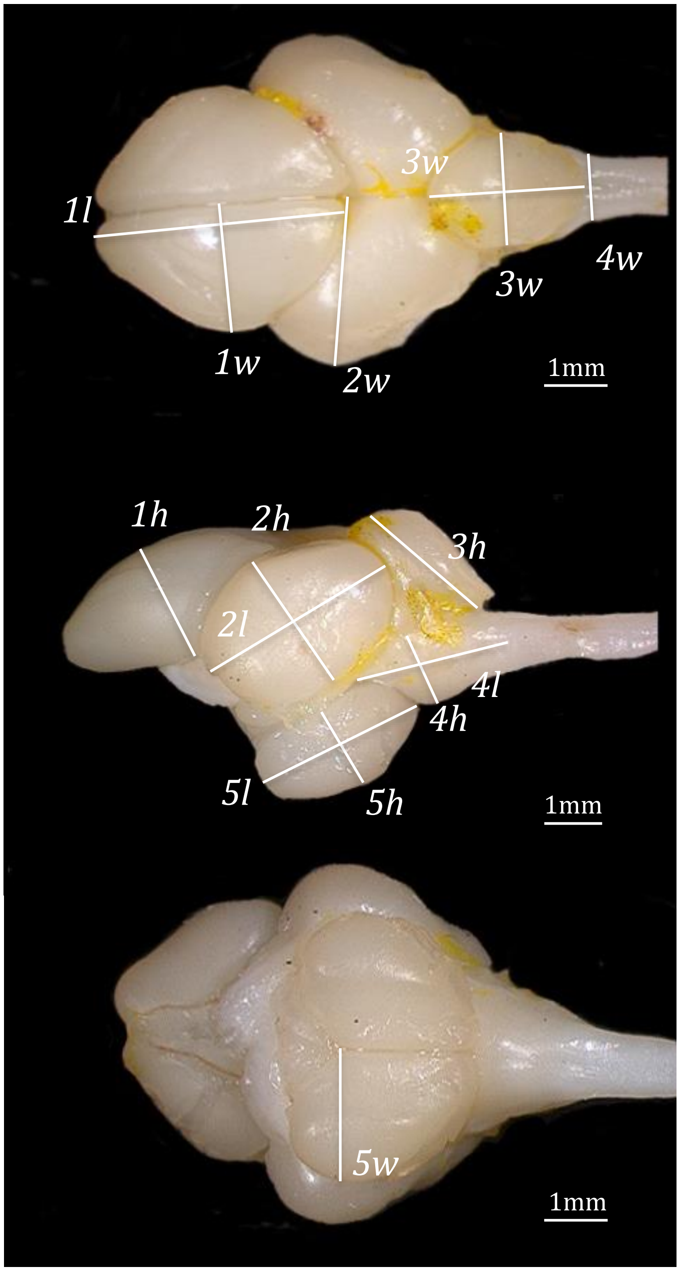
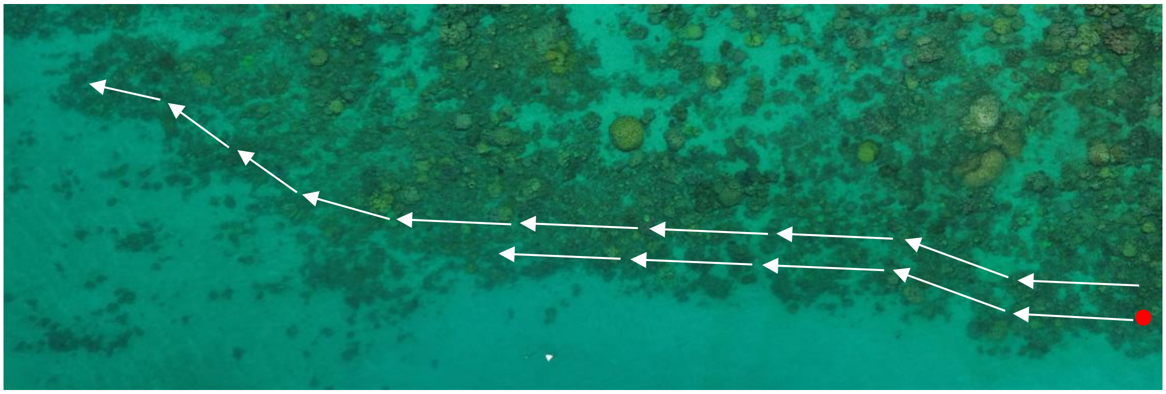


Fig. S3. Aerial view of A) North Horsehoe, B) Horseshoe, C) Clam Gardens and D) Corner Beach. The starting point of our transects is marked with a red dot. The arrows represent direction and approximate location of the ten transects. Since the reefs at corner beach are patchy, transects as described in methods were performed on patches 2 and 3 while the small size of patches 1, 4 and 5 allowed us to quantify all fishes in that area without using a stratified method.



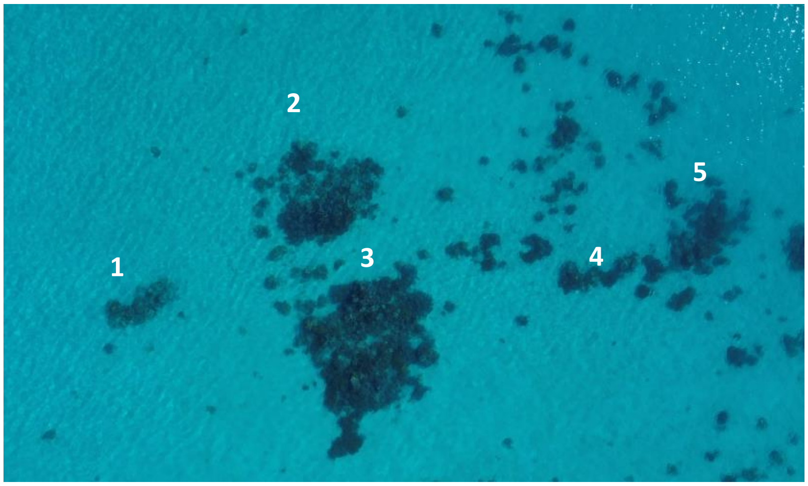

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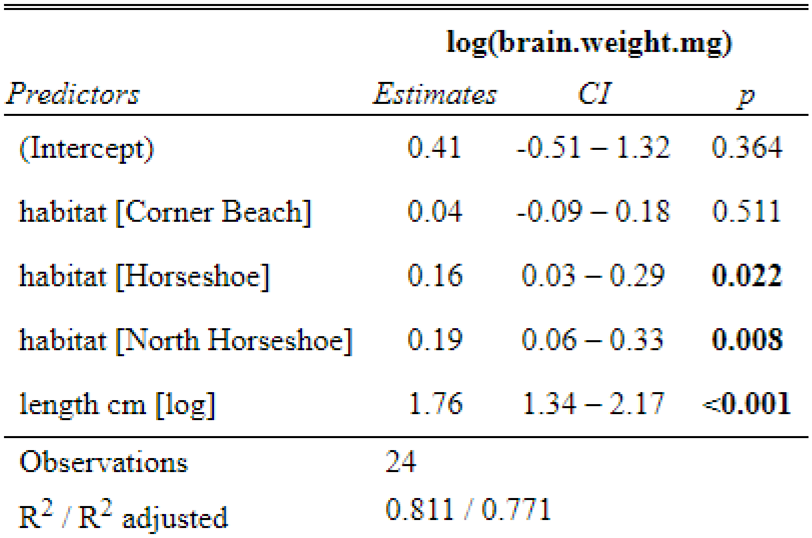
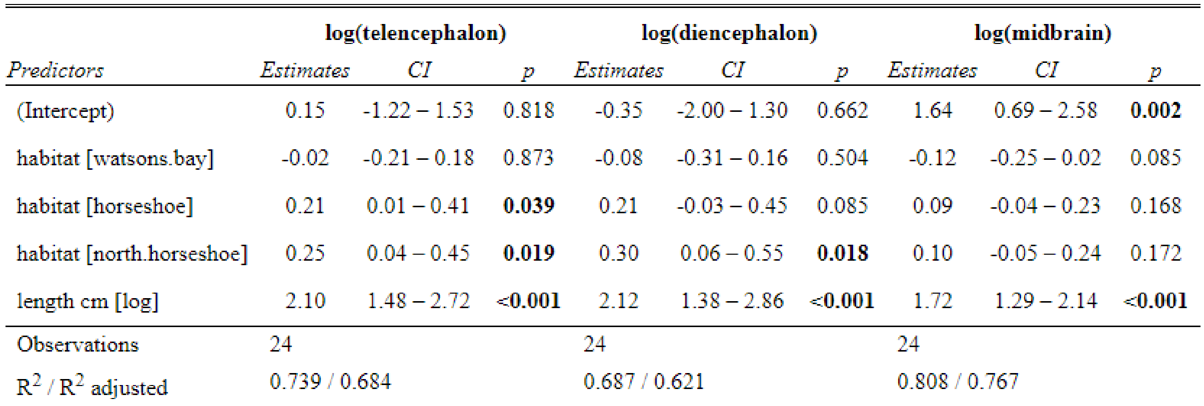
Table S1. Differences in relative brain weights between habitats. Results from linear model examining relationship between habitat and relative brain weight (mg). Brain weight and body length were both log-transformed. Body length is included as covariate to control for body size. Significant values are represented in bold.

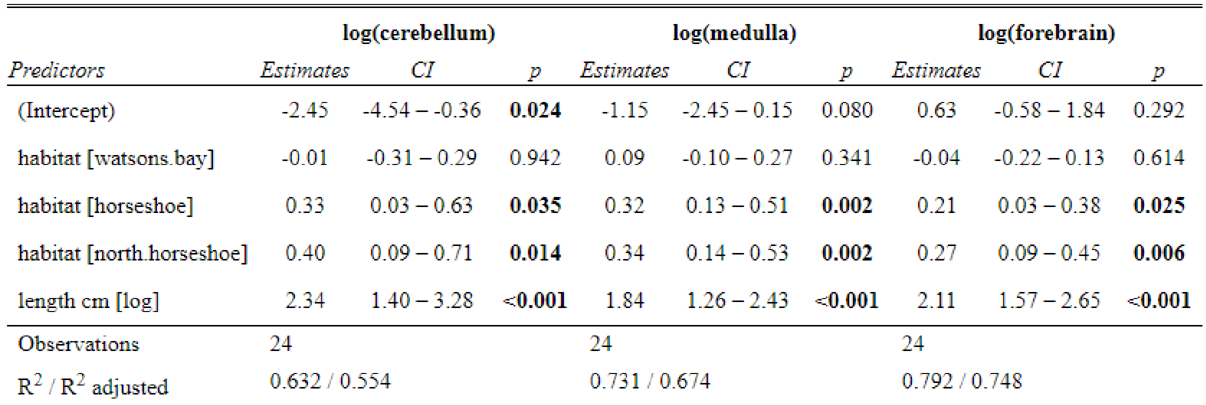


	log(brain.weight.mg)		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	0.41	-0.51 – 1.32	0.364
habitat [Corner Beach]	0.04	-0.09 – 0.18	0.511
habitat [Horseshoe]	0.16	0.03 – 0.29	**0.022**
habitat [North Horseshoe]	0.19	0.06 – 0.33	**0.008**
length cm [log]	1.76	1.34 – 2.17	**<0.001**
Observations	24		
R^2 / R^2 adjusted	0.811 / 0.771		

Table S2. Summary of main statistical effects for each brain region size differences between habitats, corner beach as reference. Each model includes n=24, significance threshold was set at alpha ≤ 0.05 confidence intervals *CI* at 95% and goodness of fit measure estimated by R^2 and Adjusted R^2. This table uses Corner beach as the reference habitat and not Clam Garden's like in the main text.



	log(telencephalon)			**log(diencephalon)**			**log(midbrain)**		
Predictors	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*
(Intercept)	0.15	-1.22 – 1.53	0.818	-0.35	-2.00 – 1.30	0.662	1.64	0.69 – 2.58	**0.002**
habitat [watsons.bay]	-0.02	-0.21 – 0.18	0.873	-0.08	-0.31 – 0.16	0.504	-0.12	-0.25 – 0.02	0.085
habitat [horseshoe]	0.21	0.01 – 0.41	**0.039**	0.21	-0.03 – 0.45	0.085	0.09	-0.04 – 0.23	0.168
habitat [north.horseshoe]	0.25	0.04 – 0.45	**0.019**	0.30	0.06 – 0.55	**0.018**	0.10	-0.05 – 0.24	0.172
length cm [log]	2.10	1.48 – 2.72	**<0.001**	2.12	1.38 – 2.86	**<0.001**	1.72	1.29 – 2.14	**<0.001**
Observations	24			24			24		
R^2 / R^2 adjusted	0.739 / 0.684			0.687 / 0.621			0.808 / 0.767		



	log(cerebellum)			**log(medulla)**			**log(forebrain)**		
Predictors	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*
(Intercept)	-2.45	-4.54 – -0.36	**0.024**	-1.15	-2.45 – 0.15	0.080	0.63	-0.58 – 1.84	0.292
habitat [watsons.bay]	-0.01	-0.31 – 0.29	0.942	0.09	-0.10 – 0.27	0.341	-0.04	-0.22 – 0.13	0.614
habitat [horseshoe]	0.33	0.03 – 0.63	**0.035**	0.32	0.13 – 0.51	**0.002**	0.21	0.03 – 0.38	**0.025**
habitat [north.horseshoe]	0.40	0.09 – 0.71	**0.014**	0.34	0.14 – 0.53	**0.002**	0.27	0.09 – 0.45	**0.006**
length cm [log]	2.34	1.40 – 3.28	**<0.001**	1.84	1.26 – 2.43	**<0.001**	2.11	1.57 – 2.65	**<0.001**
Observations	24			24			24		
R^2 / R^2 adjusted	0.632 / 0.554			0.731 / 0.674			0.792 / 0.748		

Table S3. Relationship of each of each brain region to the rest of the brain. Results from linear model examining selective enlargement of each brain region. Relationship of each brain region compared to the rest of the brain. Each brain region and "rest of the brain" regions were log-transformed. Significant values are represented in bold.

	tel.ratio			**dien.ratio**			**mid.ratio**		
Predictors	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*
(Intercept)	0.78	0.77 – 0.80	**<0.001**	0.70	0.68 – 0.72	**<0.001**	0.88	0.87 – 0.88	**<0.001**
habitat [corner beach]	-0.00	-0.03 – 0.02	0.766	0.01	-0.03 – 0.04	0.641	0.01	0.00 – 0.02	**0.009**
habitat [horseshoe]	0.00	-0.02 – 0.03	0.744	0.02	-0.02 – 0.05	0.325	0.00	-0.01 – 0.01	0.747
habitat [north horseshoe]	0.00	-0.02 – 0.03	0.760	0.02	-0.01 – 0.06	0.145	-0.00	-0.01 – 0.00	0.286
Observations	24			24			24		
R^2 / R^2 adjusted	0.026 / -0.120			0.114 / -0.018			0.459 / 0.378		

	cer.ratio			**med.ratio**			**fb.ratio**		
Predictors	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*
(Intercept)	0.44	0.40 – 0.48	**<0.001**	0.49	0.47 – 0.52	**<0.001**	0.86	0.85 – 0.87	**<0.001**
habitat [corner beach]	0.00	-0.05 – 0.06	0.901	-0.02	-0.05 – 0.02	0.298	-0.00	-0.02 – 0.01	0.894
habitat [horseshoe]	0.03	-0.02 – 0.09	0.268	0.01	-0.02 – 0.05	0.403	0.00	-0.01 – 0.02	0.493
habitat [north horseshoe]	0.03	-0.02 – 0.09	0.236	0.01	-0.02 – 0.04	0.525	0.01	-0.01 – 0.02	0.226
Observations	24			24			24		
R^2 / R^2 adjusted	0.112 / -0.021			0.183 / 0.061			0.111 / -0.022		

Figure S4. Differences in brain region ratios across by habitat complexity. The x-axis shows the habitats and the y-axis indicates the ratios of each brain region (volume of the region of interest to the volume of the rest of the brain). a) telencephalon, b) hypothalamus, c) optic tectum, d) cerebellum, e) dorsal medulla and f) forebrain. This scaling method is used to explore for selective enlargement (table in suppl S2). Boxplots show the median, interquartile ranges and upper and lower quartiles as whiskers, and outliers.

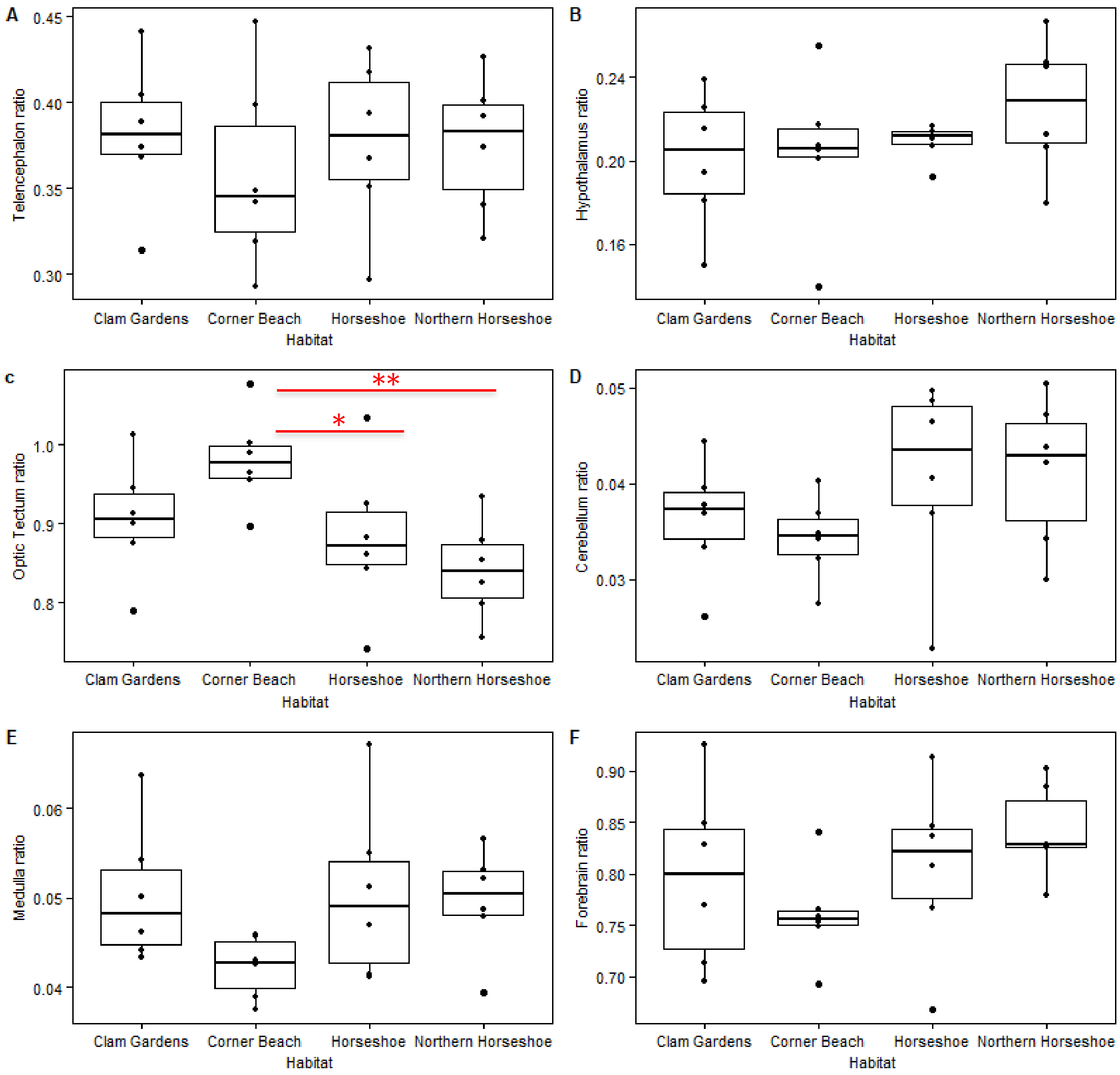


Figure S6. The allometric relationship of each brain region relative to the length for (a) telencephalon (b) hypothalamus (c) optic tectum (d) cerebellum and (e) medulla oblangata. Each color corresponds to each habitat. The x-axis shows the log-transformed length (cm) and the y-axis shows the log-transformed volumes (mm^3) of each brain region.