

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

3

4 Adam R. Reddon^{1,#,*}, Laura Chouinard-Thuly¹, Ioannis Leris^{1,2}, Simon M. Reader¹

5

6 1. Department of Biology, McGill University, Montreal, Quebec, Canada

7 2. Department of Biology and Helmholtz Institute, Utrecht University, Utrecht, The Netherlands

8

9 *Author for correspondence: a.r.reddon@ljmu.ac.uk

10 #Current address: School of Natural Sciences and Psychology, Liverpool John Moores University,
11 Liverpool, UK

12 Abstract

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

29
30 Keywords: brain size, development, plasticity, *Poecilia reticulata*, sex differences

31 **1 | INTRODUCTION**

32 Brains vary considerably in volume and organisation both within and between species (de
33 Winter & Oxnard, 2001; Gonda et al., 2013; Striedter 2005). Such variation is often understood as
34 the result of a trade-off between costs and benefits, balancing for example the energetic or
35 developmental costs of brain enlargement against proposed benefits such as increased efficacy of
36 perception, cognition, or motor skills (Barton, 1998; Benson-Amram et al., 2016; MacLean et al.,
37 2014). Identifying the factors that shape brain evolution and development furthers our
38 understanding of these costs and benefits (Sherry, 2006).

39 Predation poses a major challenge for many species (Edmunds, 1974; Lima & Dill, 1990)
40 and may have a substantial influence on brain evolution (van der Bijl & Kolm, 2016). Animals
41 faced with frequent predation threats may be selected for greater investment in neural tissues
42 that help them to sense, integrate or act upon information from the environment in order to
43 evade predators (Gonda et al., 2012). For example, birds with larger brains have shorter flight
44 initiation distances, potentially reflecting superior predator monitoring abilities (Møller &
45 Erritzøe, 2014). Larger brained bird species have lower adult mortality (Sol et al., 2007) and have
46 reduced depredation of their nests (Öst & Jaatinen, 2015). Mammalian predators capture smaller
47 brained prey more often than expected by their abundance (Shultz & Dunbar, 2006) and the
48 presence of predators is associated with larger brains in mammalian prey species (Jerison, 1973).
49 In fish, prey species tend to have a larger relative brain size than do their predators and there is a
50 positive association between the brain sizes of predators and prey (Kondoh, 2010). By contrast,
51 Walsh et al. (2016) found that in the Trinidad killifish, *Rivulus hartii*, males from high predation
52 populations had smaller brains than those from low predation populations. The authors
53 speculated that killifish with fewer predators might be selected for larger brains because of the
54 greater competition for food and mates in these populations. Similarly, a recent study on

55 threespine stickleback, *Gasterosteus aculeatus*, found that experimental exposure to predators
56 selected for fish with smaller rather than larger brains (Samuk et al. 2018). Collectively, these
57 results illustrate that the drivers of brain size variation are complex, and the effect of predation
58 on relative brain size and may depend on multiple interacting ecological and social pressures
59 (Dunbar & Shultz, 2017).

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

68 While the brain is shaped by evolution, it is also a highly malleable organ and phenotypic
69 plasticity may also play a key role in generating individual variation in brain size (Gonda et al.,
70 2013; Healy & Rowe, 2007). For example, environmental complexity during early life increases
71 relative brain size in rodents (Diamond et al., 1966; Rozenzweig & Bennett, 1969), insects
72 (Heisenberg et al., 1995) and fish (DePasquale et al., 2016; Gonda et al., 2011), while low oxygen
73 during development decreases relative brain size in fish (Chapman et al., 2008).

74 The Trinidadian guppy, *Poecilia reticulata*, is a small livebearing freshwater fish that
75 experiences pronounced interpopulation variation in predation threat (Magurran, 2005), and
76 thus provides a valuable system to study how predation shapes the brain. Throughout Trinidad,
77 guppies have repeatedly colonized independent river reaches above natural waterfall barriers,
78 where aquatic predators are scarce, while simultaneously living below the same barriers where

79 abundant aquatic predators impose substantial mortality (Magurran, 1998). As a result, there has
80 been repeated parallel evolution of distinct behavioural, morphological, and life-history traits
81 among guppy populations that are heavily depredated compared to those that are relatively free
82 from predation pressure (Magurran, 2005).

83 A recent series of papers has examined the effect of artificially selecting guppies for large
84 or small relative brain mass, finding that increased investment in brain tissue can provide
85 antipredator benefits, but also carry costs. Female guppies artificially selected for larger brains
86 exhibited greater survival under predation and altered predator responses compared to small-
87 brained individuals (Kotrschal et al., 2015a; van der Bijl et al., 2015). However, larger-brained
88 female guppies also had smaller guts, produced fewer offspring, and had reduced innate immune
89 responses, suggesting a trade-off between neural investment and other fitness-relevant
90 parameters (Kotrschal et al., 2013, 2015b, 2016). If antipredator advantages were sufficient to
91 overcome the costs of maintaining a larger brain, then we would expect that guppies from high
92 predation populations would consistently have larger brains for their body size than guppies
93 from low-predation environments. Indeed, female guppies under greater threat from predatory
94 prawns have larger relative brain sizes than do females under lesser threat from these predators
95 (Kotrschal et al., 2017a). Artificial selection on brain size in guppies has consistently revealed
96 differing effects in males and females, suggesting that sex may be a key modulator of the
97 relationship between brain size and performance in this species (e.g., Kotrschal et al., 2012, 2013
98 2015a; van der Bijl et al., 2015), and therefore, it is important to examine both males and females.

99 Guppies also show plasticity in brain size; for example, guppies raised in the laboratory
100 have smaller brains than fish born in the wild (Burns & Rodd, 2008; Burns et al., 2009; Eifert et
101 al., 2015). Furthermore, guppy males that cohabitated with females have larger brains than those
102 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

127 *Crenicichla* sp. and *Aequidens pulcher* in the Aripo River; and eleotrids such as *Eleotris pisonis* and
128 *Gobiomorus dormitor* in the Marianne River; Magurran, 2005; Reznick et al., 1996), which is
129 consistent across years (Schwartz & Hendry, 2010). Guppies were collected from each site using
130 butterfly nets and then were transported to the William Beebe Research Station near Arima,
131 Trinidad. Each fish was euthanized with an overdose of tricaine methanesulfonate (Finquel MS-
132 222; Argent Chemical Laboratories, USA) buffered to a neutral pH with NaHCO₃. We measured
133 each fish for standard length (SL; from the tip of the snout to the caudal peduncle) and then
134 dissected out the brains using a portable stereomicroscope (Ken-a-vision VisionScope 2) at 10x
135 magnification. Care was taken to sever the spinal cord and optic nerves at a consistent position
136 on each brain. We placed the brains in RNAlater (Sigma Aldrich) and incubated them for 24 h at
137 room temperature before transferring them to -20°C. We transported the samples back to McGill
138 University (Montreal, Canada) where we removed them from RNAlater and gently dabbed them
139 dry. Blind to the population of origin, we weighed each whole brain to the nearest 0.1 mg using
140 an analytic laboratory balance (Mettler Toledo ME104E). Because all brains were treated
141 identically, any storage effects on brain mass should affect all samples similarly. Following
142 measurement, the brains were used in another study.

143

144 **2.2 | Developmental experiment**

145 We exposed developing guppies to cues of predation threat during the first 45 days of life. The
146 parental generation were guppies from a laboratory-reared population, descended from a
147 mixture of fish captured in high predation sites in the Aripo and Quare Rivers of Northern
148 Trinidad in 2009 and 2010. Parental fish were housed in mixed sex groups of ~10 adults in 18 L
149 aquaria. We maintained the water at 26±1°C and fed the fish ad libitum daily on a mixture of
150 dried prepared tropical fish flakes (TetraMin, Tetra, Germany) and rehydrated decapsulated

151 brine shrimp eggs (Brine Shrimp Direct, Inc., Ogden, Utah, USA). Lights were on from 07:00 to
152 19:00 h, with a 30 min dawn/dusk period. To collect fry for the experiment, we moved groups of
153 10-12 visibly gravid females into separate aquaria, which we checked daily for newborn fry. We
154 mixed fry born to different females and randomly assigned them to one of two treatments:
155 Exposure to cues of predation risk or a control condition. Fry in both treatments were held at
156 densities of 30 individuals per 18 L aquarium during the treatment period. We had three
157 replicate aquaria in each experimental condition (six aquaria total). The experimental aquaria
158 contained 1 cm of white coral sand and were furnished with an artificial plant to serve as a
159 refuge. Water and light conditions were the same as for the parental generation, but the
160 developing fry were fed twice daily.

161 Five days per week during the 45-day treatment period, the fish in the predator cue
162 condition were visually exposed to a sympatric cichlid fish predator (*Crenicichla* sp.) living in an
163 adjacent aquarium by removing an opaque barrier between them for 5 minutes. Concurrent with
164 the visual exposure, we infused 5 ml of water previously collected from aquaria housing live
165 *Crenicichla* that had recently been fed freshly euthanized guppies (following Brown et al., 2000).
166 Guppies respond to the odour of damaged conspecifics and predator dietary cues with
167 antipredator responses (Brown & Godin, 1999). On four of the five weekly cue exposure days, we
168 also added 5 ml of odour cue harvested from the skin and muscle tissue of adult guppies in
169 addition to the predator housing water. To collect this cue, we sacrificed adult guppies of both
170 sexes by briefly immersing them in an ice water bath and then swiftly decapitating them
171 (Matthews & Varga, 2012). We then homogenized skin and muscle tissues with dH₂O, filtered the
172 solution with cotton floss, and diluted it with dH₂O until we obtained a concentration of 0.1 cm²
173 of tissue per ml of cue (following Brown & Godin, 1999). We exposed the guppies in the control
174 condition to the sight and housing water of a non-predatory suckermouth catfish

175 (*Pterygoplichthys* sp.) that had been fed blanched spinach leaves. In lieu of the damaged
176 conspecific cue, the control fish received blank dH₂O. We exposed the guppies to these
177 heterospecific fish stimuli at a randomly chosen time (between 10:00 and 16:00 h) on each
178 exposure day.

179 After 45 days, we ceased all heterospecific stimuli exposures. On day 50, we reduced the
180 housing density of the experimental fish by splitting each group into 3 separate 18 L aquaria with
181 ~10 individuals of mixed-sex in each, resulting in a total of 18 housing aquaria, nine per
182 treatment. We also reduced the feeding frequency to once per day to match the standard adult
183 husbandry protocols in our laboratory. The experimental fish were held in these conditions until
184 approximately 300 days of age, during which time behavioural and hormonal measures were
185 taken for other studies (Chouinard-Thuly et al., 2018; Leris, 2016). We then sacrificed 73
186 individuals (22 predator exposed males, 27 control males, 11 predator exposed females, and 13
187 control females), by briefly immersing them in an ice water bath and then swiftly decapitating
188 them. We then dissected out their brains using a stereomicroscope (Leica EZ4W) at 10x
189 magnification. Care was taken to sever the spinal cord and optic nerves at a consistent position
190 on each brain. We weighed the fresh brains to the nearest 0.1 mg on an analytic laboratory
191 balance (Mettler Toledo ME104E). Brain mass and body size values were taken blind to
192 treatment. Following measurement, the brains were used in another study.

193

194 **2.3 | Analysis**

195 We used linear models to investigate the relationship between brain mass and body size with
196 exposure to predation both naturally in the field, and in our developmental experiment. To
197 account for the allometric relationship between brain mass and body size (Brandstätter &
198 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 \pm 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

222 We found that, for an average body size, males collected from high predation sites had brains
223 11.3% heavier in the Marianne River and 16.5% heavier in the Aripo River than males collected
224 from low predation sites in the same rivers ($p = 0.052$; Figure 1a; Table 2). Males from the
225 Marianne River had 14.7% heavier brains than males from the Aripo River, but the interaction
226 between predation regime and river was not significant (Figure 1a; Table 2). We found no
227 evidence that predation regime or river was associated with relative brain mass in female
228 guppies (Figure 1b; Table 2). Males from high predation sites were significantly smaller bodied
229 than low predation males (Mean SL \pm s.e.: high predation males = 12.08 ± 0.14 ; low predation
230 males = 13.61 ± 0.14 ; $p < 0.001$; Table 3), but there was no similar significant difference in female
231 body length (Mean SL \pm s.e.: high predation females = 16.31 ± 0.36 ; low predation females =
232 14.80 ± 0.48 ; $p = 0.062$). Supplementary Figure S1 illustrates the allometric relationships
233 between brain mass and body length in the wild caught fish.

234

235 **3.2 | Developmental experiment**

236 We found that, for an average body size, males exposed to predation cues during development
237 had brains 21.2% heavier than males exposed to control cues ($p = 0.011$; Figure 2a; Table 4). We
238 found no evidence that exposure to predation cues during development influenced the relative
239 brain mass of female guppies (Figure 2b; Table 4). Males exposed to predation cues were
240 significantly larger bodied than males exposed to control cues (Mean SL \pm s.e.: predator cue
241 exposed males = 14.51 ± 0.23 ; control cue males = 13.80 ± 0.17 ; $p = 0.014$; Table 5), but there was
242 no significant difference in female body length (Mean SL \pm s.e.: predator cue exposed females =
243 28.13 ± 0.80 ; control cue females = 28.39 ± 0.49 ; $p = 0.65$). Supplementary Figure S2 illustrates
244 the allometric relationships between brain mass and body length in the laboratory reared fish.

245

246 4 | DISCUSSION

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

257 Male guppies could hypothetically benefit from greater neural investment under
258 predation threat if heavier brains relative to body size provide sensory, cognitive or motor
259 benefits. It is possible that having a heavier brain may allow guppies to detect, assess, react to, or
260 learn about predation threats better, and/or allow for simultaneous monitoring of predator
261 threats while engaged in alternative activities such courtship or foraging, similar to reports in
262 birds (Møller & Erritzøe, 2014; Sol et al. 2007). A heavier brain may also allow individual guppies
263 to better address social demands, such as group cohesion or coordination with conspecifics
264 (Dunbar & Shultz, 2017). Guppies from high predation populations do form more cohesive and
265 coordinated groups (Ioannou et al., 2017) and group cohesion has antipredator benefits in prey
266 fishes (Krause & Ruxton, 2002; Ioannou et al., 2012). Interestingly, predation seems to select for
267 a reduction in brain size in some other fish species, and the putative advantages of increased
268 brain size in the face of predation risk thus certainly merit closer examination (Walsh et al. 2016;
269 Samuk et al. 2018). Samuk et al. (2018) suggest that differences between studies could result

270 from the type of antipredator responses employed and local ecological conditions, such as the
271 availability of shelter. An experimental evolution study on guppies, tracking the effects of
272 different predators on brain size, cognitive performance, social behaviour and antipredator
273 defences across generations, with different antipredator responses available, would be a large
274 undertaking but highly informative in this regard.

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

293 Why should predation affect brain mass in male guppies but not females? In guppies,
294 males are more conspicuous, less social, bolder, and are poorer swimmers than females (Houde,
295 1997), and thus are more vulnerable to predation (Kotrschal et al., 2015a). As a result, males may
296 have more to gain from investment in neural tissues under predation threat. Alternatively,
297 because females are slower to mature and longer lived (Magurran, 2005) they may show greater
298 neural investment regardless of predation risk. Relative brain size has been linked to life history
299 strategy in birds and mammals, with slower developing and longer-lived animals typically having
300 larger brains for their body size (Bennett & Harvey, 2009; Iwaniuk & Nelson, 2003).

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

316 al., 2013, 2014, 2015a, 2017b), these data suggest that relative brain mass is a relevant trait in
317 guppies, encouraging more fine-scaled work in the future.

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

328 Our field comparisons of high and low predation guppies came from only two replicate
329 rivers. The parallel results in the two rivers, in the same sex, and the qualitative match with the
330 developmental manipulation, again in the same sex, suggests however that predation is likely a
331 key driver of the differences we observed in male brain mass. However, sampling of a greater
332 number of rivers and a variety of other ecological conditions would clearly be a valuable follow-
333 up. Additionally, the laboratory study was conducted on only a single lab population, descended
334 from a mixture of high predation fish from two different rivers. It would be interesting to
335 examine whether the developmental effects of predation cues differ between populations. We
336 also note that the balance we used to weigh the brains was relatively coarse (0.1 mg listed
337 repeatability, with more error likely at lower masses) given the small size of guppy brains (1.3 –
338 9.2 mg in our samples). However, any measurement error introduced by our instrument would
339 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.

ACKNOWLEDGEMENTS

We thank Pierre-Olivier Montiglio, Lea Blondel and Andrew Hendry for assistance in the field. We thank Kiyoko Gotanda for providing parental fish for the developmental experiment. This

364 research was funded by grants from the Natural Sciences and Engineering Research Council of
365 Canada (NSERC; grants #418342-2012, #429385-2012) and the Canadian Foundation for
366 Innovation (grant #29433), awarded to SMR. ARR was supported by a Banting Postdoctoral
367 Fellowship. IL was funded by a Utrecht University Stimulus grant to SMR.

368

369 COMPETING INTERESTS

370 We have no competing interests to declare.

371

372 AUTHOR CONTRIBUTIONS

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

377

378 DATA ACCESSIBILITY

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

380

381 REFERENCES

382 Barton, R. A., & Harvey, P. H. (2000). Mosaic evolution of brain structure in mammals. *Nature*,
383 405, 1055–1058. doi:10.1038/35016580
384
385 Barton, R. A. (1998). Visual specialization and brain evolution in primates. *Proceedings of the*
386 *Royal Society B*, 265, 1933–1937. doi:10.1098/rspb.1998.0523
387
388 Bennett, P. M., Harvey, P. H. (2009). Brain size, development and metabolism in birds and
389 mammals. *Journal of Zoology*, 207, 491–509. doi:10.1111/j.1469-7998.1985.tb04946.x
390
391 Benson-Amram, S., Dantzer, B., Stricker, G, Swanson, E. M., Holekamp, K. E. (2016). Brain size
392 predicts problem-solving ability in mammalian carnivores. *Proceedingsofthe National Academy*
393 *of Sciences*, 113, 2532–2537. doi:10.1073/pnas.1505913113

- Botham, M. S., Hayward, R. K., Morrell, L. J., Croft, D. P., Ward, J. R., Ramnarine, I. & Krause, J. (2008). Risk-sensitive antipredator behavior in the trinidadian guppy, *Poecilia reticulata*. *Ecology*, 89, 3174–3185. doi:10.1890/07-0490.1
- Brandstätter, R., Kotrschal, K. (2008). Brain growth patterns in four European cyprinid fish species (Cyprinidae, Teleostei): roach (*Rutilus rutilus*), bream (*Abramis brama*), common carp (*Cyprinus carpio*) and sabre carp (*Pelecus cultratus*). *Brain Behavior and Evolution*, 35, 195–211. doi:10.1159/000115867
- Brown, G. E., Godin, J.-G. J. (1999). Chemical alarm signals in wild Trinidadian guppies (*Poecilia reticulata*). *Canadian Journal of Zoology*, 77, 562–570. doi:10.1139/z99-035
- Brown, G. E., Paige, J. A., Godin, J.-G., J. (2000) Chemically mediated predator inspection behaviour in the absence of predator visual cues by a characin fish. *Animal Behaviour*, 60, 315–321. doi:10.1006/anbe.2000.1496
- Burns, J. G. & Rodd, F. H. (2008). Hastiness, brain size and predation regime affect the performance of wild guppies in a spatial memory task. *Animal Behaviour*, 76, 911–922. doi:10.1016/j.anbehav.2008.02.017
- Burns, J. G., Saravanan, A., & Rodd F. H. (2009) Rearing environment affects the brain size of guppies: lab-reared guppies have smaller brains than wild-caught guppies. *Ethology*, 115, 122–133. doi:10.1111/j.1439-0310.2008.01585.x
- Chapman, L., Albert, J., & Galis, F. (2008). Developmental plasticity, genetic differentiation, and hypoxia-induced trade-offs in an African cichlid fish. *The Open Evolution Journal*, 2, 75–88. doi:10.2174/1874404400802010075
- Chittka, L., Niven, J. (2009). Are bigger brains better? *Current Biology*, 19, R995–R1008. doi:10.1016/j.cub.2009.08.023
- Chouinard-Thuly, L., Reddon, A. R., Leris, I., Earley, R. L. & Reader, S. M. (2018). Developmental plasticity of the stress response in female but not in male guppies. *Royal Society Open Science*, 5, 172268–10. doi:10.1098/rsos.172268
- de Winter W. & Oxnard, C. E. (2001). Evolutionary radiations and convergences in the structural organization of mammalian brains. *Nature*, 409, 710–714. doi:10.1038/35055547
- DePasquale, C., Neuberger, T., Hirrlinger, A. M., & Braithwaite, V. A. (2016). The influence of complex and threatening environments in early life on brain size and behaviour. *Proceedings of the Royal Society B*, 283, 20152564. doi:10.1098/rspb.2015.2564
- Diamond, M. C., Law, F., Rhodes, H., Lindner, B., Rosenzweig, M. R., Krech, D., Bennett, E. L. (1966). Increases in cortical depth and glia numbers in rats subjected to enriched environment. *Journal of Comparative Neurology*, 128, 117–125. doi:10.1002/cne.901280110
- Dunbar, R. I. M. & Shultz, S. (2017). Why are there so many explanations for primate brain

- evolution? *Philosophical Transactions of the Royal Society B*, 372, 20160244.
doi:10.1098/rstb.2016.0244
- Edmunds, M. (1974). *Defence in animals*. New York: Longman Inc.
- Eifert, C., Farnworth, M., Schulz-Mirbach, T., Riesch, R., Bierbach, D., Klaus, S., Wurster, A., Tobler, M., Streit, B., Indy, J.R., Arias-Rodriguez, L. & Plath, M. (2015). Brain size variation in extremophile fish: local adaptation versus phenotypic plasticity. *Journal of Zoology*, 295, 143–153.
- Elvidge, C. K., Ramnarine, I & Brown, G. E. (2014). Compensatory foraging in Trinidadian guppies: effects of acute and chronic predation threats. *Current Zoology*, 60, 323–332.
- Finlay, B. & Darlington, R. (1995). Linked regularities in the development and evolution of mammalian brains. *Science*, 268, 1578–1584. doi:10.1126/science.7777856
- Gonda, A., Herczeg, G., & Merilä J. (2011). Population variation in brain size of nine-spined sticklebacks (*Pungitius pungitius*) - local adaptation or environmentally induced variation? *BMC Evolutionary Biology*, 11, 75. doi:10.1186/1471-2148-11-75
- Gonda, A., Herczeg, G., & Merilä, J. (2013). Evolutionary ecology of intraspecific brain size variation: a review. *Ecology & Evolution*, 3, 2751–2764. doi:10.1002/ece3.627
- Gonda, A., Valimaki, K., Herczeg, G., Merila, J. (2012). Brain development and predation: plastic responses depend on evolutionary history. *Biology Letters*, 8, 249–252.
doi:10.1098/rsbl.2011.0837
- Gotanda, K. M., Delaire, L. C., Raeymaekers, J. A. M., Pérez-Jvostov, F., Dargent, F., Bentzen, P., Scott, M. E., Fussmann, G. F., Hendry, A. P. (2013). Adding parasites to the guppy-predation story: insights from field surveys. *Oecologia*, 172, 155–166. doi:10.1007/s00442-012-2485-7
- Harris, R. M., O'Connell, L. A., Hofmann, H. A. (2016). Brain evolution, development, and plasticity. In *The Wiley Handbook of Evolutionary Neuroscience*, pp. 422–443. Chichester, UK: John Wiley & Sons, Ltd. doi:10.1002/9781118316757.ch15
- Healy, S. D. & Rowe, C. (2007). A critique of comparative studies of brain size. *Proceedings of the Royal Society B*, 274, 453–464. doi:10.1098/rspb.2006.3748
- Heisenberg, M., Heusipp, M., & Wanke, C. (1995). Structural plasticity in the *Drosophila* brain. *Journal of Neuroscience* 15, 1951–1960.
- Houde, A. E. (1997). *Sex, color, and mate choice in guppies*. Princeton: Princeton University Press. doi:10.1046/j.1439-0310.2000.00565.x
- Ioannou, C. C., Guttal, V., & Couzin, I. D. (2012). Predatory fish select for coordinated collective motion in virtual prey. *Science*, 337, 1212–1215. doi:10.1126/science.1218919
- Ioannou, C. C., Ramnarine, I. W., Torney, C. J. (2017). High-predation habitats affect the social dynamics of collective exploration in a shoaling fish. *Scientific Advances*, 3, 1–8.

doi:10.1126/sciadv.1602682

Iwaniuk, A. N., Nelson, J. E. (2003). Developmental differences are correlated with relative brain size in birds: a comparative analysis. *Canadian Journal of Zoology*, 81, 1913–1928.

doi:10.1139/z03-190

Jerison, H. (1973). *Evolution of the brain and intelligence*. New York: Academic Press.

Kondoh, M. (2010). Linking learning adaptation to trophic interactions: a brain size-based approach. *Functional Ecology*, 24, 35–43. doi:10.1111/j.1365-2435.2009.01631.x

Kotrschal, A., Lievens, E.J., Dahlbom, J., Bundsen, A., Semenova, S., Sundvik, M., Maklakov, A.A., Winberg, S., Panula, P. & Kolm, N. (2014). Artificial selection on relative brain size reveals a positive genetic correlation between brain size and proactive personality in the guppy. *Evolution*, 68, 1139–1149.

Kotrschal, A., Buechel, S. D., Zala, S. M., Corral-Lopez, A., Penn, D. J., Kolm, N. (2015). Brain size affects female but not male survival under predation threat. *Ecology Letters*, 18, 646–652. doi:10.1111/ele.12441

Kotrschal, A., Corral-Lopez, A., Szidat, S., & Kolm, N. (2015). The effect of brain size evolution on feeding propensity, digestive efficiency, and juvenile growth. *Evolution*, 69, 3013–3020. doi:10.1111/evo.12784

Kotrschal, A., Deacon, A. E., Magurran, A. E., & Kolm, N. (2017a). Predation pressure shapes brain anatomy in the wild. *Evolutionary Ecology*, 31, 619–633. doi:10.1007/s10682-017-9901-8

Kotrschal, A., Zeng, H. L., van der Bijl, W., Öhman-Mägi, C., Kotrschal, K., Pelckmans, K., & Kolm, N. (2017b). Evolution of brain region volumes during artificial selection for relative brain size. *Evolution*, 71, 2942–2951.

Kotrschal, A., Kolm, N., & Penn, D. J. 2016 Selection for brain size impairs innate, but not adaptive immune responses. *Proceedings of the Royal Society B*, 283, 20152857. doi:10.1098/rspb.2015.2857

Kotrschal, A., Rogell, B., Bundsen, A., Svensson, B., Zajitschek, S., Brännström, I., Immler, S., Maklakov, A. A., & Kolm, N. (2013). Artificial selection on relative brain size in the guppy reveals costs and benefits of evolving a larger brain. *Current Biology*, 23, 168–171. doi:10.1016/j.cub.2012.11.058

Kotrschal, A., Rogell, B., Maklakov, A. A., Kolm, N. (2012). Sex-specific plasticity in brain morphology depends on social environment of the guppy, *Poecilia reticulata*. *Behavioral Ecology and Sociobiology* 66, 1485–1492. doi:10.1007/s00265-012-1403-7

Krause, J. & Ruxton, G. D. (2002). *Living in Groups*. Oxford, UK: Oxford University Press
doi:10.1093/sysbio/sys022

Leris, I. (2016). Early environment and the development of social behaviours in the Trinidadian

- 538 guppy. PhD thesis. Utrecht University, 1–169.
539
- 540 Lima, S. L. & Dill, L. M. (1990). Behavioral decisions made under the risk of predation: A review
541 and prospectus. *Canadian Journal of Zoology*, 68, 619–640. doi:10.1139/z90-092
542
- 543 Logan, C. J., Avin, S., Boogert, N., Buskell, A., Cross, F. R., Currie, A., Jelbert, S., Lukas, D., Mares, R.,
544 Navarrete, A. F., Shigeno, S., Montgomery, S. H. (In press). Beyond brain size: uncovering the
545 neural correlates of behavioral and cognitive specialization. *Comparative Cognition & Behavior*
546 *Reviews*.
547
- 548 MacLean, E. L., Hare, B., Nunn, C.L., Addessi, E., Amici, F., Anderson, R. C., Aureli, F., Baker, J. M.,
549 Bania, A. E., Barnard, A. M., Boogert, N. J., Brannon, E. M., Bray, E. E., Bray, J., Brent, L. J. N., Burkart,
550 J. M., Call, J., Cantlon, J. F., Cheke, L. G., Clayton, N. S., Delgado, M. M., DiVincenti, L. J., Fujita, K.,
551 Herrmann, E., Hiramatsu, C., Jacobs, L. F., Jordan, K. E., Laude, J. R., Leimgruber, K. L., Messer, E. J.
552 E., de A Moura, A. C., Ostojić, L., Picard, A., Platt, M. L., Plotnik, J. M., Range, F., Reader, S. M., Reddy,
553 R. B., Sandel, A. A., Santos, L. R., Schumann, K., Seed, A. M., Sewall, K. B., Shaw, R. C., Slocombe, K. E.,
554 Su, Y., Takimoto, A., Tan, J., Tao, R., van Schaik, C. P., Virányi, Z., Visalberghi, E., Wade, J. C.,
555 Watanabe, A., Widness, J., Young, J. K., Zentall, T. R. & Zhao, Y. (2014). The evolution of self-
556 control. *Proceedings of the National Academy of Sciences*, 111, E2140–E2148.
557
- 558 Magurran, A. E. (1998). Population differentiation without speciation. *Philosophical Transactions*
559 *of the Royal Society B*, 353, 275–286. doi:10.1098/rstb.1998.0209
560
- 561 Magurran, A. E. (2005). *Evolutionary ecology: The Trinidadian guppy*. London: Oxford University
562 Press.
563
- 564 Matthews, M., Varga, Z. M. (2012). Anesthesia and euthanasia in zebrafish. *ILAR Journal*. 53, 192–
565 204. doi:10.1093/ilar.53.2.192
566
- 567 Møller, A. P. & Erritzøe, J. (2014). Predator-prey interactions, flight initiation distance and brain
568 size. *Journal of Evolutionary Biology*, 27, 34–42. doi:10.1111/jeb.12272
569
- 570 Öst, M., Jaatinen, K. (2015). Smart and safe? Antipredator behavior and breeding success are
571 related to head size in a wild bird. *Behavioural Ecology*, 26, 1371–1378.
572 doi:10.1093/beheco/arv093
573
- 574 R Core Development Team. (2016). R: A language and environment for statistical computing,
575 3.3.0. <http://www.r-project.org>. doi:10.1017/CBO9781107415324.004
576
- 577 Reznick, D., Callahan, H., Llauredo, R. (1996). Maternal effects on offspring quality in poeciliid
578 fishes. *American Zoologist*, 36, 147. doi:10.1093/icb/36.2.147
579
- 580 Reznick, D., Endler, J. A. (1982). The impact of predation on life history evolution in Trinidadian
581 guppies (*Poecilia reticulata*). *Evolution*, 36, 160–177. doi:10.2307/2408156
582
- 583 Rosenzweig, M. R. & Bennett, E. L. (1969). Effects of differential environments on brain weights
584 and enzyme activities in gerbils, rats, and mice. *Developmental Psychobiology*, 2, 87–95.
585 doi:10.1002/dev.420020208

- Roth, T. C., LaDage, L. D., Pravosudov, V. V. (2010). Learning capabilities enhanced in harsh environments: A common garden approach. *Proceedings of the Royal Society B* 277, 3187–3193. doi:10.1098/rspb.2010.0630
- Roth, T. C. & Pravosudov, V. V. (2009). Hippocampal volumes and neuron numbers increase along a gradient of environmental harshness: A large-scale comparison. *Proceedings of the Royal Society B*, 276, 401–405. doi:10.1098/rspb.2008.1184
- Samuk, K., Xue, J. & Rennison, D. J. (2018). Exposure to predators does not lead to the evolution of larger brains in experimental populations of threespine stickleback. *Evolution*, 511, 307–14. doi:10.1111/evo.13444
- Schwartz, A. K. & Hendry, A. P. (2010). Testing the influence of local forest canopy clearing on phenotypic variation in Trinidadian guppies. *Functional Ecology*, 24, 354–364. doi:10.1111/j.1365-2435.2009.01652.x
- Sherry, D. F. (2006). Neuroecology. *Annual Reviews in Psychology*, 57, 167–197. doi:10.1146/annurev.psych.56.091103.070324
- Shultz, S. & Dunbar, R. I. (2006). Chimpanzee and felid diet composition is influenced by prey brain size. *Biology Letters*, 2, 505–508. doi:10.1098/rsbl.2006.0519
- Sol, D., Szekely, T., Liker, A., Lefebvre, L. (2007). Big-brained birds survive better in nature. *Proceedings of the Royal Society B*, 274, 763–769. doi:10.1098/rspb.2006.3765
- Striedter, G. F. (2005). *Principles of brain evolution*. Sunderland: Sinauer Associates.
- van der Bijl, W., Kolm, N. (2016). Why direct effects of predation complicate the social brain hypothesis. *BioEssays*, 38, 568–577. doi:10.1002/bies.201500166
- van der Bijl, W., Thyselius, M., Kotrschal, A., Kolm, N. (2015). Brain size affects the behavioural response to predators in female guppies (*Poecilia reticulata*). *Proceedings of the Royal Society B*, 282, 20151132. doi:10.1098/rspb.2015.1132
- Walsh, M. R., Broyles, W., Beston, S. M., Munch, S. B. (2016). Predator-driven brain size evolution in natural populations of Trinidadian killifish (*Rivulus hartii*). *Proceedings of the Royal Society B* 283, 20161075. doi:10.1098/rspb.2016.1075
- Wickham, H. (2009). *ggplot2: Elegant graphics for data analysis*. New York: Springer-Verlag. doi:10.1007/978-0-387-98141-3

629 **Figure captions**

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

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

648 **Table captions**

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

651

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

658

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

664

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

671

672 **Table 5.** Estimates and standard error of fixed parameters and their interaction for the linear
673 model with log-transformed standard length for the guppy laboratory developmental study.
674 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 \leq 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.

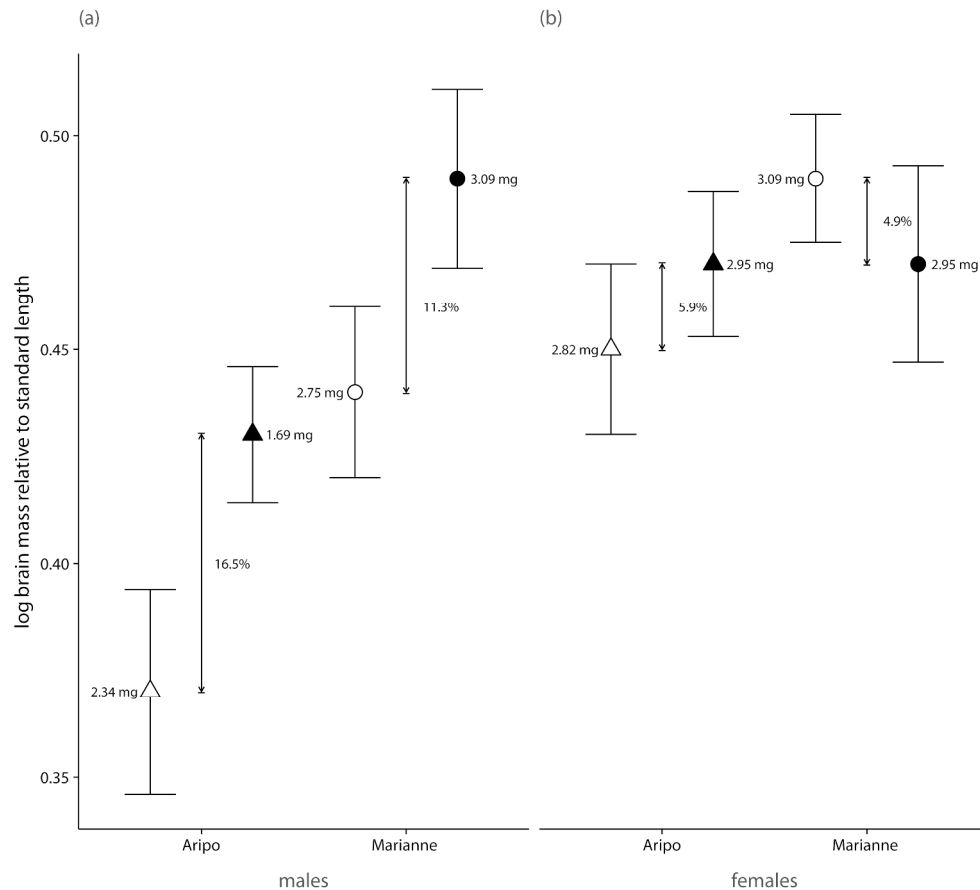
| Site | UTM coordinates (x, y) | Predation regime | <i>n</i> males | <i>n</i> females |
|-------------|------------------------|------------------|----------------|------------------|
| Aripo 1 | 693 188, 1 181 605 | Low | 15 | 15 |
| Aripo 2 | 694 231, 1 177 709 | High | 27 | 20 |
| Marianne 10 | 686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

| Parameter | Estimate | Std. Error | <i>t</i>-value | <i>p</i>-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 | <i>t</i> -value | <i>p</i> -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 |

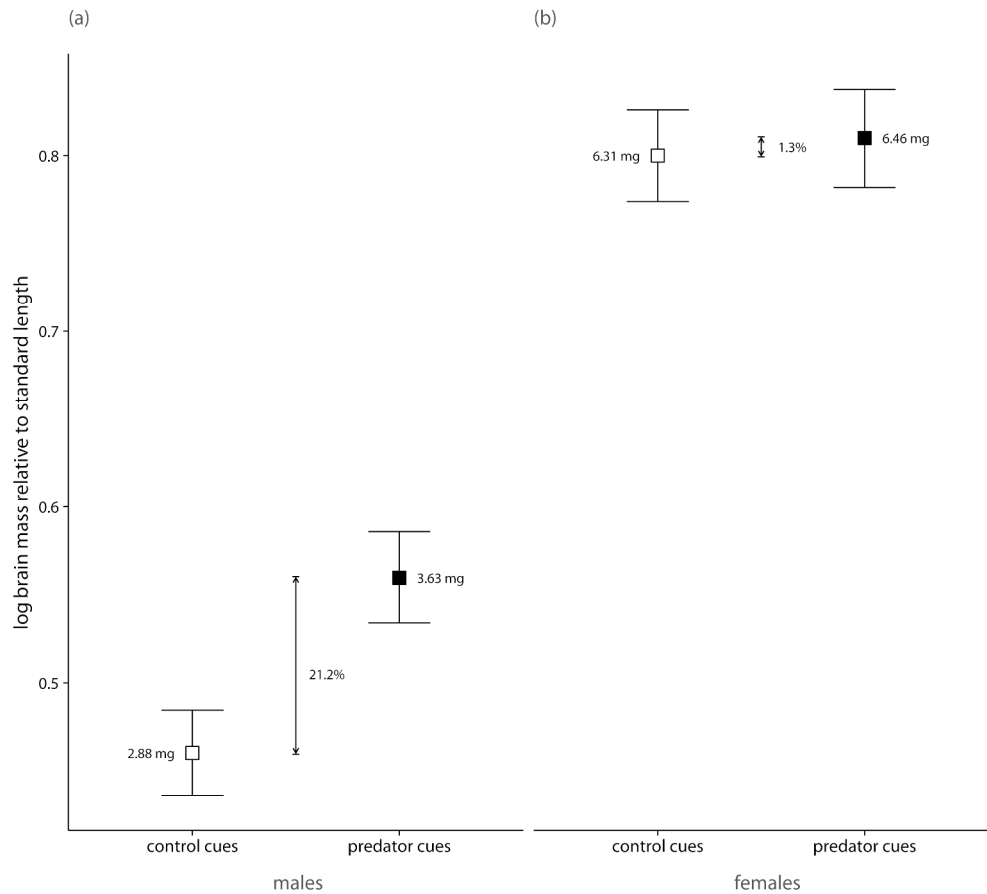
| Parameter | Estimate | Std. Error | <i>t</i>-value | <i>p</i>-value |
|----------------------------|-----------------|-------------------|-----------------------|-----------------------|
| Males (d.f. = 46) | | | | |
| Intercept | 0.56 | 0.026 | 21.16 | <0.0001 |
| Standard length | 1.11 | 0.59 | 1.89 | 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 | <i>t</i> -value | <i>p</i> -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 |



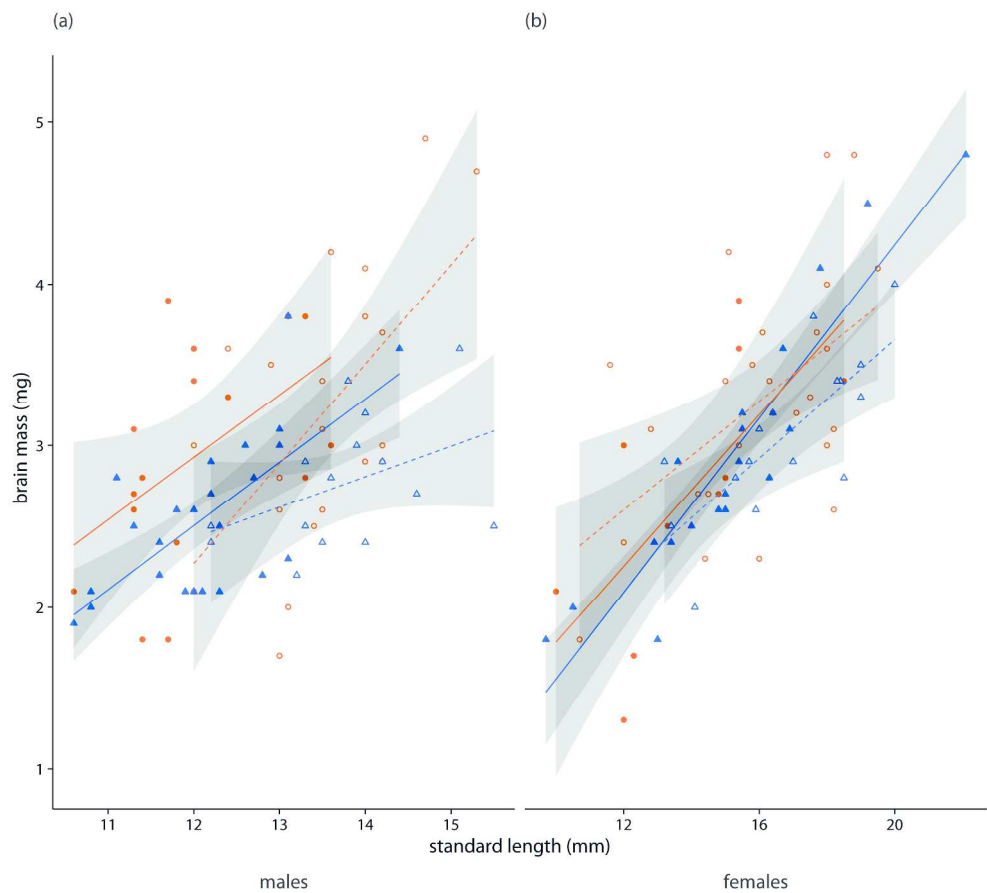
Expected (log transformed) brain mass (\pm s.e.) of guppies captured in the wild. Expected values are for the mean standard length for each sex. Mean brain masses for each group in mg are alongside the points, percent differences between groups are indicated with arrows. Males (a) from high predation 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.

275x243mm (300 x 300 DPI)

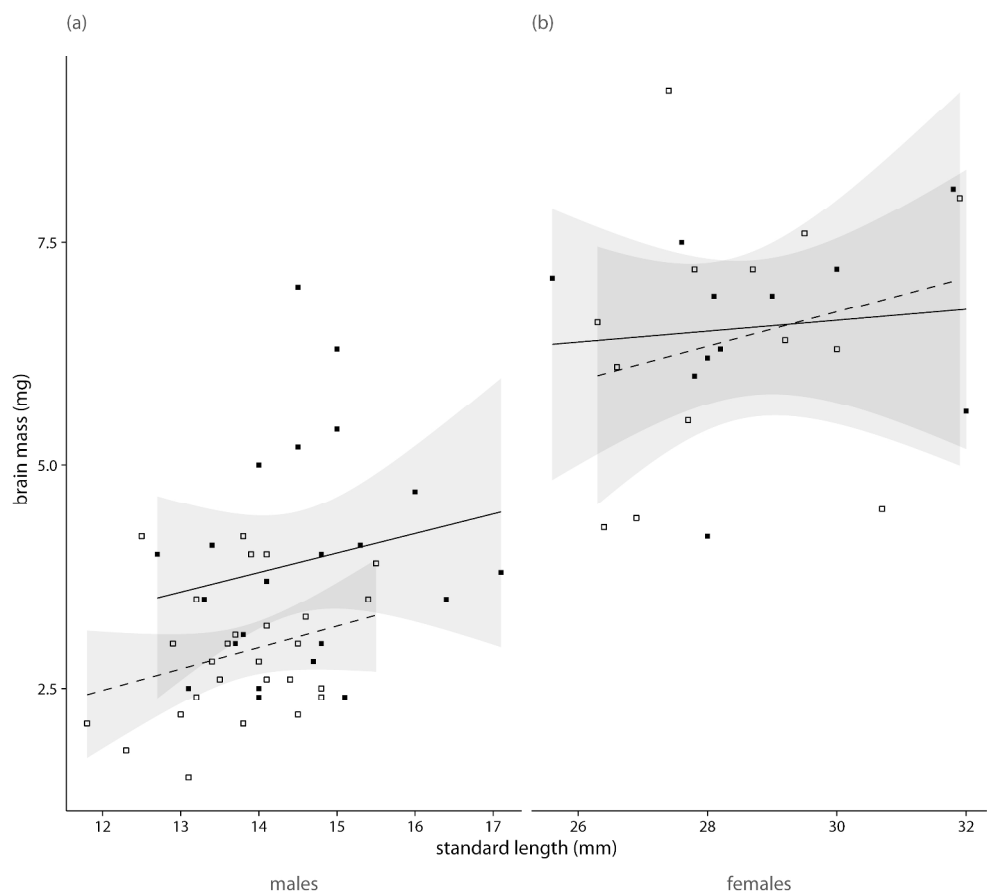


Expected (log transformed) brain mass (\pm s.e.) of guppies experimentally exposed to predator or control cues during development in captivity. Expected values are for the mean standard length for each sex. Mean brain masses in mg are alongside the points, percent differences between groups are indicated 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.

275x243mm (300 x 300 DPI)



282x249mm (300 x 300 DPI)



282x249mm (300 x 300 DPI)