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Predator-driven brain size evolution in natural populations of Trinidadian killifish (*Rivulus hartii*)

Matthew R. Walsh[1], Whitnee Broyles[1], Shannon M. Beston[1] and Stephan B. Munch[2]

[1]Department of Biology, University of Texas at Arlington, Arlington, TX 76019, USA
[2]National Marine Fisheries Service, 110 Shaffer Road, Santa Cruz, CA 95060, USA

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Author for correspondence:
Matthew R. Walsh
e-mail: matthew.walsh@uta.edu

Vertebrates exhibit extensive variation in relative brain size. It has long been assumed that this variation is the product of ecologically driven natural selection. Yet, despite more than 100 years of research, the ecological conditions that select for changes in brain size are unclear. Recent laboratory selection experiments showed that selection for larger brains is associated with increased survival in risky environments. Such results lead to the prediction that increased predation should favour increased brain size. Work on natural populations, however, foreshadows the opposite trajectory of evolution; increased predation favours increased boldness, slower learning, and may thereby select for a smaller brain. We tested the influence of predator-induced mortality on brain size evolution by quantifying brain size variation in a Trinidadian killifish, *Rivulus hartii*, from communities that differ in predation intensity. We observed strong genetic differences in male (but not female) brain size between fish communities; second generation laboratory-reared males from sites with predators exhibited smaller brains than *Rivulus* from sites in which they are the only fish present. Such trends oppose the results of recent laboratory selection experiments and are not explained by trade-offs with other components of fitness. Our results suggest that increased male brain size is favoured in less risky environments because of the fitness benefits associated with faster rates of learning and problem-solving behaviour.

1. Introduction

Organisms exhibit extensive variation in brain size [1]. It has long been hypothesized that brain size variation is driven and maintained by contrasting ecological selective pressures. This is, in part, because there are clear costs and benefits associated with shifts in brain size. Increased brain size is associated with increased cognitive function and learning capabilities ([2–10], but see [11]). Yet, there are often reproductive and energetic trade-offs associated with allocation towards brain tissue [12–17]. This large body of research demonstrating fitness costs and benefits of brain size variation primarily stems from correlations across species [6]. Far less is known about intraspecific variation in brain size or the ecological forces that drive evolutionary shifts in brain size (but see [18–22]).

Recent laboratory selection experiments have begun to address the conditions that favour brain size evolution. Kotrschal *et al*. [16] artificially selected for large and small brain size using replicate populations of guppies (*Poecilia reticulata*). This work revealed rapid shifts in brain size in response to directional selection; brain size increased by 10% after two generations of selection. Increased brain size was associated with increased cognitive ability [23–25], shifts in personality and anti-predator behaviour [26,27], and decreased susceptibility to predation [28]. This leads to the hypothesis that variation in rates of predation may be a key selective force on the evolution of brain size and that increased predation should select for increased brain size. Artificial selection experiments, however, do not directly assess the influence of ecologically driven mortality on the evolution of brain size nor do they allow

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for additional ecological interactions within the community. This is important because comparisons among natural populations have shown that increased predation is often, but not always, correlated with increased risk-taking behaviour (i.e. 'boldness') ([29–36], but see [37–40]). Increased boldness enhances fitness (i.e. increased foraging and mating success) [29,41,42] apparently at the expense of spatial learning ability and cognitive function [43,44]. Given that interspecific comparisons clearly show that increased brain size is associated with increased cognition [6], these comparisons among natural populations foreshadow that the connection between predation and brain size is more complicated and that increased predation may actually select for declines in brain size.

Fish communities on the island of Trinidad present the opportunity to test the factors that select for variation in brain size. A killifish, *Rivulus hartii*, is found in localities in which they are the only species present ('*Rivulus*-only' sites) and in 'high predation' sites that contain several species of piscivorous fish (i.e. *Crenicichla alta* and *Hoplias malabaricus*) [29,30,45–48]. These communities are located within a close proximity to one another, exhibit similar physical habitats, and do not differ in variables such as water temperature and dissolved oxygen [49]. In high predation sites, *Rivulus* suffer increased mortality, are less abundant, and exhibit much faster rates of individual growth compared with *Rivulus* from sites in which they are alone [46,47]. These differences in growth probably reflect higher *per capita* levels of food availability that are indirect effects of predation [46,48–51]. Research has shown that these ecological differences are associated with phenotypic shifts in *Rivulus* life-history traits [49] and behaviour [29,30]. In particular, *Rivulus* are bolder and exhibit greater movement in sites with predators compared with streams that lack predators [29,30].

Walsh & Reznick [48] evaluated *Rivulus* from high predation and *Rivulus*-only sites for genetically based differences in life-history traits. This work tested the influence of the direct (i.e. mortality) and indirect (i.e. increased resources) effects of predators on life-history evolution by rearing second generation laboratory born fish under two resource levels that match the known differences in growth between the high predation and *Rivulus*-only communities. *Rivulus* from high predation sites matured at an earlier age, were smaller at maturation, and allocated more energy to reproduction [48,49]. This pattern of divergence is consistent with theoretical predictions that consider predator-induced extrinsic mortality [48]. Yet, the observed differences in life-history traits were more pronounced under high food levels and often disappeared under low food conditions. These contrasting responses to controlled food indicate that evolution in *Rivulus* is also shaped by the indirect consequences of predation [48].

Here, we tested the influence of increased predation on the evolution of brain size and trade-offs between brain size and other components of fitness. We compared the brain sizes and gut lengths of *Rivulus* from two high predation and two *Rivulus*-only communities using preserved specimens from our previous common garden experiments (see [48]). Based upon the results of the artificial selection experiments [16,28], we predict that *Rivulus* from high predation sites will exhibit larger brain sizes than individuals from *Rivulus*-only sites but smaller gut lengths. A failure to observe such patterns would indicate that the connection between predatory selection and the evolution of brain size is more complex. If differences in brain size are associated with the known differences in behaviour in this system (i.e. high predation fish are bolder) [29,30], then high predation fish will exhibit a smaller brain than *Rivulus* from *Rivulus*-only sites.

2. Material and methods

The collection, maintenance, and experimental design for this study were previously published [48] and are summarized here. Wild-caught *Rivulus* were obtained from *Rivulus*-only and high predation populations from the Arima and Guanapo rivers in July 2005 (i.e. two high predation and two *Rivulus*-only sites). We established laboratory stocks from approximately 10 wild-caught males and females per population (72 fish total). To generate the first common garden reared generation, wild-caught females were placed in 9 l aquaria supplied with artificial spawning substrate and randomly paired with a male from the same locality. Eggs from these pairings were incubated in Petri dishes and newly hatched larvae from each pairing were then reared in aquaria on an ad libitum diet of liver paste and brine shrimp *nauplii* (at a maximum density of eight fish per tank).

To generate the second generation, mature females from each lineage in the first generation were mated to mature males from the same locality but different lineage. The common garden experiment included offspring from six unique pairings per population. Similar to the first laboratory generation, all offspring per pairing were reared at densities of eight fish per 9 l aquarium and fed ad libitum. At an age of 20 days, eight fish per unique pairing were individually placed in 9 l aquaria. The structural complexity of aquaria was uniform across populations/treatments as each tank lacked gravel but was provided with a clay pot for refuge and an artificial spawning substrate. All fish were allocated equally between two food treatments: (i) a high food level that matches growth in high predation sites and (ii) a low food level that approximates growth in sites with just *Rivulus* [48]. Each day all fish were given quantified portions of liver paste in the morning and brine shrimp *nauplii* in the afternoon. All individuals were then reared until maturity (see [48] for details regarding quantification of life-history traits). Upon attaining maturation, males were immediately euthanized and preserved in 5% formalin. Eggs were collected from females for two weeks after maturation and all females were then preserved in 5% formalin. All fish were preserved in formalin for approximately eight years prior to being dissected for assessments of brain size and gut length.

(a) Brain and gut size quantification

We assessed all preserved specimens for differences in brain size beginning in August 2015. We removed the brain from each preserved specimen by cutting from the top of each gill slit and then removing the lower jaw and the tissue between the roof of the mouth and the braincase. Each brain was blotted dry and measured for total wet weight. We subsequently photographed the dorsal surface of each brain. We then measured the length of the optic tectum (a structure that is highly correlated with total brain size) via ImageJ (NIH) [16]. We removed the gut from each fish by cutting at the point where the oesophagus meets the stomach and then at the anus at the posterior end. We blotted each gut dry and measured the wet weight of each gut. It is important to note that all fish in the common garden experiment were euthanized and preserved each morning after assessments for maturation and egg production [48]. This occurred prior to the daily morning feeding and approximately 16 h after the previous afternoon feeding. This duration of time allows for sufficient processing of food to minimize bias associated with differences in the amount of food present in the gut when making comparisons among treatments.

(b) Statistical design and analyses

Differences in absolute and relative brain size, optic tectum length, and relative gut size were analysed using general linear models (SPSS v. 23 IBM Corporation) with fish community (high predation, *Rivulus* only), food level (high, low), river (Arima, Guanapo), and sex (male, female), and all statistical interactions entered as fixed effects. We ln transformed all traits to improve normality and homogeneity of variances. All analyses included age at maturation as a covariate. The analyses for relative brain size and gut size included fish length as a covariate. To better linearize the data, we ln transformed the data for fish size. In our analyses, we first evaluated the full model that included all interactions. We then removed interactions with little statistical influence (F-value < 1.0) and re-ran all analyses using this reduced model. The removal of these non-significant interactions did not alter the significant trends. We also evaluated allometric relationships between fish length (ln transformed) and brain and gut size by examining the 'predator × length' and 'predator × sex × length' interactions for statistical significance and confirmed that there are similar scaling relationships for all traits (see Results). For all analyses that included a covariate, treatment effects were tested at the mean of the covariate.

(c) Trait correlations

We performed Pearson correlations between brain size versus gut size and brain size versus age at maturation for fish from high predation and *Rivulus*-only sites (for each river separately) to explore trade-offs between allocation towards brain tissue and other traits. Note that the data for age at maturation were published previously [48]. The data for brain and gut size were corrected for body size by outputting the residuals from the general linear model. Because we pooled data across food treatments and the differences between the food treatments were linear, we corrected all trait estimates for differences in feeding regime by including food level as a covariate when generating the residuals. We tested for differences in allocation strategies by comparing the correlation coefficients of high predation and *Rivulus*-only sites via a one-way analysis of variance.

(d) Sample sizes

The original common garden experiment yielded data on 181 fish. Between 2007 and 2015 several preserved specimens dried out. In addition, the brains and/or guts of several individuals were destroyed during dissection. The final sample sizes of fish for this study were (total $n = 163$): brain size: Arima high predation = 42, Arima *Rivulus*-only = 39, Guanapo high predation = 42, Guanapo *Rivulus*-only = 40; gut size: Arima high predation = 41, Arima *Rivulus*-only = 38, Guanapo high predation = 43, Guanapo *Rivulus*-only = 40 (see the electronic supplementary material, table S1).

3. Results

We statistically compared the relationships between brain and gut size versus body size. Allometric scaling relationships were similar and did not differ significantly ($p < 0.05$) between populations (predator × fish length interaction: brain size: $F_{1,141} = 0.001$, $p = 0.97$; gut size: $F_{1,142} = 0.37$, $p = 0.54$) or between populations within each sex (predator × sex × fish length interaction: brain size: $F_{1,141} = 1.6$, $p = 0.2$; gut size: $F_{1,142} = 0.46$, $p = 0.5$).

(a) Relative brain size

Differences in brain size between high predation and *Rivulus*-only sites depended upon sex as we observed a significant 'predation × sex' interaction (table 1 and figure 1; electronic supplementary material, table S1). Male *Rivulus* from high predation sites exhibited a relative brain size that was 19% smaller than *Rivulus* from *Rivulus*-only sites but such differences were minor in females (figure 1). We also observed overall significant differences in relative brain size between our focal populations (12% smaller in high predation fish) and between males and females (approx. 32% larger in females) (table 1). The results for optic tectum length parallel those observed for relative brain size (see table 1). The predator × sex interaction was significant as there were strong differences in male optic tectum length between high predation and *Rivulus*-only sites (male average optic tectum length ± 1 standard error (s.e.): high predation = 0.29 ± 0.024, *Rivulus*-only = 0.38 ± 0.012) but small differences in females (female average optic tectum length ± 1 s.e.: high predation = 0.41 ± 0.016, *Rivulus*-only = 0.40 ± 0.022).

(b) Absolute brain size

We evaluated high predation and *Rivulus*-only sites for absolute differences in brain size to explore the consistency of the trends when fish size is not taken into account. Similar to the analyses for relative brain size, differences in absolute brain size between fish communities depended upon sex (table 1 and figure 1; electronic supplementary material, table S1). We observed a significant ($p < 0.05$) predation-by-sex interaction for absolute brain size. Males from high predation communities exhibited an absolute brain size that was 33% smaller than fish from sites where *Rivulus* is alone (figure 1). Differences in absolute brain size between females were smaller (figure 1).

(c) Gut size

We observed a significant ($p < 0.05$) predation × sex interaction for gut size (table 1 and figure 2; electronic supplementary material, table S1). The gut size of males from high predation sites was 9% smaller than males from *Rivulus*-only sites but such differences were reversed in females; high predation females exhibited a gut size that was 5% larger than *Rivulus*-only females (figure 2). Gut size differences between fish communities or food levels were not significant ($p < 0.05$) although the 'predator × food' interaction was marginally non-significant (table 1); *Rivulus*-only fish responded to decreased food by developing larger guts (6% increase) while responses in high predation were minor and in the opposite direction (gut size averages ± 1 s.e.: high predation, high food = 2.25 ± 0.054, high predation, low food = 2.21 ± 0.048, *Rivulus*-only, high food = 2.21 ± 0.053, *Rivulus*-only, low food = 2.34 ± 0.058).

(d) Tests of simple main effects

Because we observed significant predation × sex interactions, we performed tests of simple main effects to statistically evaluate the nature of the differences between fish communities within each sex. In these tests, we used the Bonferroni correction to adjust our p-values for multiple tests. As these tests made two comparisons, we considered p-values less than 0.025 as 'significant' (p-value correction: $0.05/2 = 0.025$). These analyses revealed non-significant ($p > 0.025$) differences between high predation and *Rivulus*-only sites for female relative brain size ($F_{1,71} = 0.84$, $p = 0.36$), absolute brain size ($F_{1,72} = 0.14$, $p = 0.71$), and gut size ($F_{1,71} = 0.01$, $p = 0.92$). Differences

Table 1. Analyses of brain and gut size data. *F*, *F*-values; *p*, *p*-values; d.f., numerator degrees of freedom; error d.f., denominator degrees of freedom. Significant terms are indicated in italics. The *F*-statistics and *p*-values for the significant terms are from the reduced model following the removal of non-significant ($F < 1$) interactions. The *F*-statistics and *p*-values for the interactions with little explanatory power ($F < 1$) are from analyses performed using the full model (see Methods).

		absolute brain size (mg)		relative brain size (mg)		optic tectum length (mm)		gut weight (mg)	
	d.f.	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*
covariates:									
fish length	1	—	—	*48.31*	*<0.001*	3.23	0.074	*44.28*	*<0.001*
age at maturation	1	*16.34*	*<0.001*	2.45	0.12	*4.64*	*0.033*	0.01	0.92
main effects:									
predation	1	*27.78*	*<0.001*	*25.47*	*<0.001*	*8.41*	*0.004*	0.39	0.53
food	1	*30.76*	*<0.001*	0.2	0.89	2.34	0.13	0.52	0.47
river	1	0.04	0.84	0.5	0.83	3.6	0.059	0.57	0.45
sex	1	*47.86*	*<0.001*	*7.72*	*0.006*	*4.42*	*0.037*	0.36	0.55
predation × food	1	*4.48*	*0.036*	1.12	0.29	1.45	0.23	3.85	0.052
predation × river	1	*4.04*	*0.046*	1.06	0.31	0.35	0.56	0.22	0.64
predation × sex	1	*76.24*	*<0.001*	*21.33*	*<0.001*	*15.84*	*<0.001*	*11.55*	*0.001*
food × river	1	0.01	0.91	0.18	0.67	0.75	0.39	0	0.99
food × sex	1	0.69	0.41	0.89	0.35	0.05	0.82	3.6	0.06
river × sex	1	*16.84*	*<0.001*	*5.31*	*0.023*	*7.76*	*0.006*	*5.1*	*0.026*
predation × food × river	1	1.14	0.32	1.8	0.17	2.09	0.13	3.02	0.052
predation × food × sex	1	*3.27*	*0.041*	2.09	0.13	0.12	0.73	0.19	0.66
predation × river × sex	1	0.26	0.61	0.01	0.91	1.04	0.31	3.58	0.061
food × river × sex	1	0.3	0.58	0.54	0.46	0.02	0.88	0.18	0.68
predation × food × river × sex	1	0.76	0.52	0.03	0.86	0.02	0.88	0.001	0.97
error d.f.		145		144		145		143	

between communities for male relative brain size ($F_{1,71} = 7.31$, $p = 0.009$) and absolute brain size ($F_{1,72} = 15.84$, $p < 0.001$) were significant ($p < 0.025$) but mean differences in gut size were not significant ($F_{1,70} = 3.49$, $p = 0.066$).

(e) Trait correlations

The correlations between brain size versus age at maturation and brain size versus gut size were non-significant ($p > 0.05$) for males in high predation and *Rivulus*-only sites (figure 3; see also electronic supplementary material, figure S1). Differences in correlation coefficients between high predation and *Rivulus*-only sites were not significant for the brain size versus age at maturation ($F_{1,4} = 2.36$, $p = 0.26$) and brain size versus gut size comparisons ($F_{1,4} = 0.95$, $p = 0.43$).

4. Discussion

Our results revealed genetically based sex-specific differences in brain size between fish communities that differ in predator-induced mortality (figure 1). Male *Rivulus* from localities where they co-occur with piscivores exhibited significantly smaller brain sizes than corresponding upstream communities that lack predators. Females exhibited a similar pattern of divergence but such differences between high predation and *Rivulus*-only sites were smaller in magnitude (and not statistically significant). These differences oppose our *a priori* predictions based upon the results of recent selection experiments showing that selection for a larger brain size yields higher fitness in risky environments [16,28]. There is also little evidence to suggest that trade-offs between brain size and gut size (figure 2) or maturation schedules underlie the observed differences in brain size; observed correlations between brain size and gut size or age at maturation were not significant and did not differ between fish communities (figure 3). Such results thus beg two questions: (i) what new insights do our results provide in regards to the evolution of brain in nature? (ii) Why have *Rivulus* evolved a smaller brain in high predation versus *Rivulus*-only sites and why are such differences observed only in males?

Studies investigating inter-population variation in brain size including sex-specific brain size differences are accumulating [18–22,52–56]. Crispo & Chapman [22] observed differences in brain size (and brain size plasticity) in cichlids from sites that differed in oxygen availability but the adaptive significance of these differences is unclear. Kolm *et al.* [52] revealed phenotypic differences in brain size between divergent mating strategies in brown trout (*Salmo trutta*) as non-migratory forms exhibited a larger relative brain size than individuals that migrate to the sea and delay maturation

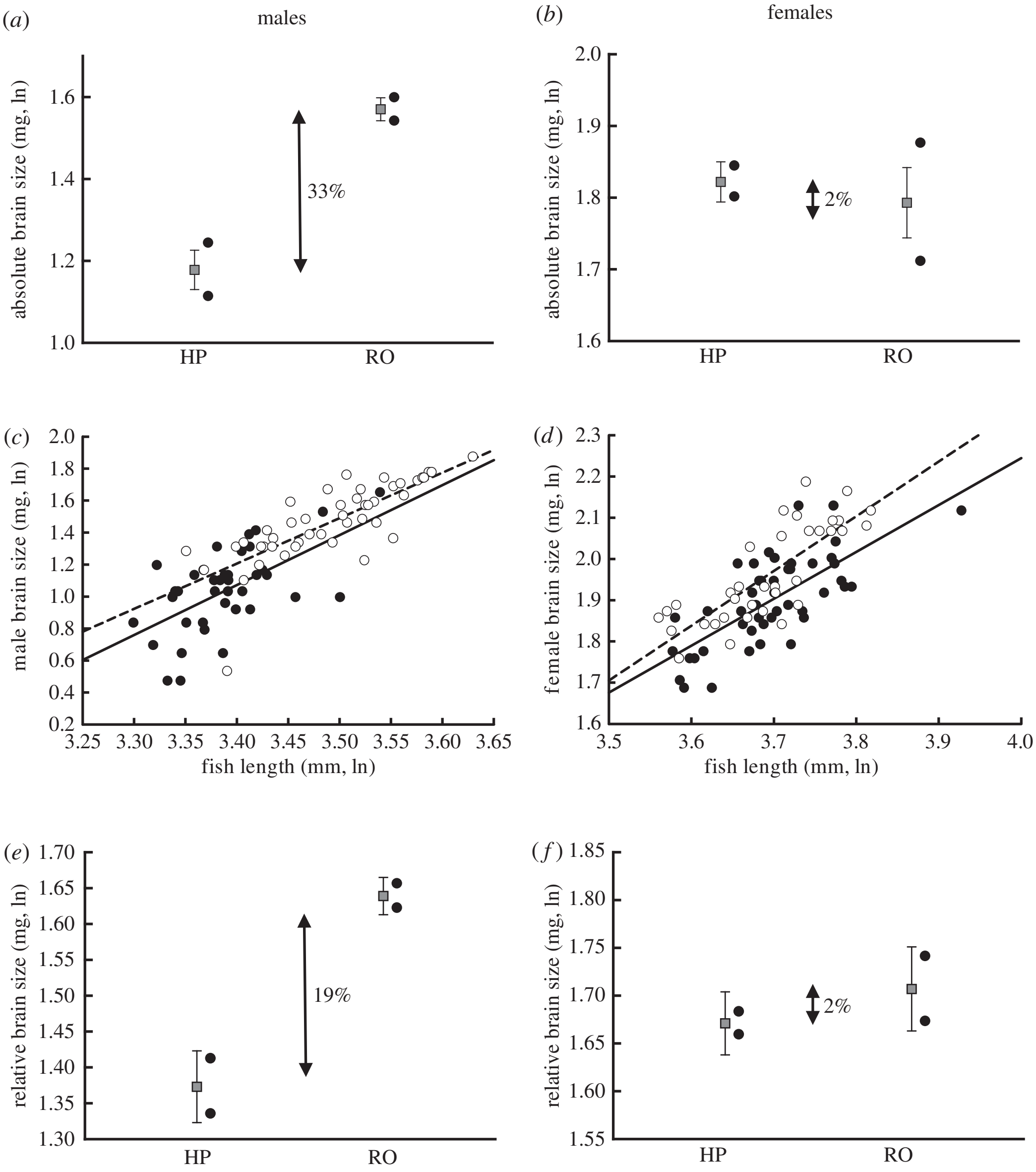

Figure 1. Variation in brain size between predator communities. (*a*) Male absolute brain size, (*b*) female absolute brain size, (*c*) male length versus brain size, (*d*) female length versus brain size, (*e*) male relative brain size, and (*f*) female relative brain size. Panels (*a,b*) and (*e,f*): grey squares indicate population means, filled circles indicate replicate river means. Panels (*c,d*): filled circles (solid regression line)—high predation fish, open circles (dashed regression line)—*Rivulus*-only fish. The regression lines between fish size and brain size are based upon the raw data. We observed significant ($p < 0.05$) interactions between sex and predation for absolute and relative brain size. Small differences were observed between *Rivulus* from high predation (HP) and *Rivulus*-only (RO) sites for females but such differences were much larger in males. The data points for male and female relative brain size reflect the estimated marginal means at the mean of the covariate. Error = ± 1 s.e.

until later in life. Eifert *et al*. [46] did not find genetically based differences in brain size in *Poecilia mexicana* from sites that differ in light or toxicity. Work by Gonda *et al*. [18–20] (see also [54,56]) provides the closest parallel to the current set of results. This work compared sticklebacks from marine and derived pond populations for differences in brain size [18–20]. Marine environments are characterized by higher predation levels and enhanced structural complexity, but lower densities of sticklebacks. Similar to the results obtained in this study, wild-caught specimens from marine environments (which have higher predation) exhibited a smaller brain size than fish from small ponds [20]. Yet, these population-level differences in relative brain size were not consistently observed in first generation common garden reared fish [18] nor were there sex-specific differences in brain size between marine and pond environments [54]. In a separate study on sticklebacks, Kotrschal *et al*. [53] did, however, find that male sticklebacks from a site characterized by high intraspecific density exhibited a larger relative brain size than males from a site with lower densities but female brain size did not differ between these locales.

Our results extend our current understanding of brain size evolution because all fish were reared for multiple generations in the laboratory to remove confounding environmental effects (and maternal effects) and we have illustrated a link between predation and evolutionary shifts in brain size (figure 1). Based upon previous research on natural populations [18–22,52–57] and the results of this study, it is becoming increasingly clear that variation in ecological properties are often correlated with intraspecific shifts in brain size [18–22,52–57]. Yet, our understanding of the generality of these results is currently limited. For example, the recent guppy selection experiments showed that selection for larger brains led to enhanced anti-predator behaviour and survival in females but not males [27,28]. In this

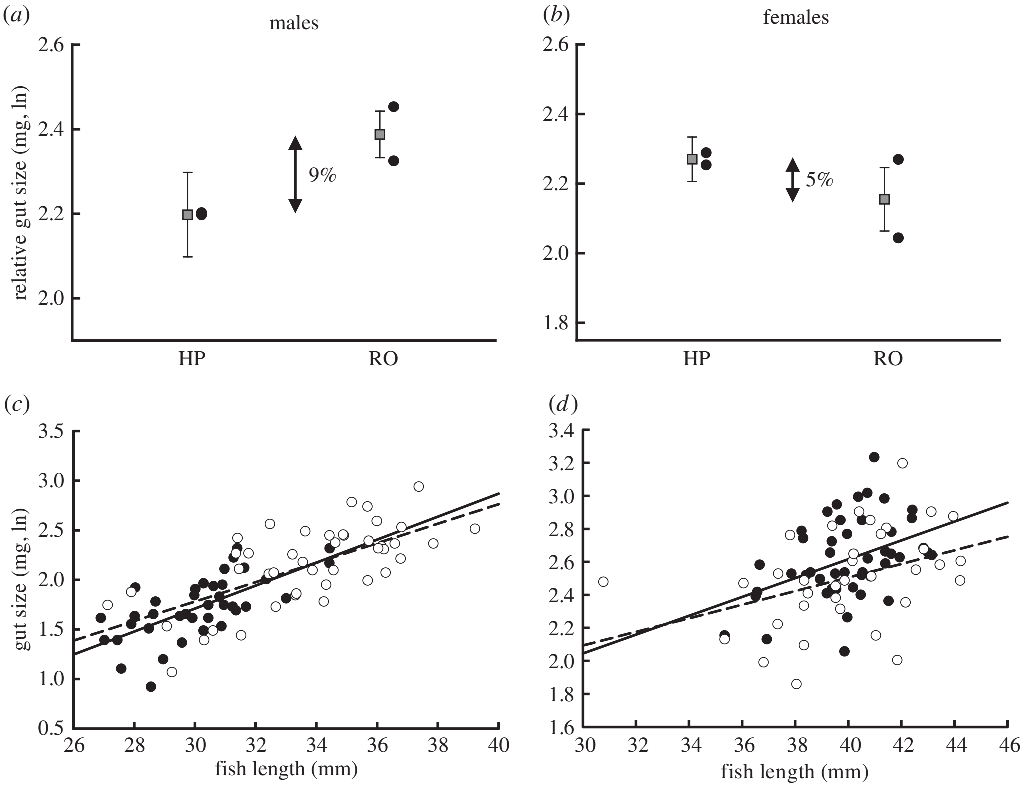

Figure 2. Variation in gut size between predator communities. (*a*) Male relative gut size, (*b*) female relative gut size, (*c*) male length versus gut size, and (*d*) female length versus gut size. Panels (*a*,*b*) and (*e*,*f*): grey squares indicate population means, filled circles indicate replicate river means. Panels (*c*,*d*): filled circles (solid regression line)—high predation fish, open circles (dashed regression line)—*Rivulus*-only fish. The regression lines between fish size and brain size are based upon the raw data. The predation × sex was significant ($p < 0.05$). Males from high predation sites exhibited a smaller gut size than *Rivulus* from *Rivulus*-only sites but such trends were opposite in females. The data points for male and female relative gut size reflect the estimated marginal means at the mean of the covariate. Error = ±1 s.e.

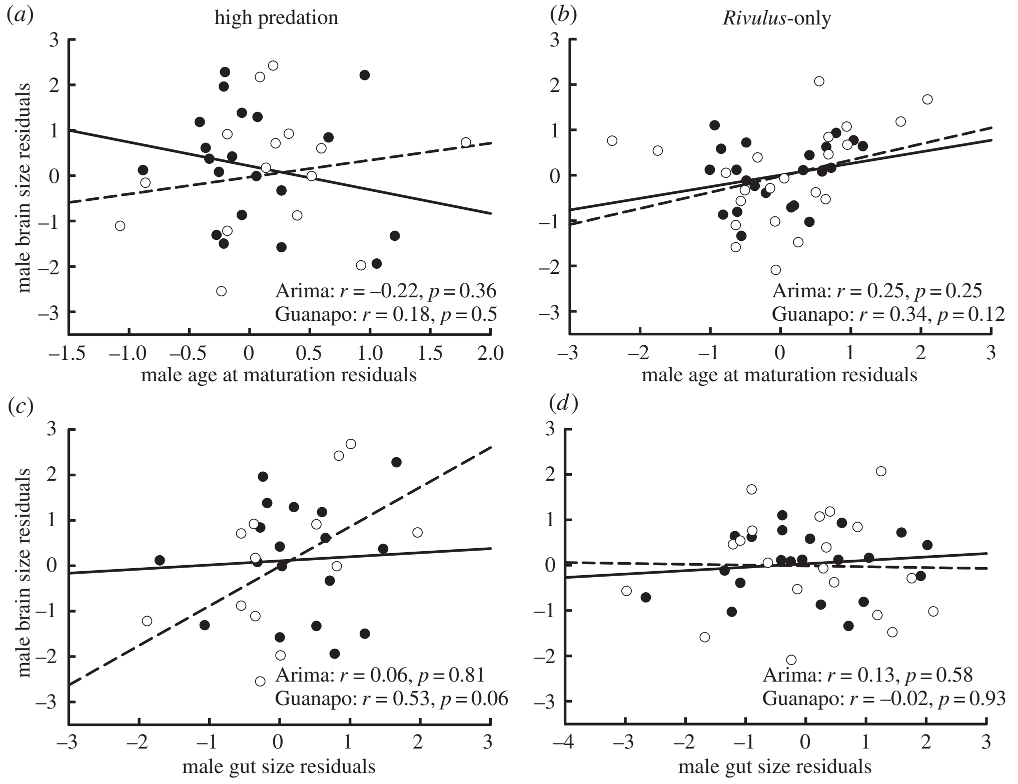

Figure 3. Correlations between male brain size, gut size, and age at maturation. (*a*) Brain size versus age at maturation for high predation males, (*b*) brain size versus age at maturation for *Rivulus*-only males, (*c*) brain size versus gut size for high predation males, and (*d*) brain size versus gut size for *Rivulus*-only males. Open circles (solid regression line), Arima River; filled circles (dashed regression line), Guanapo River. All correlations were not significant ($p > 0.05$).

study, increased predation was associated with shifts in male brain size only. Thus, the extent to which predators favour the evolution of a smaller brain (in males) or whether brain size patterns are species specific or depend upon life history or reproductive characteristics (i.e. egg layer versus livebearer) is unclear. Moreover, the consistency of the link between specific ecological forces (i.e. predation) and brain size evolution is not well resolved. Future research needs to continue to leverage naturally occurring variation in ecological properties to test for evolved differences in brain size.

(a) Ecological drivers of brain size evolution

To understand brain size evolution in *Rivulus*, we need to consider: why larger brains might be favoured in the absence of predators *and* why small brains might be selected for in risky environments. Research that has compared species of fish from contrasting predation regimes for differences in learning and cognitive function revealed an inverse relationship between predation intensity and rates of learning; fish from sites with weak predation levels often exhibit faster rates of learning and an increased ability to solve spatial tasks compared with fish from sites with predators [43,44]. Variation in learning is especially important for males as faster rates of learning enhance male mating success in fish [58]. In the absence of predators, *Rivulus* are commonly observed in the open water and are not restricted to stream margins as in sites with predators (M Walsh 2006, personal observation). It thus seems likely that reductions in predation allows for increased foraging opportunities, aggressive interactions with males, and courting opportunities with females. Given that brain size is widely known to correlate positively with learning and problem solving across species [6], one possibility is that a larger brain size is favoured in males in *Rivulus*-only sites because it translates into improved learning and, in turn, mating success (via improved foraging success and/or due to an enhanced ability to attract mates and encounter females). Perhaps, selection for spatial learning is weak in risky environments because enhanced levels of *per capita* food availability or high mortality rates lessen the fitness benefits of cognition (and yields selection for a smaller brain).

Variation in predation is also associated with shifts in the behaviour of prey. The longstanding assumption is that increased predation should favour prey that forage less and engage in less risky behaviour ([59]; see [37–40]). This prediction depends upon the characteristics of predator and prey as it also possible that the fitness benefits of a larger size in prey override the survival costs of risky behaviour. This is important because there are numerous empirical examples that illustrate the opposite trend; the propensity to take risks, or the ‘boldness’ of individuals is higher in organisms that coexist with predators [29–36]. Such behavioural differences have been documented in Trinidadian streams as *Rivulus* from sites with predators are bolder and exhibit greater movement than *Rivulus* from sites that lack predators [29,30]. Research on other species of fish have also shown that males are typically ‘bolder’ than females [33,36,60]. Increased male boldness is correlated with increased growth, foraging success, predator escape response, ability to attract mates, and mating success [29,41,42]. Thus, males that co-occur with predators are bolder but such increased boldness is negatively correlated with rates of learning. The extent to which the evolved differences in brain size observed in this study are also connected to differences in behaviour between high predation and *Rivulus*-only sites [29,30] require further exploration.

(b) Gut size variation and life-history trade-offs

Gut size reflects a balance between the benefits associated with the ability to digest food versus the cost of maintaining these tissues [61]. Gut size often varies as a function of resource availability [61–64]; decreased food levels or food quality are typically associated with larger guts because they increase the potential for absorption [65]. Overall, declines in resources did not significantly influence *Rivulus* gut size (table 1) although responses to reduced food did vary between populations; *Rivulus*-only fish responded to declines in food by developing larger guts while high predation fish did not (see Results). These divergent responses to changes in resource availability are small in magnitude but may indicate that selection for a rapid life history in high predation sites (see [48,49]) is associated with selection for a gut size that ensures maximal conversion of resources into growth and/or reproduction.

Much work has shown that investment in brain tissue is expensive (i.e. expensive tissue hypothesis) [66] and can incur costs [12–17]. Yet, at the individual level, our results revealed little evidence for trade-offs between brain size and gut size or age at maturation in either sex (figure 3 and electronic supplementary material, figure S1). The lone evidence for a trade-off associated with allocation towards brain tissue is at the population level; males from high predation sites mature early [48] perhaps, at the expense of brain size (figure 1). However, we note that investment in brain tissue may coincide with declines in components of fitness not measured in this study such as metabolism [12], muscle mass [13], or fat storage [15]. Furthermore, trade-offs between brain size and gut size or reproductive traits are not universal (see [13,15]). In general, trade-offs associated with brain tissue do not appear to be easily predicted *a priori*.

5. Conclusion

Here, we leveraged fish communities on the island of Trinidad that experience divergent patterns of predator-induced mortality [49] to evaluate the influence of predation on the evolution of brain size. We found that increased predation was associated with evolution of a smaller relative brain size in males but not females (figure 1). These results clearly show that contrasting ecological conditions can drive genetically based shifts in brain size in natural populations (see also [18–22]). We hypothesize that this variation in brain size is connected to known differences in rates of learning and risk-taking behaviour observed in prey between sites that vary in predation intensity [29–36,43,44]. Tests of the link between varying ecological conditions (i.e. predation, habitat complexity, and competition), brain size, and behaviour are now needed.

Data accessibility. Data available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.5gb67.

Authors' contributions. M.R.W. designed the study, M.R.W., W.B., and S.B. collected all data, M.R.W. and S.B.M. analysed the data, and all authors wrote the manuscript.

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