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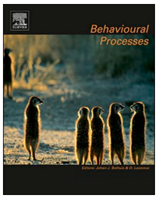

Boldness is affected by recent experience with predation cues and body size in mosquitofish

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ABSTRACT

Adjusting behaviour can be crucial to prey surviving a predator encounter. How any one individual modifies their behaviour in response to predation risk might be affected by their previous experience with danger and their own vulnerability. Using western mosquitofish, we examined how boldness in different contexts was affected by an individual's recent experience with predation risk. Individuals were repeatedly chased by a largemouth bass model and encountered alarm cue to mimic conditions of high risk (cues twice on 2 days), low risk (cues twice on 1 day), or no risk (water only). We then measured boldness and avoidance behaviour under three different contexts: in a novel tank, with a shoal of conspecifics, and with alarm cues and a model predator. We found that how recent experiences influenced boldness in a novel tank depended on body size. Smaller fish from the no and low risk treatments were more likely to emerge from a shelter into a novel environment than larger individuals. When individuals had recently experienced high levels of risk however, this pattern was reversed. We also found that individuals who had experienced any recent risk (low and high) were more likely to leave the safety of a shoal and approach a novel object compared to individuals who had not experienced any recent danger. Avoidance behaviour across the three assays was not affected by recent experiences but was affected by body size to varying degrees. For example, larger fish were more likely to stay in the plants, away from the cues of predation compared to smaller fish. Overall, our results suggest that how recent experiences with risk influence subsequent behaviour can depend on a variety of interacting factors including the intensity of recent experiences, the particular behaviour examined, and an individual's body size.

1. Introduction

Prey can use a variety of cues to infer the level of predation risk (Brown, 2003; Kelley and Magurran, 2003). By adjusting their behaviour in response to these cues, prey can increase their likelihood of surviving a predator encounter and can often escape detection by the predator altogether (Kelley and Magurran, 2003; Lima and Dill, 1990; Sih, 1992). For example, seeing a predator nearby causes individuals to increase refuge use and shoaling while decreasing foraging and courtship behaviours (Abrahams and Cartar, 2000; Candolin, 1997; Lima, 1998; Lima and Dill, 1990). Similarly, encountering chemical cues of predators or of injured conspecifics (i.e. alarm cues) causes individuals to reduce movement, increase refuge use and shoaling, and avoid the area with the cues (Brown, 2003; Brown and Godin, 1999; Brown et al., 2009; Ferrari et al., 2010a; Stephenson, 2016). Prey can also combine information from both visual and chemical cues to assess predation risk (Brown, 2003; Kelley and Magurran, 2003; Stephenson, 2016; Ward and Mehner, 2010). A nearby predator, whether determined from visual and/or chemical cues, clearly indicates danger in the current environment and prey can adjust their behaviour accordingly once the danger has been detected.

Experiencing cues of risk for an extended period of time might predict the likelihood of prey encountering predators in the future (Crane and Ferrari, 2017; Lima and Bednekoff, 1999; Lima and Dill, 1990). For example, studies have found that repeatedly encountering alarm cues over several days causes individuals to be neophobic and less active (Brown et al., 2013, 2006; Chivers et al., 2014). One interpretation of these patterns is that individuals behave more cautiously in order to minimize their encounters with novel potential dangers. However other studies have shown the opposite pattern with individuals from high predation populations being bolder and more exploratory than individuals from low-predation populations (Archard and Braithwaite, 2011; Brown et al., 2005, 2007; Brown, Burgess, & Braithwaite, 2007; Elvidge et al., 2014; Harris et al., 2010; Kortet, Sirkka, Lai, Vainikka, & Kekäläinen, 2015; but see Hellström and Magnhagen, 2011, 2017; Sommer-Trembo et al., 2017). Experiencing a

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high level of background risk might result in prey becoming habituated to these cues, or stress inoculated (Lyons and Parker, 2007). For example, encountering the daily stress of being chased by a net for > 10 days increases boldness and activity in both poeciliids and cichlids (Brown et al., 2007; Moscicki and Hurd, 2015). Similarly, rats encountering stressors over extended time periods are bolder and take more foraging risks (Chaby et al., 2015). This pattern of stress inoculation allows individuals to perform better under conditions of stress and make appropriate behavioural trade-offs between antipredator behaviour and other key behaviours such as foraging and courtship (Lima and Bednekoff, 1999; Lima and Dill, 1990; Sih, 1992).

How any one individual adjusts their behaviour to indicators of risk might vary due to many factors. For example, prey of different ages and sizes may not be equally vulnerable to predation risk. There is evidence for this in aquatic systems where small individuals are often at higher risk than large individuals due to gape-limited predators and/or slower swimming speeds (Lundvall et al., 1999; Sogard, 1997). In support of this, several studies have found that smaller individuals are less likely to take risks and are less exploratory than large individuals (Abrahams and Cartar, 2000; Ioannou et al., 2008). However, other studies have found the opposite pattern with smaller individuals being bolder, more willing to take risks, and emerging more quickly from a shelter than large individuals (Brown et al., 2007; Brown and Braithwaite, 2004; Brown et al., 2005; Dowling and Godin, 2002; Krause et al., 1998a, 2000; Polverino et al., 2016; Wilson et al., 2010). These conflicting patterns might be due to differential vulnerability to different types of predators and differences in the most appropriate behavioural responses (Templeton and Shriner, 2004). It is also possible that these conflicting patterns could arise from size dependent trade-offs between foraging and avoiding risk. For example, the higher metabolic demands of smaller individuals make them more willing to take risks in order to resume foraging compared to large individuals who are better able to cope with reduced foraging (Krause et al., 1998b). Smaller individuals might also resort to risky behaviour in order to increase their growth rate and escape the size-dependent risk of predation (Biro et al., 2005; Urban, 2007). It is possible that the trade-off between vulnerability to predation and vulnerability to starvation varies with respect to the level of risk perceived in the environment. How recent experiences with predation risk influence the willingness of different sized individuals to take risks remains unclear.

In this study, we examined whether recent experiences with predation risk affect the boldness and avoidance behaviour of different sized individuals across different contexts. Individuals were measured for exploration in a novel tank, response to conspecifics, and reaction to a predator, all of which might assess boldness, or the tendency to take risks. Specifically, if recent experience with risk makes individuals more cautious, they should be less likely to emerge from a shelter into a novel tank, leave the safety of a shoal, and approach a predator. If instead, recent experience with risk makes individuals stress inoculated, we would expect the opposite pattern.

2. Methods

2.1. Fish collection & housing

We collected western mosquitofish (*Gambusia affinis)* using dipnets from Lake Cheston, Sewanee, TN in May 2016. Mosquitofish are prey to many different predators and Lake Cheston has many piscivorous fishes (largemouth bass, *Micropterus salmoides*; green sunfish, *Lepomis cyanellus*), birds, insects, and reptiles. Mosquitofish were housed in mixed sex 38 L tanks (50 × 25 × 27 cm; length × width × height) with a sponge filter and artificial plants for at least one week before being used in the experiment. Fish were fed frozen daphnia and bloodworms and Tetramin flake food daily, and were held at approximately 22 C with a 14:10 light:dark cycle.

Each week during the summer of 2016, we moved groups of three females (except for the first week, when we set up two females per tank) into new 38 L tanks with artificial plants, a sponge filter, and black plastic on all sides to prevent interactions between tanks. Females were in these tanks for three days before receiving their treatment (see *Recent Predation Risk* below). Only sexually-mature (and likely pregnant) females were used to allow comparison with other studies on body size, boldness, and predation risk in female poecillids (e.g. Brown et al., 2014; Brown and Braithwaite, 2004; Brown and Godin, 1999; Elvidge et al., 2014), as well as to attempt to standardize foraging and reproductive demands across tested individuals. Females were assigned to treatment tanks randomly with respect to size and there were 24 treatment (home) tanks used over the 8 weeks of the experiment. Overall, risk treatments did not differ significantly from one another in their standard length (overall average: 33.2 +/- 0.8 mm; None: 33.0 +/- 1.3 mm, Low: 33.4 +/- 1.5 mm, High: 33.2 +/- 1.5 mm; $F_{2,\,24.5} = 0.002$, $P = 0.998$, n = 68).

2.2. Alarm cue

Following protocols from other studies (Brown and Godin, 1999; Brown et al. 2014; Brown et al., 2009; Stephenson, 2016), alarm substance was made by euthanizing field-caught females by decapitation with sharp scissors. To minimize pain and stress, females were housed in water that was slowly cooled by surrounding the holding container with ice in a darkened box prior to decapitation. We removed all gut and reproductive organs, and sonicated the entire body (skin, skeletal muscles, and bone but excluding organs and head) with DI water. Ground tissue and water were filtered through polyester floss fitted in a funnel. DI water was added to the alarm cue substance so that the concentration of the alarm substance was approximately 0.04 cm^2 of tissue per ml. In total, 16 females were euthanized (average standard length +/- SD = 3.9 +/- 0.6 cm) over the course of the experiment with fresh alarm cue from two females made each week. Alarm cue was frozen in 10 ml tubes and held in a -20 C freezer until needed. As a control, 10 ml tubes of DI water were also made at the same time as the alarm cue, and held in the same -20 C freezer.

2.3. Recent predation risk

After three days in their home tank, we manipulated the recent experiences with predators over two days. Female tanks were randomly assigned to one of three predation risk treatments: no risk, low risk, and high risk. Cues of predation risk involved the addition of 10 mL of thawed alarm substance as well as a 30 s bout of being chased by a realistic plastic model of a largemouth bass (manufactured by *Rainbow Island*: length = 29 cm, height = 12 cm). Each group received cues twice a day for two days at a random time each morning and afternoon to prevent habituation. The 'no risk' group received a thawed water cue twice (am and pm) on both days 1 and 2. The 'low risk' group received a thawed water cue twice (am and pm) on day 1, and predation cues (10 mL alarm substance plus bass chase) twice (am and pm) on day 2. The 'high risk' group received predation cues (10 mL alarm substance plus bass chase) twice (am and pm) on both days 1 and 2. To minimize the accumulation of alarm cues over time, each home tank continued to have constant filtration with a sponge filter over the treatment time.

This approach allows us to examine different levels of recent risk but also lets us distinguish between the effects of risk the day before testing (predation cues on day 2) which both low risk and high risk groups experienced, and the accumulated effects of risk cues over more than 1 day (predation cues on both day 1 and day 2) which only high risk groups experienced. For example, if individuals are most sensitive to cues of risk within the past 24 h, then we should expect similar behavioural responses to high and low risk treatments. Alternatively, if individuals use experiences over several days to shape their perception of risk, we should expect high and low risk treatments to show different behavioural responses. Unfortunately, this design does not allow us to examine the consequences of the temporal sequence of cues. For example, we cannot examine how the order of risk and safety cues across (or within) days affects behaviour.

After two days of their respective predation treatments (days 1 and 2), we measured the behaviour of each individual female in three different assays over the subsequent days: exploration assay on day 3, sociality assay on day 4, and predator assay on day 5. Assays were always conducted in the same fixed order, rather than in a randomized order. This was done because the behavioural response to the assays are specific to the order in which they are conducted and to minimize unwanted carryover effects (Bell, 2013). For example, the testing tank is only "novel" in the first encounter therefore the exploration assay was conducted first. Similarly, the addition of the alarm cues in the predator assay persist in the water therefore the predator test was conducted last. It is important to note that the testing assays extended over 3 days (see below) and individuals received no cues of risk over that time. Thus, these days of "safety" following the manipulation could be informative to individuals and change their perception of risk over time. New treatment and assay tanks were set-up each week with new females and focal females were not reused.

2.4. Exploration assay

Exploratory behaviour was measured in a 19 L tank (40 × 20 cm with 10 cm water depth) that was positioned over a 4 cm^2 grid and was covered on all sides with black opaque plastic. A shelter was constructed from a plastic cup (height = 12.5 cm) with the bottom cut out. The cup was positioned with the wide top end (diameter = 9 cm) flat against the ground and the cut bottom facing upwards allowing us to gently release a fish into the cup from above. The shelter consisted of two such cups, one on top of the other. The bottom cup had a small "doorway" cut into the side (width = 4 cm, height = 5 cm, 3 cm from bottom) and the top cup was whole on all sides with no doorway. By positioning this top cup over the bottom cup, we could prevent the focal fish from escaping out the door during acclimation. We released a single focal fish into the shelter cups and placed a cover on the top (darkening the inside of the shelter). After 30 s, the top cup was gently removed, revealing the door. We recorded (1) how quickly the focal fish completely emerged from the shelter with a maximum time of 10 min. Once the focal fish exited the shelter (or after 10 min), the shelter cup was gently removed and behaviour in the novel tank was measured for an additional 10 min. A video camera was positioned above the tank and from the videos, we measured (2) the time spent along the edge of the tank (as a measure of thigmotaxis).

2.5. Sociality assay

After completing the exploration assay, focal fish were moved to a novel 19 L tank (water depth = 12 cm) that was covered on all sides with black opaque plastic, had an artificial plant and a sponge filter. Focal fish acclimated in this tank overnight. The next day (day 4), boldness and social behaviour of the focal fish was measured in this same tank. Prior to testing, the front black cover of the tank, the plant, and sponge filter were removed. The focal fish was gently covered in a plastic acclimation cup (as above without the doorway) in the centre of the tank for ˜30 s while the assay was set up. Two glass beakers (GROSCHE French press: diameter = 9.5 cm; water depth = 12 cm) were placed on each side of the tank (touching opposite walls). One beaker contained a shoal of three unfamiliar field-collected mosquitofish and the second beaker contained water as a control. Beaker sides were alternated between trials. We gently released the focal fish and removed the acclimation cup. A video camera was positioned in front of the tank and recorded the focal fish for 5 min.

From the videos, we measured (1) the latency to first approach the shoal within a body length and the time spent with it, and (2) whether or not the empty beaker on the opposite side of the tank was approached. Two new groups of shoal individuals were used each week and alternated for each trial. After completing the sociality assay, the plant and sponge filter were returned, the front was covered, and the focal fish remained in the testing tank overnight.

2.6. Predator assay

The next day (day 5), behaviour of the focal fish in response to predation risk was measured in the same testing tank as above. We used both visual (plastic model of largemouth bass) and chemical cues (10 mL alarm cue) to simulate predation risk. Prior to testing, the front black cover of the tank and the sponge filter were removed. The focal fish (who had spent the last 2 days in the tank) was gently covered in a plastic acclimation cup (as above without the doorway) in the centre of the tank for ˜30 s while the assay was set up. The artificial plant was moved to one side of the tank and the plastic bass model was positioned outside the opposite side of the tank and looking into the tank. Cues were also added to both sides simultaneously: 10 mL of alarm cue (concentration 0.04 cm^2) was added to the bass side and 10 mL of water was added to the plant side as a control. Lines were drawn on the outside of the tank to indicate 10 cm on each side (10 cm for plant side and 10 cm for bass side) with each side separated by 13 cm. We gently released the focal fish and removed the acclimation cup. A video camera was positioned in front of the tank and recorded the focal fish for 5 min.

From the videos, we measured (1) the latency to first approach the bass model within 10 cm, and (2) the time spent in the 10 cm plant area opposite to the model bass. Preliminary trials with water and food colouring showed that 10 ml of coloured liquid dispersed throughout the bass side within 30 s, halfway through the tank within 60 s and throughout the tank within 90 s.

After completion of all assays, we measured the standard length of each fish from the tip of the snout to end of the caudal peduncle using laminated graph paper to the nearest 0.5 mm. Videos were watched using Jwatcher (Blumstein et al., 2010) by N.A.D. in a random order with respect to treatment and test-week and blind to fish treatment. We measured the behaviour of 69 females total. One female was excluded from all analyses because she died after giving birth during the experiment. Additionally, due to a video camera malfunction in the exploratory assay after recording emergence time ($N = 1$) and accidental death of one female in the predator assay ($N = 1$), our sample size varies among the analyses ($N = 67–68$ females).

2.7. Data analyses

We examined how recent predation risk (none, low, high) affected boldness and avoidance behaviour within each of the three assays. To examine boldness, we compared recent predation risk treatments in whether or not an individual (1) left the safe shelter to explore the novel environment (*exploration assay:* emerge – yes/no), (2) left the safety of the shoal to approach the area with the empty beaker (*sociality assay:* approach beaker – yes/no), and (3) entered the bass area to inspect the predator (*predator assay:* approach bass – yes/no). These measures were all yes-no measures rather than latencies since approximately half of the individuals did not show the behaviour (see results). We examined these binomial measures (yes or no) of boldness with separate generalized linear mixed models (with a binomial distribution, a logit link, and the Laplace method for likelihood approximation).

To examine avoidance (or safety) behaviour, we used separate general linear mixed models (GLMs) to compare recent predation risk treatments in the proportion of time individuals spent (1) along the edge of the tank in the *exploration assay* (thigmotaxis), (2) with the shoal beaker in the *sociality assay*, and (3) in the safety of the plants opposite to the model bass in the *predator assay*. The proportion of time individuals spent in these avoidance behaviours during each of the assays was calculated of a total of 10 min in the exploration assay and 5 min in the sociality and predator assays.

To account for potential differences in vulnerability, we included body size as a covariate in all analyses (GLMs and GLMM), and initially interactions with size were also included but the covariate and interactions were removed if nonsignificant. In all analyses (GLMs and GLMM), we included 'home tank' (i.e. the groups in which individuals received their treatment each week, n tanks = 24) as a random effect. We specified REML estimation and the Satterthwaite method for estimating the degrees of freedom. Model assumptions were validated by examining residuals. All variables were left untransformed except for the latency to approach the shoal which was natural log-transformed after adding 1 to all values. We used Spearman rank correlations to explore any relationships among behavioural measures and standard length.

All analyses were conducted in R, version 3.4.4 (R Core Team, 2018), with packages lme4 (Bates et al., 2015), lmerTest (Kuznetsova et al., 2017), and with figures made in ggplot2 (Wickham, 2016). Means +/- SE are given throughout.

2.8. Ethical note

All animal collections were approved by the Tennessee Wildlife Resources Agency (TWRA permit# 3896) and all experiments were approved by the ACUC at the University of the South (protocol# McGhee 1–2016). Field-collections and housing conditions in the lab adhered carefully to guidelines for ethical animal treatment (ASAB/ABS, 2018). In addition to daily care and *ad libitum* feeding with diverse food types (including during the behavioural testing period), all tanks had enrichment (e.g. plastic plants) and constant water movement (e.g. sponge filters), animals were housed in groups unless being tested in an assay, and stressors (chemical and visual) were designed to be brief and ecologically relevant. Tennessee scientific collection permits prohibit release of any wild-caught aquatic animal after 12 h. Thus, the animals that were euthanized to make alarm cue in this experiment could not have been released and had been living in our "retirement" tanks from earlier field collections. We also could not release the field-collected females used in this experiment and they were transferred to multiple large stock tanks for future experiments.

3. Results

In the exploration assay, 41 of 68 fish emerged from the shelter into the novel tank within the 10 min time period (number emerging/total: None: 15/23, Low: 13/23, High: 13/22). Whether or not an individual emerged from the shelter depended on an interaction between their recent experience with risk and their body size (Fig. 1; Table 1). If recent experiences suggested low risk or no risk, larger fish were more cautious than smaller fish and were less likely to emerge from the shelter in the exploration assay. In contrast, if recent experiences suggested high risk, smaller fish were less likely to emerge from the shelter than larger fish. Although smaller fish spent a slightly greater proportion of time along the tank edge than larger fish (r_S = -0.20, $P =$ 0.113, n = 67) this was not affected by treatment (None: 0.45 +/- 0.06, Low: 0.50 +/- 0.06, High: 0.45 +/- 0.05; Table 2).

In the sociality assay, regardless of their recent experience treatment, all individuals approached the shoal very quickly (46 +/- 8 s; treatment $F_{2,\,68}$ = 0.591, $P =$ 0.557) and spent most of their time with the shoal (69.4 +/- 3% of time; Table 2). However, recent experience with predation risk did affect whether individuals were willing to leave the shoal and approach the area with the empty beaker within the 5 min time period (43 of 68 individuals). Specifically, individuals who had experienced any recent predation risk (low or high risk treatments) were nearly twice as likely to approach the empty beaker on the opposite side of the tank than individuals who had experienced no recent risk (number of approachers/total: None = 9/17, Low = 17/23, High = 17/22; Table 1).

In the predator assay, 49 of 67 fish approached the bass area with the alarm cue within the 5 min time period (number of approachers/total: None = 14/22, Low = 16/23, High = 19/22). Risk treatment did not significantly affect whether or not an individual was willing to approach the area with the visual and chemical cues of predation (Table 1). Similarly, the proportion of time spent in the plants opposite to the model bass and the alarm cues was not affected by treatment but was affected by body size (None: 0.56 +/- 0.08, Low: 0.56 +/- 0.08, High: 0.53 +/- 0.06; Table 2). Larger fish spent significantly more time in the plants than smaller fish (Fig. 2; r_S = 0.45, $P <$ 0.0001, n = 67).

All data has been deposited in the Mendeley digital depository (doi:10.17632/ry8d4jnb47.1).

4. Discussion

We found that boldness was affected by recent experience with cues of predation risk but this depended on body size and the context under which the behaviour was measured. First, the willingness to emerge into a novel environment depended on an interaction between recent experience with risk and body size (Fig. 1). Second, individuals were twice as likely to leave the safety of a shoal and approach an empty beaker on the opposite side of the tank if they had recently experienced cues of predation risk (i.e. either low and high predation risk treatments) compared to individuals who had experienced no cues of risk. Finally, the tendency to avoid cues of predation risk and stay in the safety of plants was not affected by treatment but was strongly affected by body size with larger individuals spending more time in the plants than smaller individuals (Fig. 2).

Boldness can differ between populations and while some of the differences between high and low predation populations might have a heritable component, boldness can also be sensitive to cues of predation risk experienced over various time periods. Multiple studies have found that fish from high predation populations tend to be bolder and emerge from a shelter faster than fish from low predation (Brown et al., 2007a, b, 2005; Elvidge et al., 2014; Harris et al., 2010; Kortet et al., 2015; but see Hellström and Magnhagen, 2011, 2017; Sommer-Trembo et al., 2017). In some cases, these behavioural differences persist in the lab-reared animals (e.g. Brown et al., 2007a), but in other cases, the predation risk experienced early in life and over an extended time underlay the differences (e.g. Hellström and Magnhagen, 2011, 2017, reviewed in Kelley and Magurran, 2003). Additionally, behaviour can be modified by more recent experience over a much shorter time period. For example, chasing fish with a net for 2 min every day for over a week increases boldness and fish emerge faster into a novel environment (Brown et al., 2007a; Moscicki and Hurd, 2015) regardless of whether the fish had high- or low-predation population parents (Brown et al., 2007a). Similarly, we found that experiencing recent and brief cues of danger affected boldness in both a novel environment and with a shoal of conspecifics, even in field-collected adult mosquitofish that had presumably had many experiences with predators, including largemouth bass, throughout their lives. Exploring how the intensity of predator cues experienced recently combines with cues received early in life or those encountered multiply through time would be interesting, particularly since animals seem to be able to distinguish between different levels of predation risk (Brown et al., 2006, 2009; Ferrari et al., 2010b) as well as extrapolate risk to other habitats (Mitchell et al., 2018), other predators (Ferrari et al., 2007), and future time points (Crane and Ferrari, 2017).

Our results suggest that perceived risk might explain the conflicting patterns of how body size affects boldness. Specifically, we found that small individuals were bolder than larger individuals in the novel tank, but only when they had recently experienced low levels of risk or no risk. This pattern of smaller fish being bolder than larger fish is consistent with multiple studies (Brown and Braithwaite, 2004; Brown et al., 2005, 2007b; Dowling and Godin, 2002; Krause et al., 1998a,

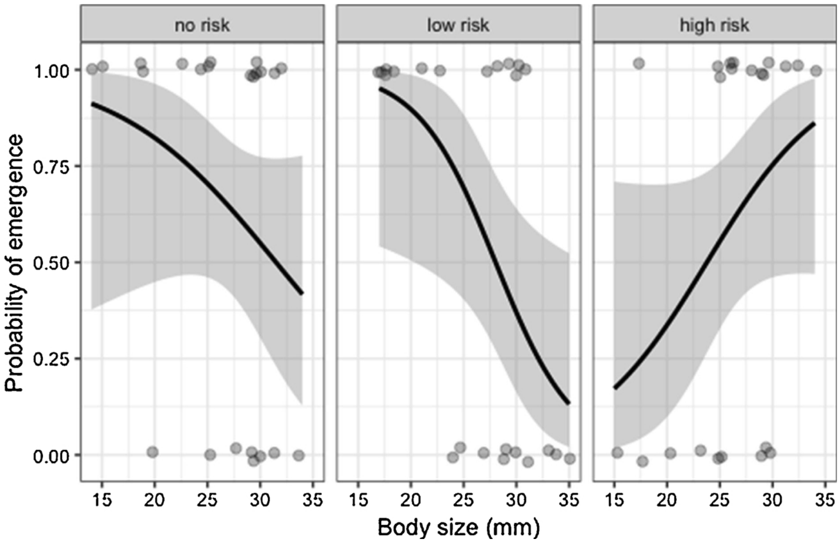

Fig. 1. Recent experience with predation risk interacts with body size to affect boldness in the exploration assay (i.e. willingness to emerge from shelter into a novel tank within 10 min). Predicted lines with confidence intervals are shown for each treatment with each point indicating a single individual (n = 67: no risk n = 22, low risk n = 23, high risk n = 22).

Table 1
Results from generalized linear mixed models examining how boldness was affected by recent risk experiences across the three assays (n = 68, 68, 67). Statistically significant factors are in bold.

Parameter	Estimate	SE	χ^2	Df	F-value	P-value
Day 1 – exploration assay: emerge from container (yes/no)						
(Intercept)	4.20	2.85				
Risk treatment			0.296	2	0.280	0.862
None vs Low:	3.39	4.67				
None vs High:	−8.46	4.01				
Size	−0.13	0.10	1.884	1	0.911	0.170
Treatment X Size			**10.450**	**2**	**4.173**	**0.005**
None vs Low:	−0.14	0.17				
None vs High:	0.31	0.15				
Day 2 – sociality assay: approach empty beaker (yes/no)						
(Intercept)	−0.45	0.45				
Risk treatment			**7.728**	**2**	**4.035**	**0.021**
None vs Low:	1.69	0.72				
None vs High:	1.50	0.68				
Day 3 – predator assay: approach model predator (yes/no)						
(Intercept)	4.57	2.23				
Risk treatment			2.800	2	1.408	0.246
None vs Low:	0.67	0.95				
None vs High:	1.84	1.09				
Size	−0.15	0.08	3.498	1	3.658	0.061

2000; Wilson et al., 2010) and the underlying mechanism proposed has been the elevated metabolism of these smaller fish (Krause et al., 1998a). In fishes with indeterminate growth, body size is associated with age and another explanation for larger individuals being more cautious than smaller individuals is that larger older individuals have increased experience with risk. However, in support of the importance of metabolism in boldness, studies have found that food deprivation and unpredictability increases risk-taking behaviour (Biro et al., 2005; Chapman et al., 2010; Killen et al., 2011) and risk-taking itself is associated with increased food consumption and growth (Biro et al., 2005; Ioannou et al., 2008). Our results are consistent with the hypothesis that, for small individuals, the cost of lost foraging opportunities can outweigh the relatively low chance of encountering a predator when recent experiences suggest low or no predation risk. Interestingly, in line with our findings, some studies comparing populations have also found that smaller individuals were bolder than large individuals but only in low predation populations (Brown and Braithwaite, 2004; Roy and Bhat, 2018; but not Brown et al., 2005).

Our results also suggest that when predation risk is high (i.e. fish had received visual and chemical cues of risk four times over the past two days), the relationship between boldness and body size reverses. Under these conditions, small fish are no longer willing to take more risks than large fish potentially due to smaller fish being more vulnerable to gape limited predators, outweighing the costs of any lost

Table 2
Results from general linear mixed models examining whether recent risk experiences affect the proportion of time individuals spend showing avoidance/safety behaviour across the three assays (n = 67, 68, 67). Statistically significant factors are in bold.

Parameter	Estimate	SE	χ^2	Num df, Den df	F-value	P-value
Day 1 – exploration assay: proportion of time spent on outer edges (thigmotaxis)						
(Intercept)	0.77	0.16				
Risk treatment			0.559	2, 23.2	0.344	0.713
None vs Low:	−0.001	0.08				
None vs High:	0.05	0.08				
Size	**−0.01**	**0.006**	**4.057**	**1, 59.2**	**4.202**	**0.045**
Day 2 – sociality assay: proportion of time spent with the shoal						
(Intercept)	0.76	0.05				
Risk treatment			2.15	2, 20.9	1.115	0.347
None vs Low:	−0.10	0.08				
None vs High:	−0.10	0.08				
Day 3 – predator assay: proportion of time spent in the plants						
(Intercept)	0.05	0.20				
Risk treatment			0.039	2, 18.3	0.040	0.961
None vs Low:	−0.01	0.12				
None vs High:	−0.03	0.12				
Size	**0.02**	**0.01**	**4.94**	**1, 66.5**	**7.538**	**0.008**

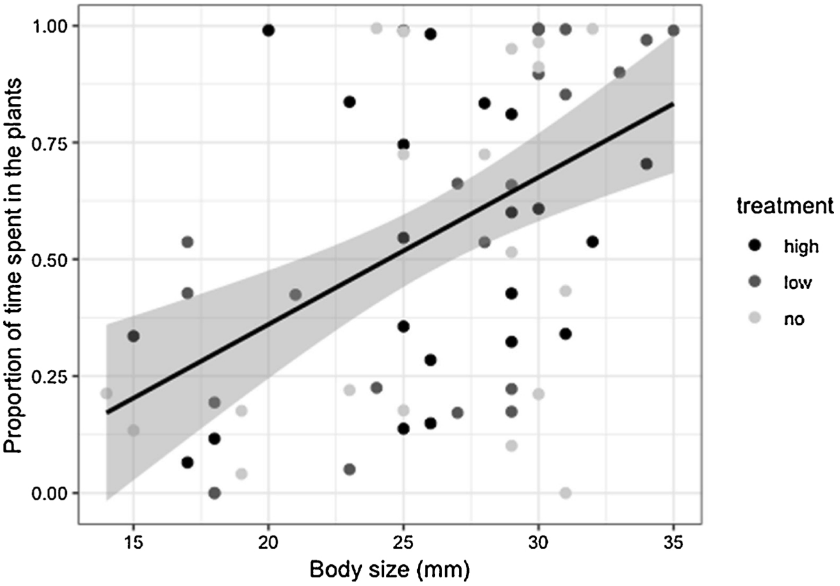

Fig. 2. The proportion of time individuals spent in the plants when cues of predation risk were present (Day 3: Predator assay) was positively correlated with body size (no risk n = 23, low risk n = 23, high risk n = 22).

foraging opportunities. This pattern is consistent with studies finding no clear association between body size and boldness (Archard and Braithwaite, 2011; Harris et al., 2010; Ingley et al., 2014; Ioannou et al., 2008; Moscicki and Hurd, 2015), or no association in high predation populations (Brown and Braithwaite, 2004; Roy and Bhat, 2018). A recent study manipulating background risk throughout early life (> 80 days) in fathead minnows found a strikingly similar pattern of boldness being affected by background risk in combination with body size (Meuthen et al., 2019). Specifically, cues of low risk resulted in smaller fish being bolder than larger fish while cues of high risk caused this size-dependent pattern to disappear (Meuthen et al., 2019). Our results suggest that these size-dependent relationships are flexible even into adulthood and that even brief periods of risk have the potential to change how boldness is affected by body size. The possibility that recent stressful experiences can change the trade-offs between predation risk and foraging needs is intriguing and further studies would be worthwhile, particularly across predation regimes.

As cues of risk become frequent, animals are predicted to decrease their antipredator behaviour and prioritize foraging during periods of low risk in order to balance their conflicting needs (predation risk allocation hypothesis: Lima and Bednekoff, 1999). We found that individuals in the high- and low-risk treatments were twice as likely to leave the shoal and approach the area with the empty beaker on the other side of the tank compared to the no risk treatment (% approaching the flask: high risk = 77%, low risk = 74%, no risk = 39%). If high and low risk individuals perceive the environment as dangerous, it is possible that they are taking advantage of the period of safety during testing (i.e. no current cues of risk) by increasing their foraging effort and decreasing their antipredator shoaling behaviour (or relying on vigilance from other group members). Shoaling is a well-known antipredator behaviour but can increase competition for food among group mates (Pitcher and Parrish, 1993). It is also possible that moving away from the shoal might be an attempt by individuals to position themselves at the front of the shoal, in order to reap the foraging benefits of the front position (Krause et al., 1998b). Exploring how brief predator encounters influence subsequent social behaviours over time would be an interesting research avenue, particularly since social interactions and collective behaviours are affected by population differences in predation risk (e.g. Herbert-Read et al., 2017; Ioannou et al., 2017).

In contrast to boldness in the exploration and sociality assays, recent risk did not affect behaviour in the predator assay, whether the willingness of an individual to approach a potential predator or the time spent in the safe plant area. It is possible that when both visual and chemical cues of risk are detected, current information about danger outweighs that of past experiences (Lima and Dill, 1990). Thus, past experiences with risk induced by our treatments might no longer be informative when the current conditions indicate the presence of a potential predator. Alternatively, the effects of our treatment might have decayed over the 3 days of testing and been updated with new information about risk (Ferrari et al., 2010a). Specifically, over the testing period (Days 1–3) individuals received no cues of risk and these several days of safety might have changed their perception of risk. If all individuals (regardless of initial risk treatment) are indeed perceiving the environment to be generally safe by Day 3, that smaller individuals are spending less time hiding in the plants compared to larger individuals is consistent with smaller fish being less able to cope with the metabolic costs of reduced foraging and more willing to take risks (Krause et al., 1998a). Similarly, we found that regardless of risk treatment, the majority of individuals approached the area with the model predator and alarm cues (60%–86%). There are benefits to predator inspection, such as information about predator threat and motivation (reviewed in Dugatkin and Godin, 1992; Pitcher and Parrish, 1993), and if individuals now perceive the environment to be low risk, this information might be particularly important. Testing individuals in the predator assay on the day following the last treatment cue, rather than three days later, could help distinguish between these possibilities. It would be particularly interesting to explore whether recent experience with risk affects the relationship between body size and the tendency to avoid the predator cues (i.e. stay in plants), similar to how risk treatment affected the relationship between body size and emergence (Day 1: exploration assay).

Overall, our study suggests that the influence of recent experiences on subsequent behaviour can depend on a variety of interacting factors including the intensity of recent predator encounters, the particular behaviour examined, and an individual's body size. Exploring the fitness consequences of this behavioural plasticity in response to recent experiences and body size would be promising for future studies.

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