Predation impacts brain allometry and offspring production in female guppies (*Poecilia reticulata*)

Regina Vega-Trejo[1,2*], David J Mitchell[1], Catarina Vila Pouca[1,3], Alexander Kotrschal[1,3]

[1]Department of Zoology, Stockholm University, Svante Arrhenius väg 18B, 10691, Stockholm, Sweden

[2]Department of Zoology, Edward Grey Institute, University of Oxford, Oxford OX1 3SZ, UK

[3]Department of Animal Sciences: Behavioural Ecology, Wageningen University & Research, 6708 WD Wageningen, Netherlands

[*]corresponding author: reginavegatrejo@gmail.com

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Abstract

Survivorship under predation exerts strong selection on reproductive traits as well as on brain anatomy of prey. However, how exactly predation and brain evolution are linked has not been resolved as current empirical evidence is inconclusive. This may be due to predation pressure having different effects across life stages and/or due to confounding factors in ecological comparisons of predation pressure. Here, we used adult guppies (*Poecilia reticulata*) to experimentally test the impact of a period of strong predation on brain anatomy and reproduction of surviving individuals. We compared the survivors to control fish, which were exposed to visual and olfactory predator cues but could not be predated on, and found that predation impacted the relative size of female brains. This effect was dependent on body size as larger female survivors showed relatively larger brains, while smaller survivors showed relatively smaller brains when compared to control animals. There were no differences in male relative brain size between the treatments, nor for any specific relative brain region sizes for either sex. Moreover, survivors produced more offspring, but did not show shorter interbrood intervals than controls. Our results corroborate the important, yet complex, role of predation as an important factor behind variation in brain anatomy.

Introduction

Predation pressure is a key ecological factor in shaping the evolution of morphological, physiological, behavioural, and life-history traits (Reznick & Endler 1982; Lima & Dill 1990; Heinen-Kay & Langerhans 2013). Predators drive prey trait evolution over generations through non-random mortality (natural selection) or elicit changes in morphology, behaviour, or decision-making processes within a generation (phenotypic plasticity; Lima & Dill 1990; Kondoh 2010). One key trait for which predation has been identified as an important evolutionary selective force is vertebrate brain size (Burns & Rodd 2008; Kotrschal *et al.* 2015; van der Bijl *et al.* 2015; Walsh *et al.* 2016). This is because individuals may differ in their ability to assess predators due to differences in cognitive abilities associated with brain size (Striedter 2005; Moller & Erritzoe 2014; Samuk *et al.* 2018). Larger-brained prey could be more effective at avoiding predators given their likely better ability to alter their behavioural responses to specific predator encounters (Shultz & Dunbar 2006). Conversely, predicting the likelihood of a predator attack requires gathering and processing information, at which individuals with larger brains might be better (Moller & Erritzoe 2014; van der Bijl *et al.* 2015). Correlational studies in birds have shown that species with larger brains experience lower rates of adult mortality when compared to smaller brained species (Sol *et al.* 2007). Another link between predation and brain anatomy in birds is flight initiation distance. This indicator for assessing and evading predation threat is shorter in species with relatively larger brains (Moller & Erritzoe 2014). Potentially, larger-brained birds benefit from being able to better assess the risk, reducing the costs associated with fleeing too often, while remaining safe (Sol *et al.* 2005).

The relationship between predation and the brain is not straightforward, with ecological comparisons showing evolutionary effects varying in magnitude, direction, heritability, and with sex (Gonda *et al.* 2012; Gonda *et al.* 2013; Kotrschal *et al.* 2015; Walsh *et al.* 2016; Samuk *et al.* 2018). For instance, in guppies artificially selected for large and small relative brain size (i.e. brain size relative to body size), large-brained females survived longer under predation in a semi-natural setting, while no effect was found for males (Kotrschal *et al.* 2015). However, male but not female guppies exposed to cues of predation

risk in the laboratory or actual predation risk in the wild developed heavier brains (Reddon *et al.* 2018). Predator-prey comparisons across different fish species have also revealed that larger brained predators tend to prey on larger-brained prey, but that prey's relative brain size is larger than that of the predator (Kondoh 2010).

On the other hand, large brains may not always be beneficial in a predatory environment as high metabolic costs (Niven & Laughlin 2008) can outweigh the cognitive benefits of larger brains. Indeed, in Trinidadian killifish (*Rivulus hartii*), males from sites with large piscivores, which predate on adults, have evolved smaller brains when compared with fish from sites that lack piscivorous fish (Walsh *et al.* 2016). In contrast, when comparing sites that differ in juvenile predation, there was no association between predation and adult brain size (Beston *et al.* 2017). Moreover, sticklebacks from complex marine environments characterized by high levels of predation and lower prey densities have smaller brain size than fish from simple pond environments that lack predators (Gonda *et al.* 2011). Similarly, experimental exposure to predators in sticklebacks resulted in smaller relative brain sizes when compared to control populations (Samuk *et al.* 2018). One cause of variation in the effect of predation in ecological comparisons may be other environmental factors. For instance, in studies of the guppy (*Poecilia reticulata*), high- and low-predation populations are usually separated by waterfalls which larger predatory fish cannot cross. Predation also drives population demographics by reducing densities, and by changing intra and interpecific competition dynamics (Magurran & Phillip 2001; Reznick *et al.* 2001; Reznick *et al.* 2012). These factors are likely to affect brain size through energetic trade-offs (Isler & van Schaik 2006), and provide an alternate explanation for the higher growth rates and reproductive productivity shown on high predation fish (Arendt & Reznick 2005). Hence, despite a wealth of data showing correlations between predation and aspects of brain anatomy, empirical evidence for a direct effect of predation on the brain is currently lacking.

We examined how predation impacts brain morphology and reproductive traits, while controlling for non-lethal effects of visual and olfactory predation cues. All individuals were bred and raised in similar conditions and were sexually mature young adults when exposed to a predator for the first time. We focused on the effect of direct removal by predators while controlling for potential foraging effects by providing food '*ad libitum*',

allowing us to test the link between survival and brain size driven by direct predation in the adult stage. Guppies are livebearing fish that naturally occur in streams where the presence and abundance of predators differ, mainly cichlids and killifish (Reznick & Endler 1982), and where predation pressure has been shown to correlate with brain anatomical differences (Kotrschal *et al.* 2017c). While we expected predation to impact brain anatomy in our experiment, predation likely also impacts the life-history traits of surviving animals. For example, killifish from high predation populations allocate more energy to reproduction than those from populations that lack predators (Walsh & Reznick 2008). Guppies are a classic system for investigating how predation pressure impacts life history traits (Reznick & Endler 1982), and it is well established that guppies from high predation sites mature at an earlier age, have higher reproductive effort per pregnancy, and have more but smaller offspring per brood in high compared to low predation populations (Reznick *et al.* 1990; Reznick *et al.* 1996). Moreover, larger brain sizes may be negatively associated with reproductive effort due to the costs associated with maintaining a large brain (Isler & van Schaik 2006). However, which aspects of predation underlie differences in reproductive output, and how they may relate to brain size investment, are not well resolved.

We predicted that fish surviving predation events would reproduce faster and have more offspring, in line with previous life-history evidence in guppies (Reznick & Endler 1982). We also predicted survivors would have a larger relative brain size, and more specifically larger structures related to perception or learning (in particular telencephalon and optic tectum), than fish from the control treatment. This is because the telencephalon is associated with spatial learning and memory in fish (Broglio *et al.* 2003), which could increase the accuracy or speed of decisions. Similarly, elelctrical stimulation in the the optic tectum elicits coordinated body movements and motor patterns (Broglio *et al.* 2003), that would allow individuals to have a better response to avoid predators. Both brain regions have been shown to be positively associated with predator pressure in the wild (Kotrschal *et al.* 2017b).

Methods

We examined the effect of direct predation on relative brain size, as well as on reproductive traits (number of offspring and interbrood interval), by comparing fish that were exposed to visual and olfactory cues but could not be predated (control treatment) with fish that survived exposure to a predator (predation treatment).

We used laboratory descendants of Trinidadian guppies originated from large high predation populations, but that have not been exposed to predators for 16 years. We set up 110 breeding pairs, from which individuals for three replicates were produced, and we additionally included 100 fry from the stock population. Fry were kept in 4L tanks until their sex could be identified (females by their gravid spot, and males by the presence of a modified anal fin called a gonopodium). Mature individuals were kept in single-sex 50L tanks until the start of the experiment. For each replicate, a total of 200 mature individuals of each sex were used; 180 individuals were randomly selected for the predation treatment and 32 individuals for the control treatment, and we conducted three replicates per sex.

The experimental tank was 120×110×70 cm, filled with 220L of water, with a bottom layer of multicoloured limestone gravel (3-8 mm grain size) with which we crafted areas of different depths, so that the water depth ranged from 5-17 cm (Fig. 1). The shallow area provided a refuge for the guppies where the predator could not hunt. One cichlid (*Chrenicicla alta*) was placed at the deepest area of the tank and provided a clay pipe as a shelter (Fig. 1). The cichlids used were acquired through the aquarium trade and fed with live guppies prior to the experiment. Note that this is a sister species to *C. frenata*, the guppy's natural predator. Control fish (16 individuals per tank) were held in two 11L transparent tanks which were located at each side of the experimental tank. We installed two Eheim filter pumps (60L $\times$ h^{-1} per pump) outside the 11L tanks and directed the water flow into each of these 11L tanks to provide olfactory cues for the control fish. Thus, control fish had visual exposure to the cichlid, to the behaviour and density of guppies in the full tank, and were exposed to the same water. This setup had the potential limitation that control fish were in a more constrained area, which *may* affect their growth and development. However, we prioritised standardising the visual and olfactory cues of predation which are known to have strong developmental effects on guppies (Torres-Dowdall *et al.* 2012; Ghalambor *et al.* 2015) , including on their relative brain size (Reddon

166*et al*. 2018). Thus we effectively controlled for these effects to better focus on selective
167survival.
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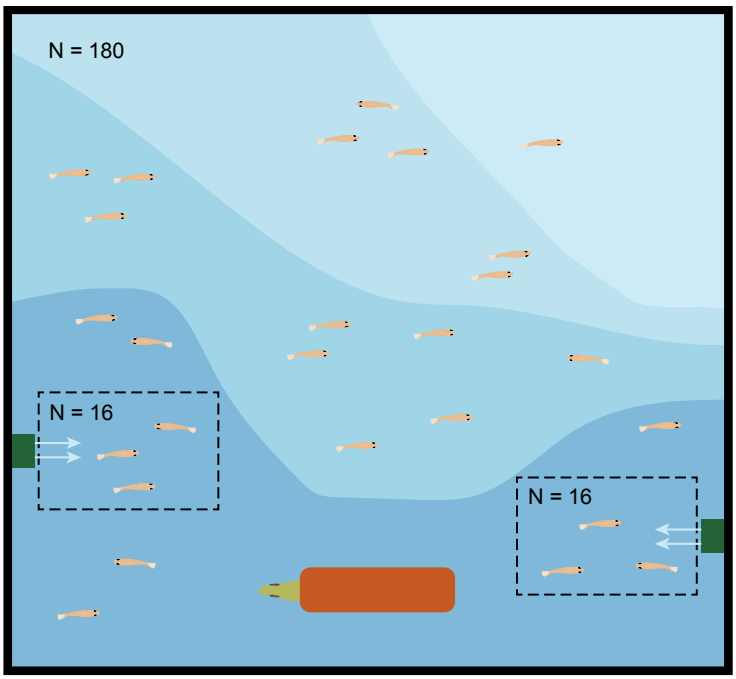

169
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171**Figure 1.** Set up of the experimental tank (view from above). Fish from the predation
172treatment were allowed to swim freely in the tank (N=180), whereas control fish were
173placed in 11L transparent tanks (N=16 each, shown in dashed squares) to provide visual
174cues, with a filter pump that allowed water to get into the tank to provide olfactory cues.
175Water flow from the pumps is indicated with arrows. A predatory cichlid was placed at the
176deepest area with a clay pipe for shelter. Different shades of blue represent different
177depths.
178
179 During the first two days of the experiment, we placed a mesh enclosure around the
180cichlid so guppies could escape easily, acclimatise, and learn about the position and
181potential danger of the predator. This mesh was removed on the third day. We visually
182monitored the amount of fish in the tanks daily. When the number of surviving fish seemed
183to have reached our target (15% of the group, ~ 30 individuals), they were captured with a
184net to be counted. If more than the desired number of survivors was counted, they were
185returned to the tank. Due to logistical constraints, the final percentage of survivors varied
186from 13 to 23% between replicates. The number of weeks fish were in the treatment varied

from 3 to 7 weeks for males, and from 11 to 14 weeks for females. See Supplementary material Section 1 for details. Because one predator showed signs of stress (hiding and very little feeding), it was replaced with another cichlid after 28 days. Fish were kept at 24˚C under a 12:12 light:dark cycle and fed flake food daily and freshly hatched brine shrimp at least three times per week.

Reproductive traits

Once a replicate for each sex was completed, a male and a female from the same treatment (control or predation) were paired up to produce fry. Individuals were placed in a 4L tank with a mesh in the front to allow fry to shelter. Tanks were then checked daily (or every other day due to logistical constraints) and the number of fry and date of birth was noted. Fish were allowed to breed between 15 and 24 weeks. Note that the variation on time allowed to breed depended on the number of fry produced, as a minimum number of offspring were needed for another experiment. After breeding, fish were euthanised with an overdose of benzocaine and fixed with 4% formalin in buffered phosphate buffer saline (PBS) solution.

Body size and brain measurements

To test for differences in body size between treatments, photographs of fish were taken before and after the predation event by placing 30 fish at a time in a 4L tank with 2cm of water and photographed from above with a Nikon DSLR camera. Images were then measured using Image J software (Abramoff *et al*. 2004) to obtain individual's standard length (from the tip of the snout to the end of the caudal peduncle).

To test for differences in relative brain size and relative brain regions, 12 individuals from each sex, replicate, and treatment group (N=144) were randomly selected and their standard length was measured to the nearest 0.01 mm using a digital calliper. Fish were then placed under a dissection microscope (Leica MZFLIII) and brains dissected and stored in PBS. To quantify brain region volumes, brains were photographed from the dorsal, ventral, left, and right side under the dissection microscope with an attached digital camera (Leica

DFC 490) and then weighed to the nearest 0.01 mg (VWR SM-425i-C precision scale). The length, width, and height of the olfactory bulbs, telencephalon, optic tectum, hypothalamus, cerebellum, and dorsal medulla were then measured using Image J following (Kotrschal *et al.* 2012). See Supplementary material Section 7.2.1 for details. The volume of each of the brain regions was estimated using: $V = (L \times W \times H)\frac{\pi}{6}$. All body size and brain measurements were taken blind to treatment.

Statistical analyses

We first tested whether the likelihood of producing offspring varied between survivor fish from the predation treatment and control fish using a generalised linear model (GLM, binomial distribution) using *lme4* (Bates *et al.* 2015), with treatment and replicate as predictors. We then evaluated whether the number of offspring differed between treatments using a GLM (Poisson distribution) for both the first brood and when including all broods. We tested the first brood separately as we expected an immediate response after fish had been exposed to the predator (or its cues for the control fish). To test whether interbrood interval differed between the treatments we ran two linear models with the same predictors as above. We ran one for the time to produce the first brood, and another one for interbrood interval for fish that had more than one brood. Note that for interbrood interval we present models where time between broods was log transformed as this improved normality. When we tested the likelihood of producing offspring across all broods, the number of broods was also added as a predictor, where a maximum of four was included as pairs that produced more than four were very unlikely.

To test whether body size before and after the predation event differed between treatments we calculated the standardised mean difference for each group (SMD, Hedges & Olkin 1985), which is the difference in body size between the time fish started the experiment and when they finished the experiment. Note that fish were not individually marked, and that sample sizes were the same in the control treatment before and after the experiment but different in the predation treatment as at the end of the experiment we could only measure survivors. SMDs for each observation with their associated variances

were used as dependant variables in a random meta-regression model using the *metaphor* package (Viechtbauer 2010). We included treatment and replicate as fixed effects, and observation ID as a random effect. We ran these models separately for males and females as sex differences in guppies are considerable.

To test whether female reproductive success was associated with body size, we used a subsample of 72 females and evaluated whether the number of offspring differed between treatments using a GLM (Poisson distribution), with body size (log-transfomed), treatment, replicate, and total number of broods as predictors. We note that because individuals were not individually marked when fish went into the treatment, the body size measurements were obtained when dissecting the females for brain measurements.

To test for differences in relative brain size and relative brain regions between treatments, we log-transformed body size (mm), brain weight (mg), and brain region volume (mm^3) before the analyses. These analyses were fitted separately for males and females as treatment duration (the time in the predator tank) varied between the sexes (est males = -651, se= 10.970, F = 21.615, p = 0.009), and the highly pronounced sexual dimorphisms make sex comparisons not too meaningful.

To test for differences in relative brain size, we ran a linear model for brain weight, with the predictors of treatment, body size, replicate, and all two-way interactions. Log-body size was fitted as a covariate to account for allometry and focus on treatment differences in *relative* brain size. We checked whether replicate interacted with treatments or allometries, but as these were not parameters of biological interest we removed non-significant interactions from the model (all $p>0.2$).

To test for an effect of predation treatment on relative brain region volumes, we fitted a multivariate linear model for each sex with the predictors of treatment, brain weight, replicate, and all two-way interactions. All interactions in this model were non-significant and uninformative for our research question and therefore removed (all $p>0.9$). We also ran univariate models for each brain region (details for those models and results are available in the Supplementary Material). All statistical analyses were performed in R

v.3.6.1 (R Development Core Team 2012) and model terms were tested for significance using the ANOVA function in the *car* package (Fox & Weisberg 2011) specifying Type III Wald chi-square tests. The model results and code are available in the Supplementary Material – https://osf.io/42cpt/?view_only=540a73672a594597b51d9345d9fea1a1.

Results

Effects on reproductive traits

Surviving fish from the predation treatment were more likely to breed than control fish (97% vs 90%, est = 1.288, se= 0.686, χ^2 = 4.072, p = 0.044), which was consistent across the three replicates (Anova replicate: χ^2 = 0.708, p = 0.702). Across all broods, survivors produced more offspring than controls (est predation treatment = 0.123, se= 0.055, χ^2 = 2955.018, p = 0.025), with a non-significant tendency to vary between replicates (χ^2 = 5.863, p = 2960.053). The number of offspring also differed between broods (χ^2 = 36.322, p <0.001), with an increasing number of offspring in broods two and three. This tendency of survivors producing more fry appeared consistent when considering the first brood only, albeit non-significant likely due to lower power (est predation treatment = 0.128, se= 0.067, χ^2 = 3.645, p = 0.056). The number of offspring differed between replicates (χ^2 = 22.002, p < 0.001). The time to produce a first brood and the interbrood interval when considering all broods did not differ between treatments (χ^2 = 0.158, p = 0.691; χ^2 = 3.695, p = 0.054, respectively). However, these varied between replicates (χ^2 = 14.547, p <0.001; χ^2 = 23.399, p <0.001, respectively). Interbrood interval differed between broods (χ^2 = 308.610, p <0.001), with fish producing broods more frequently with time.

Effects on body size

We found sex-dependent effects of predation on body size. In females, body size differed between treatments, with surviving females being larger than control females (est = 1.322, s.e. = 0.505, Z = 2.619 p = 0.008; Fig. 2a; Suppl. 5.2.2 & 5.2.3). The magnitude of this

312difference in body size varied between replicates (est replicate 2 = 1.094, s.e. = 0.602, Z =
3131.817, p = 0.069; est replicate 3 = 2.673, s.e. = 0.625, Z = 4.277, p <0.001).
314In males, body size between survivors and controls did not differ (est = 0.303, s.e. = 0.187, Z
315= 1.622, p = 0.105; Fig. 2b; Suppl. 5.2.2 & 5.2.4), nor was it affected by replicate (Suppl.
3165.2.4).
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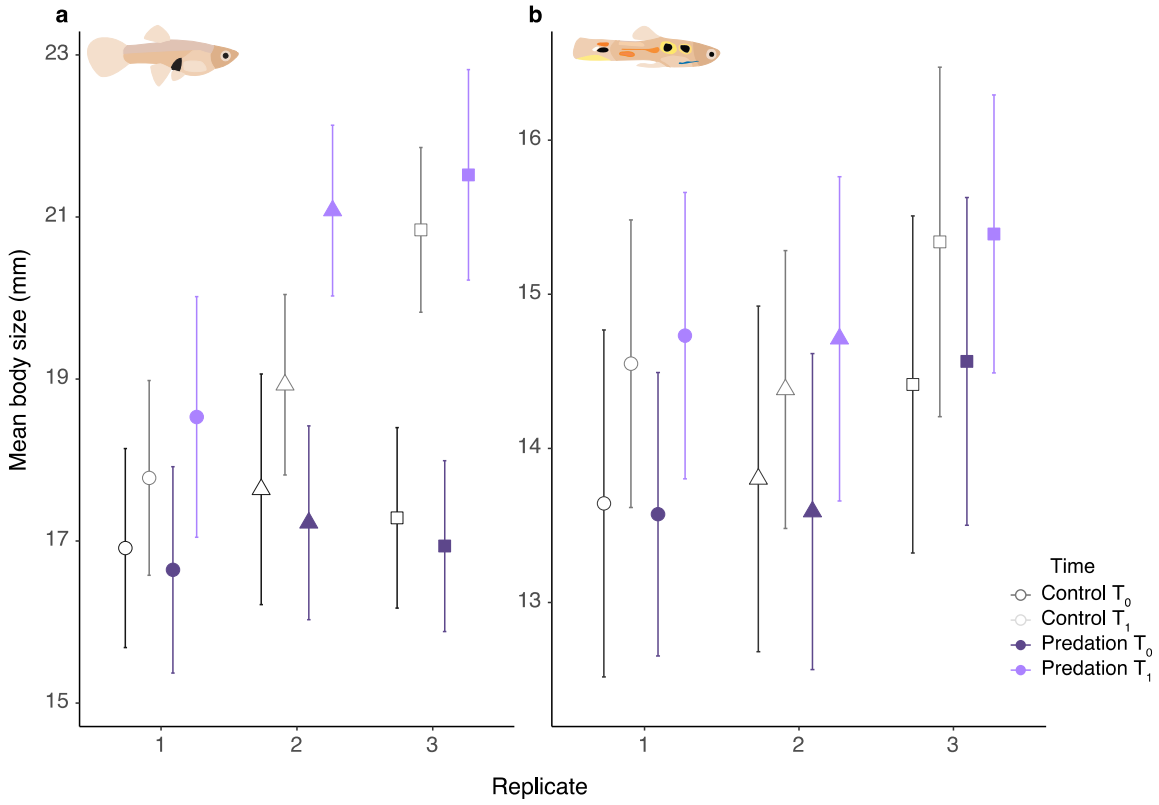

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320**Figure 2.** Raw data for mean body size ± SD for a) females and b) males for control (open
321symbols) and predation (filled symbols) treatment fish. Three replicates were measured for
322control and predation fish before (T_0) and after (T_1) the predation event. Each replicate is
323shown with a different symbol.
324
325*Linking body size and reproductice traits*
326
327Bigger females had more offspring (body size est= 1.397 s.e. = 0.478, χ^2 = 8.572, p = 0.003;
328Suppl. 6), although there was no difference in the allometry between the treatments (est=
3290.067, s.e. = 0.058, χ^2 = 1.343, p = 0.246; Suppl. 6). The total number of offspring produced

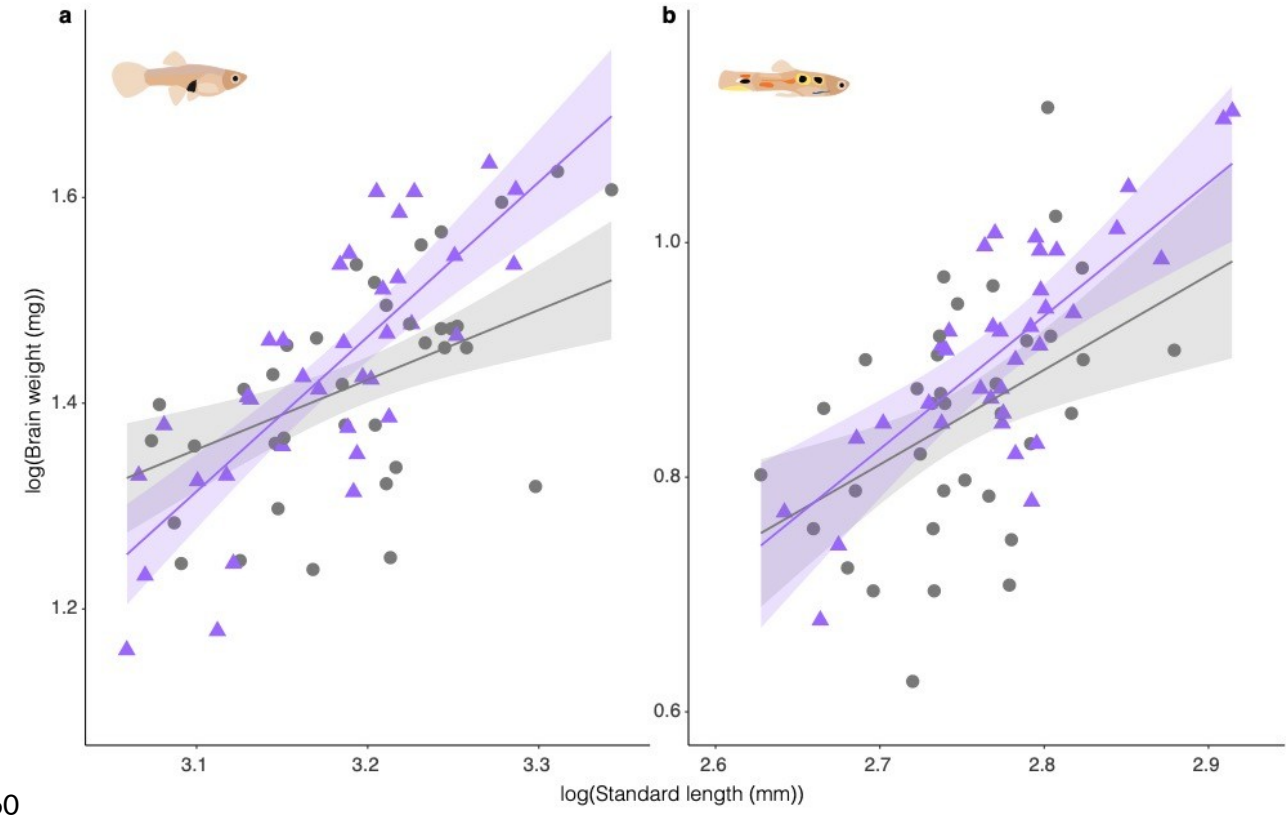
differered between replicates (χ^2 = 12.418, p = 0.002), but in the same direction across replicates. As expected, females having more broods had more offspring (est= 0.523, s.e. = 0.039, χ^2 = 247.5556, p <0.001; Suppl. 6).

Effects on relative brain size

In females, we found that predation impacted the allometry between brain weight and body size, with surviving females having relatively larger brains than controls at large body sizes and relatively smaller brains at smaller body sizes (treatment × body size est = 0.825, s.e. = 0.260, $F_{1,66}$= 19.051, p = 0.002; Fig. 3a). Note that at smaller to intermediate sizes, the difference between surviving females and controls was not as large (Fig. 3a). These results varied between replicates ($F_{2,66}$ = 17.378, p <0.001). In males, we found no difference in the allometry between brain weight and body size between treatment groups (treatment × body size est = 0.328, s.e. = 0.327, $F_{1,66}$= 1.006, p = 0.319; Fig. 3b). Additionally, overall relative brain size was similar between treatments (treatment est = - 0.871, s.e. = 0.902, $F_{1,66}$= 0.933, p = 0.338; Suppl. Section7). We observed a body size effect on relative brain independent of treatment; larger males had relative bigger brains (body size est = 0.807, s.e. = 0.239, $F_{1,66}$= 11.429, p = 0.001; Suppl. Section7). These results varied between replicates ($F_{2,66}$ = 3.672, p = 0.031), but note there was no replicate by treatment interaction.

Effects on relative brain region size

The multivariate analyses of brain region volumes (olfactory bulb, telencephalon, optic tectum, hypothalamus, cerebellum, and dorsal medulla) for females and for males after accounting for the allometries associated with brain weight (females: $F_{6,62}$ = 25.030, p < 0.001; males: $F_{6,62}$ = 13.449, p < 0.001) revealed no effect of treatment on relative brain region volumes for either sex (females: $F_{6,62}$ = 0.479, p = 0.821; males: $F_{6,62}$ = 1.130, p = 0.356). Univariate models of each brain region supported the lack of differences and are available at the Suppl. Section 7.



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362**Figure 3.** Differences in brain size between the control (grey circles) and predation (purple
363triangles) treatments for a) females and b) males. Model predictions are plotted as the best
364fit line.

365

366Discussion

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368We compared fish that survived a predation event (survivors) to fish that were exposed to
369olfactory and visual predator cues only (controls), and found that survivors were more likely
370to reproduce and had overall more offspring in an equal number of broods than controls.
371Importantly, survivor females were bigger and showed a body-size-dependent difference in
372brain size compared to controls. There were no such differences in males associated with
373the treatments; and in both sexes, relative brain region volumes were similar between the
374two treatment groups. Our study provides experimental evidence on the impacts of direct
375predation on brain size and reproductive output.

376

We assessed brain anatomy in adult guppies exposed to strong predation and in control fish where visual and olfactory cues were provided, in an effort to account for phenotypic plasticity to test for an effect of selection. Given the nature of the experiment, several weeks passed between the predation event and the assessment of body size and brain anatomy, and so we cannot fully disentangle the effects of natural selection and phenotypic plasticity. Did non-random predation according to female brain and body size lead to our results, or did the period of actual predation threat trigger alternative developmental pathways? The literature provides evidence for both those mechanisms. For instance, life history traits, growth, and brain anatomy have all been shown to respond rapidly to natural selection (Roff 1992; Stearns 1992; de Winter & Oxnard 2001), and adult guppies show plasticity in all three traits (Reznick 1990; Reznick & Yang 1993; Burns & Rodd 2008). More importantly, plasticiy and evolution/natural selection may not necessarily work in the same direction. Plasticity for instance could lead to lower somatic growth on body size, while the natural selection could increase somatic growth. Evidence suggests however, that at least for brain size in guppies, both plasticity and natural selection seem to be in the same direction (Reddon *et al.* 2018). Partitioning evolutionary from plastic effects was not the aim of this study. Yet, the strength of our approach is that it revealed, independently of ecological confounding factors present in the wild, how life history decisions and brain anatomy 'depend' on an episode of predation. Moreover, this event occurred during adulthood, be it via non-random predation or divergent development. In the following we offer several non-mutually exclusive explanations for our results, including the apparent lack of response in male traits.

The body size difference in survivor and control females that we found can be explained by size-selective predation if the pike cichlid preferentially preyed on smaller females. Although 'high predation' guppies are typically smaller in body size than 'low predation' fish (Reznick *et al.* 2001; Reddon *et al.* 2018), this effect is likely due to the strong selection for maturing early and at smaller size in risky habitats rather than due to size-selective predation (Reznick 1990; Reznick *et al.* 1996). In fact, the relationship between body size and predation seems complex. For instance, males guppies from high predation sites in the wild are smaller than those from low predation sites, but such differences are not apparent for female body size (Reddon *et al.* 2018). Yet, males exposed to predation risk

409cues in the laboratory during development were bigger than those exposed to control cues,
410with female body size showing no response (Reddon *et al*. 2018). In another study, a
411comparison of a laboratory-born generation of guppies from high and low predation
412localities showed that females from high-predation sites grew faster than those from low-
413predation sites (Arendt & Reznick 2005). While *Crenicichla* cichlids often prefer larger prey
414(Johansson *et al*. 2004), predation across prey sizes by cichlids, rather than selective
415predation on large guppies has also been described (Mattingly & Butler 1994). Moreover,
416other predators present in wild populations target smaller size classes (Rodd & Reznick
4171997). In our case, smaller females may have been easier to catch than larger ones by the
418cichlid, since body size is a key factor influencing swimming parameters (Rubio-Gracia *et al*.
4192020), and in our study females were virgin and so were not compromised by swimming
420performance (Banet *et al*. 2016). This hypothesis may also explain why in our study males
421were predated faster than females. Cichlids would have required more (small) males than
422(large) females to reach satiation (as seen by Mattingly & Butler 1994), thus explaining the
423increased consumption rate of males. The lack of body size differences in males between
424the treatments may be explained by the fact that male guppies show almost determinate
425growth with little additional growth after maturation, while females continue to grow
426substantially during adulthood (Constantz 1989; Arendt & Reznick 2005). Due to the large
427number of animals necessary for this experiment, breeding all animals took several months.
428This means that, while all animals were adults, they were between four and seven months
429old. This produced a larger range in female compared to male body size and hence a
430stronger potential to detect size-selective mortality. Alternatively, in a scenario relying on
431phenotypic plasticity, survivor females may have simply grown faster during the time in the
432predation treatment tank. This could be due to the fact that controls and survivors, although
433designed to only differ in the potential for physical contact between guppies and pike
434cichlid, also differed in the space they could utilize. Controls were restricted to smaller tanks
435within the predator tanks whereas survivors could use the larger tank. As growth in fish can
436depend on tank size (Espmark *et al*. 2017), this may have contributed to our results. While
437the lacking body size difference in males may indicate such a secenario is unlikely, the near-
438determinate male growth explained above may render this counter-argument invalid. It is
439hence evident that dedicated growth experiments in the set up used here, but without a

predator, are needed to conclusively clarify the mechanism by which females that survive predation are larger than controls.

The brains of female guppies were affected by the predation treatment. This resembles results from a previous study where a large-scale survival experiment under semi-natural conditions showed that relative brain size determined survival in females, but not in males (Kotrschal *et al.* 2015). However, the results we report here deviate from this previous study and from our predictions as we did not find a clear effect of predation on relative brain size. Instead, predation changed the slope of the regression between female brain and body size, which resulted in relatively smaller brains in small survivors but relatively larger brains in large survivors, compared to controls. This was unexpected but interesting, as it suggests that brain-size derived cognitive advantages (e.g. Kotrschal *et al.* 2013; Benson-Amram *et al.* 2016; Buechel *et al.* 2016) may be size-dependent under predation pressure. Relatively larger brains might indeed provide cognitive advantages if indeed a relatively larger brain helps to avoid getting eaten (Moller & Erritzoe 2014), but may also be costly to maintain. Thus, differential selection of predators (e.g. Johansson *et al.* 2004), or different escape strategies used by small and large fish may be causing the allometric effects we found. Such body size-dependent effect of predation on brain size has been shown in male killifish when comparing high predation sites versus sites with no predators (Dunlap *et al.* 2019), but seems absent in other studies relating predation pressure to brain size (Walsh *et al.* 2016; Reddon *et al.* 2018; Mitchell *et al.* 2020). This may be due to a mix of differently-sized predators in the wild, either across predatory species or from changes with age/size of gape-size limited predators like *Crenicichla*.

Variation in specific brain regions may play a fundamental role in how animals respond to their environment, and indeed it has been shown that changes in specific brain regions can be associated with predation risk (Joyce & Brown 2020). Despite predicting differences between treatments on specific brain regions such as the telencephalon and optic tectum as they are associated with learning and motor functions (Broglio *et al.* 2003), we found no such effect. There is indeed contradictory evidence on the link between predation and brain size (Gonda *et al.* 2009; Kotrschal *et al.* 2015; Walsh *et al.* 2016; Beston *et al.* 2017). Indirect ecological consequences such as density, or food availability were not

expected to influence our results, and these have likely contributed to both negative and positive results in previous studies, highlighting the multiple mechanisms by which predation may shape the brain and its regions' size.

We found a higher likelihood to reproduce and higher reproductive output of surviving females compared to controls. Our results support, at least partially, an increased reproductive effort under high predation pressure. This may be a direct consequence of the body size differences in females of those groups, as body size is a strong predictor of reproductive output in fish (Lim *et al.* 2014). However, high mortality rates in guppy localities predated by the pike cichlid have been associated with a higher investment in reproduction (Reznick 1990; Reznick *et al.* 1996). It is possible that we did not find more differences in reproductive effort between treatments because the risk of predation was no longer present when fish were allowed to breed. This is because the costs of behaviours related to acquiring a mate, conspicuous displays, or the costs of gravid females having lower agility (Magnhagen 1991) were removed when fish were moved to the breeding tanks. Interestingly, larger surviving females had bigger brains, and although we cannot disentangle fully male and female contribution to reproductive effort, it seems like large females with larger brains do not exhibit a larger brain investment at the cost of reproductive effort.

Predation may lead to differences in brain size across species (Moller & Erritzoe 2014), and even to a large degree of variation among similar populations on the same species (Burns & Rodd 2008; Walsh *et al.* 2016; Kotrschal *et al.* 2017a). Here we show that predation impacts brain size likely through direct lethal effects, and uncover a sex- and body-size dependent effect. Our results highlight the need to explore the complex effect of predation on brain evolution further, and ultimately incorporate cognitive assays to understand whether individuals evolve larger brains and better learning capacities to avoid predators.

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Conflicts of interest

We declare we have no conflict of interest.

Ethics approval

This research was approved by the Stockholm Ethical Board (Dnr: 11627-2019).

Availability of data and code

Data and code ara available at https://osf.io/42cpt/?view_only=540a73672a594597b51d9345d9fea1a1.

Authors' contributions

R.V-T and A.K conceived the study, R.V-T collected the data aided by D.J.M and C.V.P. R.V-T analysed the data and wrote the first version of the manuscript. All authors provided feedback on earlier versions of the manuscript.

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