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Increases in predation favour evolutionary shifts in behavioural plasticity in Trinidadian killifish *(Anablepsoides hartii)*

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Abstract

Behavioural plasticity is expected to be favoured in risky environments, such as when prey species coexist with predators because prey must alternate between fitness-related foraging/mating behaviours and antipredator behaviours that enhance survival. We compared behavioural plasticity in Trinidadian killifish that are found in sites with and without predators. We quantified aggressive and antipredator behaviours via a mirror assay in second-generation lab-reared and wild-caught killifish before and after exposure to predator cues. We compared 2 types of aggression including: overt aggression (ramming, biting, lunging, and tail-slapping) and display aggression (spine arching, bending into an *s*-shape, and opercular flaring). We additionally compared the amount of time the fish spent frozen as a proxy for antipredator behaviour. We show clear differences in plasticity between populations with and without predators. Killifish from sites with predators decreased overt aggression in response to exposure to predator chemical cues. Plastic responses to the predator cue were lower in killifish from sites that lack predators. Interestingly, wild fish from sites without predators did respond to the predator cue by decreasing overt aggression and increasing time spent frozen, though to a lesser degree compared to the fish from sites with predators. Our results support the expectation that development in a risky environment favours evolutionary changes in predator-mediated behavioural plasticity.

Keywords: behavioural plasticity, eco-evo dynamics, natural selection, predator–prey interactions

Introduction

Phenotypic plasticity occurs when a change in the environment alters developmental trajectories and the expression of traits (Scheiner, 1993). Behavioural plasticity is a form of phenotypic plasticity that allows an individual to respond to environmental cues integrated through past experience and learning (Baldwin, 1896). Organisms under the threat of predation face the challenge of a tradeoff between evading predators versus meeting energy requirements (Clark, 1994; Houston et al., 1993; Lima & Dill, 1990). This is because antipredator behaviours (i.e., avoidance, freezing, or hiding) reduce the ability to effectively obtain resources (Dall, 2010; Sih, 1992; Sih et al., 2004). Thus, behavioural plasticity can provide a fitness advantage for prey species that must avoid predation but also continue to forage and move about the environment (Sih et al., 2000). Research has demonstrated that prey species respond to the presence of predators with varying degrees of intensity depending on immediate predation risk and past experience (Brown et al., 2006). Therefore, behavioural plasticity has numerous fitness implications and indeed has been shown to improve survival in variable and novel environments (Schlichting & Pigliucci, 1998; Snell-Rood, 2013; West Eberhard, 2003). Plasticity, in general, is expected to be favoured when selection varies spatially and temporally in a predictable manner (Lively, 1986; Scheiner, 1993). Previous research on guppies (Dugatkin & Godin, 1992; Seghers, 1974) and sticklebacks (Fraimout et al., 2022; Herczeg et al., 2009; Wund et al., 2015) have provided evidence that differences in predation intensity can drive shifts in behaviour. This includes predator-driven divergence in antipredator behaviour (Wund et al., 2015), shoaling behaviour (Seghers, 1974), foraging and predator inspection (Dugatkin & Godin, 1992, and female mate choice (Breden & Stoner, 1987; Godin & Briggs, 1996). However, more research is needed on the evolutionary drivers of predator-mediated behavioural plasticity (Mery and Burns 2010).

In Trinidad, killifish (*Anablepsoides hartii*) are found in locations where they coexist with predators and in locations where they are the only fish species present (Fraser et al., 1995, 1999; Gilliam et al., 1993). Populations that coexist with predators (i.e., high predation) are found in the downstream drainages of watersheds while ‘killifish-only’ sites are found at higher elevations above barrier waterfalls. This is because killifish are able to jump above the barrier waterfalls that prevent the upstream movement of piscivorous predators, such as the wolf fish (*Hoplias malabaricus*). High-predation fish experience increased mortality due to the presence of predators and are therefore found at relatively low densities with higher per capita resource availability (Furness et al., 2012; Gilliam et al., 1993). Killifish-only fish experience high population densities, lower per capita resources, and thus, intense competition for resources (Fraser et al., 1999;

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Walsh & Reznick, 2008, 2009). These divergent patterns of natural selection have led to evolutionary shifts in life history traits (Walsh & Reznick, 2008, 2009), physiology (Oufiero et al., 2011), and brain and eye size (Beston et al., 2017; Walsh et al., 2016). The differences in predation intensity have also been shown to influence the behaviour of killifish. In the wild, high-predation fish are bolder and exhibit increased dispersal distances in the streams (Fraser et al., 2001; Gilliam & Fraser, 2001). Lab assays revealed that the absence of predators and subsequent increased competition has favoured the evolution of increased aggression in killifish-only fish (Korte & Walsh, 2024). The differences in predator presence and their associated predator cues provide the opportunity to examine the interplay between environmental signals (i.e., predator cues) and the evolution of behavioural plasticity.

Here we tested for differences in behavioural responses to predator cues in killifish from killifish-only and high-predation sites across multiple independent rivers. We quantified aggressive and antipredator behaviours before and after exposure to predator cues in wild-caught and second-generation lab-reared fish. The goal of the assays that use second-generation lab-reared fish was to eliminate confounding environmental and maternal effects to quantify any potential genetic differences amongst the populations. We also compared the results of the assays using the second-generation lab-reared fish with trends observed in the wild-caught fish to determine the importance of environmental versus genetic effects on the expression of behavioural plasticity. We utilized a mirror aggression assay to quantify several aspects of aggressive behaviour. We additionally measured time spent frozen, an antipredator behaviour that reduces the chances of being seen by a predator (Eilam, 2005). In general, we expect that exposure to predator cues will lead to declines in aggression and increased time spent frozen. If increased predator-induced mortality selects for shifts in behavioural plasticity, then we expect that the responses to predator cues will be larger in high-predation vs. killifish-only fish. This should manifest in greater increases in time spent frozen and/or more drastic decreases in aggression in high-predation fish following exposure to predator cues when compared to killifish-only fish.

Methods

Our protocols were approved and permitted by the Institutional Animal Care and Use Committee under permit numbers A17.001 (laboratory) and A17.004 (field).

Sampling and lab-rearing

In January 2020, we collected Trinidadian killifish *(Anablepsoides hartii)* from high-predation and killifish-only sites in the Aripo and Arima rivers in the Northern Range mountains of Trinidad, West Indies. We housed them in our field facility for 1 week in 9-L aquaria with artificial spawning substrate and arranged mating pairs of males and females from the same populations ($n = 15$ pairs). To supplement these numbers and enhance genetic diversity we additionally collected eggs directly from the streams by attaching bunches of yarn to rocks and roots throughout the natural habitat and leaving them overnight for 3 days to act as an artificial spawning substrate in all four locations (Aripo killifish-only, Aripo high predation, Arima killifish-only, and Arima high predation). All eggs were collected daily and brought back to the University of Texas at Arlington laboratory facilities to be placed in Petri dishes until they hatched ($n = 166$). Fry were then transferred to aquaria at a maximum density of one fish per litre and fed a mixture of flake food and live or frozen brine shrimp ad libitum until they reached sexual maturity at approximately 6 months of age. Males can be distinguished from females by their distinct white bars along the top and bottom of the caudal fin that develops when they are approximately 30 mm in total length (Walsh & Reznick, 2008).

From these first-generation fish, we created mating pairs to generate second-generation lab-reared fish. We paired up males and females from the first generation of fish from the same populations, taking care to avoid inbreeding by mating individuals that were derived from lineages from different locations of collection or from different known genetic backgrounds. We collected eggs daily and placed them in Petri dishes until they hatched. These fry were reared on the same feeding routine as their parents. Once mature, a subset of fish was selected for an aggression assay (total $n = 61$, 17 Arima high predation, 15 Arima killifish-only, 15 Aripo high predation, and 14 Aripo killifish-only). First, we anaesthetized the fish in a solution of 0.3 g MS-222 Tricaine methane-sulfonate with 0.2 g bicarbonate (pH buffer) dissolved in 333 ml water (Topic Popovik et al., 2012). Once the fish were immobilized, we measured total length and determined the sex of the fish. After recovery, the fish were placed individually in aquaria within a flow-through system designed by Aquaneering Incorporated that yielded standardized water quality parameters, thereby minimizing random tank effects. They were allowed to acclimate to their new tanks for at least 1 week before behavioural assays were conducted.

In December of 2022, we collected a wolf fish (*Hoplias malabaricus*) from the Arima river in the Northern Range of Trinidad. We brought the wolf fish back to the University of Texas at Arlington laboratory facility and housed it individually in a 38-L tank with a centimetre of multicoloured gravel as a substrate. We included plastic plants and PVC pipes for shelter and life enrichment. We fed the wolf fish daily a combination of frozen blood worms and frozen silversides. Water changes (30%) were performed weekly. On 3rd January 2023, 7 days after the last water change, we collected a 2-L sample from the wolf fish tank. We passed the water through a vacuum filter apparatus with a particle retention of 1.2 µm to remove extraneous organic material. We separated the sample into 50-ml aliquots and stored them at −20 °C for later use.

Behavioural assay

We used second-generation lab-reared killifish to test for evolved differences in plasticity of aggression and antipredator behaviour between high-predation and killifish-only sites. Behaviour was quantified via a commonly used mirror test in a 10-L glass aquarium with a centimetre of multicoloured gravel as substrate (Balzarini et al., 2014; Reichmann et al., 2022). The mirror was fixed to the left end of the aquarium while the fish acclimated for 2 min in the opposite end with an opaque divider to keep it out of sight. We subjected each fish to a pre- and post-cue trial design with respect to the addition of the predator cue. We controlled for stress due to handling the fish by netting the fish both before the pre-trial and before the post-trial. We also allowed equal amounts of acclimation time after netting under the same parameters for each trial. Therefore, the only difference between the pre- and post-trial is the presence or absence of the predator cue. Wolf fish predator cues were thawed half an hour before initiation of the assays. Each fish was gently captured with a dip net in its home tank and moved to the assay tank to acclimate for 2 min behind the opaque divider. To initiate the pre-trial, the divider was removed and a front-facing camera recorded the movement of the fish for 5 min. After the 5-min pre-trial, the fish was corralled back into the right end of the tank with a dip net and the opaque divider was replaced to block the mirror. The fish were allowed another 2-min acclimation period, during which the wolf fish predator cue was added to the opposite end of the tank. After the acclimation period had been completed, the opaque divider was removed and the 5-min post-trial was initiated to allow comparisons of behaviour before and after sensing the presence of the predator. After each individual fish, we thoroughly washed the tank and performed a 100% water and substrate change to avoid lingering predator cue for the next pre-trial.

We subsequently analyzed videos for aggressive and antipredator behaviours. Behaviours that were considered aggressive included biting, lunging, ramming, tail-slapping, arching of the spine, flaring of the operculum, as well as bending the body into an *s*-shape. We counted the number of aggressive interactions the fish made with the mirror. We categorized types of aggressive behaviours based on whether they were direct attempts to fight and make contact with the mirror or whether they were defensive posturing displays. Overt aggression included biting, lunging, ramming, and tail-slapping while display aggression included postures such as spine arching, bending the body into an *s*-shape and opercular flaring. For these variables, we used total counts of each behaviour type. We also analyzed videos for total time spent frozen on the bottom of the tank as a variable to represent antipredator behaviour.

Wild fish sampling

In June 2023, we returned to Trinidad to perform the behavioural assay described above on wild fish to further investigate variation in behavioural plasticity. We hand-captured fish from high-predation and killifish-only sites in the Arima, Aripo, El Cedro, and Turure rivers in Trinidad. Note that we did not access a true killifish-only site in the Turure river, but we sampled a locality where killifish co-occur with guppies that predate only on killifish fry (Walsh & Reznick, 2010), thus this site is considered low predation. We selected fish which were near or above 30 mm in total length, which is the stage at which they reach sexual maturity distinguished by the white or gold bars that develop on the top and bottom of the caudal fin in males only. Our sample size was a total of 163 fish (20 Turure high predation, 20 Turure low predation, 22 El Cedro high predation, 18 El Cedro killifish-only, 22 Aripo high predation, 19 Aripo killifish-only, 22 Arima high predation, and 20 Arima killifish-only).

After capture, we brought the fish to our field laboratory facilities in Arima, Trinidad. We housed the fish individually in plastic containers and included rocks from the natural environment as shelter and enrichment. Fish were fed daily with flake food ad libitum during their stay in the field facilities. The fish were allowed to acclimate overnight and the following morning we anaesthetized them in a solution of MS-222 and sodium bicarbonate. Once anaesthetized, we identified each fish's sex, and all fish were weighed and measured for total length. The fish were allowed to recover for at least 24 hr following anaesthesia before the behavioural assays were conducted. We used the same methodology for the behavioural assay as described above for the lab-reared fish. As we were unable to effectively transport predator cues without degradation, we collected a new wolf fish to obtain predator cues. The wolf fish was captured from the Arima river and kept in a 38-L aquarium with rocks and PVC pipes for shelter and life enrichment. The wolf fish was fed cichlid pellets daily ad libitum. Predator cues were collected after the wolf fish presided in the tank for 3 days without water changes. We took 50-ml aliquots directly from the tank as needed during assays. After assays were completed, the fish were brought to university laboratory facilities to continue studies.

Statistical analyses

We used SPSS v. 29 software (IBM Corporation) to conduct all statistical analyses and SigmaPlot v. 12.3 (Systat Software Incorporated) to produce graphs. To determine if fish size influenced behaviours, we ran regressions between log natural transformed total length and each behavioural variable (i.e., time spent frozen, overt aggression, and display aggression). None of the variables were significantly influenced by fish size, therefore, we did not include total length in our analyses. To account for zero inflation in our behavioural variables, we fit the models with negative binomial distributions.

To compare changes in behaviour between the populations, we used linear mixed models with river, population, and sex included as fixed effects. We entered individual identity as the subjects term and trial (pre- aggression vs. post-aggression or pre-time spent frozen vs. post-time spent frozen) as the repeated measure for each subject. This allows us to explore how different types of behaviour may shift in the presence of predators. The dependent variables in the models included time spent frozen, overt aggression, and display aggression. We used a model design that included the main effects and the following interactions: river*population, trial*river, trial*population, trial*sex, and trial*river*population. We were specifically interested in the trial × population, and trial × river × population interactions because they test for differences in the responses to predator cues between the populations and whether any such differences are consistent between replicate rivers. When a main effect or interaction was significant, we output estimated marginal means with standard error from the model to compare values across groups. We used tests of simple main effects to investigate significant interactions (Winer, 1971). We used one-way ANOVAs with the associated treatment group as a fixed effect and used the split file function in SPSS to attain statistical differences between treatment groups. Below, we use these to interpret trends in the models and determine how the fish population of origin influences the response to predator cues.

Results

Lab-reared fish

We observed a significant ($p < .05$) interaction between trial and population for overt aggression ($F_{1,112} = 4.62$; $p = .035$) in the lab-reared fish but not for time spent frozen or display aggression (Table 1). The populations did not differ in overt aggression in the pre-trial ($F_{1,59} = 0.358$; $p = .552$), but in the post-trial—after the addition of the predator cue—the high-predation fish decreased overt aggressive behaviour while the killifish-only fish increased overt aggression ($F_{1,59} = 4.193$; $p = .045$) (Figure 1A).

Table 1. F statistics resulting from the linear mixed model on second-generation lab-reared killifish ($n = 61$) behavioural variables in a mirror aggression assay. Fixed factors included river, population, and sex. Fish identity was included as the subjects term. The repeated measure was trial, pre and post addition of a predator cue. Df (num) indicates numerator degrees of freedom. Df (error) indicates error degrees of freedom. Significant terms are in bold font. Significance level is indicated by: $.05 \geq p \geq .01$*, $.01 \geq p \geq .001$**, $.001 \geq p \geq .0001$***.

Effects and interactions	df (num)	df (error)	Time spent frozen	Overt aggression	Display aggression
River	1	112	**9.06****	0.05	**29.70*****
Population	1	112	0.01	**5.89***	0.01
Sex	1	112	2.73	0.01	0.50
River*Population	1	112	0.57	1.20	**10.36****
Trial	1	112	2.08	0.97	1.29
Trial*River	1	112	0.01	1.81	0.07
Trial*Population	1	112	1.14	**4.56***	1.65
Trial*Sex	1	112	0.22	2.25	0.37
Trial*River*Population	1	112	1.75	0.29	0.06

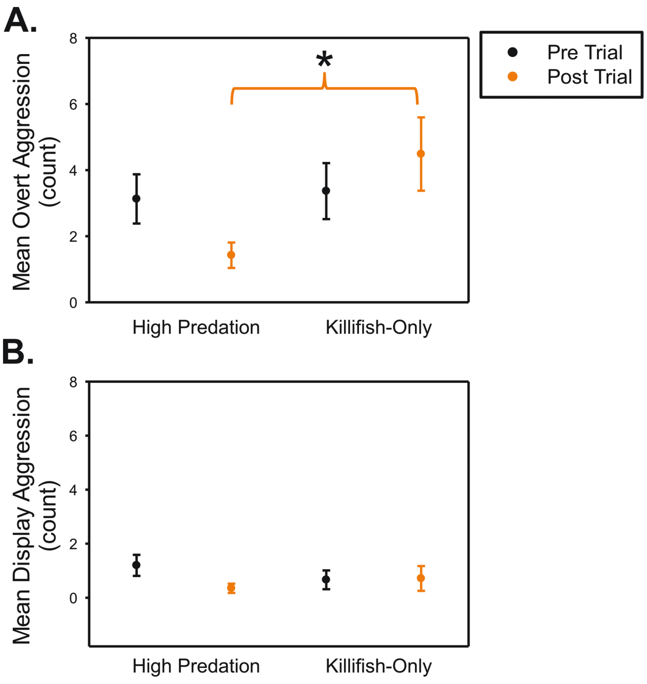

Figure 1. Estimated marginal means of second-generation lab-reared killifish ($n = 61$) in (A) overt aggression (biting, ramming, lunging, and tail-beating) and (B) display aggression (opercular flaring, spine arching, and bending into an s shape) between populations for the trial before and after addition of a predator cue in a mirror aggression assay over a period of 5 min. Error bars represent SEM. Significance between populations in the post-trial is indicated by * ($p = .045$).

We also observed a significant interaction between river and population for display aggression ($F_{1,112} = 10.355$; $p = .002$), but not for overt aggression or time spent frozen. Killifish-only fish from the Arima river showed higher levels of display aggression compared to high-predation fish from the same river ($F_{1,62} = 7.896$; $p = .007$). Meanwhile, fish from the Aripo river showed no difference in display aggression frequency between high-predation and killifish-only fish ($F_{1,56} = 3.402$; $p = .070$) (Supplementary Figure S1). We observed a significant effect of river for time spent frozen. Aripo fish spent approximately 4× the amount of time frozen in the assay compared to Arima fish (Aripo mean = 32.87 s, SEM = 10.93; Arima mean = 8.16 s, SEM = 2.65). The effect of river was not significant for overt aggression. The main effect of trial was not significant for any variables. Neither was the main effect of sex nor the interaction between trial and sex significant. The trial*river and trial*river*population interactions were also non-significant.

Wild-caught fish

We observed a significant interaction between trial and population for overt aggression ($F_{2,304} = 3.815$; $p = .023$) in the behavioural assay (Table 2). All populations decreased overt aggression after the predator cue was added (Figure 2A). The populations did not differ in expression of overt aggressive behaviour in the pre-trial ($F_{1,158} = 0.289$; $p = .749$), but in the post-trial high-predation and low-predation fish exhibited lower levels of overt aggression than killifish-only fish ($F_{1,158} = 2.613$; $p = .077$). After the predator cue was added, fish from the low-predation and high-predation locales exhibited stronger decreases in overt aggression compared to killifish-only fish—high-predation fish decreased by 78% and low-predation fish decreased by 80%, respectively. Meanwhile, killifish-only fish also decreased overt aggression in the post-trial but showed a weaker response (47%) compared to the low-predation and high-predation populations. We also observed a significant interaction between trial and river for overt aggression ($F_{3,304} = 3.072$; $p = .028$). The rivers showed differences in overt aggression in the pre-trial ($F_{3,157} = 4.066$; $p = .008$) with Aripo fish showing the highest average overt aggression followed by Turure, El Cedro, and Arima, respectively. After the predator cue was added in the post-trial the rivers did not differ in overt aggression ($F_{3,157} = 0.643$; $p = .588$) (Supplementary Figure S2A). The trial-by-population and trial-by-river interactions were not significant for time spent frozen or display aggression and the three-way interaction between trial, river, and population was not significant for any variables.

The main effect of trial was significant for all variables including time spent frozen ($F_{1,304} = 27.194$; $p < .001$), overt aggression ($F_{1,304} = 35.735$; $p < .001$), and display aggression ($F_{1,304} = 7.329$; $p = .007$). Estimated marginal means for time spent frozen in the pre-trial were at 58.78 s (SEM = 6.23) which increased to 114.10 s (SEM = 7.87) after the predator cue was added. Overt aggression started at a mean of 2.32 instances (SEM = 0.25) in the pre-trial and decreased to 0.70 instances (SEM = 0.12) in the post-trial. Display aggression also decreased from an initial average of 0.85 instances

Table 2. F statistics resulting from the linear mixed model on wild killifish (*n* = 163) behavioural variables in a mirror aggression assay. Fixed factors included river, population, and sex. Fish identity was included as the subjects term. The repeated measure was trial, pre and post addition of a predator cue. Df (num) indicates numerator degrees of freedom. Df (error) indicates error degrees of freedom. Significant terms are in bold font. Significance level is indicated by: .05 ≥ *p* ≥ .01*, .01 ≥ *p* ≥ .001**, .001 ≥ *p* ≥ .0001***.

Effects and interactions	df (num)	df (error)	Time spent frozen	Overt aggression	Display aggression
River	3	304	**4.46***	**4.45****	0.47
Population	2	304	0.76	**5.91****	0.21
Sex	1	304	0.94	2.21	0.18
River*Population	2	304	**4.13***	1.88	0.51
Trial	1	304	**27.19*****	**35.74*****	**7.33****
Trial*River	3	304	0.52	**3.07***	0.39
Trial*Population	2	304	1.37	**3.82***	0.17
Trial*Sex	1	304	1.37	0.34	0.04
Trial*River*Population	2	304	2.29	1.70	0.07

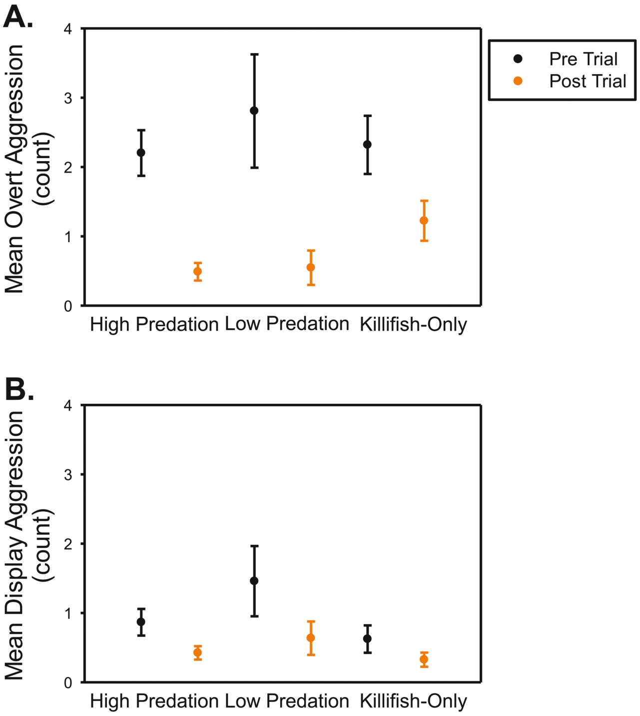

Figure 2. Estimated marginal means of wild-caught killifish (*n* = 163) in (A) overt aggression (biting, ramming, lunging, and tail-beating) and (B) display aggression (opercular flaring, spine arching) between populations for the trial before and after addition of a predator cue in a mirror aggression assay over a period of 5 min. Error bars represent SEM.

(SEM = 0.14) in the pre-trial to 0.41 instances (SEM = 0.07) in the post-trial.

We also found a significant interaction between river and population for time spent frozen regardless of predator cue presence (see Supplementary Table S1). High-predation fish from the Aripo river spent more time frozen than killifish-only fish in the Aripo ($F_{1,76} = 7.648$; $p = .007$). The populations did not differ in time spent frozen in the Arima, El Cedro, or the Turure rivers (Supplementary Table S1). The interaction between river and population was not significant for overt aggression or display aggression. There were also significant main effects due to river for time spent frozen and overt aggression. For time spent frozen, El Cedro fish exhibited the highest mean at 119.97 s (SEM = 14.88), followed by the Turure fish at 73.89 s (SEM = 9.12), while the Arima and Aripo fish showed practically no difference (Arima mean = 71.02 s, SEM = 8.59; Aripo mean = 71.42 s, SEM = 9.35). For overt aggression, Arima fish exhibited the lowest mean frequency at 0.80 instances (SEM = 0.18), while Aripo, El Cedro, and Turure fish showed higher frequencies at 1.48 (SEM = 0.30), 1.38 (SEM = 0.25), and 1.62 (SEM = 0.29), instances of overt aggression, respectively. The effect of river was not significant for display aggression. We additionally observed a significant effect due to population for the overt aggression variable only. Killifish-only fish showed the highest mean instances of overt aggression (mean = 1.69, SEM = 0.25) compared to low-predation (mean = 1.24, SEM = 0.33) and high-predation fish (mean = 1.04, SEM = 0.16). The effect of sex and its interaction with trial were not significant for any variables.

Discussion

Our results provide evidence that increases in predation are associated with evolutionary shifts in behaviour and predator-induced behavioural plasticity. Our second-generation high-predation fish responded to the predator cues by exhibiting decreased overt aggression (ramming, biting, lunging, and tail-slapping). The comparative responses in second-generation killifish-only fish were to increase overt aggression, though this was a weak response (Figure 1). In our wild fish, all fish regardless of population of origin decreased overt aggression and display aggression and increased time spent frozen. However, high- and low-predation fish showed stronger decreases (78%–80%) in overt aggression compared to killifish-only fish (47%) (Figure 2). It is worth noting that, on average, the frequency of occurrence of aggressive behaviours observed in our results seems low—up to approximately five instances of overt aggression per 5 min in lab-reared fish and three instances in wild-caught fish. However, given that aggressive altercations with conspecifics increase the likelihood of injury, or even death, the fitness implications of such behaviours may be substantial (e.g., Mehlman et al., 1994, 1995). Though, our study did not directly measure fitness so we can only speculate on the fitness implications of these behaviours. Additionally, aggression displayed towards a mirror may not be representative of aggression towards a conspecific in a natural setting (Balzarini et al., 2014). Below we consider why predators are linked to these specific differences in behaviour and the implications of the contrasting trends between assays performed using wild-caught vs. lab-reared fish. We also discuss the fitness implications of shifts in behaviour depending on predator presence.

Predation has well-known effects on animal behaviour including shifts in boldness, activity levels, and aggression (Sih et al., 2004). One might initially expect declines in boldness and activity with predator presence due to the increased likelihood of mortality when leaving refugia. However, animals must be able to continue to forage to meet energy requirements in the presence of predators (Lima & Dill, 1990; Sih, 1992). In an environment where resources and predators are patchily distributed, it is intuitive that high-predation fish would evolve the ability to detect predator presence and respond with shifts in behaviour. By having a refined predator detection system, high-predation fish gain an increased ability to better assess risks associated with foraging and dispersal. Our results support this hypothesis as wild killifish derived from sites with predators exhibit clear decreases in overt aggression after being exposed to predator cues. Furthermore, our lab-reared killifish derived from high-predation sites exhibited decreased overt aggression compared to killifish from sites with no predators. This is a logical response as research has shown that aggressive displays can decrease prey vigilance and make them more conspicuous to predators (Caraco & Pulliam, 1984; Jakobssen et al., 1995; Sih et al., 2004).

On the other hand, shifts in aggression following exposure to predator cues were weaker or absent in killifish-only fish. Contrary to high-predation fish, killifish-only fish face intense competition for resources due to higher population densities (Gilliam et al., 1993; Walsh et al., 2011). Aggression is expected to be favoured in high-competition environments (Abesamis & Russ, 2005; Cubaynes et al., 2014; Endler, 1995; Holekamp & Strauss, 2016). Furthermore, recent perturbation experiments in Trinidadian killifish have shown that decreases in predation and the corresponding increase in competition in killifish-only sites favour the evolution of increased aggressive behaviour (Korte & Walsh, 2024). It appears that adaptation in sites that lack predators is associated with the loss of predator-induced plasticity. The alternative perspective is that fish from killifish-only sites no longer recognize predator odour cues because they have evolved in the absence of piscivorous fish. However, it is important to highlight that, similar to the high-predation and low-predation fish, wild-caught killifish-only fish also decreased aggression and increased time spent frozen in the post-predator cue trial. Therefore, it would seem they do adaptively respond to the predator cue. More research is needed to understand why behavioural plasticity shifts between high-predation and killifish-only sites.

An interesting facet of our results is that the specific responses to predator cues differed between second-generation lab-reared and wild-caught fish. All wild killifish regardless of population of origin responded to the predator cue by decreasing aggression and increasing time spent frozen, but the lab-reared fish did not show this trend. This raises the question: why do the results differ between lab-reared vs. wild-caught fish? One possible explanation is that confounding environmental effects mask the genetic differences in behaviour between the populations. In natural settings, a wide array of variation in environmental conditions generate background “noise” that can make it difficult to assess and distinguish environmental signals. Another possibility is that behavioural plasticity is largely environmentally induced. In a review of behavioural plasticity, Snell-Rood (2013) identified two main types of behavioural plasticity: activational and developmental plasticity. Activational plasticity (also termed “innate behavioural plasticity” or “behaviour as plasticity”) is defined by an already existing neural framework that produces contextualized responses triggered by environmental cues. On the other hand, developmental plasticity requires learning over a developmental period to develop different neural networks in different environments (see Figure 1 in Snell-Rood, 2013). With this framework in mind, our lab-reared high-predation fish—which show evolved differences from KO fish in behavioural plasticity in aggression—seem to exhibit activational plasticity because these fish retain the ability to sense and respond to a predator cue despite lacking prior experience with predators. This notion is further supported because we performed these assays using second-generation lab-reared fish, which minimizes maternal effects on offspring behaviour.

A surprising aspect of our results was the more dramatic decrease in overt aggression for the fish from the low-predation population compared to the high-predation populations. (Figure 2A). This is unexpected considering the only predators killifish encounter in low-predation sites are guppies, which prey on juvenile killifish (Walsh & Reznick, 2010). These results signal that early life exposure to predation by guppies may have long-lasting effects into adulthood since our sample contained only mature killifish. Our high-predation sample also decreased overt aggression, though at a slightly lesser degree than the low-predation fish. However, the comparison between the trends for high- and low-predation sites should be viewed with caution because the low-predation sample only came from one river and is not a repeated result. Repeated sampling in low-predation sites across watersheds is needed to confirm the influence of guppies as predators on the evolution of predator-mediated behavioural plasticity in killifish.

Additionally, we found contrasting trends between rivers for display aggression (second-generation killifish) (Supplementary Figure S1B) and for time spent frozen (wild-caught killifish) (Supplementary Table S1). For instance, second-generation lab-reared killifish-only fish from the Arima river showed heightened display aggression (spine arching, opercular flaring, and bending the body into an *s*-shape) compared to high-predation fish from the Arima river. On the other hand, Aripo killifish-only fish exhibited slightly lower frequencies of display aggression compared to high-predation Aripo fish. In the wild fish, time spent frozen in the assay was comparatively higher for high-predation fish in the Aripo and El Cedro rivers, but lower in the Arima and Turure rivers to the killifish-only fish. These nuanced differences in behaviour may be occurring across fish communities due to various abiotic and biotic differences. For example, factors such as canopy cover and light availability vary spatially across fish communities. In addition, the rivers can vary in stream morphology. Our experimental design did not allow us to disentangle these effects which may alter shifts in behaviour and productivity in the streams.

Conclusion

Our results support the theory that development in a risky environment due to the threat of predation favours behavioural plasticity. We show clear differences in the plasticity of behaviour between populations with predators and those that lack predators. Specifically, fish from sites with predators respond to exposure to a predator cue by decreasing their overt aggression which, in turn, may decrease the chances of being spotted by a predator. In contrast, fish from sites that lack predators showed a higher degree of canalization in aggressive behaviours presumably due to the high competition they face in those locales. Interestingly, wild fish from sites without predators retain the ability to detect the predator cue and respond by decreasing overt aggression, though at a lesser degree compared to the fish from high-predation and low-predation sites. Collectively, these results indicate that predation selects for increased behavioural plasticity.

Supplementary material

Supplementary material is available at *Journal of Evolutionary Biology* online.

Data availability

The data underlying this manuscript are available in the Dryad Digital Repository doi:10.5061/dryad.xwdbrv1pt

Author contributions

Meghan Korte (Conceptualization [equal], Data curation, Formal analysis [lead], Methodology [equal], Visualization, Writing—original draft [lead], Writing—review & editing [equal]), and Matthew Walsh (Conceptualization [equal], Funding acquisition [lead], Methodology [equal], Supervision [lead], Writing—review & editing [equal])

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Conflict of Interest

The authors claim no conflict of interest that would affect the objectivity of this research.

Ethical Approval

Our research, which involves comparative studies of a vertebrate species has been approved and permitted by the University of Texas at Arlington's Institutional Animal Care and Use Committee under permit numbers A17.001 (laboratory protocols) and A17.004 (field protocols).

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