Visual and olfactory cues of predation affect body and brain growth in the guppy

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Abstract

1. Phenotypic plasticity requires animals to acquire reliable environmental information. When multiple sources of information agree, cues should be perceived as reliable and induce a relatively strong response. Conversely, where stimuli conflict, animals must weigh the accuracy of the sources of information and responses should be reduced.
2. Availability of reliable information is often considered a limitation on plasticity, yet how animals integrate seemingly contradictory or incomplete information remains enigmatic, as empirical tests are scarce.
3. We tested how incomplete information determines phenotypic plasticity by simulating predation risk during early ontogeny of guppies (*Poecilia reticulata*). We exposed guppy fry to a combination of visual and/or olfactory cues of the predatory pike cichlid (*Crenicichla alta*), and monitored growth of the body and brain. After five weeks of exposure, guppies were returned to common no-risk conditions and their activity rates were monitored for four weeks post-treatment.
4. Visual predator exposure more strongly affected development; reducing body size of adult males and increasing brain size in females. However, there was little evidence for the hypothesised additive effect, with the combined treatment not inducing a larger effect than when only receiving olfactory or visual treatments.
5. While there was consistent individual variation in activity rates, this was unaffected by developmental risk and uncorrelated with the growth parameters.
6. Our results demonstrate the differential reliability of cues during development. Visual exposure to a predator was a highly reliable environmental cue, while environmental certainty was unaffected by combined stimuli.

Key words: Behavioural plasticity, developmental plasticity, animal personality, pace of life syndrome, U-model.

Introduction

Environmental conditions are constantly changing. While many factors change predictably (e.g. seasonal change), other factors are much less predictable, such as movement and density of predators. Accordingly, animals survey the environment to gather information which informs the development of various phenotypic traits, including behaviour (Stein et al., 2018; Urszán Tamás et al., 2018), life-history (Torres-Dowdall et al., 2012), and morphology (Agrawal, 2001). Information often comes from multiple sources which may conflict temporally, for example when parental and individual experience differs (Salinas and Munch, 2012; Stein et al., 2018) or if information is sampled from different cues (e.g. visual, olfactory, auditory, and social cues) (Munoz and Blumstein, 2012). Animals must therefore gauge the accuracy of acquired information, as mismatches between the environmental conditions and expressed phenotype can have high fitness costs (Nussey et al., 2005).

Environmental information processing can be conceptualised as a Bayesian updating process (Stamps and Frankenhuis, 2016). Animals begin life with a prior ‘belief’ of the state of the environment, derived from their ancestors (genetic and transgenerational plasticity), which is updated in response to information from the current environmental state – represented by a likelihood distribution (Stamps and Bell, 2020; Stamps and Krishnan, 2014). Where multiple stimuli indicate the same conditions, this could be represented as high certainty in the environmental state, and thus induce a stronger phenotypic response relative to a solitary cue. Conversely, when stimuli conflict, animals must weigh the relative reliability that the stimulus (or absence of the stimulus) conveys to inform the plastic response (Stamps and Bell, 2020). For example, a recent study found that direct visual cues of a predator had a greater effect on behaviour than indirect alarm and mobbing calls in adult black-capped chickadees (*Poecile atricapillus*) – indicating visual cues were perceived as more reliable (Arteaga-Torres et al., 2020). Such evaluation of the relative certainty of stimuli should become even more important over developmental timeframes and in traits where plasticity is non-reversible, as responses are predictive of future adult environmental state.

Developmental studies of predation usually manipulate one stimulus – often olfactory cues in aquatic systems (Agrawal, 2001; Brönmark and Pettersson, 1994; Urszán Tamás et al., 2018). This stimulus is assumed to provide information about the state of the environment, to estimate the density or relative risk of predators (Stamps and Bell, 2020). By extension, the absence of cues is implicitly assumed to convey low density (or absence) of predators (e.g. Ghalambor et al., 2015), which is often used as the control. Such olfactory cues can also be multifaceted, conveying different pieces of information. In crucian carp (*Carassius carassius*), olfactory cues of a predator lead to a plastic response of increased body depth as a defence against gape-limited predators (Brönmark and Pettersson, 1994), though skin extract cues isolated from tissue extract cues failed to replicate the effect (Stabell et al., 2010). Alternative study designs have manually chased fish to simulate risk (Edenbrow and Croft, 2013), added visual cues (Reddon et al., 2018; Stein et al., 2018), or replicated the social environment to resemble contrasting predation regimes (Rodd et al., 1997). In natural systems, while these cues are not independent (e.g. olfactory cues will positively correlate with visual encounter rate), different life-stages or individuals may occupy different areas and not always receive complete information. Visual encounter rates or olfactory cue concentrations alone may therefore be insufficient to predict the wider environment. Multiple cues could add greater certainty to the perceived environmental state, while also providing subtly different information. Studies which contrast multiple sources of environmental information have been well considered in the immediate environment (Munoz and Blumstein, 2012), though are relatively lacking in developmental plasticity.

In this experiment, we aimed to quantify the effect of conflicting or incomplete information on development, both from different sensory modalities and a temporal change in environmental state. We make use of the guppy system, where there are known effects of high predation – commonly defined as the presence of the Trinidadian pike cichlid (*Crenicichla sp.*) (Magurran, 2005). Pike cichlids target larger guppies (Johansson et al., 2004) which adds a strong selection pressure on life-history traits, increasing growth rates while decreasing size at birth, size at maturity and adult size (Reznick and Endler, 1982; Reznick et al., 1990). Predation risk also has broad behavioural effects, increasing gregarious behaviour (Seghers, 1974), and affecting social structure (Herbert-Read et al., 2017). Fish from high predation environments are slower to habituate to a novel stimulus (Brown et al., 2013) and make decisions slower than low predation fish (Burns and Rodd, 2008).

Accordingly, there are associated changes in brain morphology (though reported effects are variable) (Kotrschal et al., 2017; Mitchell et al., 2020b; Reddon et al., 2018). Artificial selection on brain size has revealed that large-brained fish out-perform small-brained fish in various cognitive tasks (Buechel et al., 2018; Kotrschal et al., 2013), are better able to assess risk (van der Bijl et al., 2015) and survive longer when faced with predation (Kotrschal et al., 2015).

Beyond the pronounced geographic variation in predation regimes (Endler, 1978), there is also considerable temporal variation (Deacon et al., 2018), and dispersal of individuals across predator barriers (Crispo et al., 2006). Accordingly, guppies incorporate environmental information during early development into their phenotypes (Handelsman et al., 2013; Torres-Dowdall et al., 2012), though effects often counter the evolved response (Ghalambor et al., 2015). A previous study to evaluate the effect of predation on development of brain anatomy found an increase in relative brain size – consistent with the ecological comparisons of the same study – yet this difference was limited to males (Reddon et al., 2018). Such alterations in relative brain size can result from broader selection or alterations in life-history – either growth rates or adult size (Rogell et al., 2020). These brain anatomy and life-history traits are energetically costly, with predicted associations with activity and other behaviours which underly acquisition of resources and energy expenditure (Careau et al., 2008).

We exposed guppies to two different sensory cues of the predatory pike cichlid (*C. alta*), so that cues were either combined, or in isolation. We quantified growth in body and brain size throughout the experiment. Fish were then returned to risk free conditions, causing a temporal change in environmental state. We measured activity rates after fish were returned to risk free conditions, to assess if behavioural differences were retained. These data allowed us to test whether olfactory and visual predation cues additively affected developmental plasticity. We expected predator exposed fish to have decreased growth rates and adult size (Handelsman et al., 2013), an increase in brain size (Reddon et al., 2018) and a decrease in activity rates to reduce encounter rates with predators (Stamps, 2007). More specifically, we predicted the combination of visual and olfactory risk to provide greater environmental certainty and therefore elicit a stronger phenotypic response. In the behavioural assays, where change is likely reversible, we expected treatment groups to converge following the completion of the treatment phase of the experiment.

Methods

Experimental design

Wild-type Trinidadian guppies (*Poecilia reticulata*) were sampled from a stock bred population at XXXX. The parents of the focal animals were housed in pairs in nanotanks (12×20×13cm; 4L of water). Each of these tanks contained gravel substrate and Java moss (*Taxiphyllum barbieri*) to provide environmental enrichment, snails (*Planorbis sp.*), and a constant stream of air. Plastic mesh was provided on one side of the tank to provide refuge for fry. Fish were fed twice daily with either flake or live brine *nauplii*.

We checked for fry daily, and when found recorded the date of birth and moved fry to a holding nanotank. Once enough fry were available, fry were photographed (see below) and split into the four treatments. No more than one fry from a mother was allocated to each treatment combination to avoid pseudoreplication.

Developmental treatment

Fish were exposed to the cues of predation for five weeks. Experimental aquaria were designed to expose fry to visual and olfactory stimuli in a full-factorial design (see Fig. 1 for schematic). Fry were held in 16 nanotanks within two pairs of larger tanks (55L). One of the tanks housed a pike cichlid (*C. alta*) that was fed one freshly culled guppy per day to provide predator chemical cues and possibly information on its diet, and the other was vacant (Fig. 1a). The predator was provided shelter in the middle of the tank (shelter also present in the vacant tank). The four nanotanks within the predator tank had visual cues from the predator, while the four nanotanks in the vacant tank were not exposed to visual cues of predation. The two main tanks were connected via two tubes and a pump circulating water between the two tanks, to allow olfactory cues to flow into the vacant tank. Nanotanks from the olfactory treatment had a 1cm hole drilled on the side to allow water to pass, and a fine mesh was glued over the hole to keep fry in. The control nanotanks for olfactory cues were left undrilled. Nanotanks contained between four and seven fish, snails, Java moss, and gravel as substrate.

Fry were reared in this setup for five weeks (see Fig. 1c for timeline), where they were fed daily with hydrated flake food, supplemented with live brine *nauplii* every second day. After five weeks, fish were returned to standard housing conditions and held in groups of 3-4. Fish were sexed based on morphological traits and colouration and kept in single sex groups. A total of four replicates were run from the two setups. 95 fish were used and distributed in the four treatments (Control-Control n = 23; Visual-Control n =24; Control-Olfactory n =24; Visual-Olfactory n =24).

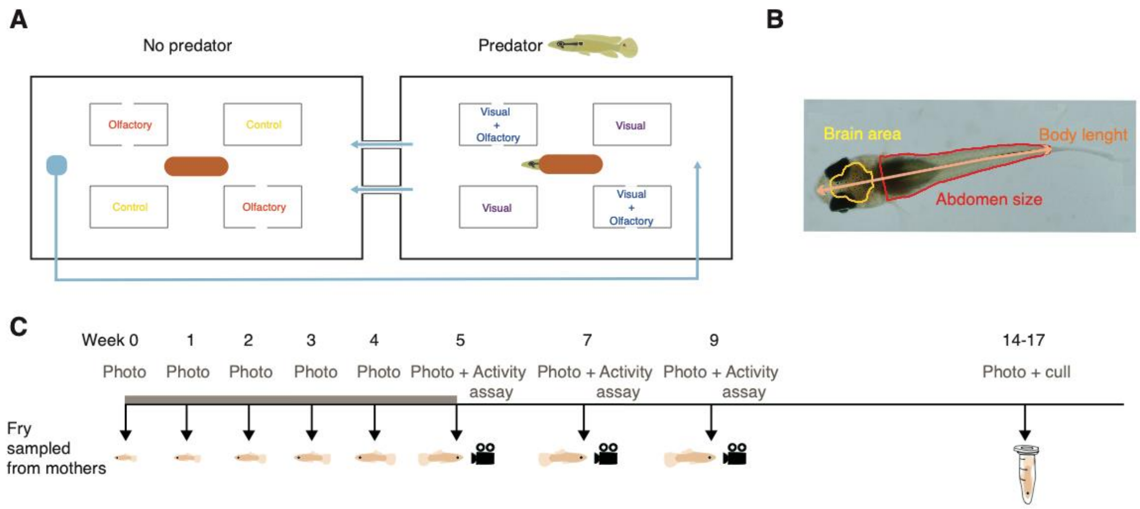

Figure 1. Schematic of the experimental setup (a), with treatments indicated in the nanotanks. The predator was allowed to move freely in its tank. Water was pumped between main tanks (blue arrows) to circulate predator olfactory cues, and holes were drilled in the side of olfactory treatment nanotanks. We quantified growth by measuring body length, abdomen area, and brain area (b). Fish were exposed to the treatment for 5 weeks, we photographed fish nine times across 14-17 weeks to obtain measurements, and recorded activity three times per burst at weeks 5, 7 and 9 (c).

Body and brain growth

To quantify growth rates, photos were taken weekly with a Nikon DSLR camera equipped with a Tamron 90mm macro lens. Fish were lit from underneath and photographed from above to enhance contrast of the brain. A picture was taken prior to putting the fry in the setup and then weekly during the manipulation phase (Fig. 1c). Photos were then taken biweekly during the behavioural phase, and a final photo was taken at the end of the experiment (14-17wks). From each photo, we measured standard length, abdomen area (area of the body from the pectoral fins to the tail fin) and brain area with ImageJ (Schneider et al., 2012)(Fig. 1b). Abdomen area aimed to capture variation in body condition and growth not captured by the length measurements. Individual guppies were identified by manually their characteristic melanic spots on their brain and body (see Castillo et al., 2018). Measurements and identification were all performed by a single experimenter (XX)

Activity assays

After conclusion of the treatment phase (week 5), fish were removed from the set-up to record their activity. To do so, fish were placed individually in small arenas (11×11×9cm) with 200mL of water with Java moss for enrichment and left overnight. The activity assay started at 2pm the next day; Java moss was removed, and the arenas were placed onto a filming stage, backlit with infrared light and left to acclimate for 15 minutes. After acclimation, fish were recorded with a USB camera (ELP-USB 100w05MT-SFV) for 30 minutes. At the end of the trial, fish were returned to the holding rack still in their activity arena. Moss was placed in the arena again and fish were fed with flake. Videos were recorded for three consecutive days, after which the fish were put back into their holding nanotanks in small groups (3-4 fish). Recordings were filmed at 10fps (using OBS Studio) and videos were later tracked with EthoVision XT 10.

This protocol of three activity measures was then repeated for weeks 7 and 9 (i.e. three "bursts" of trials, Fig. 1c). Observations of behaviour taken closely together in time can inflate estimations of individual variation (Mitchell et al., 2020a), so this burst design allows us to better account for potential lack of independence. In the first replicate, 10 fish jumped out of the setup overnight, and subsequently transparent covers were placed on top of the boxes at all times.

Activity rates were measured as the total distance moved for the trial. We analysed a total of N=85 individuals that were recorded during three bursts of observation ($N_{ID:Burst}$ = 255) with three observations per burst (N_{obs} = 765).

Statistical analysis

Growth data

To assess the effect of predation treatments on growth rates, we fit the data to a Unified-Richards growth curve. This equation is a re-parameterisation of the Richards-Bertalanffy model which removes the mathematical dependencies of the different growth parameters (Tjørve and Tjørve, 2017). This facilitates comparability of the estimated coefficients between groups, and correlations of parameters to be assessed among individuals. The change in size (S) as a function of time (t) is given by the equation:

$$S = S_{\infty}\left(1 + \left(\left(\frac{S_0}{S_{\infty}}\right)^{-3} - 1\right) * exp\ \left(-4^{\frac{4}{3}}k * t\right)\right)^{\frac{1}{3}} \qquad (1)$$

where S_0 is the size at birth and S_{∞} is the asymptotic adult size. The growth coefficient is given by k, and in a unified-model can be interpreted as the maximum relative growth rate at the point of inflexion (Thorley and Clutton-Brock, 2019; Tjørve and Tjørve, 2017). This growth curve is selected from Tjørve and Tjørve (2017) and has a shape parameter set as a constant which moves the point of inflexion higher than the standard von Bertalanffy growth equation – a characteristic which offered a much better fit to the data at hand.

The growth model was specified for body length, abdomen area and brain area, with time being the age of the fish in weeks (t). This was fit as a non-linear multivariate mixed model in the Bayesian package brms 3.6.1 (Bürkner, 2017). The S_{∞} and k parameters were fit with the fixed predictors of sex, olfactory treatment, visual treatment and all interactions. As S_0 corresponds to size before any exposure to the treatment, this was fit with the fixed predictor of sex. To evaluate individual variance, identity was fit as a random effect for all three parameters. Correlations between growth parameters were assessed with an unstructured correlation matrix that evaluates all correlations of the three coefficients for the three traits (i.e. a 9×9 matrix).

As all three coefficients are bound by 0 (negative size or growth is impossible), coefficients were log-linked to constrain the model to possible values, and *post hoc* diagnostic plots confirmed this log-normal distribution. Priors were diffuse and directly set on the group means by fixing the global intercept of 0 (see supplementary material for priors and parameterisation). Posteriors were then reorganised to give treatment group comparisons. Residual error was assumed to be normal, though there was a clear pattern of variance expanding. Accordingly, we fit a model for the residual dispersions, with the fixed effect of age.

Behavioural data

Activity rates were log-transformed to achieve normality, then standardised to a mean of 0 and standard deviation of 1. This was fit to the predictors of sex, olfactory treatment, visual treatment, burst, and all interactions. Individual identity (ID) was fit as a random intercept as was the interaction of individual identity by burst (ID:Burst). The separate random effects at ID and ID:Burst thus account for temporal dependence of observations taken closely together in time. This temporal dependence is thus placed in the denominator of the repeatability equation to yield a "long-term" repeatability; $R = \frac{\sigma_{ID}^2}{\sigma_{ID}^2 + \sigma_{ID:Burst}^2 + \sigma_{\varepsilon}^2}$ (sensu Araya-Ajoy et al., 2015).

Growth and behaviour correlations

Finally, we aimed to assess whether activity rates correlated with the growth parameters. Equivalent growth coefficients were highly correlated across the three size variables (see Results), so analyses were limited to correlations of body length and activity rates. We used a character-state model for the activity measures, which fits three separate intercepts of activity (one for each burst), which allowed us to test whether the hypothesised correlations with growth were temporally dependent. This model aimed to quantify the phenotypic correlations, so we removed treatment effects. These three burst observations are correlated to the three growth parameters (see above) yielding a 6×6 matrix.

Results

Predator cues impact growth

The different predator cues impacted male and female growth differently, and appeared limited to the asymptotic size coefficients. In males, the combined effect of the visual and olfactory treatments reduced adult body length, and abdomen area, relative to the control males (no cues). The incomplete information groups (i.e. only visual or only olfactory) were intermediate in body length, but did not differ significantly from control-control or visual-olfactory treatments (Table 1a). This makes it difficult to discern whether the effect was additive (as hypothesised), or constrained by statistical power. Brain area was unaffected by predation risk in males.

Females exposed to the visual stimulus of the pike cichlid had larger brain areas, while there was no effect of the olfactory treatment on brain area. There were additionally only small effects of the treatments on the body growth parameters; the combined treatments increased body size, while there was an insignificant increase in visual only treatment. The olfactory stimulus when presented alone had no effect on female growth relative to the control. However, they were different from when the visual cue was presented. This indicated that the olfactory treatment was perceived as less reliable than the visual treatment.

Combined effects present in body size and brain size indicated that for both sexes, there was an increase in relative brain size. In males, this was driven by a decrease in body size of predator exposed fish, while brain size was unaffected. In females, this was due to an increase in absolute brain size, while effects on body size were small.

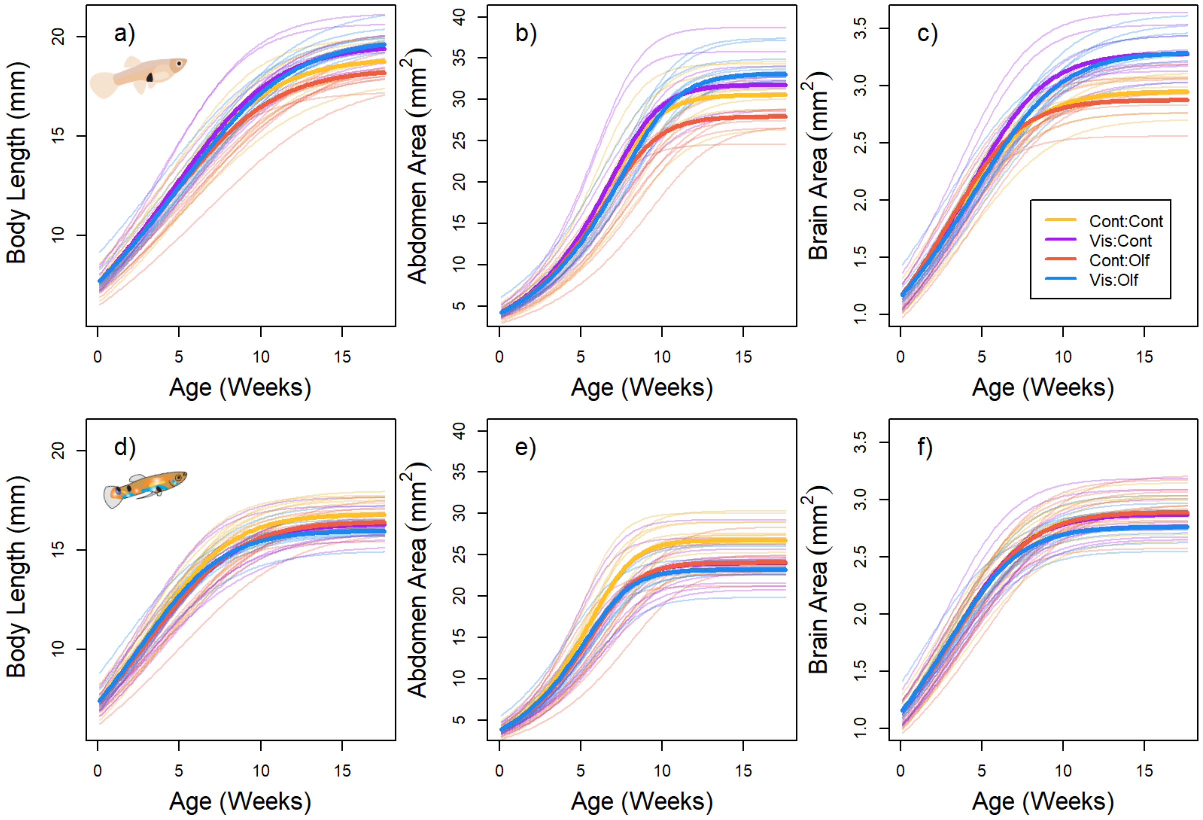

Figure 2: Growth curves are shown for body length (a,d), abdomen area (b,e) and brain area (c,f), with females shown on top (a-c) and males on the bottom (d-f). Group geometric mean trajectories are overlaid in bold, with thin light colours representing predicted individual growth.

	Parameter		Body Length			Abdomen Area			Brain Area		
			Est	Lower	Upper	Est	Lower	Upper	Est	Lower	Upper
Females	S_0		2.03	2.007	2.052	1.432	1.382	1.482	0.145	0.113	0.176
	k	Int	-2.856	-2.926	-2.785	-2.161	-2.225	-2.096	-2.577	-2.668	-2.488
		Vis:Cont	-0.004	-0.104	0.093	0.004	-0.088	0.097	0.009	-0.11	0.127
		Cont:Olf	-0.035	-0.145	0.07	-0.042	-0.145	0.056	0.087	-0.044	0.215
		Vis:Olf	-0.069	-0.175	0.036	-0.041	-0.181	0.101	-0.094	-0.228	0.04
	S_∞	Int	2.939	2.907	2.971	3.42	3.35	3.49	1.081	1.022	1.142
		Vis:Cont	0.035	-0.01	0.079	0.039	-0.062	0.138	**0.107**	**0.024**	**0.192**
		Cont:Olf	-0.03	-0.075	0.018	-0.09	-0.196	0.017	-0.026	-0.111	0.061
		Vis:Olf	**0.05**	**0.003**	**0.099**	0.081	-0.026	0.189	**0.11**	**0.015**	**0.208**
Males	S_0		1.989	1.968	2.01	1.338	1.29	1.385	0.13	0.101	0.159
	k	Int	-2.655	-2.736	-2.575	-2.009	-2.082	-1.936	-2.523	-2.628	-2.421
		Vis:Cont	-0.063	-0.168	0.045	-0.073	-0.171	0.027	-0.006	-0.137	0.127
		Cont:Olf	-0.072	-0.17	0.029	-0.077	-0.171	0.017	-0.029	-0.153	0.098
		Vis:Olf	0.012	-0.096	0.118	-0.045	-0.144	0.055	0.019	-0.117	0.155
	S_∞	Int	2.821	2.789	2.854	3.287	3.216	3.357	1.057	0.994	1.125
		Vis:Cont	-0.029	-0.074	0.015	**-0.109**	**-0.21**	**-0.013**	-0.003	-0.089	0.083
		Cont:Olf	-0.023	-0.064	0.019	**-0.106**	**-0.197**	**-0.016**	0.004	-0.08	0.085
		Vis:Olf	**-0.051**	**-0.094**	**-0.008**	**-0.143**	**-0.239**	**-0.047**	-0.042	-0.127	0.043

Table 1: Displayed are the fixed effect predictions with 95% credible intervals of the growth equations. The intercept is the reference control ('Cont') for both the visual ('Vis') and olfactory ('Olf') treatments, with the three other combinations of treatments given in relation to this reference group. Parameters that do not overlap with 0 are presented in bold.

Correlations between growth parameters

There were very strong correlations between equivalent parameters in body length and abdomen area (Table 2), which was expected as they are physically and mathematically linked. Correlations of these variables' starting size (S_0) with starting brain size were very high, though for the growth coefficient (k), and adult size (S_∞) estimates were comparatively weak, with correlations much lower than 1. This indicated the potential for brain size to respond independently of body size. Finally, while there were few significant correlations of S_0, k and S_∞ parameters, we found that starting brain size correlated significantly with growth rates (k) of the body size variables.

		Body Length			Abdomen Area			Brain Area		
		k	S_∞	S_0	k	S_∞	S_0	k	S_∞	S_0
Body Length	k	σ = 0.110 0.086 0.136								
	S_∞	-0.078 -0.327 0.195	σ = 0.045 0.035 0.056							
	S_0	0.112 -0.134 0.355	0.167 -0.059 0.38	σ = 0.066 0.056 0.077						
Abdomen Area	k	**0.947** **0.878 0.986**	0.009 -0.262 0.296	0.068 -0.176 0.311	σ = 0.102 0.079 0.128					
	S_∞	0.033 -0.253 0.323	**0.865** **0.737 0.95**	0.109 -0.14 0.347	0.098 -0.204 0.409	σ = 0.096 0.074 0.121				
	S_0	0.179 -0.081 0.418	0.129 -0.101 0.356	**0.966** **0.93 0.988**	0.09 -0.168 0.349	0.115 -0.139 0.353	σ = 0.142 0.119 0.168			
Brain Area	k	**0.595** **0.19 0.859**	0.118 -0.286 0.525	0.297 -0.121 0.672	**0.532** **0.108 0.833**	0.113 -0.302 0.535	0.368 -0.04 0.711	σ = 0.073 0.03 0.115		
	S_∞	0.167 -0.147 0.465	**0.474** **0.191 0.708**	-0.177 -0.448 0.107	0.259 -0.06 0.554	**0.532** **0.257 0.756**	-0.223 -0.486 0.058	-0.095 -0.527 0.398	σ = 0.075 0.055 0.097	
	S_0	**0.384** **0.132 0.61**	0.059 -0.195 0.302	**0.805** **0.686 0.895**	**0.366** **0.117 0.593**	0.127 -0.144 0.381	**0.792** **0.653 0.894**	0.272 -0.156 0.687	0.077 -0.227 0.368	σ = 0.086 0.071 0.103

Table 2: Displayed are the mean correlations with 95% credible intervals of the growth parameters for the three size variables. Parameters in bold denote no overlap of the correlation coefficients with 0. Among-individual standard deviations are presented on the diagonal.

Juvenile predator cues show no strong effects on adult activity rates

Activity rates showed moderate repeatability from weeks 5-9 (*R* = 0.431 [0.327, 0.541]), demonstrating individual variation was stable. Activity generally decreased with time, though there were no clear effects of the treatments (Fig. 3). The control fish appeared to have lower activity rates, but differences among the treatment groups were inconsistent, with the combined treatment (i.e. Visual:Olfactory) being intermediate for females and the visual only intermediate for males. Also counter to our predictions, this effect was restricted to later trials – as such this effect needs to be interpreted with care.

The alternative character state model found variance increased in the last burst, while individual differences were largely maintained – consistent with the reported repeatability. This rank order consistency was very strong for females (for all combinations r > 0.71), though more modest for males (r = 0.37 – 0.57). There were additionally no among-individual correlations of activity rates with the growth parameters for body length in the alternative character-state model (correlation coefficients 0 ± 0.3 NS; see supplementary table).

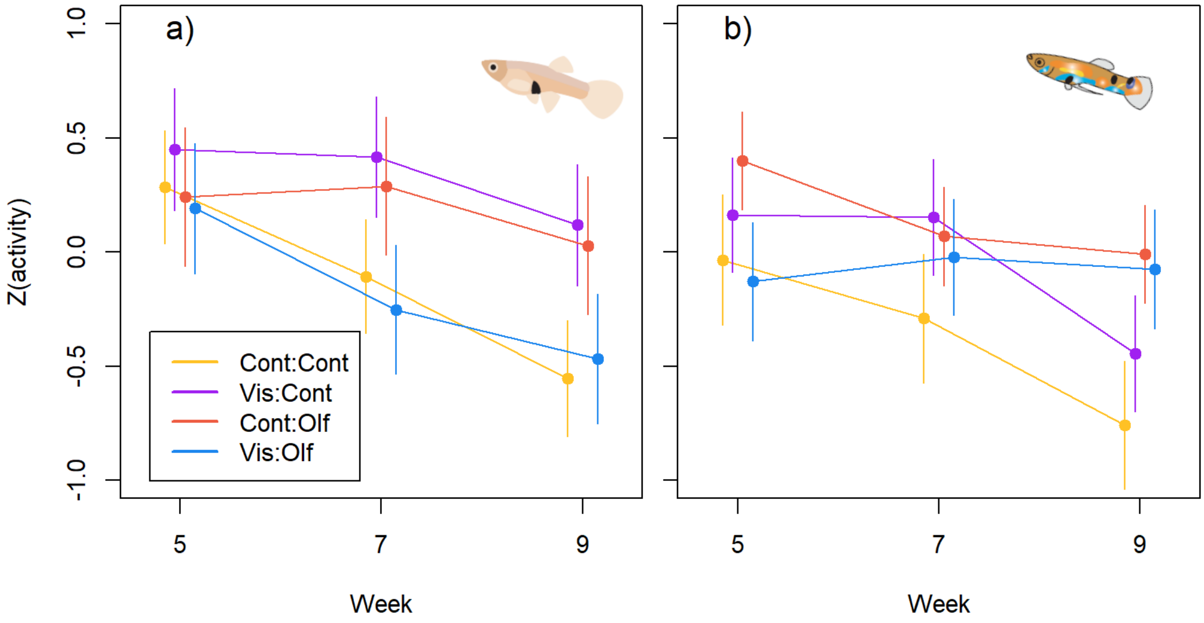

Figure 3: Displayed are mean activity rates for treatment and temporal blocks with error bars representing the standard deviation of the credible distribution.

Discussion

We tested the effect of contradictory or incomplete information on informational state, and resulting developmental plasticity. Exposure to a predator altered adult size in all three of the measured variables, with the effect of the visual cues being stronger than the olfactory cues, indicating this was perceived as a more reliable source of information. However, the treatments did not have the predicted additive effect; we found no clear pattern of a larger response when the visual and olfactory stimuli were combined. The effect of predation on brain size was largely independent of overall body size, though appeared to lead to greater relative size in both sexes. In contrast, there was no effect of the predation treatments on activity rates, which also did not correlate with the growth parameters.

Effect of predation on growth

The visual cue had a strong effect on the development of guppies, increasing female brain size and reducing male body size. This indicates that the visual stimulus was perceived as a highly reliable source of information, which was not enhanced by the addition of olfactory cues. By contrast, the olfactory cue had a smaller effect, reducing male abdomen size, with no effect on females. This low reliability may help to explain the maladaptive effects when olfactory cues are presented alone (Ghalambor et al., 2015).

In manipulations of predator cues, there is an implicit assumption that the absence of cues (i.e. control) indicates that predators are at a low density and the environment is at low risk. Such information would likely be highly valuable to guppies from populations where larger predators are absent face other challenges owing to density regulations, competition and predation of juveniles by killifish (*Anablepsoides hartii*) (Travis et al., 2014). However, the absence of a cue may not be as reliable as the presence of a cue (Stamps and Bell, 2020). This is especially pertinent in cases such as predation, where events and exposure are infrequent and unpredictable, but clustered in time (Taborsky et al., 2020). Accordingly, we found that the visual stimulus had a strong effect – consistent with results over contextual time periods (Arteaga-Torres et al., 2020) – while the absence of complimentary olfactory cues did not reduce the response. Had we have provided a stimulus which indicated no-risk with greater certainty, a reduction of the response may have been more likely. For instance, population demographics which reflect low predation environments can provide developmental cues of a low predation environment (Rodd et al., 1997).

Contrary to previous work showing that exposure to predation risk through development reduces somatic growth rates in guppies (Handelsman et al., 2013), none of our treatments affected the growth parameter (k). This meant that during the predator exposure phase of the experiment, there was no observed difference in size – divergence among treatments occurred after all individuals were returned to no-risk conditions. While compensatory changes in growth trajectories often occur in response to other environmental stressors (e.g. resources shortages or extreme temperatures) (Ali et al., 2003), predation in early ontogeny appeared to canalise later-life phenotype, while not affecting the measured phenotype during exposure. However, comparison to Handelsman et al. (2013) does require a caveat – while all effects of predation on growth rate were insignificant, best estimates were typically negative and we may not be able to reject a false-negative. Further, adult abdomen area appeared more sensitive than length, indicating effects of juvenile developmental stress may have reduced condition.

While studies of the evolved response of brain anatomy to predation pressure have provided variable results (Burns and Rodd, 2008; Kotrschal et al., 2017; Mitchell et al., 2020b; Reddon et al., 2018; Walsh et al., 2016), only one study has previously examined the developmental effects of predation risk (Reddon et al., 2018). These studies have focussed on differences in brain size after being standardised for body size, though results can reflect differences in starting size and growth of body size (Rogell et al., 2020). Here we explored growth in body and brain size as separate but correlated traits. Our results showed that while brain size was very highly correlated with body length and abdomen area at birth (r = 0.81 & 0.79 respectively), brain growth and adult size were less strongly correlated with the body size variables (r = 0.47 – 0.6), potentiating independent responses to predation risk.

Under exposure to predation, females had increased absolute brain size, while body size was largely unaffected – equating to an increase in relative brain size. This is likely an adaptive response, as previous experiments with brain size selected guppies have demonstrated benefits to antipredator behaviour and survival in large-brained females (Kotrschal et al., 2015; Kotrschal et al., 2013; van der Bijl et al., 2015). Males had smaller bodies when exposed to risk – consistent with evolved change from introduction experiments (Reznick et al., 1990) – though brain size was unaffected. This also equates to an increase in *relative* brain size, and is consistent with results previously reported by Reddon et al. (2018). However, due to the lack of behavioural and survival advantages in the face of predation for males (Kotrschal et al., 2015; van der Bijl et al., 2015), and plasticity apparently limited to body size, differences in relative brain size among treatments seems unlikely to be adaptive for males.

No effect of juvenile predator cues on behaviour

We predicted a decrease in activity rates of predator exposed fish, so as to reduce vulnerability and encounter rates (Stamps, 2007), followed by convergence through time as fish updated their behaviour to match the standardised no-risk conditions. While predation risk affected adult size variables, and there was clear evidence for consistent among-individual variation in activity, juvenile exposure to risk did not affect activity rates at 5 weeks old. Given this lack of an initial effect, we did not expect to see treatments affect temporal change through time. However, it was possible we would see divergence after the exposure to predation had ended. Pike cichlids target larger guppies (Johansson et al., 2004), while also reducing population sizes of killifish that prey on juvenile guppies (Travis et al., 2014). Accordingly, the perceived relative risk of our treatments may not have been high for five-weeks old fish. We observed some treatment level differences later in the experiment with control fish being more sedentary, though this did not seem intuitively meaningful as effects were counter to predictions and inconsistent among treatment groups. Further, contrary to the Bayesian models which predict convergence of phenotypes when animals are moved to standardised conditions (Stamps et al., 2018; Stamps and Krishnan, 2017), our character-state model revealed increased among-individual variance at nine weeks old.

Individual variation in activity was largely independent of the growth parameters. In addition to the lack of treatment differences, we found no correlations between activity rates and body length growth. Growth rates slow with age, and correlations may therefore be expressed at younger ages when animals are growing fastest – limiting hypothesised links of growth rates to earlier weeks. For our study, growth was fastest at 5-6 weeks old (burst 1) for males before slowing through time; for females, high growth rates were maintained through to the second burst of assays at 7-8 weeks old. Accordingly, we complimented our analyses of treatment differences with a character-state model which allows us to address potential age-dependence of behaviour-growth correlations (Mitchell and Houslay, 2021). This analysis revealed no correlations between activity and growth parameters. Proposed correlations of life-history productivity and behaviour are built on an assumption that behaviours underly acquisition of resources (Biro and Stamps, 2008; Réale et al., 2010), though alternative allocation models may instead predict negative correlations due to energetic costs (Careau et al., 2008). These two factors are likely to be in balance (Laskowski et al., 2020), but as fish were fed routinely and were non-constrained by resources, the significance of activity to acquisition may have been dissociated.

Concluding remark

In this experiment, we aimed to test the effect of incomplete or contradictory information given by visual and olfactory cues of predation. Both cues affected the growth and morphology of guppies, reducing male body size and increasing female brain size, with effects of visual exposure appearing stronger. However, there was no evidence for a larger response when the stimuli were combined. Further, later life activity rates were also unaffected by exposure to predation. Together, our results indicate that visual and olfactory cues of predation were perceived as highly reliable sources of environmental information, while the absence of these cues as an indicator of low predator density was likely perceived as an unreliable source of information. Future work aiming to reduce the reliability of cues by altering frequency and/or concentration of stimuli, or working in systems where there is more evenly balanced perceived reliability of contrasting cues will be necessary to understand how informational state affects plasticity.

Data availability: Raw data and analysis code are deposited on OSF and will be made available on publication.

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References

Agrawal AA, 2001. Phenotypic plasticity in the interactions and evolution of species. Science 294:321. doi: 10.1126/science.1060701.

Ali M, Nicieza A, Wootton RJ, 2003. Compensatory growth in fishes: a response to growth depression. Fish and Fisheries 4:147-190. doi: 10.1046/j.1467-2979.2003.00120.x.

Araya-Ajoy YG, Mathot KJ, Dingemanse NJ, 2015. An approach to estimate short-term, long-term, and reaction norm repeatability. Methods in Ecology and Evolution 6:1462–1473. doi: 10.1111/2041-210X.12430.

Arteaga-Torres JD, Wijmenga JJ, Mathot KJ, 2020. Visual cues of predation risk outweigh acoustic cues: a field experiment in black-capped chickadees. Proceedings of the Royal Society of London 287:20202002. doi: 10.1098/rspb.2020.2002.

Biro PA, Stamps JA, 2008. Are animal personality traits linked to life-history productivity? Trends in Ecology and Evolution 23:361-368. doi: 10.1016/j.tree.2008.04.003.

Brönmark C, Pettersson LB, 1994. Chemical cues from piscivores induce a change in morphology in crucian carp. Oikos 70:396-402. doi: 10.2307/3545777.

Brown GE, Ferrari MCO, Elvidge CK, Ramnarine I, Chivers DP, 2013. Phenotypically plastic neophobia: A response to variable predation risk. Proceedings of the Royal Society of London 280:20122712. doi: 10.1098/rspb.2012.2712.

Buechel SD, Boussard A, Kotrschal A, van der Bijl W, Kolm N, 2018. Brain size affects performance in a reversal-learning test. Proceedings of the Royal Society of London 285. doi: 10.1098/rspb.2017.2031.

Bürkner P-C, 2017. brms: An R package for Bayesian multilevel models using Stan. Journal of statistical software 80:1-28. doi: 10.18637/jss.v080.i01.

Burns JG, Rodd FH, 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.

Careau V, Thomas D, Humphries M, Réale D, 2008. Energy metabolism and animal personality. Oikos 117:641-653. doi: 10.1111/j.0030-1299.2008.16513.x.

Castillo GC, Sandford ME, Hung T-C, Tigan G, Lindberg JC, Yang W-R, Van Nieuwenhuyse EE, 2018. Using natural marks to identify individual cultured adult delta smelt. North American Journal of Fisheries Management 38:698-705. doi: 10.1002/nafm.10066.

Crispo E, Bentzen P, Reznick DN, Kinnison MT, Hendry AP, 2006. The relative influence of natural selection and geography on gene flow in guppies. Molecular Ecology 15:49-62. doi: 10.1111/j.1365-294X.2005.02764.x.

Deacon AE, Jones FAM, Magurran AE, 2018. Gradients in predation risk in a tropical river system. Current Zoology 64:213-221. doi: 10.1093/cz/zoy004.

Edenbrow M, Croft DP, 2013. Environmental and genetic effects shape the development of personality traits in the mangrove killifish *Kryptolebias marmoratus*. Oikos 122:667-681. doi: 10.1111/j.1600-0706.2012.20556.x.

Endler JA, 1978. A predator's view of animal color patterns. Evolutionary Biology 11:319-364. doi: 10.1007/978-1-4615-6956-5_5.

Ghalambor CK, Hoke KL, Ruell EW, Fischer EK, Reznick DN, Hughes KA, 2015. Non-adaptive plasticity potentiates rapid adaptive evolution of gene expression in nature. Nature 525:372-375. doi: 10.1038/nature15256.

Handelsman CA, Broder ED, Dalton CM, Ruell EW, Myrick CA, Reznick DN, Ghalambor CK, 2013. Predator-induced phenotypic plasticity in metabolism and rate of growth:

rapid adaptation to a novel environment. Integr Comp Biol 53:975-988. doi: 10.1093/icb/ict057.

Herbert-Read JE, Rosén E, Szorkovszky A, Ioannou CC, Rogell B, Perna A, Ramnarine IW, Kotrschal A, Kolm N, Krause J, Sumpter DJT, 2017. How predation shapes the social interaction rules of shoaling fish. Proceedings of the Royal Society of London 284:20171126. doi: 10.1098/rspb.2017.1126.

Johansson J, Turesson H, Persson A, 2004. Active selection for large guppies, *Poecilia reticulata*, by the Pike cichlid, *Crenicichla saxatilis*. Oikos 105:595-605. doi: 10.1111/j.0030-1299.2004.12938.x.

Kotrschal A, Buechel SD, Zala SM, Corral A, Penn DJ, 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, Deacon AE, Magurran AE, Kolm N, 2017. Predation pressure shapes brain anatomy in the wild. Evolutionary Ecology 31:619-633. doi: 10.1007/s10682-017-9901-8.

Kotrschal A, Rogell B, Bundsen A, Svensson B, Zajitschek S, Brännström I, Immler S, Maklakov Alexei 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.

Laskowski KL, Moiron M, Niemelä P, 2020. Integrating behavior in life-history theory: Allocation versus acquisition? Trends in Ecology and Evolution. doi: 10.1016/j.tree.2020.10.017.

Magurran AE, 2005. Evolutionary ecology: the Trinidadian guppy. Oxford: Oxford University Press.

Mitchell DJ, Dujon AM, Beckmann C, Biro PA, 2020a. Temporal autocorrelation: a neglected factor in the study of behavioral repeatability and plasticity. Behavioral Ecology 31:222–231. doi: 10.1093/beheco/arz180.

Mitchell DJ, Houslay TM, 2021. Context-dependent trait covariances: how plasticity shapes behavioural syndromes. Behavioral Ecology. doi: 10.1093/beheco/araa115.

Mitchell DJ, Vega-Trejo R, Kotrschal A, 2020b. Experimental translocations to low predation lead to non-parallel increases in relative brain size. Biology Letters 16:20190654. doi: 10.1098/rsbl.2019.0654.

Munoz NE, Blumstein DT, 2012. Multisensory perception in uncertain environments. Behavioral Ecology 23:457-462. doi: 10.1093/beheco/arr220.

Nussey DH, Postma E, Gienapp P, Visser ME, 2005. Selection on heritable phenotypic plasticity in a wild bird population. Science 310:304-306. doi: 10.1126/science.1117004.

Réale D, Garant D, Humphries MM, Bergeron P, Careau V, Montiglio P-O, 2010. Personality and the emergence of the pace-of-life syndrome concept at the population level. Philosophical Transactions of the Royal Society B: Biological Sciences 365:4051-4063. doi: 10.1098/rstb.2010.0208.

Reddon AR, Chouinard-Thuly L, Leris I, Reader SM, 2018. Wild and laboratory exposure to cues of predation risk increases relative brain mass in male guppies. Functional Ecology 32:1847-1856. doi: 10.1111/1365-2435.13128.

Reznick D, Endler JA, 1982. The impact of predation on life history evolution in Trinidadian guppies (*Poecilia reticulata*). Evolution 36:160-177. doi: 10.1111/j.1558-5646.1982.tb05021.x.

Reznick DA, Bryga H, Endler JA, 1990. Experimentally induced life-history evolution in a natural population. Nature 346:357-359. doi: 10.1038/346357a0.

Rodd FH, Reznick DN, Sokolowski MB, 1997. Phenotypic plasticity in the life history traits of guppies: Responses to social environment. Ecology 78:419-433. doi: 10.1890/0012-9658.

Rogell B, Dowling DK, Husby A, 2020. Controlling for body size leads to inferential biases in the biological sciences. Evolution Letters 4:73-82. doi: 10.1002/evl3.151.

Salinas S, Munch SB, 2012. Thermal legacies: transgenerational effects of temperature on growth in a vertebrate. Ecology Letters 15:159-163. doi: 10.1111/j.1461-0248.2011.01721.x.

Schneider CA, Rasband WS, Eliceiri KW, 2012. NIH Image to ImageJ: 25 years of image analysis. Nature Methods 9:671. doi: 10.1038/nmeth.2089.

Seghers BH, 1974. Schooling behavior in the guppy (*Poecilia reticulata*): An evolutionary response to predation. Evolution 28:486-489. doi: 10.1111/j.1558-5646.1974.tb00774.x.

Stabell OB, Faeravaag AC, Tuvikene A, 2010. Challenging fear: Chemical alarm signals are not causing morphology changes in crucian carp (*Carassius carassius*). Environmental Biology of Fishes 89:151-160. doi: 10.1007/s10641-010-9707-9.

Stamps JA, 2007. Growth-mortality tradeoffs and “personality traits” in animals. Ecology Letters 10. doi: 10.1111/j.1461-0248.2007.01034.x.

Stamps JA, Bell AM, 2020. The information provided by the absence of cues: insights from Bayesian models of within and transgenerational plasticity. Oecologia. doi: 10.1007/s00442-020-04792-9.

Stamps JA, Biro PA, Mitchell DJ, Saltz JB, 2018. Bayesian updating during development predicts genotypic differences in plasticity. Evolution 72:2167-2180. doi: 10.1111/evo.13585.

Stamps JA, Frankenhuis WE, 2016. Bayesian Models of Development. Trends in Ecology and Evolution 31:260-268. doi: 10.1016/j.tree.2016.01.012.

Stamps JA, Krishnan V, 2014. Combining information from ancestors and personal experiences to predict individual differences in developmental trajectories. The American Naturalist 184:647-657. doi: 10.1086/678116.

Stamps JA, Krishnan VV, 2017. Age-dependent changes in behavioural plasticity: insights from Bayesian models of development. Animal Behaviour 126:53-67. doi: 10.1016/j.anbehav.2017.01.013.

Stein LR, Bukhari SA, Bell AM, 2018. Personal and transgenerational cues are nonadditive at the phenotypic and molecular level. Nature Ecology & Evolution 2:1306-1311. doi: 10.1038/s41559-018-0605-4.

Taborsky B, English S, Fawcett TW, Kuijper B, Leimar O, McNamara JM, Ruuskanen S, Sandi C, 2020. Towards an evolutionary theory of stress responses. Trends in Ecology & Evolution. doi: 10.1016/j.tree.2020.09.003.

Thorley J, Clutton-Brock TH, 2019. A unified-models analysis of the development of sexual size dimorphism in Damaraland mole-rats, *Fukomys damarensis*. Journal of Mammalogy 100:1374-1386. doi: 10.1093/jmammal/gyz082.

Tjørve KMC, Tjørve E, 2017. A proposed family of Unified models for sigmoidal growth. Ecological Modelling 359:117-127. doi: 10.1016/j.ecolmodel.2017.05.008.

Torres-Dowdall J, Handelsman CA, Reznick DN, Ghalambor CK, 2012. Local adaptation and the evolution of phenotypic plasticity in Trinidadian guppies (*Poecilia reticulata*). Evolution 66:3432-3443. doi: 10.1111/j.1558-5646.2012.01694.x.

Travis J, Reznick D, Bassar RD, López-Sepulcre A, Ferriere R, Coulson T, 2014. Do eco-evo feedbacks help us understand nature? Answers from studies of the Trinidadian guppy. In: Moya-Laraño J, Rowntree J, Woodward G, editors. Advances in Ecological Research: Academic Press. p. 1-40. doi: 10.1016/B978-0-12-801374-8.00001-3.

Urszán Tamás J, Garamszegi László Z, Nagy G, Hettyey A, Török J, Herczeg G, 2018. Experience during development triggers between-individual variation in behavioural plasticity. Journal of Animal Ecology 87:1264-1273. doi: 10.1111/1365-2656.12847.

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 of London 282:20151132-20151132. doi: 10.1098/rspb.2015.1132.

Walsh MR, Broyles W, Beston SM, Munch SB, 2016. Predator-driven brain size evolution in natural populations of Trinidadian killifish (*Rivulus hartii*). Proceedings of the Royal Society of London 283. doi: 10.1098/rspb.2016.1075.

Supplementary Table 1: Univariate activity model.

The following table corresponds to the activity analysis, which underlies Figure 3 in the main text.

				Est	SD[est]	Q2.5	Q97.5
Females	Control	Contol	BurstA	0.285	0.248	-0.206	0.761
	Visual	Contol	BurstA	0.450	0.268	-0.076	0.978
	Control	Olfactory	BurstA	0.241	0.303	-0.353	0.842
	Visual	Olfactory	BurstA	0.191	0.286	-0.377	0.749
	Control	Contol	BurstB	-0.108	0.249	-0.593	0.383
	Visual	Contol	BurstB	0.418	0.264	-0.093	0.938
	Control	Olfactory	BurstB	0.289	0.301	-0.301	0.878
	Visual	Olfactory	BurstB	-0.252	0.283	-0.805	0.300
	Control	Contol	BurstC	-0.554	0.255	-1.052	-0.050
	Visual	Contol	BurstC	0.118	0.265	-0.399	0.641
	Control	Olfactory	BurstC	0.029	0.303	-0.563	0.623
	Visual	Olfactory	BurstC	-0.468	0.284	-1.031	0.098
Males	Control	Contol	BurstA	-0.034	0.284	-0.590	0.517
	Visual	Contol	BurstA	0.163	0.250	-0.330	0.656
	Control	Olfactory	BurstA	0.400	0.215	-0.017	0.824
	Visual	Olfactory	BurstA	-0.129	0.259	-0.653	0.371
	Control	Contol	BurstB	-0.291	0.281	-0.856	0.259
	Visual	Contol	BurstB	0.152	0.253	-0.344	0.644
	Control	Olfactory	BurstB	0.070	0.217	-0.348	0.498
	Visual	Olfactory	BurstB	-0.022	0.255	-0.516	0.483
	Control	Contol	BurstC	-0.759	0.280	-1.317	-0.209
	Visual	Contol	BurstC	-0.445	0.253	-0.953	0.045
	Control	Olfactory	BurstC	-0.009	0.215	-0.430	0.414
	Visual	Olfactory	BurstC	-0.076	0.261	-0.594	0.435
		σ	ID	0.654	0.067	0.533	0.799
		σ	ID:Burst	0.378	0.045	0.291	0.468
		σ	e	0.645	0.020	0.607	0.686

Supplementary Table 2: Character-state model

The following is the table for the full output from the character-state model assessing covariances between the growth parameters for body length and activity rates at 5, 7 and 9 weeks old. Best estimates are given as the mean coefficient with the standard deviation and 95% credible intervals as quantiles.

				Est	SD[est]	Q2.5	Q97.5
Body length	Female		S_0	2.034	0.012	2.010	2.057
	Male		S_0	1.985	0.012	1.961	2.010
	Female		k	-2.905	0.025	-2.954	-2.856
	Male		k	-2.677	0.022	-2.720	-2.633
	Female		S_∞	2.969	0.012	2.944	2.993
	Male		S_∞	2.792	0.008	2.776	2.808
		$ln(\sigma)$	int	-1.186	0.066	-1.314	-1.056
		$ln(\sigma)$	Age	0.022	0.013	-0.002	0.046
	Female	σ	S0	0.069	0.009	0.052	0.089
		σ	k	0.129	0.022	0.091	0.177
		σ	S_∞	0.065	0.010	0.047	0.087
	Male	σ	S_0	0.075	0.010	0.059	0.097
		σ	k	0.123	0.020	0.087	0.166
		σ	S_∞	0.049	0.007	0.037	0.065
Activity	Female		BurstA	0.300	0.128	0.049	0.550
	Male		BurstA	0.138	0.113	-0.087	0.361
	Female		BurstB	0.081	0.134	-0.179	0.343
	Male		BurstB	0.000	0.102	-0.203	0.197
	Female		BurstC	-0.223	0.162	-0.537	0.096
	Male		BurstC	-0.272	0.144	-0.556	0.006
	Female	σ	S_0	0.705	0.100	0.529	0.923
		σ	k	0.734	0.101	0.558	0.951
		σ	S_∞	0.901	0.117	0.698	1.155
	Male	σ	S_0	0.656	0.096	0.486	0.865
		σ	k	0.579	0.090	0.418	0.772
		σ	S_∞	0.899	0.114	0.704	1.146
Correlations	**Females**	S_0	k	-0.071	0.181	-0.412	0.287
		S_0	S_∞	0.279	0.167	-0.069	0.585
		k	S_∞	0.021	0.197	-0.358	0.408
		S_0	BurstA	0.008	0.171	-0.327	0.343
		k	BurstA	0.134	0.183	-0.234	0.473
		S_∞	BurstA	0.006	0.181	-0.346	0.358
		S_0	BurstB	0.078	0.170	-0.258	0.408
		k	BurstB	0.068	0.180	-0.289	0.409
		S_∞	BurstB	-0.039	0.176	-0.374	0.307
		BurstA	BurstB	0.856	0.084	0.656	0.971
		S_0	BurstC	0.162	0.163	-0.165	0.469

	k	BurstC	0.274	0.166	-0.072	0.574
	S_∞	BurstC	-0.139	0.174	-0.469	0.200
	BurstA	BurstC	0.715	0.117	0.448	0.901
	BurstB	BurstC	0.803	0.094	0.583	0.942
Males	S_0	k	0.418	0.176	0.053	0.744
	S_0	S_∞	0.077	0.168	-0.255	0.392
	k	S_∞	-0.255	0.173	-0.565	0.110
	S_0	BurstA	-0.147	0.167	-0.466	0.183
	k	BurstA	-0.222	0.180	-0.557	0.146
	S_∞	BurstA	0.042	0.179	-0.309	0.388
	S_0	BurstB	-0.176	0.168	-0.492	0.160
	k	BurstB	-0.102	0.190	-0.464	0.275
	S_∞	BurstB	-0.129	0.177	-0.464	0.233
	BurstA	BurstB	0.579	0.153	0.246	0.833
	S_0	BurstC	-0.006	0.159	-0.315	0.299
	k	BurstC	0.016	0.176	-0.333	0.359
	S_∞	BurstC	-0.076	0.165	-0.392	0.251
	BurstA	BurstC	0.369	0.157	0.044	0.647
	BurstB	BurstC	0.570	0.143	0.262	0.816