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Article

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Wild and laboratory exposure to cues of predation risk increase relative brain mass in male guppies

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Abstract

1. There is considerable diversity in brain size within and among species, and substantial dispute over the causes, consequences and importance of this variation. Comparative and developmental studies are essential in addressing this controversy.
2. Predation pressure has been proposed as a major force shaping brain, behaviour and life history. The Trinidadian guppy, *Poecilia reticulata*, shows dramatic variation in predation pressure across populations. We compared the brain mass of guppies from high and low predation populations collected in the wild. Male but not female guppies exposed to high predation possessed heavier brains for their body size compared to fish from low predation populations.
3. The brain is a plastic organ, so it is possible that the population differences we observed were partly due to developmental responses rather than evolved differences. In a follow-up study, we raised guppies under cues of predation risk or in a control condition. Male guppies exposed to predator cues early in life had heavier brains relative to their body size than control males, while females showed no significant effect of treatment.
4. Collectively our results suggest that male guppies exposed to predation invest more in neural tissue, and that these differences are at least partly driven by plastic responses.

Keywords: brain size, development, plasticity, *Poecilia reticulata*, sex differences

1 | INTRODUCTION

Brains vary considerably in volume and organisation both within and between species (de Winter & Oxnard, 2001; Gonda et al., 2013; Striedter 2005). Such variation is often understood as the result of a trade-off between costs and benefits, balancing for example the energetic or developmental costs of brain enlargement against proposed benefits such as increased efficacy of perception, cognition, or motor skills (Barton, 1998; Benson-Amram et al., 2016; MacLean et al., 2014). Identifying the factors that shape brain evolution and development furthers our understanding of these costs and benefits (Sherry, 2006).

Predation poses a major challenge for many species (Edmunds, 1974; Lima & Dill, 1990) and may have a substantial influence on brain evolution (van der Bijl & Kolm, 2016). Animals faced with frequent predation threats may be selected for greater investment in neural tissues that help them to sense, integrate or act upon information from the environment in order to evade predators (Gonda et al., 2012). For example, birds with larger brains have shorter flight initiation distances, potentially reflecting superior predator monitoring abilities (Møller & Erritzøe, 2014). Larger brained bird species have lower adult mortality (Sol et al., 2007) and have reduced depredation of their nests (Öst & Jaatinen, 2015). Mammalian predators capture smaller brained prey more often than expected by their abundance (Shultz & Dunbar, 2006) and the presence of predators is associated with larger brains in mammalian prey species (Jerison, 1973). In fish, prey species tend to have a larger relative brain size than do their predators and there is a positive association between the brain sizes of predators and prey (Kondoh, 2010). By contrast, Walsh et al. (2016) found that in the Trinidad killifish, *Rivulus hartii*, males from high predation populations had smaller brains than those from low predation populations. The authors speculated that killifish with fewer predators might be selected for larger brains because of the greater competition for food and mates in these populations. Similarly, a recent study on threespine stickleback, *Gasterosteus aculeatus,* found that experimental exposure to predators selected for fish with smaller rather than larger brains (Samuk et al. 2018). Collectively, these results illustrate that the drivers of brain size variation are complex, and the effect of predation on relative brain size and may depend on multiple interacting ecological and social pressures (Dunbar & Shultz, 2017).

The majority of studies that examine the evolution of brain size have made use of cross species comparisons, however, these analyses can be complicated by phylogenetic relationships and unaccounted for ecological or life-history factors (Harris et al., 2016; Healy & Rowe, 2007; Logan et al., in press). Intraspecific studies across populations are valuable as they can partially control for some of the potentially confounding variables that inherently complicate the interpretation of interspecies comparisons (Gonda et al., 2012; Logan et al., in press). Leveraging natural variation in ecological conditions among populations represents a powerful approach to the study of brain evolution (Walsh et al., 2016).

While the brain is shaped by evolution, it is also a highly malleable organ and phenotypic plasticity may also play a key role in generating individual variation in brain size (Gonda et al., 2013; Healy & Rowe, 2007). For example, environmental complexity during early life increases relative brain size in rodents (Diamond et al., 1966; Rozenzweig & Bennett, 1969), insects (Heisenberg et al., 1995) and fish (DePasquale et al., 2016; Gonda et al., 2011), while low oxygen during development decreases relative brain size in fish (Chapman et al., 2008).

The Trinidadian guppy, *Poecilia reticulata*, is a small livebearing freshwater fish that experiences pronounced interpopulation variation in predation threat (Magurran, 2005), and thus provides a valuable system to study how predation shapes the brain. Throughout Trinidad, guppies have repeatedly colonized independent river reaches above natural waterfall barriers, where aquatic predators are scarce, while simultaneously living below the same barriers where abundant aquatic predators impose substantial mortality (Magurran, 1998). As a result, there has been repeated parallel evolution of distinct behavioural, morphological, and life-history traits among guppy populations that are heavily depredated compared to those that are relatively free from predation pressure (Magurran, 2005).

A recent series of papers has examined the effect of artificially selecting guppies for large or small relative brain mass, finding that increased investment in brain tissue can provide antipredator benefits, but also carry costs. Female guppies artificially selected for larger brains exhibited greater survival under predation and altered predator responses compared to small-brained individuals (Kotrschal et al., 2015a; van der Bijl et al., 2015). However, larger-brained female guppies also had smaller guts, produced fewer offspring, and had reduced innate immune responses, suggesting a trade-off between neural investment and other fitness-relevant parameters (Kotrschal et al., 2013, 2015b, 2016). If antipredator advantages were sufficient to overcome the costs of maintaining a larger brain, then we would expect that guppies from high predation populations would consistently have larger brains for their body size than guppies from low-predation environments. Indeed, female guppies under greater threat from predatory prawns have larger relative brain sizes than do females under lesser threat from these predators (Kotrschal et al., 2017a). Artificial selection on brain size in guppies has consistently revealed differing effects in males and females, suggesting that sex may be a key modulator of the relationship between brain size and performance in this species (e.g., Kotrschal et al., 2012, 2013 2015a; van der Bijl et al., 2015), and therefore, it is important to examine both males and females.

Guppies also show plasticity in brain size; for example, guppies raised in the laboratory have smaller brains than fish born in the wild (Burns & Rodd, 2008; Burns et al., 2009; Eifert et al., 2015). Furthermore, guppy males that cohabitated with females have larger brains than those that lived with only males (Kotrschal et al., 2012). If guppies can adjust their investment in neural tissue to local conditions during development, it is possible that plastic responses to cues of predation risk may at least partially explain any observed population differences in brain size. In order to understand the expression of a quantitative phenotypic trait, evolutionary studies on interpopulation differences in trait expression should be combined with studies of phenotypic plasticity (Gonda et al., 2013).

Our study aimed to help illuminate the importance of predation in shaping within-species variation in brain mass and to elucidate the potential role of plasticity in generating these differences. Specifically, we had two objectives: First, we aimed to determine whether there are differences in relative brain mass between wild guppies collected from high and low predation populations. We predicted that guppies from high predation populations would have relatively heavier brains. Second, we aimed to determine whether guppies show brain mass plasticity in response to cues of predation risk during development. We conducted a laboratory experiment in which guppies were exposed to multisensory cues of predation risk or a control condition during the first 45 days of life. We predicted that guppies exposed to cues of predation risk would show increased relative brain mass.

2 | MATERIALS AND METHODS

2.1 | Field collections

In March 2016, we collected 151 adult guppies (79 males and 72 females) from four sites, one high predation and one low predation site in each of two rivers (Aripo and Marianne) in the Northern mountain range of Trinidad (Table 1). These rivers belong to independent drainages, and therefore are subject to a distinct suite of biotic and abiotic conditions (Gotanda et al. 2013). Assignment of predation regime followed previous studies at these sites (Gotanda et al., 2013) and was based on the presence or absence of dangerous fish predators (e.g., cichlids such as

Crenicichla sp. and *Aequidens pulcher* in the Aripo River; and eleotrids such as *Eleotris pisonis* and *Gobiomorus dormitor* in the Marianne River; Magurran, 2005; Reznick et al., 1996), which is consistent across years (Schwartz & Hendry, 2010). Guppies were collected from each site using butterfly nets and then were transported to the William Beebe Research Station near Arima, Trinidad. Each fish was euthanized with an overdose of tricaine methanesulfonate (Finquel MS-222; Argent Chemical Laboratories, USA) buffered to a neutral pH with $NaHCO_3$. We measured each fish for standard length (SL; from the tip of the snout to the caudal peduncle) and then dissected out the brains using a portable stereomicroscope (Ken-a-vision VisionScope 2) at 10x magnification. Care was taken to sever the spinal cord and optic nerves at a consistent position on each brain. We placed the brains in RNAlater (Sigma Aldrich) and incubated them for 24 h at room temperature before transferring them to -20°C. We transported the samples back to McGill University (Montreal, Canada) where we removed them from RNAlater and gently dabbed them dry. Blind to the population of origin, we weighed each whole brain to the nearest 0.1 mg using an analytic laboratory balance (Mettler Toledo ME104E). Because all brains were treated identically, any storage effects on brain mass should affect all samples similarly. Following measurement, the brains were used in another study.

2.2 | Developmental experiment

We exposed developing guppies to cues of predation threat during the first 45 days of life. The parental generation were guppies from a laboratory-reared population, descended from a mixture of fish captured in high predation sites in the Aripo and Quare Rivers of Northern Trinidad in 2009 and 2010. Parental fish were housed in mixed sex groups of ~10 adults in 18 L aquaria. We maintained the water at 26±1°C and fed the fish ad libitum daily on a mixture of dried prepared tropical fish flakes (TetraMin, Tetra, Germany) and rehydrated decapsulated brine shrimp eggs (Brine Shrimp Direct, Inc., Ogden, Utah, USA). Lights were on from 07:00 to 19:00 h, with a 30 min dawn/dusk period. To collect fry for the experiment, we moved groups of 10-12 visibly gravid females into separate aquaria, which we checked daily for newborn fry. We mixed fry born to different females and randomly assigned them to one of two treatments: Exposure to cues of predation risk or a control condition. Fry in both treatments were held at densities of 30 individuals per 18 L aquarium during the treatment period. We had three replicate aquaria in each experimental condition (six aquaria total). The experimental aquaria contained 1 cm of white coral sand and were furnished with an artificial plant to serve as a refuge. Water and light conditions were the same as for the parental generation, but the developing fry were fed twice daily.

Five days per week during the 45-day treatment period, the fish in the predator cue condition were visually exposed to a sympatric cichlid fish predator (*Crenicichla* sp.) living in an adjacent aquarium by removing an opaque barrier between them for 5 minutes. Concurrent with the visual exposure, we infused 5 ml of water previously collected from aquaria housing live *Crenicichla* that had recently been fed freshly euthanized guppies (following Brown et al., 2000). Guppies respond to the odour of damaged conspecifics and predator dietary cues with antipredator responses (Brown & Godin, 1999). On four of the five weekly cue exposure days, we also added 5 ml of odour cue harvested from the skin and muscle tissue of adult guppies in addition to the predator housing water. To collect this cue, we sacrificed adult guppies of both sexes by briefly immersing them in an ice water bath and then swiftly decapitating them (Matthews & Varga, 2012). We then homogenized skin and muscle tissues with dH_20, filtered the solution with cotton floss, and diluted it with dH_20 until we obtained a concentration of 0.1 cm^2 of tissue per ml of cue (following Brown & Godin, 1999). We exposed the guppies in the control condition to the sight and housing water of a non-predatory suckermouth catfish

(*Pterygoplichthys* sp.) that had been fed blanched spinach leaves. In lieu of the damaged conspecific cue, the control fish received blank dH_20. We exposed the guppies to these heterospecific fish stimuli at a randomly chosen time (between 10:00 and 16:00 h) on each exposure day.

After 45 days, we ceased all heterospecific stimuli exposures. On day 50, we reduced the housing density of the experimental fish by splitting each group into 3 separate 18 L aquaria with ~10 individuals of mixed-sex in each, resulting in a total of 18 housing aquaria, nine per treatment. We also reduced the feeding frequency to once per day to match the standard adult husbandry protocols in our laboratory. The experimental fish were held in these conditions until approximately 300 days of age, during which time behavioural and hormonal measures were taken for other studies (Chouinard-Thuly et al., 2018; Leris, 2016). We then sacrificed 73 individuals (22 predator exposed males, 27 control males, 11 predator exposed females, and 13 control females), by briefly immersing them in an ice water bath and then swiftly decapitating them. We then dissected out their brains using a stereomicroscope (Leica EZ4W) at 10x magnification. Care was taken to sever the spinal cord and optic nerves at a consistent position on each brain. We weighed the fresh brains to the nearest 0.1 mg on an analytic laboratory balance (Mettler Toledo ME104E). Brain mass and body size values were taken blind to treatment. Following measurement, the brains were used in another study.

2.3 | Analysis

We used linear models to investigate the relationship between brain mass and body size with exposure to predation both naturally in the field, and in our developmental experiment. To account for the allometric relationship between brain mass and body size (Brandstätter & Kotrschal, 2008) we included standard length (SL) as a covariate in the models investigating brain mass. We log transformed SL and brain mass measures before running each model, and mean-centered SL. Male and female guppies differ considerably in body size (Mean SL ± s.e.: wild males = 12.77 ± 0.13 mm, wild females = 15.66 ± 0.30 mm; Welch's $t_{96.9} = 8.76$, $p < 0.001$; laboratory males = 14.11 ± 0.15 mm, laboratory females = 28.26 ± 0.45 mm, Welch's $t_{29.19} = 29.7$, $p < 0.001$), therefore we ran separate analyses for males and females in each of our two studies. For the field-collected data, we included river (Aripo or Marianne), as well as the interaction between river and predation regime as factors. In all four models investigating brain mass, we tested for an interaction between standard length and predation exposure on brain mass to test for the possibility of different allometric relationships across populations. This interaction was not significant for any of the models (all $p > 0.22$) and was subsequently dropped from the final analyses. We examined model residuals using QQ plots to look for violations of the homogeneity of variance or normality assumptions. All statistical analyses were performed in R version 3.2.2 (R Core Development Team, 2016), and graphs produced in ggplot2 (Wickham, 2009).

2.4 | Ethics

Methods were approved by the Animal Care Committee of McGill University (Protocols 2012-7133 and 2015-7708) and were conducted in accordance with the ethical guidelines of the Canadian Council on Animal Care and ABS/ASAB. Field sampling was approved by the Ministry of Agriculture, Land and Marine Resources of the Republic of Trinidad and Tobago. Guppies are neither endangered nor threatened and were abundant at all collection sites.

3 | RESULTS

3.1 | Field collections

We found that, for an average body size, males collected from high predation sites had brains 11.3% heavier in the Marianne River and 16.5% heavier in the Aripo River than males collected from low predation sites in the same rivers ($p = 0.052$; Figure 1a; Table 2). Males from the Marianne River had 14.7% heavier brains than males from the Aripo River, but the interaction between predation regime and river was not significant (Figure 1a; Table 2). We found no evidence that predation regime or river was associated with relative brain mass in female guppies (Figure 1b; Table 2). Males from high predation sites were significantly smaller bodied than low predation males (Mean SL ± s.e.: high predation males = 12.08 ± 0.14; low predation males = 13.61 ± 0.14; $p < 0.001$; Table 3), but there was no similar significant difference in female body length (Mean SL ± s.e.: high predation females = 16.31 ± 0.36; low predation females = 14.80 ± 0.48; $p = 0.062$). Supplementary Figure S1 illustrates the allometric relationships between brain mass and body length in the wild caught fish.

3.2 | Developmental experiment

We found that, for an average body size, males exposed to predation cues during development had brains 21.2% heavier than males exposed to control cues ($p = 0.011$; Figure 2a; Table 4). We found no evidence that exposure to predation cues during development influenced the relative brain mass of female guppies (Figure 2b; Table 4). Males exposed to predation cues were significantly larger bodied than males exposed to control cues (Mean SL ± s.e.: predator cue exposed males = 14.51 ± 0.23; control cue males = 13.80 ± 0.17; $p = 0.014$; Table 5), but there was no significant difference in female body length (Mean SL ± s.e.: predator cue exposed females = 28.13 ± 0.80; control cue females = 28.39 ± 0.49; $p = 0.65$). Supplementary Figure S2 illustrates the allometric relationships between brain mass and body length in the laboratory reared fish.

4 | DISCUSSION

Male guppies exposed to cues of predation risk in the laboratory, or actual predation risk in the wild, had larger brains for their body size than did males that did not have this experience. In contrast, we did not detect a consistent difference in relative brain mass between female guppies that were or were not exposed to real or simulated predation risk, suggesting the effect of predation on relative brain mass is sex dependent in guppies and is weaker or absent in females. The population differences in males could be due to evolved differences, however, the parallel results from our laboratory experiment suggest that the difference in brain mass may be at least partially due to inducible plasticity in neural investment relative to investment in body size, triggered by exposure to predation cues during development. Notably, the effects of predator cues confined to early life were long lasting, persisting throughout life.

Male guppies could hypothetically benefit from greater neural investment under predation threat if heavier brains relative to body size provide sensory, cognitive or motor benefits. It is possible that having a heavier brain may allow guppies to detect, assess, react to, or learn about predation threats better, and/or allow for simultaneous monitoring of predator threats while engaged in alternative activities such courtship or foraging, similar to reports in birds (Møller & Erritzøe, 2014; Sol et al. 2007). A heavier brain may also allow individual guppies to better address social demands, such as group cohesion or coordination with conspecifics (Dunbar & Shultz, 2017). Guppies from high predation populations do form more cohesive and coordinated groups (Ioannou et al., 2017) and group cohesion has antipredator benefits in prey fishes (Krause & Ruxton, 2002; Ioannou et al., 2012). Interestingly, predation seems to select for a reduction in brain size in some other fish species, and the putative advantages of increased brain size in the face of predation risk thus certainly merit closer examination (Walsh et al. 2016; Samuk et al. 2018). Samuk et al. (2018) suggest that differences between studies could result from the type of antipredator responses employed and local ecological conditions, such as the availability of shelter. An experimental evolution study on guppies, tracking the effects of different predators on brain size, cognitive performance, social behaviour and antipredator defences across generations, with different antipredator responses available, would be a large undertaking but highly informative in this regard.

We found that males exposed to predators in the wild were smaller bodied than those from low predation environments, replicating previous findings (Reznick & Endler, 1982). This raises the possibility that the change in relative brain mass we observed could reflect selection by predators for decreased overall body size. This could only explain our results if the brain was not reduced to the same degree as the rest of the body under predation threat, i.e. predation caused a differential effect on body versus brain size, with the largest effect on body size. However, the results of our developmental study, in which predator-exposed males were larger than control males and yet relative brain mass was still greater, argues against a simple explanation in terms of body size. We are unsure why predator cues in the laboratory resulted in increased adult body size in male guppies while exposure to genuine predation risk in the wild decreased male body size. Although guppies from high predation populations forage less in standardized conditions (Botham et al., 2008), exposure to acute cues of high predation risk induces short-term compensatory foraging (Elvidge et al., 2014). Since fish in our developmental study were exposed to repeated acute predator cue exposures, this potentially explains the disparity between our two studies, although leaves open the question of why such an effect was not observed in females. The differential effects of predator cues versus direct predator encounters, and the effects of predation cues confined to early life compared to life-long exposure are deserving of further investigation.

Why should predation affect brain mass in male guppies but not females? In guppies, males are more conspicuous, less social, bolder, and are poorer swimmers than females (Houde, 1997), and thus are more vulnerable to predation (Kotrschal et al., 2015a). As a result, males may have more to gain from investment in neural tissues under predation threat. Alternatively, because females are slower to mature and longer lived (Magurran, 2005) they may show greater neural investment regardless of predation risk. Relative brain size has been linked to life history strategy in birds and mammals, with slower developing and longer-lived animals typically having larger brains for their body size (Bennett & Harvey, 2009; Iwaniuk & Nelson, 2003).

Some authors have been critical of studies of whole brain size (e.g., Chittka & Niven, 2009; Healy & Rowe, 2007; Logan et al., in press). We agree that a more granular examination of specific brain regions, and other subtler aspects of neuroanatomy and neural organization, as well as the costs and benefits of brain enlargement would add essential information to our understanding of neural investment in guppies. Assessing whole brain mass does however have several advantages, for instance, measuring whole brains avoids problem of correctly determining relevant homologous areas between taxa (van der Bijl & Kolm, 2016). Furthermore, while mosaic evolution of brain areas exists (Barton & Harvey, 2000), the size of different brain areas tends to correlate strongly with overall brain size (Finley & Darlington, 1995; Kotrschal et al., 2017b), so whole brain size can be a reasonable measure of neural investment, especially when the specific brain area of interest is uncertain (van der Bijl & Kolm, 2016). We argue that identifying effects on whole brain size can be a useful tool to identify relevant ecological factors affecting neural investment. Our current data shows that whole brain mass varies across populations (see also Kotrschal et al., 2017a) and responds to developmental conditions in guppies. Combined with the effects of artificial selection on brain mass in guppies (Kotrschal et al., 2013, 2014, 2015a, 2017b), these data suggest that relative brain mass is a relevant trait in guppies, encouraging more fine-scaled work in the future.

Our results contrast with some previous findings. For instance, Burns and Rodd (2008) did not find differences in size between the brains of female or male guppies collected from high versus low predation wild populations. The reason for the discrepancy in the males is unclear, but it is worth noting that different methods for estimating brain size were used: Burns and Rodd measured the dorsal surface area of the telencephalon and optic tectum rather than brain mass. Kotrschal et al. (2017a) found, as we did, that the density of fish predators across populations did not correlate with relative brain mass in female guppies, however, they did not examine males. Kotrschal et al. did find that the biomass of predatory prawns correlated positively with relative brain mass in females, suggesting that female brain mass may respond to threat from other types of predators.

Our field comparisons of high and low predation guppies came from only two replicate rivers. The parallel results in the two rivers, in the same sex, and the qualitative match with the developmental manipulation, again in the same sex, suggests however that predation is likely a key driver of the differences we observed in male brain mass. However, sampling of a greater number of rivers and a variety of other ecological conditions would clearly be a valuable follow-up. Additionally, the laboratory study was conducted on only a single lab population, descended from a mixture of high predation fish from two different rivers. It would be interesting to examine whether the developmental effects of predation cues differ between populations. We also note that the balance we used to weigh the brains was relatively coarse (0.1 mg listed repeatability, with more error likely at lower masses) given the small size of guppy brains (1.3 – 9.2 mg in our samples). However, any measurement error introduced by our instrument would not be systematic and therefore should reduce rather than increase our likelihood of detecting an effect. Indeed, it is possible that a subtle effect exists in the female brains which we failed to detect with our methodology.

It is not clear to what degree the interpopulation differences we observed in relative brain mass reflect local adaptation versus phenotypic plasticity. Environmental conditions can select for differences in neuroanatomy across populations, for example, black-capped chickadees, *Poecile atricapillus*, that live in harsher northern climates have larger hippocampal volumes than individuals from milder regions (Roth & Pravosudov, 2009), and these differences are retained in laboratory reared offspring (Roth et al., 2010). Guppies may have evolved increased brain mass under predation threat, however, our work suggests that plasticity can play an important role in determining brain mass in guppies, and therefore the population differences that have been observed (Kotrschal et al. 2017a; this study) may be partly or entirely due to a plastic response to cues of predation threat during development. Common garden experiments will be required to disentangle the contributions of selection and plasticity on relative brain mass in this species, ideally comparing plasticity across populations.

In conclusion, we found that male but not female guppies exposed to predators either naturally in the wild or experimentally in the laboratory have heavier brains for their body size than individuals that were not exposed to predators. Future work is required to determine the causes of this increased neural investment in male guppies and why this pattern is not observed in females. Our results highlight the potential importance for developmental plasticity in generating population differences in relative brain mass.

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COMPETING INTERESTS

We have no competing interests to declare.

AUTHOR CONTRIBUTIONS

ARR, IL and SMR planned the study. ARR conducted the field collections. IL conducted the developmental manipulations. ARR dissected the fish and weighed the brains. ARR and LCT analyzed the data. LCT made the figures. ARR wrote the first draft of the manuscript. All authors contributed to the final version of the paper.

DATA ACCESSIBILITY

Data are available from the Dryad Digital Repository (doi:xxx).

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Figure captions

Figure 1. Expected (log transformed) brain mass (± s.e.) of guppies captured in the wild. Expected values are for the mean standard length for each sex. Mean brain masses for each group in mg are alongside the points, percent differences between groups are indicated with arrows. Males (*a*) from high predation populations have larger brain masses for their body size than males from low predation populations ($p = 0.05$). Males from the Marianne River had significantly heavier brains for their body size than males from the Aripo River ($p = 0.02$). Females (*b*) did not show a significant effect of predation regime or river. Filled symbols, environments with predators; open symbols, environments without predators; triangles, Aripo River; circles, Marianne River.

Figure 2. Expected (log transformed) brain mass (± s.e.) of guppies experimentally exposed to predator or control cues during development in captivity. Expected values are for the mean standard length for each sex. Mean brain masses in mg are alongside the points, percent differences between groups are indicated with arrows. Males (*a*) from the predator cue exposed treatment had larger relative brain masses than males from the control treatment ($p = 0.01$). Females (*b*) did not show a significant effect of the predator cue treatment. Filled symbols, predator cue treatment; open symbols, control treatment.

Table captions

Table 1. Collection site and sample sizes for wild caught fish. Site names and predation regime classifications are based on Gotanda et al. (2013). UTM, Universal Transverse Mercator.

Table 2. Estimates and standard error of fixed parameters and their interaction for the linear model with log-transformed brain mass for the guppy field population comparison. Estimates represent the difference in log-transformed brain mass between the level of a factor (identified in parenthesis) and the reference levels for categorical factors and are mean-centered for covariates. The reference levels were high predation and Aripo River. The standard length was log-transformed and mean-centered. *P*-values ≤ 0.05 are shown in bold. D.f., degrees of freedom.

Table 3. Estimates and standard error of fixed parameters and their interaction for the linear model with log-transformed standard length for the guppy field population comparison. Estimates represent the difference in log-transformed standard length between the level of a factor (identified in parenthesis) and the reference levels. The reference levels were high predation and Aripo River. *P*-values ≤ 0.05 are shown in bold. D.f., degrees of freedom.

Table 4. Estimates and standard error of fixed parameters and their interaction for the linear model with log-transformed brain mass for the guppy laboratory developmental study. Estimates represent the difference in log-transformed brain mass between the level of a factor (identified in parenthesis) and the reference level for the categorical factor (the predator cue treatment) and are mean-centered for covariates. The standard length was log-transformed and mean-centered. *P*-values ≤ 0.05 are shown in bold. D.f., degrees of freedom.

Table 5. Estimates and standard error of fixed parameters and their interaction for the linear model with log-transformed standard length for the guppy laboratory developmental study. Estimates represent the difference in log-transformed standard length between the level of a

factor (identified in parenthesis) and the reference level (the predator cue treatment). *P*-values ≤ 0.05 are shown in bold. D.f., degrees of freedom.

Supplementary figure captions

Figure S1. The linear relationship (with 95% confidence intervals) between log-transformed brain mass and mean-centered log-transformed standard length, back-transformed into the original units, for guppies sampled from the field. Males (a) from high predation populations had larger relative brain masses than males from low predation populations (p = 0.05). Females (b) did not show a significant effect of predation regime. Orange circles and lines, Marianne River; blue triangles and lines, Aripo River; filled symbols and solid lines, environments with predators; open symbols and dashed lines, environments without predators.

Figure S2. The linear relationship (with 95% confidence intervals) between log-transformed brain mass and mean-centered log-transformed standard length, back-transformed into the original units, for guppies experimentally exposed to predator or control cues in captivity. Males (a) from the predator cue exposed treatment had larger relative brain masses than males from the control treatment (p = 0.01). Females (b) did not show a significant effect of predation cue treatment. Filled symbols and solid lines, predator cue treatment; open symbols and dashed lines, control treatment.

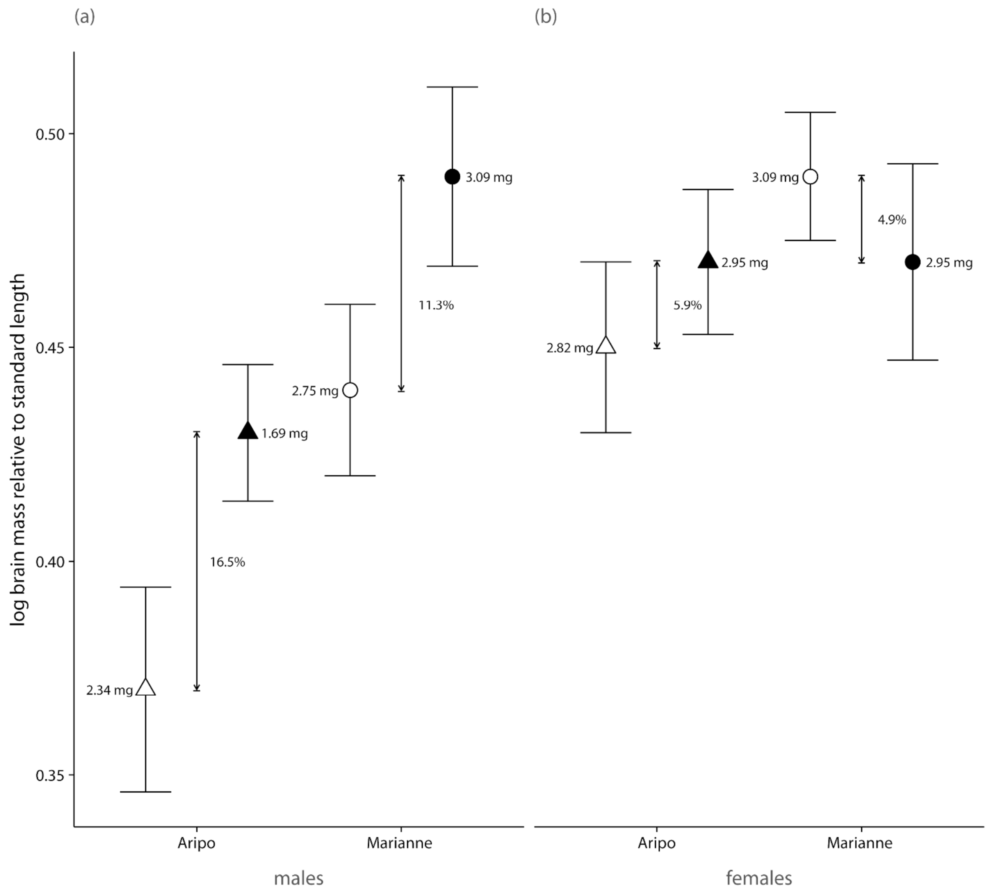
Site	UTM coordinates (x, y)	Predation regime	*n* males	*n* females
Aripo 1	693 188, 1 181 605	Low	15	15
Aripo 2	694 231, 1 177 709	High	27	20
Marianne 10	686 711, 1 191 358	Low	21	26
Marianne 14	684 934, 1 191 469	High	16	11

Parameter	**Estimate**	**Std. Error**	***t*-value**	***p*-value**
Males (d.f. = 74)				
Intercept	0.43	0.016	26.37	**<0.0001**
Standard length	1.78	0.31	5.80	**<0.0001**
Predation (low)	-0.061	0.031	1.97	**0.052**
River (Marianne)	0.063	0.025	2.48	**0.015**
River * predation	0.0046	0.047	0.124	0.902
Females (d.f. = 67)				
Intercept	0.47	0.017	27.93	**<0.0001**
Standard length	1.10	0.13	8.66	**<0.0001**
Predation (low)	-0.026	0.026	1.00	0.318
River (Marianne)	-0.0023	0.028	0.081	0.936
River*predation	0.050	0.037	1.35	0.181

Parameter	**Estimate**	**Std. Error**	***t*-value**	***p*-value**
Males (d.f. = 74)				
Intercept	1.08	0.005	186.72	**<0.0001**
Predation (low)	0.056	0.0097	5.73	**<0.0001**
River (Marianne)	-0.0036	0.0095	0.38	0.705
River * predation	-0.0045	0.014	0.33	0.743
Females (d.f. = 67)				
Intercept	1.17	0.016	73.15	**<0.0001**
Predation (low)	0.047	0.025	1.90	0.062
River (Marianne)	-0.031	0.027	1.15	0.253
River*predation	0.010	0.036	0.30	0.769

Parameter	**Estimate**	**Std. Error**	***t*-value**	***p*-value**
Males (d.f. = 46)				
Intercept	0.56	0.026	21.16	**<0.0001**
Standard length	1.11	0.59	1.89	0.065
Predation (control)	-0.098	0.037	2.66	**0.011**
Females (d.f. = 21)				
Intercept	0.81	0.028	28.86	**<0.0001**
Standard length	0.60	0.72	0.83	0.415
Predation (control)	-0.011	0.038	0.30	0.770

Parameter	**Estimate**	**Std. Error**	***t*-value**	***p*-value**
Males (d.f. = 46)				
Intercept	1.16	0.0064	182.31	**<0.00001**
Predation (control)	-0.022	0.0086	-2.54	**0.014**
Females (d.f. = 21)				
Intercept	1.46	0.0083	176.31	**<0.0001**
Predation (control)	-0.0052	0.011	-0.46	0.650



Expected (log transformed) brain mass (± s.e.) of guppies captured in the wild. Expected values are for the mean standard length for each sex. Mean brain masses for each group in mg are alongside the points, percent differences between groups are indicated with arrows. Males (a) from high predation populations have larger brain masses for their body size than males from low predation populations ($p = 0.05$). Males from the Marianne River had significantly heavier brains for their body size than males from the Aripo River ($p = 0.02$). Females (b) did not show a significant effect of predation regime or river. Filled symbols, environments with predators; open symbols, environments without predators; triangles, Aripo River; circles, Marianne River.

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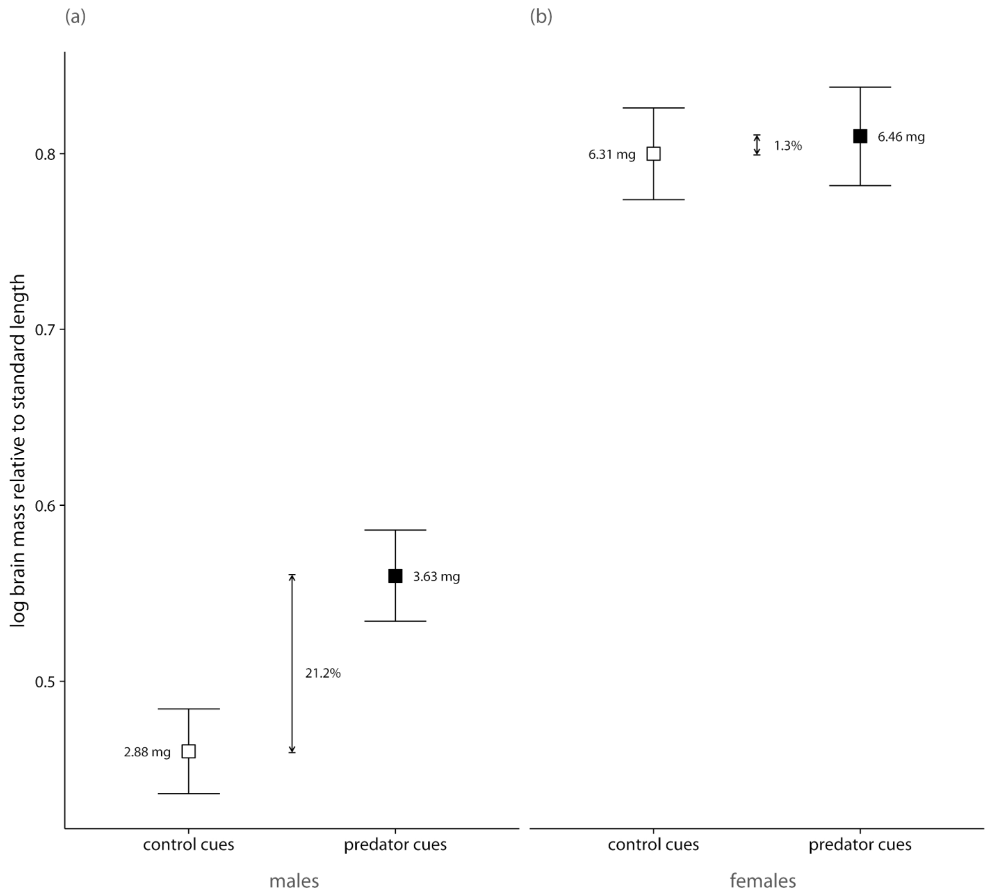

Expected (log transformed) brain mass (± s.e.) of guppies experimentally exposed to predator or control cues during development in captivity. Expected values are for the mean standard length for each sex. Mean brain masses in mg are alongside the points, percent differences between groups are indicated with arrows. Males (a) from the predator cue exposed treatment had larger relative brain masses than males from the control treatment (p = 0.01). Females (b) did not show a significant effect of the predator cue treatment. Filled symbols, predator cue treatment; open symbols, control treatment.

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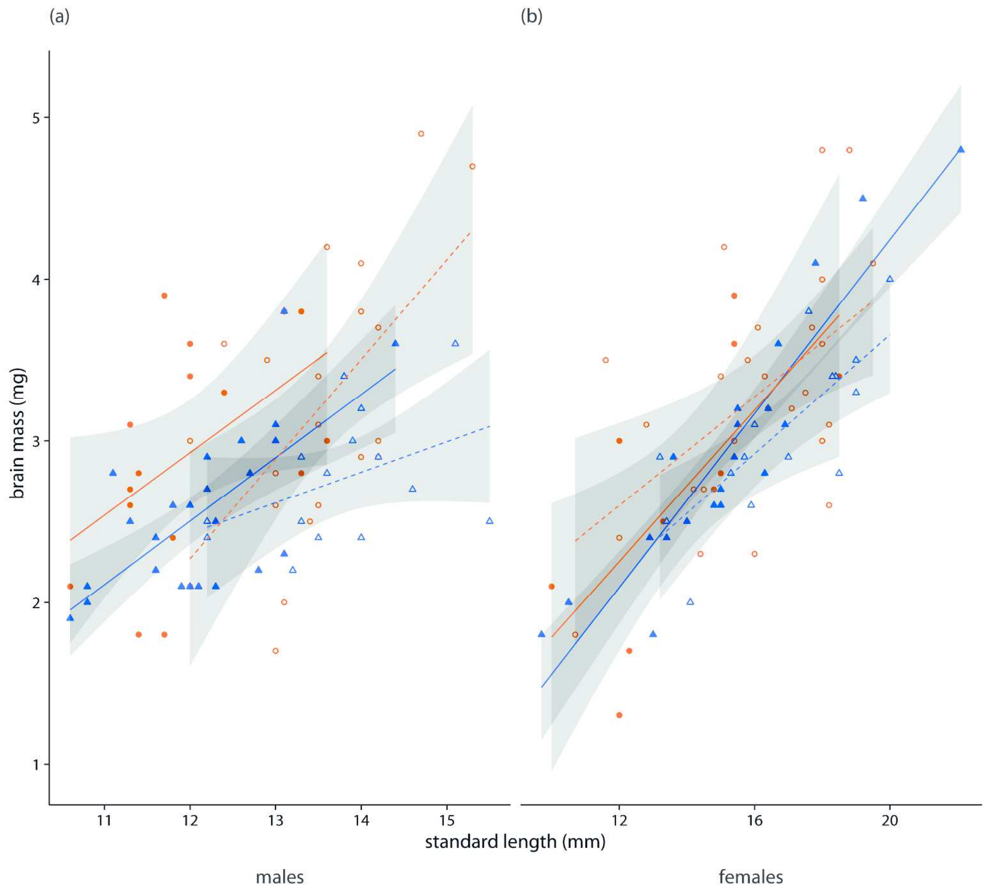

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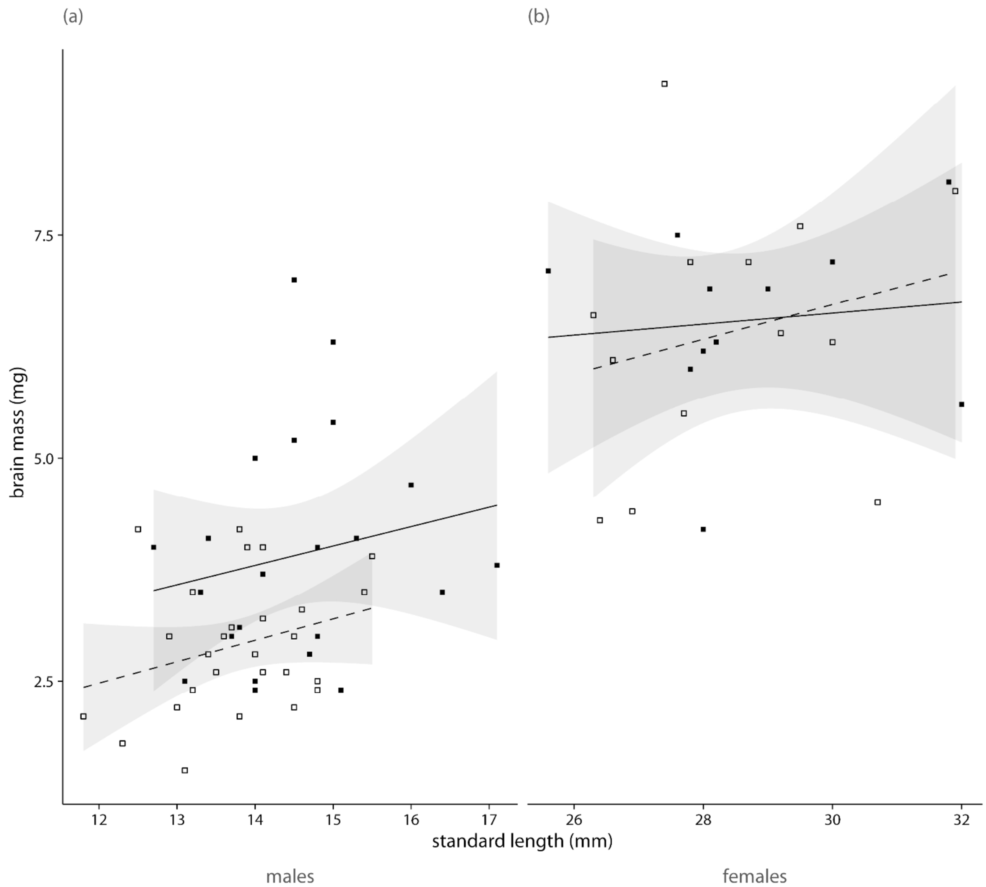

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