Evolutionary divergence of plasticity in brain morphology between ecologically divergent habitats of Trinidadian guppies

Abstract

Phenotypic plasticity is critical for organismal performance and can evolve in response to natural selection. Brain morphology is often developmentally plastic, affecting animal performance in a variety of contexts. However, the degree to which plasticity of brain morphology evolves has rarely been explored. Here we use Trinidadian guppies (*Poecilia reticulata*), which are known for their repeated adaptation to high-predation (HP) and low-predation (LP) environments, to examine the evolution and plasticity of brain morphology. We exposed second-generation offspring of individuals from HP and LP sites to two different treatments: predation cues and conspecific social environment. Results show that LP guppies had greater plasticity in brain morphology compared to their ancestral HP population, suggesting that plasticity can evolve in response to environmentally divergent habitats. We also show sexual dimorphism in the plasticity of brain morphology, highlighting the importance of considering sex-specific variation in adaptive diversification. Overall, these results may suggest the evolution of brain morphology plasticity as an important mechanism that allows for ecological diversification and adaptation to divergent habitats.

Keywords:
Phenotypic Plasticity, Brain Morphology, Rapid Evolution, Local Adaptation, Trinidadian Guppy, Sexual Dimorphism

Introduction

Phenotypic plasticity, the ability of a single genotype to manifest different phenotypes in response to environmental cues, is widespread across taxa[1]. During adaptation, plasticity can influence the strength of selective pressures and shift population responses to such pressures[2,3,4]. Plasticity in novel environments may be especially critical for mediating rapid adaptation because plasticity can change the expression of traits, and consequently individual fitness, in the new habitat. Therefore, the evolution of plasticity of key adaptive traits is hypothesized to facilitate population survival and persistence in novel environments[5,6,7,8], as well as increase population performance in highly variable environments[1,6]. As such, it is critical to study not only the evolution of trait values under ecological variation, but also the impact of trait flexibility on local adaptation. One trait that is well suited for studying the impact of plasticity on rapid evolution and adaptive diversification is brain morphology. Brain morphology is influenced by both heritable genetic variation and phenotypic plasticity. Heritable genetic variation has been demonstrated using artificial selection experiments[9,10] and common garden studies[11,12]. Variation in brain morphology can also be due to developmental plasticity. An early demonstration of brain morphology plasticity was in Diamond et al.[13], who showed that rats reared in enriched environments developed larger brains and more glial cells. Brain morphology in fish also responds plastically to predator cues[14,15], light environment[16], social environment[17], and spatial environmental enrichment[18,19,20]. Fish brain morphology in particular is expected to be highly plastic as fish brains maintain widespread neurogenesis, the generation of new neurons, well into adulthood[21,22].

Brain morphology, including brain size and the proportions of different brain regions, correlates with a range of cognitive, behavioral, and ecological characteristics of organisms[23], and can be under selection during adaptation to novel environments. For example, larger brain size is linked to greater cognition (e.g. learning and problem-solving ability) in carnivores[24], primates[25], and fish[26,9]. Variation in brain size is also linked to behavioral flexibility[27], foraging behavior[28], and mating behavior[29,30]. These links are thought to result from more neurons and neuronal connections in larger brains[31,32]. However, despite the broad benefits of larger brains, having larger brains trades off with increased energetic costs, as brains are a metabolically expensive tissue[33,34,35,36]. The proportional sizes of individual brain regions also correlate with ecologically

relevant aspects of cognition, sensory integration, and behavior[37,38,39,40,23,24,25]. The importance of brain morphology for ecological performance is further supported by observational studies showing differences in brain morphology across habitats of varying complexity and predation risk, both at the intraspecific[44,11] and interspecific[45,46,47] levels. For example, sunfish living in the complex littoral habitat of a lake show larger brain size than individuals living in the pelagic habitat of the same lake[48].

Brain morphology is considered a key characteristic influencing ecological performance under ecologically divergent conditions. The cognitive buffer hypothesis posits that larger brains allow organisms to respond to variability in their environment[49]. Comparative tests of this hypothesis have linked brain morphology to environmental variability and colonization success in birds[50,51] and primates[52]. However, a key unknown in our effort to understand the role of brain morphology in adaptation is the degree to which plasticity in brain morphology is itself an evolvable characteristic[53]. We propose that along with larger brains, more plastic brains may similarly facilitate performance when adapting to different environmental conditions. The evolution of phenotypic plasticity is hypothesized as a major contributor to phenotypic diversity[1,54], and empirical studies in bird breeding season length[2] and fruit fly heat temperature tolerance[55] demonstrate that such evolution is possible. Though rarely studied, brain morphology plasticity can evolve[56,57,58]. The evolution of brain plasticity therefore has the potential to shape brain divergence in novel or variable habitats.

We examined evolution and plasticity of brain morphology using Trinidadian guppies (*Poecilia reticulata*) collected from habitats with and without large fish predators (high versus low predation population; hereafter HP vs. LP respectively). In Trinidad, guppies from inland stream populations with large aquatic predators have repeatedly colonized low-predation areas upstream of natural waterfalls[59,60]. In adapting to their local environments the ancestral HP and derived LP guppies have diverged in ecological, morphological, life-history, and behavioral traits across streams (reviewed in Endler 1995[59]). This system is excellent for testing brain plasticity hypotheses for a few reasons. Guppy males from HP sites have larger brains than those from LP sites[15,61], suggesting that predation (and/or other characteristics correlated with it) imposes selection on brain morphology. Additionally, guppies are sexually dimorphic and sexual selection in guppies is influenced by predation. For example, male HP guppies show reduced

color ornaments and increased sexual harassment[59,62], and female HP guppies show reduced preference for colorful males[63,62]. These differences allow us to test whether sexes under different selective pressures differ in the evolution of brain morphology as well as brain morphology plasticity in ecologically divergent habitats, which is important because brain morphology can be sexually dimorphic in certain species including fish[27] . The differences in behavior between habitats also indicate differences in the social environment of HP and LP habitats that could impose additional selection on brains.

To achieve our goals, we reared both male and female guppies from LP and HP populations under two different plasticity treatments: predator chemical cues (presence or absence) and social environments (reared alone (solo), with LP adults, or with HP adults), in a full-sibling fully factorial design. The addition of the social treatment allowed us to assess how multiple environmental cues influence the development or evolution of brain morphology, as social behavior differs between HP and LP populations[59]. We used second-generation offspring of wild caught individuals to ensure any differences between populations were the result of evolved genetic differences. We measured brain mass and estimated the volumes of five brain regions and compared these across HP and LP source populations and plasticity treatments. Evolved genetic differences in brain morphology would be indicated by differences between source populations. Phenotypic plasticity in brain morphology would be indicated by differences between rearing treatments. Evolved differences in plasticity would be evidenced by different responses to plasticity treatments between source populations. Finally, differences between males and females in how brain morphology varies between source and treatments would provide evidence that selection on brain morphology or plasticity in brain morphology varies between the sexes.

Methods

Experimental Methods

We collected wild guppies from HP and LP localities on the Aripo drainage in Trinidad in March 2020 and transported them to our laboratory at Washington University in St. Louis, where they

were quarantined and treated prophylactically for a variety of parasites. We raised these wild fish in group tanks under common garden conditions to generate the first-generation (F1) lab-bred fish. We raised F1 guppies in an aquatic housing system (Aquaneering Inc., USA) and separated males and females before sexual maturity. We then randomly paired our F1 fish to create 29 unique family lines (HP: 15, LP: 14). We separated the F2 siblings at birth and reared them in isolation for 2 weeks, then randomly split them into 6 different treatments: 3 social treatments x 2 predator treatments. Families were not equally represented across treatments due to uneven numbers of births from different mothers. We kept all experimental fish in 1.8-liter tanks in the flow-through Aquaneering system.

In the social treatments, we reared individual juveniles with either adults from the HP or LP populations or on their own (HP conspecifics / LP conspecifics /solo, hereafter). In the two treatments with conspecifics, we added two adult males and one adult female from either HP or LP population into the juvenile tank at week two when juveniles were large enough to avoid adult cannibalism. We removed the conspecific fish from the tanks on day 45 to ensure that the juveniles remained unmated.

We manipulated whether guppies experienced high- or low-predation cues by raising them in water with or without predator chemical cues (pred+ and pred-, hereafter). In the predator cue treatment, we connected a tank containing a pike cichlid (*Crenicichla alta*) to the flow-through water system of the guppies. The guppies could not see the predator, but they received chemical cues through the water. Every day the cichlid was fed two guppies, so possible cues included chemical signals from the cichlid and alarm or injury cues from the predated guppies.

We maintained all fish in the lab at 25-27°C under a 12L:12D light cycle. We fed wild caught and F1 juvenile guppies live brine shrimp, and adults crushed Tetramin Tropical Flakes (Tetra Co., USA). We fed experimental F2 fish quantified amounts of brine shrimp or liver paste throughout the experiment. We chose the quantified feeding amounts so as to optimize growth, while not overfeeding, and to ensure similar levels of food across treatments. Food amounts through the experiment can be seen in Table S3.

Sample Processing

Once individuals reached sexual maturity, we euthanized all fish with an overdose of tricaine methanesulfonate (MS-222). We then photographed, weighed, and preserved the fish in 10% buffered-formalin for 1-3 months before processing. We extracted brains from each individual using dorsal dissection. All dissections and measurements were done by the same individual (C.J.A.). After dissection, we gave all brains non-identifying labels to avoid unconscious bias during brain measurements. We photographed brains from dorsal, ventral, and lateral angles using a Leica MC190 HD microscope camera. We took an additional photo with a closer zoom of the olfactory bulb to ensure accurate measurement of this region. We trimmed the excess nerves from the brains and cut the spinal cord consistently at the level of the obex. We then measured the blotted wet mass of the brains with a Mettler Toledo XPR2 microbalance at a resolution of 0.01mg.

We estimated the volumes of five external brain regions (cerebellum, optic tectum, telencephalon, olfactory bulb, and hypothalamus) using the ellipsoid formula ($V = LxWxHx\pi/6$)[64]. We measured the length, width, and depth of each region using the line measurement tool in Image J (see fig S4). Only one side of the brain was photographed, so the depth of bilaterally symmetrical lobes was assumed to be the same. Sample sizes of each group can be seen in Table S4. We use this method for estimating brain region volumes, rather than more precise methods such as magnetic resonance imaging[65], due to the feasibility of use with large numbers of individuals.

Statistical Methods

To assess the impact of evolved differences and plasticity on brain morphology in divergent populations, we used linear mixed effects models to partition variance in brain morphology between source populations, treatments, and their interaction. We used models that included brain mass or each of the five brain region volumes as the response variables and body mass (for brain mass model) or brain mass (for region models) as a covariate to control for allometric scaling of brain size and region sizes. We natural log-transformed body mass, brain mass, and region volumes to improve residual normality. We included source population (HP vs LP),

predator cue treatment (pred- vs pred+), social treatment (solo vs HP conspecifics vs LP conspecifics), and the two-way interactions between each of these as fixed effects. We subsequently removed non-significant interactions. Finally, we included family as a random effect to account for covariation among siblings. Additionally, to assess how plasticity treatments and source populations may have affected body size, we performed a separate linear mixed model with body mass as the response variable and the same predictor variables as the above models except for the scaling covariate. Final models are reported in Table 1. We further examined significant social effects and interaction using Tukey posthoc tests. For significant social treatment effects, we used posthoc tests to test for pairwise differences between the three treatments. For significant source by predator treatment or source by social treatment interactions, we report treatment effects specific to each source population. For significant predator treatment by social treatment interactions, we report both social treatment effects for each predator treatment, and predator treatment effects for each social treatment. All statistics were performed using the R program version 3.6.3[84]. We used the 'lmer' function in the 'lme4' package[85] for mixed-effects models, and the 'emmeans' function in the 'emmeans' package[86] for posthoc tests.

Finally, by testing brain region variation in separate models for each region, allowing for different patterns between regions, we are assuming that individual regions have the potential to change independently of each other. We accounted for this assumption in two ways. First, we generated correlation matrices examining how independent each of the regions are from each other. Second, we generated principal components of brain region covariation. PC1 accounted for 57% of total variance in both males and females, with all brain regions loading positively (table S6), suggesting that PC1 largely represents whole brain size. This is supported by the strong correlation between PC1 and brain mass in both males (correlation coefficient = 0.78) and females (correlation coefficient = 0.80). We therefore did not include PC1 in any further analyses because it is redundant with our analysis of brain mass. We then ran mixed effects models as described above (with no covariate) with the principal components (2-4) included as response variables to see if brain regions may change in concert across predator regimes or plasticity treatments.

We analysed males and females in separate models. We chose to separate the sexes because their evolutionary and plastic responses may be different due to sexual dimorphism. Their drastic differences in size and morphology also make direct comparisons challenging. Indeed, initial models including both sexes indicated a plethora of interactions between sex and other factors in the models (see Table S5), indicating that males and females reacted differently to divergence and the treatments. Separate models allowed for a clearer understanding of the nature of the individual effects in both sexes.

Results:

Body mass variation

Male body mass differed among social group treatments, though this effect differed depending on the predator treatment (indicated by a significant social treatment by predator treatment interaction ($F_{2,133}$=12.56, p<0.001), suggesting that plasticity cues interact to shape somatic growth in males (Table 1; Table S1; Fig 4). When reared in pred- water, individuals reared with LP conspecifics were larger than those reared with HP conspecifics and those reared alone (Table S1). When reared in pred+ water, individuals reared with conspecifics were larger than those reared alone (Table S1).

Female body mass differed among social treatments and showed evidence of evolved differences in predator cue plasticity. Females showed a significant social treatment effect on body mass (Table 1; $F_{2,141}$=3.67, p=0.029), developing larger body mass when reared with HP conspecifics than when reared with LP conspecifics (Table S2; Fig 5). Solo individuals had intermediate body mass that was not significantly different from either conspecific group (Table S2; Fig 5). Further, there was a significant interaction between source population and predator treatment on female body mass (Table 1; $F_{1,140}$=7.65, p=0.006). However, posthoc tests of this interaction did not reveal a significant predator treatment effect in either source habitat (Table S2; Fig 5).

Evolved differences in brain morphology

Males showed evolved differences in brain morphology between HP and LP populations. Relative brain mass was larger in HP males compared to LP males, regardless of rearing

treatments (Table 1; Fig. 1A; $F_{1,14}$=5.63, p=0.031). Additionally, HP males had smaller relative optic tectum (Fig 1C; $F_{1,11}$=12.51, p=0.0047) and telencephalon volumes (Fig. 1D; $F_{1,13}$=18.76, p<0.001) than LP males (Table 1). Females showed no evolved differences in brain morphology between populations.

Plasticity of brain morphology

Males showed plastic responses in brain morphology to predator cue treatment, developing larger relative brain mass (Figure 1A; $F_{1,137}$=4.61, p=0.034) and olfactory bulb volume when reared in pred+ water (Figure 1E; $F_{1,141}$=6.96, p=0.0093), and larger cerebellum (Figure 1B; $F_{1,139}$=6.97, p=0.0092), and telencephalon (Fig 1D; $F_{1,135}$=13.09, p<0.001) volume when reared in pred- water (Table 1). The plasticity in the telencephalon only occurred in LP sourced males (see *Evolution of plasticity* section below).

Males showed limited plastic responses to their social treatment. Males reared with conspecifics, regardless of the type, developed larger brains than those reared alone (Table 1; Table S1; Fig 4; $F_{2,133}$=6.83, p=0.0015). Additionally, we found an interaction of predator treatment and social treatment on male cerebellum volume (Table 1; $F_{2,139}$=3.6, p=0.03). Males developed a larger cerebellum in pred- water than in pred+ water, but only when reared alone (Table S1; Fig 4).

Female guppy brain morphology showed plasticity in response to predator cues, social cues, and their interaction. Social and predator treatments interacted to shape female brain mass (Table 1; $F_{2,135}$=3.15, p=0.046). Posthoc tests indicated that brain mass was smaller in the solo treatment than in conspecific treatments, but only when reared in pred- water (Fig 2A; Table S2). Females reared in pred+ water, regardless of social treatment, developed brain mass similar to those in pred- water with conspecifics (Fig 2A). Social and predator treatments also independently shaped telencephalon volume (Table 1; Predator: $F_{1,139}$=4.6, p=0.035; Social: $F_{2,140}$=3.9, p=0.022). Females developed smaller telencephalon volume when reared in pred+ water than in pred- water (Fig 2B; Table S2). Female telencephalon also grew largest when reared alone, smaller when reared with HP conspecifics, and smallest when reared with LP conspecifics (but only the solo-LP conspecifics comparison was significant; Table S2).

Evolution of brain morphology plasticity

Males and females both showed evolutionary divergence in brain morphology plasticity. LP males showed plasticity to predator cues in the optic tectum (Fig 1C, Fig S1; $F_{1,131}$=6.15, p=0.014) and telencephalon (Fig 1D, Fig S1; $F_{1,134}$=10.14, p=0.0018), both developing larger in pred- water than pred+ water. HP males did not show plasticity in these regions (Table S1). Female brain morphology showed evolved plastic responses to their social treatment in hypothalamus volume (Table 1, Fig S1; $F_{2,139}$=4.39, p=0.014). In LP sourced females, their hypothalamus grew largest when they were reared alone, smaller when they were reared with LP conspecifics, and smallest when they were reared with HP conspecifics. However, only the solo-HP conspecifics comparison was significant (Fig 2C; Table S2). HP sourced females did not show plasticity in hypothalamus volume.

Absolute brain size variation

Absolute brain size, though less analyzed compared to relative brain size, has also been linked to cognitive performance (Marino 2006). We found that in male guppies, absolute brain size was larger in HP fish than LP fish (Table 1, Fig 3A; $F_{1,14}$=4.8, p=0.046), and in fish reared with conspecifics than fish reared alone (Table1, Fig 3A; $F_{1,131}$=25.8, p<0.001). These patterns are similar to our results with relative brain size. Females from the LP habitat developed larger absolute brain size in the pred+ treatment than pred-, but HP females showed no difference between predator treatments (Table 1, Fig 3B; $F_{1,140}$=7.85, p=0.0058). This is consistent with our relative brain size results that indicated larger relative brain size in females from HP habitats or LP females reared in the pred+ treatment.

Brain region correlation and covariation

The volumes of all brain regions were positively correlated in both males and females, though regions also exhibited independent variation. All regions showed significant positive correlations with all other regions (Fig 6), with correlation coefficients ranging from 0.25 to 0.76. In both males and females, the optic tectum and telencephalon showed the highest correlation (M:0.75, F:0.76), and the cerebellum and olfactory bulb showed the lowest correlation (M:0.25, F:0.26).

Analyses of principal components of brain region covariation are largely consistent with the variation we found between individual brain regions. In males, PC2 largely represents variation

in olfactory bulb volume, with a small contribution of the cerebellum (Table S6). PC2 varied between predator plasticity treatments similarly to the olfactory bulb (Table S7, Fig 7A; $F_{1,131}$=19.03, p<0.001). PC3 in males was not influenced by source population or plasticity treatments (Table S7). PC4 in males represents positive covariation between the optic tectum and telencephalon, which trade off with the hypothalamus and cerebellum (Table S6). PC4 showed a significant effect of source population (Table S7, Fig 7B; $F_{1,141}$=20.28, p<0.001) and an interaction effect between source population and predator treatment. This was similar to the optic tectum and telencephalon (Table S7, Fig 7B; $F_{1,141}$=2.36, p=0.023). Female PC2 and PC3 were not influenced by source population or plasticity treatments (Table S7). Female PC4, similar to males, represents positive covariation between the optic tectum and telencephalon, which trade off with the cerebellum and hypothalamus (Table S6). Female PC4 showed a significant interaction between source population and social treatment (Table S7, Fig 7C; $F_{2,136}$=2.36, p=0.0062),

Discussion:

We examined the influence of evolutionary divergence, plasticity, and plasticity evolution on brain morphology in divergent groups of Trinidadian guppies. From our results we derive three main conclusions. First, variation in brain morphology among populations and plasticity treatments suggests that brain morphology responds evolutionarily and plastically to differences in level of predation. Second, a greater degree of plasticity in LP sourced fish compared to HP fish indicates that brain plasticity can evolve and that this evolution is associated with adaptation to a novel LP habitat. Finally, male and female guppies differ in how their brain morphology responds to ecological cues, as males respond mainly to predator cues and females respond mainly to social cues. We discuss these results in further detail below.

Evolved and plastic variation in brain morphology

We found both evolved genetic differences and plastically induced variation in brain morphology. The variation in brain morphology may stem from several potential mechanisms, including selection on cognition and behavior, energetic limitations, and selection or plasticity of correlated traits. While our study cannot discern among them, we can speculate based on

313 previous studies. All fish were fed the same amount and type of food, so differences in nutrition
314 or available energy are unlikely to explain differences in brain morphology between
315 experimental treatments, However, natural differences in food availability between HP and LP
316 sites could influence evolution of brain size differences between populations. Differences in
317 brain morphology between groups could be the result of changes in correlated traits, such as
318 cranium morphology, however we consider this unlikely because brain morphology is generally
319 not constrained by head morphology in fish[39]. Additionally, we found that brain regions, though
320 positively correlated with each other, all show some degree of independence, allowing for
321 individual responses to selection or plasticity cues. In terms of cognitive consequences of brain
322 variation, shifts in behavior and exposure to enrichment have been linked to brain size plasticity,
323 specifically in guppies[20]. Further, a previous analysis of mating behavior plasticity in the fish
324 from this experiment indicate that shifts in behavior in response to predator cues are associated
325 with plasticity in brain morphology[66]. We thus consider variation in brain morphology in our
326 study to most likely be linked to variation in cognitive, behavioral, and/or sensory functions,
327 though we cannot rule out other explanations. Below we discuss the potential cognitive links to
328 brain morphology that could explain the differences.

329 Variation in relative brain size is often thought to represent variation in general cognitive ability,
330 and larger brains are usually associated with more cognitively demanding environments[48,67]. In
331 our experiment, a larger relative guppy brain size was associated with high predation
332 environments. Both sexes showed a plastic increase in brain size when reared with predator cues.
333 Males, but not females, that were sourced from HP habitats had a larger brain compared to those
334 sourced from LP habitats, indicating an evolved response in brain size. Previous work has found
335 that in wild guppies, larger brain size is associated with greater predator threat[68]. In an artificial
336 selection study, large-brained females, but not males, were better at avoiding predation than
337 small-brained individuals[69]. This evidence and our results broadly support our hypothesis that
338 large brains (either genetically determined or plastically induced) evolved as an anti-predator
339 response, though results are inconsistent, particularly across sexes. On the other hand, smaller
340 brain size has also been linked to higher predation environments[11,70]. The benefits of increased
341 cognition afforded by a larger brain size in a high predation environment may depend on the
342 specific nature of predator-prey interactions and other aspects of the environment.

Our results further suggest that environments with increased social interactions may require larger brains in guppies as males and females developed larger brains when reared with adult conspecifics. A review of brain size variation across mammals proposed increased sociality as a mechanism of the evolution of larger brains[71]. This result is in contrast to a previous study in reptiles that found larger brain sizes are associated with solitary lifestyles[72]. It is also possible that responses in brain size to plasticity treatments does not reflect adaptation to the specific environment of the source population. For example, increased social interaction may also be viewed as a form of environmental enrichment. Enrichment has consistently been shown to lead to increased brain size[13,18,20]. Alternatively, stress caused by unknown or alarming cues from predators may impact the development of brain morphology[73,74]. Elucidating the ultimate drivers of larger brain size in high predator and high sociality environments in guppies will require further experiments that directly test the effect of brain size on the various aspects of ecological performance in HP *versus* LP habitats.

Differences in the relative sizes of specific brain regions among source populations and plasticity treatments may reflect adaptive cognitive and sensory functions of those regions. Brain regions are highly connected, with behavioral functions regulated, at least to some degree, by many regions in combination. However, distinct regions are associated with certain functions more than others. We found that both the cerebellum (motor coordination[37,38,40]) and the optic tectum (visual integration[38,40]) are smaller in males reared in predator water. The telencephalon (learning, memory, and navigation[37,40,75,76]) is similarly smaller in both males and females reared in pred+ water. This result suggests that while males develop smaller relative brain size in pred- water, they maintain a larger telencephalon. This may reflect a benefit of memory or quicker decision making when reared in the absence of predators, with other cognitive functions being less important. The olfactory bulb (olfaction[38,40,77]) is larger in males reared with predator cues, likely indicating a benefit to greater olfaction to perceive predators. Broadly, these responses to predator cues indicate that specific cognitive abilities associated with these regions may be associated with greater performance in HP and LP habitats. Finally, the hypothalamus (social behavior and endocrine control[37,40,78]) varies with the social treatment in females, supporting that this region is associated with social behavior, as previously hypothesized. The larger hypothalamus size of LP females when reared with LP compared to HP conspecifics suggests

that LP guppies interact with each other in more cognitively demanding ways, potentially an association linked with stronger sexual selection in LP guppies[62]. Differences in plastic responses in different regions suggest either that plasticity cues are specific in their effect on regions, or that certain regions are more generally plastic than others. We are unable to distinguish between these possibilities, though they raise interesting questions for future research. Broadly, these results suggest that predator and social environments are associated with specific cognitive and sensory differences in guppies. However, as with whole brain size, understanding the specific functional reason for links between brain regions and environments will require further study.

Absolute and relative brain size

Finally, the differences in relative brain size between source populations and plasticity treatments seem to result largely from changes to the development of brain size, but also from differences in fish body size. For example, male guppies develop smaller when reared in pred+ water, but have the same absolute brain size as those reared in pred- water, resulting in larger relative brain size. This raises the question of whether differences in relative brain size between these groups represent selection for plasticity only in body size, or on the maintenance of absolute brain size in a smaller body size. Due to the high metabolic costs of growth and maintenance of brain tissue[34,35,36], brains are generally expected to be just large enough to maintain necessary cognitive function. Therefore, the maintenance of absolute brain size in male guppies reared in pred+ water likely may indicate selection on plasticity to preserve function despite reductions in body size when predators are present. However, the importance of relative brain size *versus* absolute brain size in affecting cognitive and behavioral performance is still a debated topic[9]. Future studies explicitly testing this on guppies, perhaps with artificial selection, would shed light on this interesting topic.

Evolution of plasticity

Differences in plastic responses to predator and social cues between source populations indicate evolutionary divergence of brain plasticity. Males and females both show divergence in plasticity (albeit in different brain regions and in response to different cues), suggesting that brain

plasticity is an evolvable trait. When plasticity diverges between populations, our results show a trend of greater plasticity in LP compared to HP guppies. Males sourced from LP populations show plasticity in response to predator cues in optic tectum and telencephalon size, while HP males do not. Interestingly, the optic tectum and telencephalon show the highest levels of correlation of all brain regions (75% in males, 76% in females), and show similar patterns of evolution of plasticity in males. These similar patterns could therefore result from independent selection on plasticity in each region or could be due to indirect selection from one region on the other. Further, LP females show social cue plasticity in hypothalamus size, while, again, HP females do not show significant plasticity.

Two patterns of selection are typically hypothesized to result in divergence of plasticity between populations. First, colonization of novel habitats can select for increased plasticity. High predation populations are ancestral to low predation populations, as low predation habitats are colonized during upstream movement. The colonization process can select for increased plasticity if more plastic individuals have higher survival and reproductive advantage during the invasion of a novel habitat[2,4]. This hypothesis is supported by our study, which consistently showed a greater degree of plasticity in LP guppies than HP guppies. Evidence from prior research on the divergence of plasticity between HP and LP guppies is mixed, with HP guppies showing greater plasticity in reproductive traits in response to food availability cues[79], but LP guppies showing greater plasticity of body and head morphology in response to predation cues[80]. The bias towards greater plasticity in the derived LP populations in this study aligns with previous research showing greater plasticity in derived pelagic populations compared to ancestral littoral populations of pumpkinseed sunfish[58]. Further tests of divergence in brain plasticity between HP and LP guppy populations that differ in their patterns and timing of colonization could provide greater support for this hypothesis.

An alternative selective hypothesis is that habitats differ in environmental variability, which can select for increased plasticity[81]. Highly variable environments can select for greater plasticity because this permits individuals to match their traits to changing conditions. As of yet, it is not known whether HP habitats or LP habitats are more variable, or what specific ecological aspects are more variable in each habitat. If low predation habitats have greater variability in their eco-cognitive requirements, this could select for greater plasticity in brain morphology there, such as

we observed in this study. The specific aspects of environmental variability that may select for plasticity in this habitat are not yet known. Though data are consistent with colonization in a novel habitat as a selective agent on plasticity, our study cannot distinguish between these two alternative patterns of selection. Further research testing specific agents of selection across different stages of colonization will be required to fully understand why increased plasticity in brain morphology has evolved in LP guppies.

Our study has certain limitations that must be considered. First, we performed our study on one pair of HP and LP populations, and thus cannot rule out the possibility that the patterns we found are due to genetic drift. However, the central result of our study, that plasticity in brain morphology shows evolutionary divergence between habitats, is interesting regardless of the specific evolutionary mechanism generating that divergence[8]. The populations in this study are representative of HP/LP populations across Trinidad that have shown adaptive variation between habitats across several studies[15,18,60], and future studies investigating other divergent populations of guppies would clarify the generalizability of our findings. Additionally, while we focus on plasticity in brain morphology, other aspects of brain physiology and neuron structure, such as neurotransmitter levels and the strength of neuron connections, can exhibit plasticity. The evolution of these types of neuroplasticity may also be important for adaptation to novel or changing habitats[8].

Sexual dimorphism in plasticity

Male and female guppies differ in their morphology and behavior[62]. Here we show that they also differ in the types of environmental cues that induce developmental plasticity in brain morphology, with males responding more to predator cues and females responding more to social cues. Male brain morphology responded plastically in at least one population to predator cues in whole brain size, cerebellum size, optic tectum size, telencephalon size, and olfactory bulb size, while only their brain size and cerebellum size displayed any response to the social environment. Females, on the other hand, displayed plasticity in at least one population in response to the social environment in brain size, cerebellum size, telencephalon size, and hypothalamus size, while only showing responses to predator cues in brain size and telencephalon size. Males are more prone to predation than females[69], potentially due to their

more conspicuous coloration. This added challenge of predation for males could explain why their brains respond more to predator cues than do female brains. Our results indicate that females, though less prone to predation, experience more brain plasticity in response to variation in their conspecifics, perhaps indicating their greater reliance on social interaction and information. Generally, these differences provide evidence that male and female guppies experience divergent selection, and primarily evolve in response to different ecological characteristics.

Sexual dimorphism in phenotypic plasticity exists in a variety of animal species, mostly in response to temperature variation or sexual selection (reviewed in Hangartner et al. 2022[82]). However, research has not shown consistent patterns of plasticity dimorphism across species, and the ubiquity of sexual dimorphism in phenotypic plasticity is unclear. Our results suggest that divergent selection between males and females may lead to differences in brain plasticity. Guppies show sexual differences in the plasticity of sex-specific life history traits[83]. To our knowledge, this is the first study to demonstrate sexual differences in the evolution of brain plasticity, as we show that males and females both diverge in brain plasticity in different environments, but in different brain regions and in response to different cues.

Conclusion

Understanding the proximate mechanisms that shape trait variation and facilitate adaptive divergence is critical for understanding and predicting patterns of evolution and diversification[2,3,4]. Here, we show the importance of developmental plasticity and the evolution of such plasticity of brain morphology in Trinidadian guppies. Our results support that plasticity of brain morphology is itself a sexually dimorphic, evolvable trait. We also suggest that colonization of novel habitats selects for increased plasticity, indicating that brain morphology plasticity may be critical for performance during colonization. Further research is required to elucidate the functional causes and consequences of variation in brain morphology and plasticity, as well as the importance of these traits for organism fitness during adaptation to rapidly changing environments.

Table 1. Summary of analysis of variance of mixed-effects models predicting brain and body morphology. All models include source habitat (Source), predator treatment (Predator), and social treatment (Social). The model prediction brain mass also includes body mass (g) as a covariate, and the models predicting brain region volumes include brain mass (g) as a covariate. Absolute brain mass models do not include a covariate. Response variables and covariates were natural log transformed in every model. Significant two-way interactions are also included in the model. Family was included as a random effect in all models. P-values of significant predictors are bolded. Restricted maximum likelihood (REML) at convergence values for each model are listed below response variables.

Response Variable	Predictor Variable	F	Sum of Squares	p
Males				
Brain Mass REML= -147.1	Body Mass Source Predator Social	28.8 5.4 4.67 6.87	0.49 0.092 0.080 0.23	**<0.001** **0.035** **0.032** **0.0015**
Cerebellum Volume REML=-31.7	Brain Mass Source Predator Social Predator*Social	24 1.25 6.97 0.73 3.6	0.94 0.048 0.27 0.056 0.28	**<0.001** 0.27 **0.0092** 0.49 **0.03**
Optic Tectum Volume REML=-250.9	Brain Mass Source Predator Social Source*Predator	144.28 12.23 1.31 1.31 6.06	1.09 0.093 0.010 0.020 0.046	**<0.001** **0.0049** 0.25 0.27 **0.015**
Telencephalon Volume REML=-183.9	Brain Mass Source Predator Social Source*Predator	90.75 19.1 13.03 1.66 10.09	1.14 0.24 0.16 0.04 0.13	**<0.001** **<0.001** **<0.001** 0.19 **0.0019**
Olfactory Bulb Volume REML=14.8	Brain Mass Source Predator Social	47.8 0.74 6.96 0.45	2.7 0.042 0.39 0.051	**<0.001** 0.39 **0.0093** 0.64
Hypothalamus Volume REML=-82	Brain Mass Source Predator Social	28.47 0.028 1.58 0.74	0.75 0.00073 0.042 0.039	**<0.001** 0.87 0.21 0.48
Body Mass REML=-667.2	Source Predator	0.017 2.57	0.0000063 0.00095	0.90 0.11

	Social	35.1	0.026	**<0.001**
	Predator*Social	12.5	0.0093	**<0.001**
Absolute Brain Mass REML= -1770.17	Source	4.80	0.097	**0.046**
	Predator	2.10	0.042	0.15
	Social	25.83	1.04	**<0.001**
Females				
Brain Mass REML=-160.5	Body Mass	49.37	0.70	**<0.001**
	Source	0.0022	0.00003	0.96
	Predator	7.44	0.11	**0.0073**
	Social	2.85	0.081	0.061
	Predator*Social	3.15	0.089	**0.046**
Cerebellum Volume REML=-64.9	Brain Mass	61.96	1.99	**<0.001**
	Source	1.04	0.033	0.33
	Predator	0.33	0.011	0.57
	Social	1.73	0.11	0.18
Optic Tectum Volume REML=-264.9	Brain Mass	220.50	1.60	**<0.001**
	Source	0.92	0.067	0.35
	Predator	0.0081	0.00006	0.93
	Social	1.01	0.015	0.37
Telencephalon Volume REML=-196.9	Brain Mass	180.0	2.19	**<0.001**
	Source	0.53	0.0065	0.48
	Predator	4.6	0.055	**0.035**
	Social	3.9	0.096	**0.022**
Olfactory Bulb Volume REML=-27.2	Brain Mass	32.0	1.24	**<0.001**
	Source	0.38	0.015	0.54
	Predator	1.24	0.048	0.27
	Social	1.02	0.079	0.36
Hypothalamus Volume REML=-124.2	Brain Mass	38.91	0.77	**<0.001**
	Source	2.6	0.051	0.14
	Predator	0.62	0.012	0.43
	Social	1.89	0.074	0.15
	Source*Social	4.39	0.17	**0.014**
Body Mass REML=-360.73	Source	0.035	0.00013	0.85
	Predator	0.0005	0.0000019	0.98
	Social	3.67	0.014	**0.028**
	Source*Predator	7.65	0.030	**0.006**
Absolute Brain Mass REML= -1681.54	Source	0.22	0.0044	0.64
	Predator	5.29	0.10	**0.023**
	Social	2.40	0.047	0.094
	Source*Predator	7.85	0.15	**0.0058**

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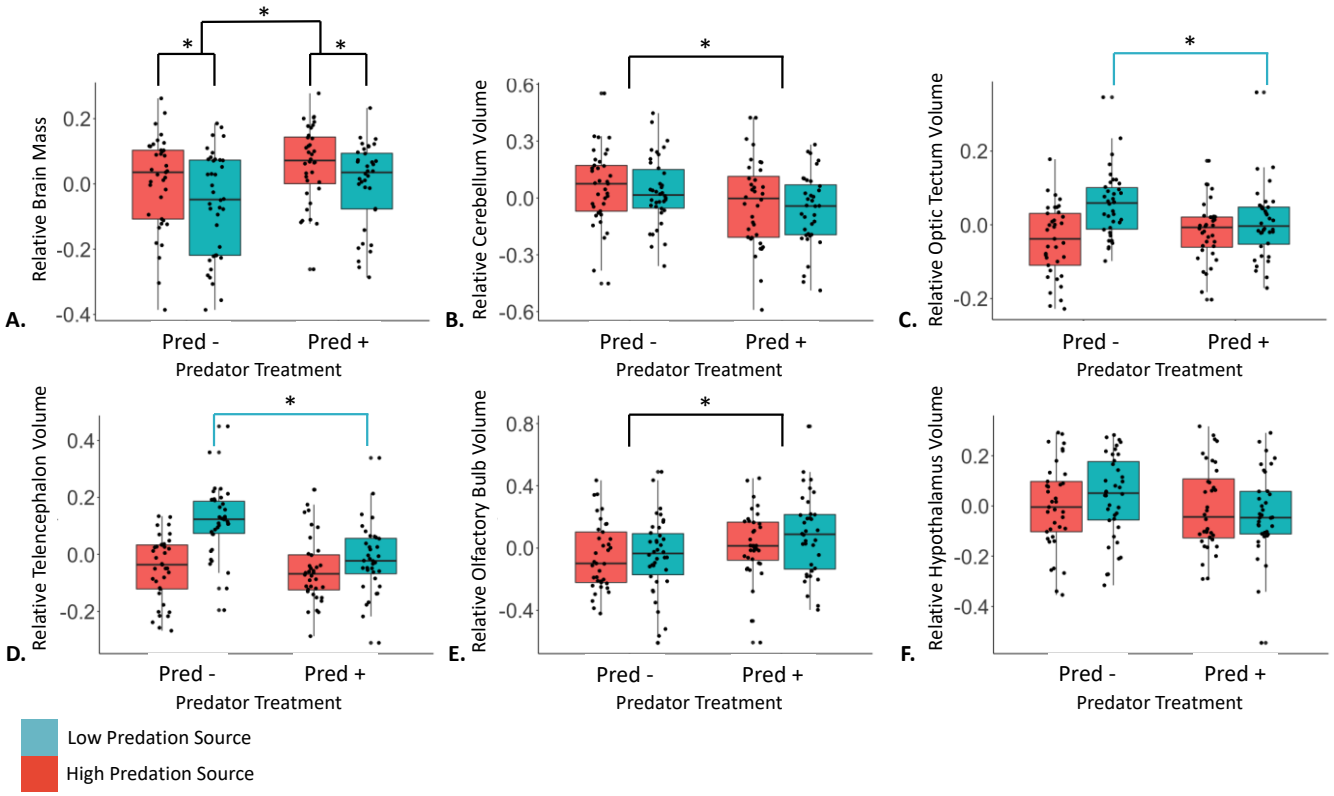

Figure 1. Box plots showing male guppy brain morphology across predator treatments and source habitats. Panels show different aspects of brain morphology: A) relative brain mass, B) relative cerebellum volume, C) relative optic tectum volume, D) relative telencephalon volume, E) relative olfactory bulb volume, F) relative hypothalamus volume. Source habitat is represented by red (HP) and blue (LP) colours. Relative brain size is estimated as the residuals from a linear model of brain mass regressed against body mass (both natural log transformed) (Fig S2). Relative brain region volumes are estimated as the residuals from a linear model of each region volume regressed against brain mass (both natural log transformed). These measures of relative brain and brain region size are used for visualization purposes only. Statistical significance indices (based on mixed-effects models) in black indicate simple differences between predator treatments or source habitats. Significance indices in color indicate that differences between predator treatments occur only in the designated source habitat. Boxes show median and interquartile range (25^{th} to 75^{th}), and whiskers show the data range. Dots show individual data points.

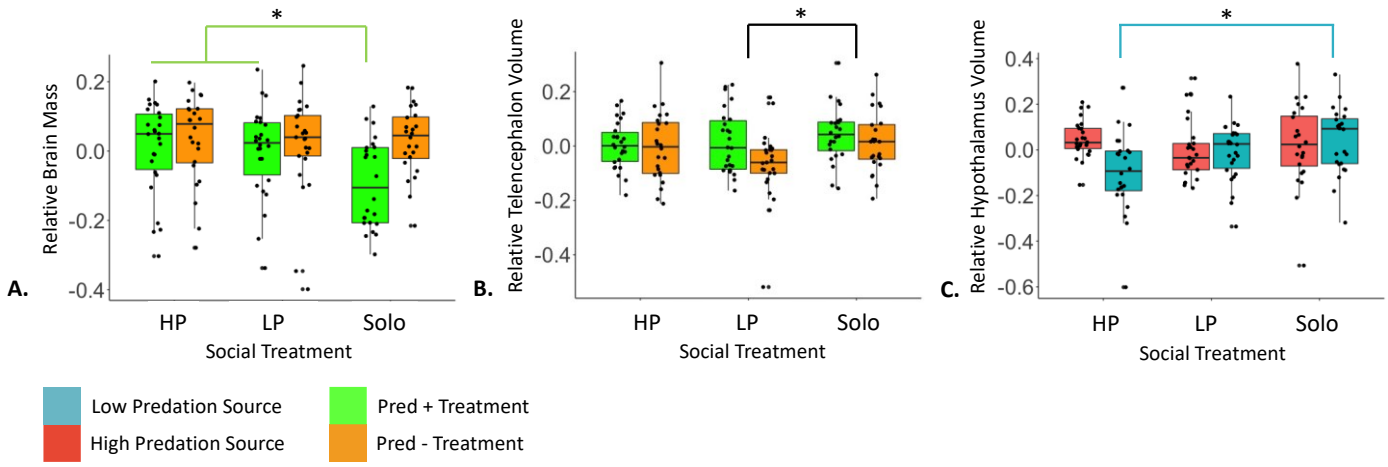

Figure 2. Box plots showing female guppy brain morphology across social treatments and predator treatments (panels A and B) or source habitats (panel C). Panels show different aspects of brain morphology: A) relative brain mass, B) relative telencephalon volume, C) relative hypothalamus volume. Predator treatment in panels A and B is represented by green (Control) and orange (Predator) color. Source habitat in panel C is represented by red (HP) and blue (LP) colours. Relative brain size is estimated as the residuals from a linear model of brain mass regressed against body mass (both natural log transformed) (Fig S3). Relative brain region volumes are estimated as the residuals from a linear model of each region volume regressed against brain mass (both natural log transformed). These measures of relative brain and brain region size are used for visualization purposes only. Statistical significance indices (based on mixed-effects models) in black indicate simple differences between social treatments. Significance indices in color indicate that differences between social treatments occur only in the designated predator treatment or source habitat. Boxes show median and interquartile range (25th to 75th), and whiskers show the data range. Dots show individual data points.

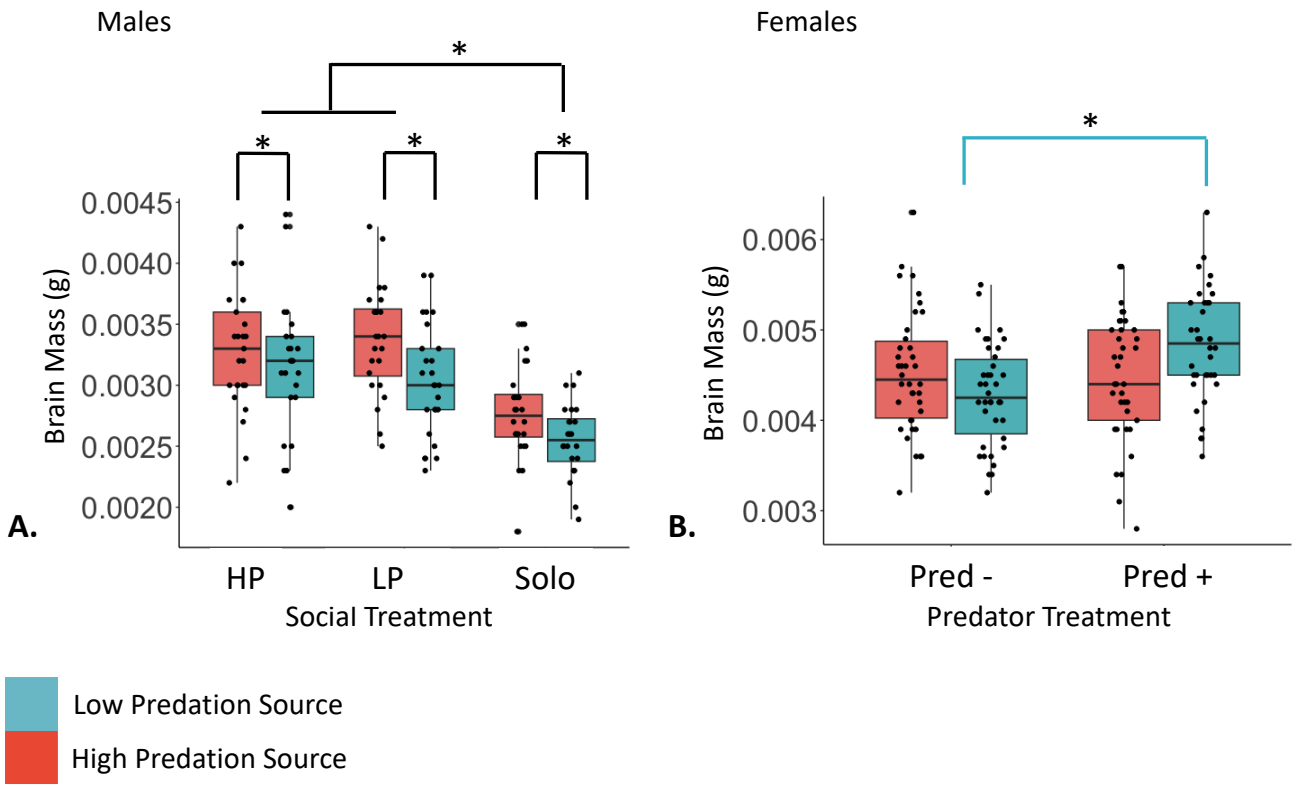

Figure 3. Box plots showing male (panel A) and female (panel B) guppy absolute brain mass across social treatments (males) and predator treatments (females). Source habitat in both panels is represented by red (HP) and blue (LP) colours. Statistical significance indices (based on mixed-effects models) in black in panel A indicate simple differences between social treatments and source populations. Significance indices in color in panel B indicate that differences between predator treatments occur only in the designated source habitat. Boxes show median and interquartile range (25th to 75th), and whiskers show the data range. Dots show individual data points.

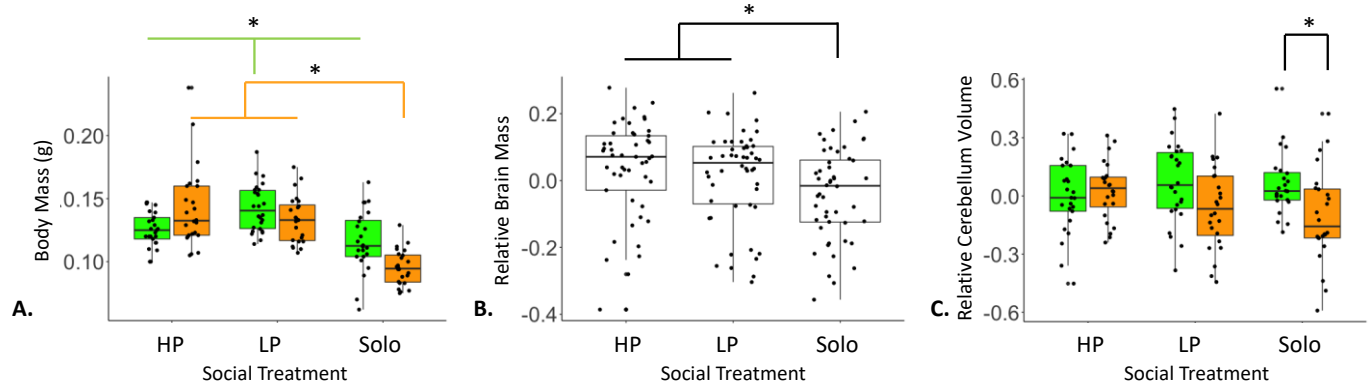

Figure 4. Box plots showing male guppy body mass (g) (panel A), relative brain mass (panel B), and relative cerebellum volume (panel C) across social treatments and predator treatments (panels A; C). Predator treatment in panels A and C is represented by green (Control) and orange (Predator) color. Relative brain size is estimated as the residuals from a linear model of brain mass regressed against body mass (both natural log transformed) (figure S2). Relative cerebellum volume is estimated as the residuals from a linear model of cerebellum volume regressed against brain mass (both natural log transformed). These measures of relative brain and brain region size are used for visualization purposes only. Statistical significance indices (based on mixed-effects models) in black indicate simple differences between social treatments in panel B, or between predator treatments within social treatments in panel C. Significance indices in color in panel A indicate that differences between social treatments occur only in the designated predator treatment (green indices in panel A indicate that the LP group is significantly different from both the HP and Solo groups. Boxes show median and interquartile range (25th to 75th), and whiskers show the data range. Dots show individual data points.

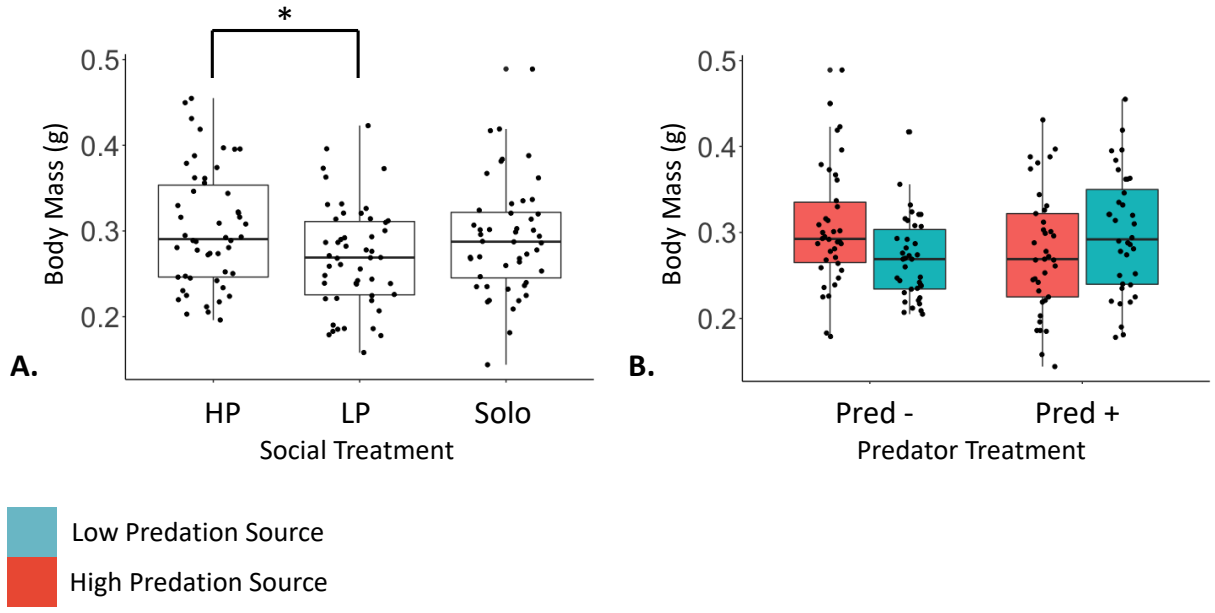

Figure 5. Box plots showing female guppy body mass (g) across social treatments (panel A) and predator treatments (panel B). Source habitat in panel B is represented by red (HP) and blue (LP) color. Statistical significance indices (based on mixed-effects models) in black indicate simple

differences between social treatments. Boxes show median and interquartile range (25th to 75th), and whiskers show the data range. Dots show individual data points.

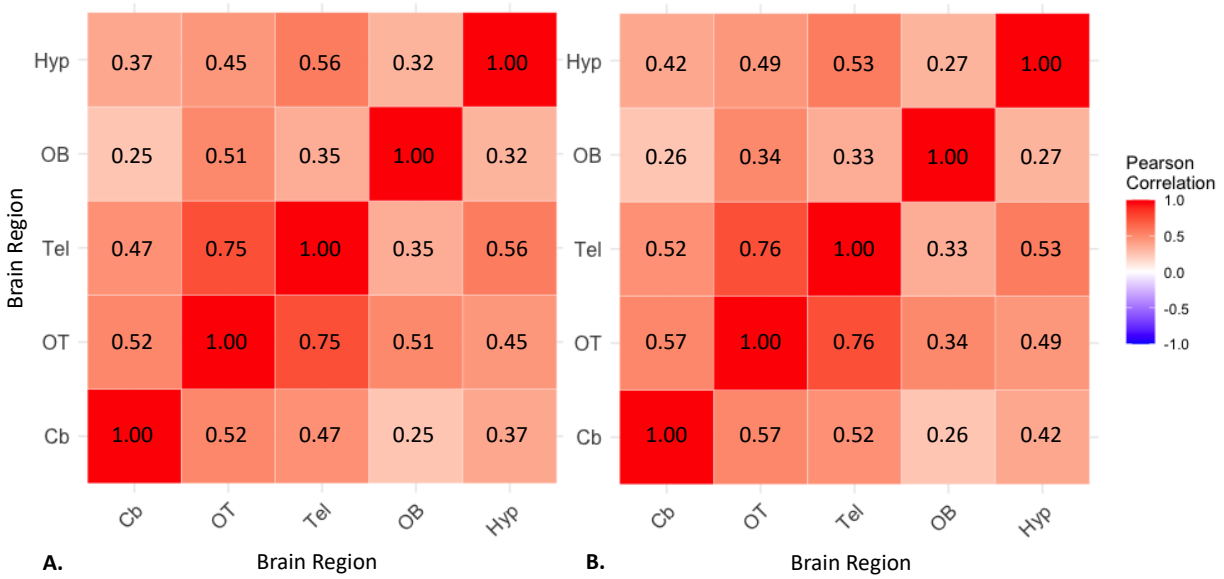

Figure 6. Correlation matrices showing Pearson correlation coefficients between the volumes of five brain regions (CB: Cerebellum, OT: Optic Tectum, Tel: Telencephalon, OB: Olfactory Bulb, Hyp: Hypothalamus) for male (panel A) and female (panel B) guppies.

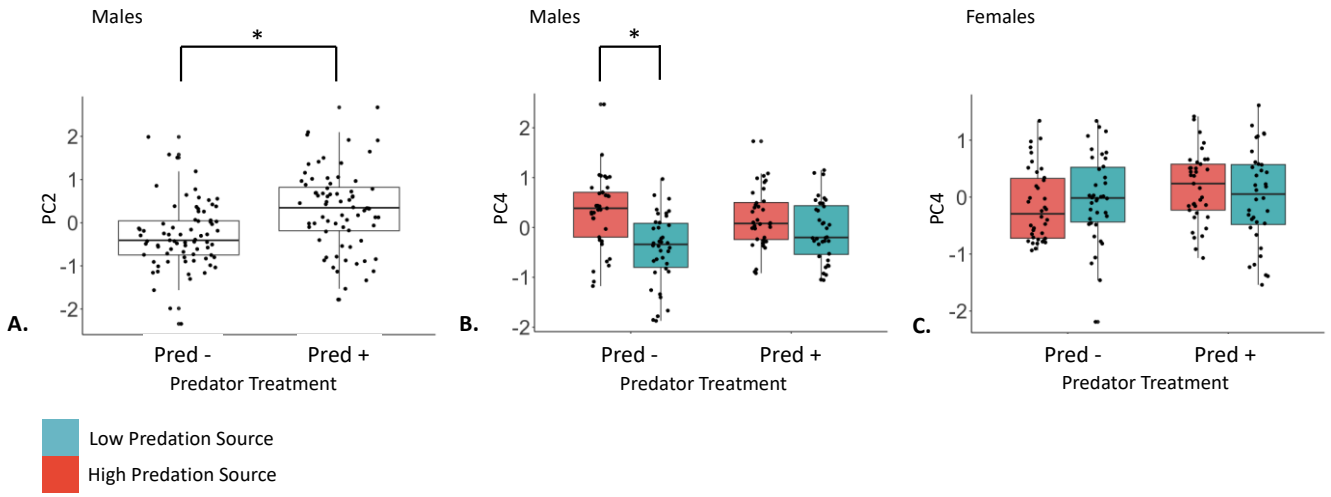

Figure 7. Box plots showing male (panels A and B) and female (panel C) guppy brain region principal components compared across predator treatments. Source habitat in panels B and C is represented by red (HP) and blue (LP) colours. Statistical significance indices (based on mixed-

effects models) in black in panel A and B indicate simple differences between predator treatments or source populations. Significance indices in color in panel B indicate that differences between predator treatments occur only in the designated source habitat. Boxes show median and interquartile range (25^{th} to 75^{th}), and whiskers show the data range. Dots show individual data points.

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