Early predation risk shapes adult learning and cognitive flexibility

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Abstract

Predation risk during early ontogeny can impact developmental trajectories and permanently alter adult phenotypes. Such phenotypic plasticity often leads to adaptive changes in traits involved in anti-predator responses. While plastic changes in cognition may increase survival, it remains unclear whether early predation experience shapes cognitive investment and drives developmental plasticity in cognitive abilities. Here, we show that predation risk during early ontogeny induces developmental plasticity in two cognitive domains. We reared female guppies (*Poecilia reticulata*) with and without predator cues and tested their adult cognitive abilities. We found that females reared under simulated predation took longer to learn a simple association task, yet outperformed animals reared without predation threat in a reversal learning task testing cognitive flexibility. These results show that predation pressure during ontogeny shapes adult cognitive abilities, which we argue is likely to be adaptive. Our study highlights the important role of predator-mediated developmental plasticity on cognitive investment in natural populations and the general role of plasticity in cognitive performance.

Keywords:

Phenotypic plasticity; behavioural flexibility; developmental stress; predator cues; guppy

Introduction

Environmental cues during early life can lead individuals to follow separate developmental trajectories and result in different adult phenotypes (Pigliucci, et al. 2006, West-Eberhard 2003), including key life-history, morphological, and behavioural traits (Agrawal 2001, Dingemanse, et al. 2009, Taborsky 2006). Phenotypic changes in traits may be permanent, where a certain trait trajectory is determined by juvenile conditions irrespective of new environmental cues. In other traits, changes may be reversible and animals can adjust their phenotype to current ecological conditions later in life (West-Eberhard 2003). For instance, in the African cichlid *Simochromis pleurospilus*, food availability during the juvenile period permanently affects major reproductive traits (Taborsky 2006) and cognitive abilities (Kotrschal and Taborsky 2010), but individual growth rates stay plastic and are flexibly adjusted to ambient food availability over time (Kotrschal, et al. 2014).

Predation pressure is one of the most pervasive ecological factors known to generate developmental plasticity in a wide range of traits (Dingemanse, et al. 2009, Ghalambor and Martin 2002, Segers and Taborsky 2012). Developmental plasticity allows individuals to develop a phenotype fine-tuned to predation risk levels perceived during ontogeny, optimising their chance of survival while avoiding the costs of investing into unnecessary anti-predator responses (Agrawal 2001, Lima and Dill 1990). Classic examples of traits that develop in prey species in response to predation pressure are the helmet and tail spine in water flea *Daphnia lumholtzi* (Agrawal 2001) and deeper bodies in crucian carp *Carassius carassius* (Brönmark and Miner 1992), both defensive morphs that impede individuals from being consumed by their predators. Behavioural traits are also likely to increase survival. For example, sticklebacks exposed to predator cues developed a strong correlation between boldness and aggressiveness which is likely adaptive in high predator density habitats since direct predation also favoured the correlation between boldness and aggressiveness (Adriaenssens and Johnsson 2013, Bell and Sih 2007). Certain cognitive abilities may also facilitate survival. Cognitive performance in associative learning or spatial learning tasks has been linked to survival in fish, reptiles, and birds (Dayananda and Webb 2017, Kotrschal, et al. 2015, Madden, et al. 2018). If better cognitive abilities indeed help prey to survive in risky environments, we would predict that animals growing up under high predator pressure should invest into enhanced cognitive abilities. However, direct tests of the effect of predation pressure on the plasticity of cognitive abilities are surprisingly scarce, but are vital to

understanding how predation and stress shape prey cognitive abilities, and ultimately impact fitness.

Plasticity of learning and memory in response to predation has only been observed over short-term experimental manipulations of perceived predation risk with adult fish (Ferrari, et al. 2005) and tadpoles (Ferrari 2014, Mitchell, et al. 2016). In these studies, individuals exposed during short windows to predator and/or conspecific alarm cues show stronger learnt antipredator responses and longer memory retention compared to individuals exposed to a no-risk condition (Ferrari 2014, Ferrari, et al. 2005, Mitchell, et al. 2016). Additionally, it has been shown that male Trinidadian guppies *Poecilia reticulata* exposed to predation cues during development invest in heavier brains relative to their body size, indicating developmental plasticity in neural investment in response to cues of predation threat (Reddon, et al. 2018). While suggesting a role for predation pressure in generating cognitive plasticity, these studies exposed individuals to highly concentrated predation risk cues during a short period of time, which is likely deviating from ecological settings in the wild where predator cues are typically moderate and long term (Clinchy, et al. 2013, Pravosudov 2003). Moreover, it remains unclear whether cognitive plasticity induced by predator experience during early ontogeny persists in adult phenotypes, or if effects are transient and reversible. A broader understanding of the environmental conditions under which different types of plasticity have evolved is highly relevant to understand plasticity and its evolution, as well as to predict how animals will respond to changing environments.

Here, we test whether exposure to predation risk cues that mimic natural conditions during early ontogeny generates developmental plasticity in cognitive abilities that is retained in the adult stage. We reared Trinidadian guppy fry in either a no-risk or a simulated predation risk environment. We then kept all female fish in similar, no-risk environments and subsequently tested their adult performance in two cognitive assays – associative learning and reversal learning. Associative learning tasks are used to test whether individuals learn a simple stimulus-response association – here, approaching a correct colour to retrieve a reward while ignoring the incorrect colour. Reversal learning tasks, commonly used to test for behavioural flexibility, are considered more cognitively demanding since the individual needs to inhibit the previously learnt response, attend to the shift in reward contingencies, and form a new association with the previously unrewarded stimulus (Sutherland and Mackintosh 2016). In addition to differing in cognitive complexity, the two cognitive traits assayed by these tasks are encoded by different brain regions (Chaves and Hodos 1997, López, et al. 2000, Sutherland and Mackintosh 2016) and performance in one task is often uncorrelated

with performance in the other (Buechel, et al. 2018). For example, guppies that were selectively bred for relatively larger and smaller brains learnt an associative task equally well, but large-brained animals were better in learning a reversal task (Buechel, et al. 2018). Environmental cues such as predation risk may therefore generate differential impacts on associative learning and reversal learning. In this study, we assessed (i) if perceived predation risk cues during early ontogeny can generate changes in cognitive abilities that are retained by adult females, and (ii) whether predation has a similar or differential impact on associative learning and reversal learning.

We predicted that predation risk cues will generate persistent changes in cognitive abilities in adult female guppies. However, predicting the exact nature of the effect for both associative learning and reversal learning is difficult. We may predict that predation risk is a moderate stressor that can have beneficial effects on cognitive abilities (Pravosudov 2003). One possibility is that the benefits of having greater cognitive processing under predation threat compensate for its energetic costs (Dunlap and Stephens 2016); thus, we might expect that fish reared under predation cues should learn faster when tested for both associative learning and reversal learning compared to no-risk fish. If animals are able to adjust their cognitive investment to particular traits, we may predict that especially cognitive flexibility is impacted by predation pressure, since flexible behavioural responses should be adaptive under predation threat (Lima and Dill 1990). Alternatively, perceived predation risk may cause chronically elevated stress levels leading to tissue damage and so negatively impact brain development (Sapolsky 1996). In such a 'non-adaptive' scenario we expect no-risk animals to outperform the predator cue exposed animals.

Methods

Developmental treatment

In this study, we make use of the guppy system, well studied for its local adaptation to vastly differing predation regimes (Magurran 2005, Reznick and Endler 1982) but that experiences relatively stable predation pressure over ecological time scales (Deacon, et al. 2018). Guppies used in this experiment were laboratory-reared descendants of fish from a high-predation population from the Quare river in Trinidad, kept in several large populations since 2005. To obtain newborn fry, we haphazardly selected adult fish from mixed-sex stock tanks and housed them as breeding pairs in 4-L aquaria with constant aeration, java moss (*Taxiphyllum* sp.), and water snails (*Planorbis* sp.). We checked daily for fry. Newborns of sufficiently

large clutches (≥ 4) were split across two treatments (no risk and predation risk), ensuring a minimum of two individuals were held together. Clutches of 2-3 siblings were randomly assigned to one treatment only to prevent keeping fry in isolation; solitary fry were returned to a stock tank. All fry were held in groups of two to six in 4-L aquaria with aeration, java moss, and water snails.

Predation risk cues consisted of chemical cues from the pike cichlid *Crenicichla alta,* a very closely related species to the Trinidadian *C. frenata,* which is a natural predator of guppies in the wild (Seghers 1973), and conspecific alarm cues. These cues were collected fresh each treatment day from a 120×110×70 cm aquarium (filled with 220 L of water) housing a cichlid that was fed either freshly culled or live guppies. This water thus contained both kairomones released from the cichlid and olfactory alarm cues from conspecifics. Note the concentrations of those cues aimed to be 'ecologically realistic' and hence were provided at levels lower than in previous guppy studies (e.g. Handelsman, et al. 2013, Torres-Dowdall, et al. 2012). We exposed developing guppies four times a week, at a variable time of day, to either 200mL of stock water (no risk) or 200mL of predation cue water (predation risk) added to their 4-L tanks during the first 101-137 days of life. Following the exposure phase, fish from both treatments were held in common garden conditions in their housing aquaria for 36-50 days (Fig. 1a). Water temperature was kept at 25 ± 1°C and lights were on a 12:12 hr dark/light cycle. Fish were fed twice daily on dried tropical fish flakes and once a week on live *Artemia salina* nauplii (brine shrimp). Experimental fish were photographed for another study, once a week during weeks 1-6 and every second week after 6 weeks old. Fish also ran a three-day open field assay for another study prior to the start of the learning assays. A group of 62 adult females (n = 31 per treatment), which were one-generation offspring of 19 breeding females, was then selected for the cognitive tasks. Only females were used in this experiment as males have been difficult to motivate with a food reward (Fuss and Witte 2019, Kotrschal, et al. 2013).

Learning and cognitive flexibility assays

Focal females were moved to experimental tanks, where they were individually housed for the duration of the learning tasks. Each experimental tank included a home compartment (25×15 cm) and an experimental compartment (15×15 cm) at the front of the tank, which was only accessible during training sessions through a guillotine door (Fig. 1b). Fish were confined to the home compartment outside of training sessions, where they had visual contact

with fish in neighbouring tanks to prevent social isolation. The experimental compartment, however, was visually isolated to avoid social learning effects.

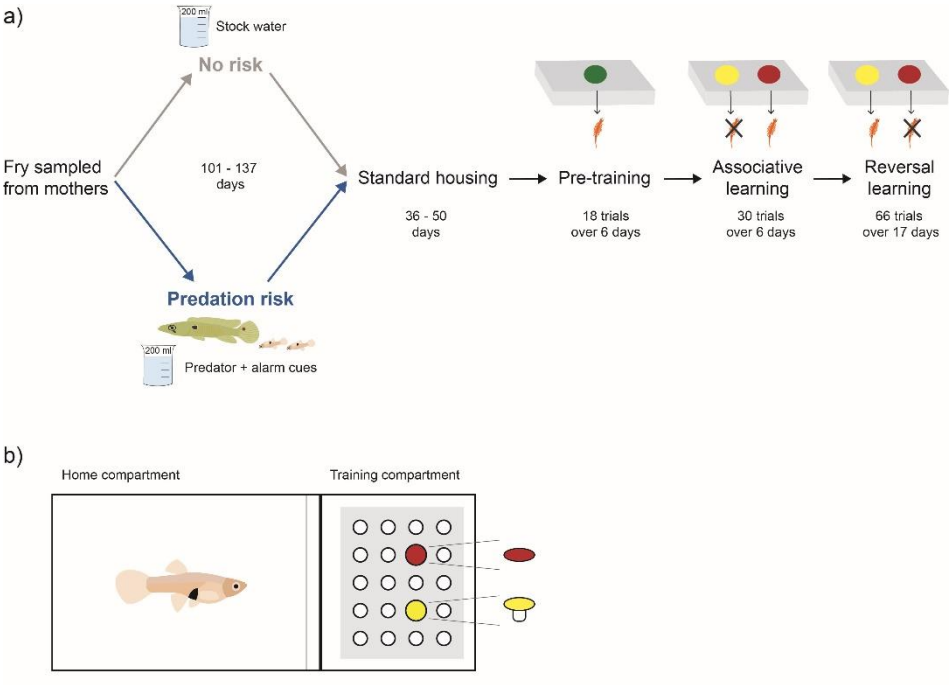

Figure 1. Schematic representation of the developmental treatment and cognitive assays. (a) We exposed developing guppies four times a week, at a variable time of day, to either 200 mL of stock water (no risk) or 200 mL of predator chemical cues and conspecific alarm cues (predation risk) during early development. Fish were then housed in common garden conditions and individually tested in two cognitive tasks, associative learning and reversal learning. (b) The experimental tank for the learning tasks consisted of a home compartment and a training compartment, where fish were given a choice between two coloured discs, one red and one yellow, both concealing a food reward in a hole underneath them. The negative stimulus (here represented in yellow) was fixed in the hole with a plastic knob and could not be moved by the fish to uncover the food item beneath it.

To test for associative learning and cognitive flexibility, we used a well-established colour discrimination and reversal learning assay for fish, using red and yellow as stimulus colours (Buechel, et al. 2018, Fuss and Witte 2019, Lucon-Xiccato and Bisazza 2014). Before the start of the experiment, female guppies (no risk, n = 31; predation risk, n = 31) were haphazardly assigned to either red or yellow as the correct stimulus, balanced across the two treatments. The experimental compartment contained a white plate with 20 identical circular holes (5 mm deep, 10 mm diameter). We started by pre-training guppies to dislodge a green disc to access a food reward (one frozen *Artemia*) hidden in one of the holes. The trial started with the opening of the opaque door; 5 s later, we opened the transparent door. The fish could

then voluntarily enter the training compartment and find the food reward. During the first trials, the disc only partially covered the hole, leaving the reward exposed. We then trained the fish to dislodge the green disc by successively moving the disc from partially to fully covering the hole. Fish ran 3 trials per day for a total of 18 pre-training trials over 6 consecutive days. Nine females were not motivated to feed or to dislodge the disc (no risk, n = 5; predation risk, n = 4) and were excluded from the experiment. It is important to note that these nine females were evenly distributed across the two treatment groups, indicating no apparent motivation differences to participate in the task between no-risk and predation-risk fish. The remaining 53 females (no risk, n = 26; predation risk, n = 27) succeeded the pre-training phase and continued the experiment.

In the associative learning task, fish now had a choice between two coloured discs, one red and one yellow, *both* concealing a food reward (to ensure fish could not be learning through olfactory cues). Only one of the discs could be dislodged by the fish to reveal the reward (positive stimulus). The negative stimulus was fixed in a different hole with a plastic knob and could not be moved to uncover the food item beneath it. For each trial, we randomised the position (left/right) of the correct colour, with the constraint of no more than two consecutive trials in the same position to avoid side biases. Choice was recorded as the first disc the fish attempted to dislodge. The fish was given 3 min to dislodge the correct coloured disc and eat the reward. For incorrect trials, we gave each female an additional 5 min to make a correct choice before we moved the rewarded disc 5 mm to the side to allow easy access to the food. This ensured that all fish experienced the same number of reinforced trials throughout the experiment. If an individual failed to make a choice, it was guided back to the holding compartment and the trial was repeated after a 1-min inter-trial interval. Trials were repeated up to a maximum of three times. The first three trials of colour discrimination training were cued; the correct coloured disc was 1 mm to the side of the hole, leaving a gap that was progressively reduced. These three cued trials were excluded from learning criterion assessments (Lucon-Xiccato and Bisazza 2014). Training for the colour discrimination task was completed over 6 training days, for a total of 30 trials with choice.

After completing the colour discrimination assay, fish started the reversal learning task. The procedure was the same except the reward was reversed between the coloured discs: fish previously trained to yellow now had red as the positive stimulus and vice-versa (Fig. 1). Fish ran 6 trials per day for a total of 66 trials over 17 days, with a day of rest every four to six training sessions to prevent overtraining and loss of motivation.

All trials were run blind to the treatment group of each fish. Individual fish were considered to have learnt the task if they reached a learning criterion of 7 out of 7 correct choices within the allocated total trials for the task, often used in colour discrimination tasks (Damas-Moreira, et al. 2018) and significant according to a binomial probability.

Data analysis

We began the experiment with 62 female guppies, but only 53 fish (no risk, n = 26; predation risk, n = 27) completed the cognitive assays. Statistical analyses were performed in R v.3.6.3 (R Core Team 2020) using '*lme4'* (Bates, et al. 2015). For each of the cognitive tasks (associative learning and reversal learning), we compared the performance of no risk and predation risk guppies in:

(a) number of fish that learnt the task (1 = learnt; 0 = failed) using a generalised linear model (GLM, binomial distribution) with Treatment and Colour as potential predictor variables;

(b) number of trials to reach learning criterion (GLM, Poisson distribution) with Treatment and Colour as potential predictor variables;

(c) learning rate, i.e., probability of success per trial (correct = 1; incorrect = 0) using a generalised linear mixed-effect model (GLMM, binomial) with Trial Number, Treatment, Colour and the interaction of Trial Number × Treatment and Trial Number × Colour as predictor variables and a random intercept and slope for fish identity, which accounts for the repeated observations of individual fish.

To examine if performance in the associative learning task had an effect on performance in the reversal task, we included the predictor variable 'Trials to learning criterion in the associative learning task' in models (b) and (c) for the reversal task.

We tested the significance of the random effects in both (c) models with likelihood ratio tests, by comparing models which culled the intercept or slope term to our final model. We chose not to run model (a) for the reversal task since most fish (>85%) reached learning criterion in each treatment and colour group. For model (c) in the reversal task, Trial Number was log-transformed to meet the assumption of linearity on the logit-scale.

Results

Most fish successfully learnt to associate a coloured disc with a food reward in the associative learning task and the number of successful fish was similar between the two treatments, with 20 out of 26 (77%) no-risk fish and 24 out of 27 (89%) predation-risk fish

reaching the learning criterion (Table 1a). However, no-risk fish reached learning criterion significantly faster (13.85 ± 5.86 days, mean ± SD) compared to predation-risk fish (16.58 ± 5.69 days; Table 1b; Fig. 2a). Both treatments increased performance with training and average learning rates were similar between no-risk and predation-risk fish (Table 1c; Fig. 2c). Individuals did not differ in their naïve probability of choosing the rewarded colour in the first trial (intercept for random effect of fish identity; Table 1c), but they did differ in their speed of acquisition over trials (random effect of slope for individuals across trials; Table 1c; see Fig. S1, Appendix S1 in Supporting Information for individual learning curves). Both treatments showed a natural preference for yellow (Fig. S3a).

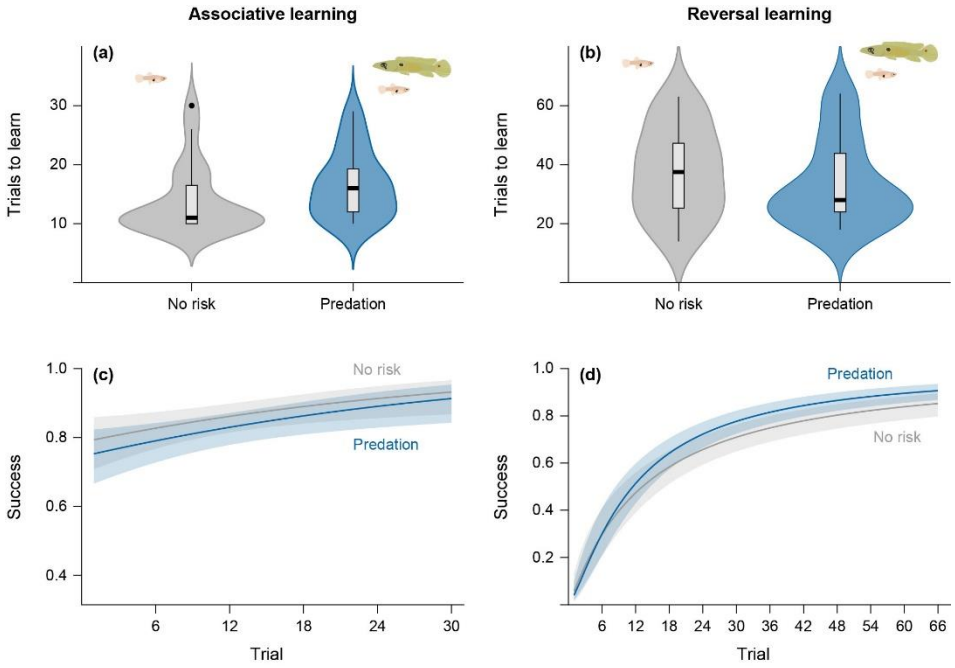

Figure 2. Performance of female guppies reared in no-risk or predation-risk environments in associative learning (left panels) and reversal learning testing cognitive flexibility (right panels). (a, b) Number of trials taken to learn each task. (c, d) Probability of correct choice over trials (lines show predicted model outputs and shaded areas indicate 95% confidence intervals).

In the reversal learning task testing behavioural flexibility, task success rates were also very high, with 22 out of 26 (85%) no-risk guppies and 26 out of 27 (96%) predation-risk guppies learning to acquire the new colour association. Contrary to the associative learning task, no-risk fish took significantly longer to reach learning criterion in the reversal (37.68 ± 15.20 days, mean ±SD) compared to predation-risk fish (33.65 ±14.47; Table 1d; Fig 2b). Performance in the associative learning task had no effect on trials needed to learn the reversal task (Table 1d; Fig. 3), indicating performance in the two tasks was independent.

Both no-risk and predation-risk guppies increasingly chose the correct colour more often and improved performance with training (Table 1e; Fig 2d). Associative learning performance had no effect on learning rates during reversal learning (Table 1e). Individuals differed both in their persistence to the previously learnt response (intercept for random effect of fish identity; Table 1e) and in the speed with which they acquired the new association over trials (random effect of slope for individuals across trials; Table 1e; see Fig. S2 in for individual learning curves).

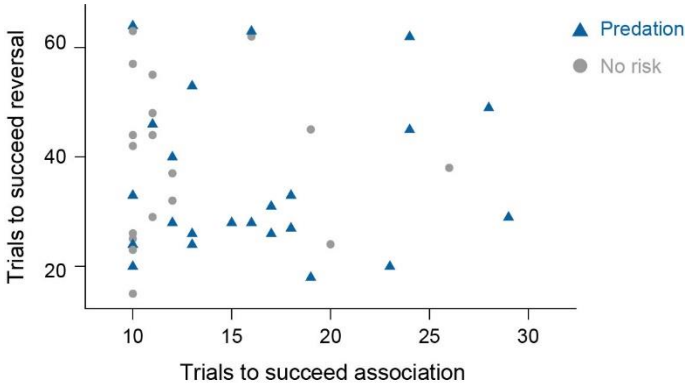

Figure 3. Relation between individual performance (trials to reach learning criterion) in the associative learning and the reversal learning task, showing a lack of correlation between the two tasks. Each point represents one fish (blue triangles, predation-risk; grey circles, no-risk).

Table 1. Outcomes of statistical models for the associative learning (a-d) and the reversal learning (e-f) tasks. N_{ind} = number of individuals; N_{obs} = number of observations; Est. = estimate; S.E. = standard error. Significant values are given in italics.

a) Number of fish that learnt association (N_{ind} = 53, N_{obs} = 53)	**Est.**	**S.E.**	***z*-value**	***P***
Intercept	0.626	0.585	1.070	0.285
Treatment (Predation Risk)	0.983	0.791	1.243	0.214
Colour (Yellow)	1.152	0.788	1.462	0.144
b) Trials to association criterion (N_{ind} = 44, N_{obs} = 44)	**Est.**	**S.E.**	***z*-value**	***P***
Intercept	*2.789*	*0.073*	*38.390*	*< 0.001*
Treatment (Predation Risk)	0.175	0.078	2.240	0.025
Colour (Yellow)	*-0.285*	*0.077*	*-3.695*	*< 0.001*
c) Probability of success in association (N_{ind} = 53; N_{obs} = 1590)	**Est.**	**S.E.**	***z*-value**	***P***
Intercept	0.101	0.279	0.362	0.717
Treatment (Predation Risk)	-0.230	0.319	-0.723	0.470
Colour (Yellow)	*2.188*	*0.325*	*6.738*	*< 0.001*

Trial Number	*0.107*	*0.021*	*5.152*	*< 0.001*
Trial Number × Treatment (Predation Risk)	-0.001	0.021	-0.051	0.959
Trial Number × Colour (Yellow)	*-0.115*	*0.022*	*-5.342*	*< 0.001*
Random effects	**Variance**	**d.f.**	**χ^2**	**P**
Fish identity intercept	0.320	2	3.247	0.197
Fish identity × Trial slope	*0.002*	*2*	*8.221*	*0.016*
d) Trials to reversal criterion (N_{ind} = 41; N_{obs} = 41)	**Est.**	**S.E.**	***z*-value**	***P***
Intercept	*3.614*	*0.038*	*94.107*	*< 0.001*
Treatment (Predation Risk)	*-0.110*	*0.055*	*-2.004*	*0.045*
Trials to association criterion	-0.0007	0.005	-0.133	0.894
Colour (Yellow)	*0.164*	*0.055*	*2.978*	*0.003*
e) Probability of success in reversal (N_{ind} = 44; N_{obs} = 2904)	**Est.**	**S.E.**	***z*-value**	***P***
Intercept	*-2.892*	*0.560*	*-5.168*	*< 0.001*
Treatment (Predation Risk)	0.064	0.770	0.083	0.934
Trials to association criterion	-0.006	0.021	-0.293	0.770
Colour (Yellow)	0.057	0.783	-0.073	0.942
Log(Trial Number)	*1.307*	*0.194*	*6.735*	*< 0.001*
Log(Trial Number) × Treatment (Predation Risk)	0.072	0.229	0.316	0.752
Log(Trial Number) × Colour (Yellow)	-0.072	0.231	-0.313	0.754
Random effects	**Variance**	**d.f.**	**χ^2**	***P***
Fish identity intercept	*4.676*	*2*	*51.918*	*< 0.001*
Fish identity × Log(Trial) slope	*0.406*	*2*	*50.289*	*< 0.001*

Both predation risk and no risk fish showed a naïve preference for the yellow discs; guppies that were assigned yellow as a positive stimulus during the initial discrimination were faster to reach criterion and had overall higher probability of success (Table 1b, c). However, fish trained to red showed a steeper learning curve and achieved similarly high performance in the final trials of the task (Table 1c; Fig. S2). Fish initially trained to red but with yellow as rewarded stimulus in the reversal task needed more trials to reach learning criterion. Colour preference did not influence probability of success over trials in the reversal learning task (Table 1e,f; Fig. S3b).

Discussion

The goal of this study was to test if exposure to predation risk during early ontogeny induces developmental plasticity in cognition that is retained later in life and whether different cognitive traits are differentially affected. As predicted, we found that adult cognitive

phenotypes of female fish that experienced predator cues during early ontogeny differed from adult cognitive phenotypes of no-risk fish. Specifically, female guppies exposed to predation risk during early development learned *more slowly* during an associative learning task but *outperformed* no-risk guppies in a reversal learning task testing behavioural flexibility. These results provide the first evidence of juvenile ecology pre-determining a suite of cognitive traits that persist in adult life, consistent with extensive research demonstrating that early life experiences can permanently alter a range of life-history, reproductive, and behavioural traits (Jonsson and Jonsson 2014, Kotrschal and Taborsky 2010, Taborsky 2006). Moreover, they demonstrate that predation pressure during early ontogeny had differential effects in two cognitive traits.

Previous studies have shown that acute exposure to predation risk can lead to plasticity of predator-related behaviours and learning abilities – namely neophobia and learned antipredator responses (Brown, et al. 2013, Ferrari 2014). Exposure to predation during early ontogeny can also lead to plasticity in neural investment; male guppies exposed to predation cues during development invest in heavier brains relative to their body size (Reddon, et al. 2018). Here, we expand these findings by showing that early exposure to long-term moderate predation cues also changes cognitive functions that are not directly linked to predator recognition, such as associative learning and behavioural flexibility, and that those effects persist in the absence of predation threat. We found that female adults from the predation-risk environment needed more trials to learn the initial colour association but were faster in reversal learning, therefore showing poorer associative learning ability but greater behavioural flexibility compared to no-risk fish. This finding cannot be explained by neophobic tendencies of predation-risk fish (Brown, et al. 2013) because each individual was pre-trained in the task until they were consistently responding, and those who failed pre-training were evenly distributed between the two treatments.

It is unlikely that our results were driven by potential differences in motivation as the pre-training phase acts as a control for motivation; only females that were motivated to feed would voluntarily enter the experimental compartment, succeed in the pre-training stage, and be included in the learning tasks. Additionally, the number of individuals not motivated to participate in the trials did not differ between predation risk and no risk fish, and all fish experienced the same food reward and the same number of reinforced trials each session.

Developmental stressors, such as predation risk, are well known to induce a wide range of physiological and phenotypic changes in developing animals (Relyea 2003, Sheriff and Love 2013). In several instances, prolonged stress can have non-adaptive, deleterious effects

on individuals, including cognitive impairment and neuronal loss, mostly mediated by glucocorticoid hormones – cortisol in most mammals and fish; corticosterone in birds, rodents, reptiles, and amphibians (Lesuis, et al. 2018, Piato, et al. 2011, Sapolsky 2015). Alternatively, brief periods of stress as well as moderate acute stress of predation during development might induce physiological and behavioural changes that prepare animals to live in harsh environments (Clinchy, et al. 2013, Giesing, et al. 2011, Pravosudov 2003). For example, a daily brief exposure to a predator for a period of 25 days elevates cortisol levels in stickleback (*Gasterosteus aculeatus*) females and their eggs and induces tighter shoaling in the fry of stressed females, an antipredator behaviour that is likely favoured under predation threat (Giesing, et al. 2011). Similarly, hormonal implants causing a long-term moderate elevation of corticosterone levels in mountain chickadees (*Poecile gambeli*) enhance food caching behaviour and spatial memory, important fitness traits for these birds as successful cache retrieval can be crucial for survival (Pravosudov 2003). Additionally, although predation risk is often associated with increased glucocorticoid levels in prey, some studies have also reported no association between predator regimes and cortisol levels (Gallagher, et al. 2019, McGhee, et al. 2020), highlighting the complexity of the link between predation risk, stress hormones, and cognitive performance. In our study, predation risk reduced performance in the simple associative learning task, which could be consistent with a "non-adaptive" scenario predicting detrimental effects of stress on cognition (Lupien, et al. 2009, Piato, et al. 2011, Sapolsky 2015). It is possible that female guppies in this study experienced high levels of stress due to exposure to predation risk cues, which impacted not only associative learning but also memory processes; in turn, poorer memory could facilitate reversal learning ability (Tello-Ramos, et al. 2019) and could have resulted in predation-risk females in this study showing slower learning of the initial association task but better reversal learning performance.

On the other hand, learning performance between the initial association and the reversal tasks at the individual level had similar range values and spread and was uncorrelated – i.e. a fish that took longer in the association task was not necessarily faster in learning the reversal. This result indicates that the strength of stimulus-reward association in the first task did not hamper the learning process during reversal learning. It is therefore likely that predation-risk fish in this study showed *enhanced* performance in the reversal learning task, unrelated to associative learning and memory. The fact that individual performance in the associative learning and reversal learning task was uncorrelated seems to support previous work showing that the cognitive traits tested by colour associative learning and reversal learning are

encoded by different brain regions (Buechel, et al. 2018, Chaves and Hodos 1997, López, et al. 2000). Several studies have examined the relationship between associative and reversal learning in a range of species and found contrasting results, as positive, negative, or no association between associative learning and reversal learning performance have been reported (Bebus, et al. 2016, Boogert, et al. 2010, Guillette, et al. 2015). This discrepancy may arise from animals using different strategies to learn reversal tasks, namely associative learning processes or rule-based learning mechanisms (e.g. win-stay lose-shift rule), or from different memory interference and forgetting mechanisms (Kumpan, et al. 2020, Tello-Ramos, et al. 2019). Behavioural and cognitive flexibility can increase survival in the face of novel predators or environments, and thus is likely to be favoured under predation risk (Sih, et al. 2010, Sol, et al. 2005). Under this scenario, our results suggest that predation risk is a moderate stressor that can enhance cognitive traits that might be adaptive. Sampling cortisol levels of fish reared under no risk and predation risk cues and linking hormonal profiles to cognitive performance is clearly an avenue for future research.

One important question that remains is, *why do predation-risk fish show poorer associative learning ability*? That is, why was performance in the associative learning task not similar between treatments? A possible explanation is that the benefits of greater overall cognitive processing under predation threat do not fully compensate its energetic costs (Dunlap and Stephens 2016), leading to a need to selectively invest in some cognitive traits at the cost of others. This implies that animals were able to adjust their cognitive investment during development independently for each trait. While investment in particular cognitive abilities has been repeatedly shown in indirect ecomorphological studies of brain anatomy (de Winter and Oxnard 2001, Kotrschal, et al. 2017, Magphail and Bolhuis 2001), our study is the first to suggest selective plasticity of different cognitive abilities in response to ecological conditions. On a more behavioural level, it is also possible that plastic cognitive responses to the environment depend on the values of other correlated behaviours. For example, predation risk can drive selection and plasticity of personality traits on the boldness–aggressiveness axis (Bell and Sih 2007, Réale and Festa-Bianchet 2003), traits that can be linked to cognitive performance (Chittka, et al. 2009, Sih and Del Giudice 2012). Future studies examining a combination of cognitive performance and individual differences in behavioural traits linked to predation should prove insightful. For instance, are the cognitive traits tested by common-place learning paradigms in the laboratory relevant to survival under natural predation?

Even though we cannot identify the exact mechanisms underlying the effects of early predation risk on cognitive abilities, our results show that perceived predation risk during

development drives phenotypic plasticity in cognitive traits which persist later in life in the absence of predation threat. These results show the potential for early predation risk affecting cognitive abilities in adults, particularly so in systems where prey individuals experience variation in predation at ecological timeframes, but some level of stability within an individual's lifetime. We therefore highlight the importance of considering plasticity in cognitive performance when investigating cognitive abilities in natural populations.

Data availability

Data publicly available at Figshare (https://doi.org/10.6084/m9.figshare.13265219.v1). R code used for analyses and figures also available (https://doi.org/10.6084/m9.figshare.13265150.v5).

Ethics

Research approved by the Stockholm Ethical Board (Dnr: 11627-2019).

Conflict of interest

We declare we have no conflict of interest.

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