Short-term environmental variation in predation risk leads to differential performance in predation-related cognitive function

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Predation produces strong selection on numerous traits affecting prey survival, such as morphology or behaviour. However, little is known about the influence of predation on behavioural traits that reflect cognitive abilities. Two studies have shown negative relationships between predation pressure and performance in foraging-related tasks in different populations of fish. Whether these differences are due to population differences or plasticity is unknown. In addition, little is known of the effect of predation risk on predator-related cognitive function. Here, I exposed woodfrog, Lithobates sylvaticus, tadpoles to a high or low level of background risk using injured conspecific cues for 4 days. Following this period, I conditioned them to recognize a novel predator as a threat. I compared the intensity of the learned response between the two groups the following day, and compared retention after 10 days. I found that high-risk tadpoles learned to respond to the predator with a greater intensity of antipredator response than low-risk tadpoles. This is the first study to demonstrate that recent history of predation risk can affect the cognition of prey, demonstrating plasticity in a relatively fixed learning mechanism. My results also raise questions regarding the existence of cross-contextual cognitive trade-offs in animals: increased cognition in predation-related tasks may come at the expense of foraging-related tasks.

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It is well known that environmental and ecological constraints shape the cognitive ecology of animals. Species with different foraging tactics will differ in the way food-related information is processed. Food-caching species, for instance, possess better spatial memory than noncaching species (Shettleworth, 1990). Species living in complex habitats are better at solving spatial tasks such as mazes (Williams et al., 2001). Cognitive abilities can also vary throughout an individual’s life, such as adaptive ontogenetic changes in cognitive performance associated with changes in habitat types (Takahashi, Masuda, & Yamashita, 2010). The potential for those cognitive traits to change according to environmental variation is known as cognitive plasticity. When it comes to the effect of predation on cognitive plasticity, a handful of studies have demonstrated differences in spatial-learning tasks between populations of fish. Brown and Braithwaite (2005) showed that poeciliids from low-predation/low-competition populations solved a maze faster than those from high-risk/high-competition populations. Similarly, Brydges, Heathcote, and Braithwaite (2008) found that sticklebacks (Gasterosteus aculeatus) from low-predation populations learned a foraging task faster and retained the information longer than fish from the high-predation populations. These results suggest that differences in environmental conditions between the populations may drive some phenotypic plasticity in cognitive traits. Specifically, the presence of predation-related stressors might lead to poorer performance in the cognitive tasks. Whether such cognitive plasticity reflects selection-mediated, population-level differences or individual plasticity is unknown. If the presence of predators can act as a stressor and in turn lead to poorer predation-related cognitive function, it could provide a negative feedback loop that would render prey living in predation-rich environments more susceptible to predation, by decreasing the amount of learning-mediated antipredator adaptations observed in the community.

My goal in this study was to investigate the effect of short-term exposure to predation on predation-related cognitive traits, namely learning and retention of predator information, and to assess the existence of individual cognitive plasticity. A recent study by Brown, Ferrari, Elvidge, Rammarine, and Chivers (2013) showed that short-term exposure to predation risk could lead to dramatic changes in responses to novel stimuli. Fish and larval amphibians maintained in a high-risk environment for 4 days showed neurophobic (fear of anything new) responses to novel odours, while those maintained in a low-risk environment did not respond to those unknown stimuli. Predation risk is therefore causing deep alteration in the way in which prey interpret what is risky, and this, in turn, could likely affect how risk-related information is learned and/or retained. I maintained woodfrog tadpoles, Lithobates sylvaticus, in a high-risk or low-risk environment using injured conspecific cues. In aquatic ecosystems, freshwater and marine alike, a wide diversity of prey, ranging from corals to amphibians, respond to injured conspecific cues with immediate antipredator responses, which in turn, gives them a survival advantage during predator–prey encounters (Chivers et al., 2013; Ferrari, Wisenden, et al., 2010). Using these cues to create a risky environment is advantageous as it does not provide prey with a predator-specific context, and hence, does not bias the learning of one type of predator over another. Following this risk exposure period, I conditioned the tadpoles from both groups to recognize a novel tiger salamander, Ambystoma tigrinum, as a predator via pairing of injured conspecific cues with salamander odour. This is a well-established learning paradigm in many aquatic species, including woodfrogs (Ferrari & Chivers, 2009). The tadpoles were then tested for their response to salamander odour alone or to a water control, either the next day or 10 days later. I compared the occurrence and intensity of the learned response to the predator 1 day postconditioning, and again after 10 days. If predation risk is acting as a negative stressor, I predicted that high-risk tadpoles would learn to respond to the predator with a lower intensity and lose their response quicker. However, if the high-risk conditions provide a stimulating environment leading to risk-aversive cognitive traits (as seen with the neophobic responses), I expected high-risk prey to learn to associate the predator with higher threat and maintain a stronger response for longer.

METHODS

Ethical Note

All work performed herein was approved by the University of Saskatchewan Animal Care protocol 20060014. All procedures took place outdoors, allowing tadpoles to experience natural conditions (temperature, precipitation, sun exposure) and other factors that may vary naturally in the field. Four weeks prior to starting the experiment, a 1900-litre tub was filled with well-water and seeded with plankton and aquatic plants (sedges, slough grasses) from a local pond. This procedure ensured that the water that would be used in the experiment contained natural pond odours, but lacked any cues from potential predators. Tadpoles were collected as eggs (six egg masses) in nearby ponds, and raised in separate pools to control for food availability and predation pressure. Tadpoles fed on algae present in the pool, along with a supplement of alfalfa chow. The salamanders were originally collected from a Saskatchewan pond and maintained in the laboratory for 3 years. They were fed earthworms, every 3 days for 2 weeks, prior to use in the experiment.

Experimental Design

Background risk

Groups of 12 tadpoles were placed in each of 36 pails containing 2 litres of water. The 36 pails were randomly allocated to one of four groups resulting from the following 2 × 2 design: tadpoles would experience either a low or high background level of risk for 4 days, and would then undergo either a pseudoconditioning (water paired with salamander odour: no learning) or a true conditioning (injured conspecific cues paired with salamander odour: learning). High- and low-risk conditions followed established protocols (Brown et al., 2013). Tadpoles in the high-risk group received an injection of injured conspecific cues three times/day for 4 days, while those in the low-risk group received a water injection on the same schedule. The injection of injured conspecific cues was obtained by placing three tadpoles in a mortar, quickly removing excess water and euthanizing them with a blow to the head using the pestle (Ferrari & Chivers, 2009). The bodies of the tadpoles were then grinded to a paste, which was then suspended in 10 ml of water. This solution was filtered and then injected in one of the pails. The injections were distributed throughout the day, from 0900 until 1800 h, with at least a 3 h delay between injections. Each pail contained an excess of alfalfa pellets to ensure that food was always available to the tadpoles. The water in each pail was changed once daily, 1 h after the last injection of the day, and food was added immediately after the water change.

Conditioning

The day following the end of the 4-day exposure, half of the pails in the high-risk and low-risk treatments received a conditioning of 10 ml of injured conspecific cues and 20 ml of salamander odour, while the other pails received a pseudoconditioning of 10 ml of water paired with 20 ml of salamander odour. Once again, the water was changed 1 h after the end of the conditioning procedure.
and food was added to the pails. The injured conspecific cue solution was prepared the same way as the solution used in the pre-exposure phase. The salamander odour solution was obtained from individually soaking six salamanders (range 11.9–14.1 cm snout–vent length) in 1.5 litres of water for 24 h, and then freezing the water until needed. I randomly selected which salamander odour to use for each trial. The salamanders were fed the day prior to odour collection, but not during the collection.

Testing
Testing took place 1 day and 10 days postconditioning, using a well-established methodology (Ferrari & Chivers, 2008, 2009; Ferrari, Manek, & Chivers, 2010). The risk background treatment was not continued during that time, as I wanted to test how the risk regime just prior to learning would affect learning and retention. Presumably, maintaining tadpoles under high- and low-risk throughout the testing period could lead to different response pattern. Tadpoles were placed individually in a 0.5-litre cup filled with water and left to acclimate for 2 h. Behavioural observations were carried out for 4 min prior to and 4 min following the stimulus injection, which consisted of a 5 ml injection of either water or salamander odour. During each observation period, I counted the total number of times the tadpole crossed the medial line of the cup, as a proxy for activity. Decreased activity is a well-established antipredator response for tadpoles. For each time period, I tested four tadpoles from each pail, two exposed to water and two exposed to salamander odour. Each tadpole was only tested once. After each trial, the tadpoles were measured and released into their native pond. There were no differences in tadpole size among groups ($P > 0.4$).

Statistical Analysis
After verifying that none of the prestimulus values differed between treatments (four-way nested ANOVA: all $P > 0.1$ for day 1, all $P > 0.3$ for day 10), pre- and poststimulus data were computed into a proportion change in line crosses ((post – pre)/pre), which was then used as dependent variable in subsequent analyses. For each time period, I ran a four-way nested ANOVA, investigating the effects of risk (low versus high), conditioning (true versus pseudoconditioning) and cue (water versus predator odour) on the behavioural activity of the tadpoles, and introducing pail as a nested factor (cue nested within pail), making pail, not tadpole, the unit of replication. Subsequent three-way nested ANOVAs were performed to investigate further the nature of the interactions.

RESULTS

1 Day Postconditioning
The four-way nested ANOVA revealed a significant three-way interaction among risk, conditioning and cue ($F_{1,1102.6} = 4.3, P = 0.04$; Fig. 1) on the behaviour of tadpoles, but no effect of pail ($F_{3,1102} = 0.9, P = 0.7$). When tadpoles were pseudoconditioned with water (no learning), I failed to find an effect of risk ($F_{1,52} = 0.7, P = 0.4$), cue ($F_{1,16} = 0.1, P = 0.9$) or any interaction ($F_{1,52} = 0.7, P = 0.4$), indicating that all the tadpoles displayed similar responses to both cues, regardless of risk. I also failed to find an effect of pail ($F_{1,52} = 0.7, P = 0.8$). When tadpoles were conditioned with injured conspecific cues (true learning), however, I found an interaction between risk and cue ($F_{1,508.5} = 5.3, P = 0.025$), but no effect of pail ($F_{1,50} = 1.2, P = 0.3$). Tadpoles showed a stronger response to predator odour than to water (low-risk: $P < 0.016$; high-risk: $P < 0.001$), indicating that both groups of tadpoles learned to recognize the predator as a threat. In addition, tadpoles exposed to water did not differ in their antipredator response ($P = 0.6$), but those exposed to predator odour did ($P = 0.006$), with high-risk tadpoles showing a stronger antipredator response than low-risk ones.

10 Days Postconditioning
The four-way nested ANOVA, once again, revealed a significant three-way interaction ($F_{1,104} = 3.4, P = 0.05$; Fig. 2), but no effect of pail ($F_{3,104} = 0.7, P = 0.8$). When tadpoles were

![Figure 1. Mean ± SE proportion change in activity for tadpoles tested 1 day after conditioning, for their response to water (empty bars) or salamander odour (solid bars). Tadpoles were exposed to a high or low level of risk for 4 days, then conditioned (or pseudoconditioned) to recognize a salamander as a predator threat.](image1)

![Figure 2. Mean ± SE proportion change in activity for tadpoles tested 10 days after conditioning, for their response to water (empty bars) or salamander odour (solid bars). Tadpoles were exposed to a high or low level of risk for 4 days, then conditioned (or pseudoconditioned) to recognize a salamander as a predator threat.](image2)
pseudoconditioned, neither risk ($F_{1,16} = 1.7, P = 0.2$) nor cue ($F_{1,52} = 0.1, P = 0.8$) affected the behaviour of the tadpoles, and I once again failed to find an interaction between these two factors ($F_{1,52} = 0.1, P = 0.8$) or an effect of pair ($F_{16,52} = 0.8, P = 0.7$). When the tadpoles were conditioned with injured conspecific cues, I found a significant two-way interaction between risk and cue ($F_{1,52} = 4.3, P = 0.043$), but no effect of pair ($F_{16,52} = 0.7, P = 0.8$). Again, tadpoles exposed to water did not differ in their responses ($P = 0.1$), and their responses did not differ from the responses of tadpoles from the low-risk group exposed to predator odour ($P = 0.2$). In addition, tadpoles from the high-risk group showed stronger antipredator responses to the predator odour than did tadpoles from the low-risk group ($P = 0.001$), indicating that tadpoles from the high-risk group were the only ones to maintain an antipredator response to the predator odour.

**DISCUSSION**

My results clearly indicate that background levels of risk affect learning and memory formation of larval amphibians. Indeed, all the tadpoles conditioned to recognize the predator as a threat displayed a significant antipredator response to the salamander odour, but tadpoles that were maintained in a high-risk environment prior to learning showed a stronger learned response to the predator and maintained that response longer than tadpoles maintained in a low-risk environment. This is the first time that an extrinsic factor like the riskiness of the environment has been shown to change the way in which information is learned and encoded by a prey animal, thereby demonstrating individual-level plasticity in learning. Such plasticity has already been demonstrated in a foraging context (Warburton, 2003), but has not been previously described in a predation context. Given that the circuitry for fear differs from that for foraging, one may or may not expect similar factors to affect learning outcomes.

Learning with injured conspecific cues has been demonstrated in a wide variety of aquatic species, flatworms and teleost fishes alike (reviewed in Ferrari, Wisenden, et al., 2010). Given its pervasiveness, even in the most primitive animals, this learning ability either stems from common ancestral structures, or has independently evolved a surprising number of times. The learning mechanism is very much Pavlovian-like, with the exception that repetition is not necessary for learning to occur. Much like conditioned taste aversion, the so-called ‘Garcia effect’ (Garcia, Lasiter, Bermudez-Rattonic, & Deems, 1985), learning something on which your survival depends should only require one trial. In contrast to nonpredation learning tasks (e.g. maze solving, choice tests or operant-conditioning tasks), the speed at which information is learned or the number of errors made to completion of a task cannot be the main performance evaluation trait. However, other traits such as the intensity of response and the retention of information related to predator risk can be evaluated. A number of factors, linked to the learning mechanism, have been shown to affect individuals’ response intensity and information retention. For instance, conditioning with increasing concentrations of injured conspecific cues leads to both a greater intensity of response at learning and a longer retention of information (Ferrari, Brown, Bortolotti, & Chivers, 2010). Changes in the prey’s intrinsic traits, such as size and/or growth rate, do not affect the intensity of the learned response, but they do affect the retention of information (Brown et al., 2011; Ferrari, Vrtelova, Brown, & Chivers, 2012). Could temporal variation in risk affect the perceived value of injured conspecific cues?

Tapping into the foraging literature, my results could be put into a ‘priming’ framework. Background levels of risk in the present study seemed to prime tadpoles to respond with a greater intensity to the predator, which may have resulted in an enhanced response. Priming is a form of implicit memory, whereby exposure to one stimulus may enhance the response to another stimulus (Meyer & Schvaneveldt, 1971). In foraging experiments, both sequential priming (repeated exposure to one type of prey) and associative priming can increase the efficacy of prey detection, both by increasing a predator’s strike accuracy and decreasing its response time (Bond & Kamil, 1999). Both mechanisms are thought to contribute to a predator’s search image ( Tinbergen, 1960). However, my results seem to differ slightly from what would be predicted in a priming context. First, repeated exposure to injured conspecific cues should have increased the tadpoles’ response to this stimulus, but instead, it decreased the tadpoles’ intensity of response, as predicted by the risk allocation hypothesis (see below). Also, the predator pairing only occurred once (during conditioning), so presentation of injured conspecific cues may not have been a good predictor of the appearance of a predator, and thus, may not fall within a priming framework. Before any conclusion can be reached, more work is needed to understand the role of priming (if any) in my results.

While the effects of spatial variation in predation risk on prey ecology have long intrigued ecologists, the effects of temporal variation in predation risk have only recently begun to be explored since the publication of Lima and Bednekoff’s (1999) risk allocation hypothesis. Lima and Bednekoff’s model allowed ecologists to understand why prey in a high-risk environment, for instance, show a lower-intensity response to predation threat than prey in a lower-risk environment. This counterintuitive result stems from the fact that prey deal with variation in predation risk in a temporal manner, affecting moment-to-moment decisions based on simple foraging/vigilance trade-offs (reviewed in Ferrari, Sih, & Chivers, 2009). For instance, constant exposure to high-risk predators does not allow prey to sustain maximal antipredator responses: their need to feed, at the expense of lower vigilance levels, might lead to seemingly suboptimal levels of antipredator responses compared to low-risk populations. Could my results be explained in the context of risk allocation? The tadpoles received no risk exposure 24 h prior to testing, and thus, they may have recouped any foraging debt (short-term loss of foraging opportunity) that they accumulated during the 4-day exposure during conditioning. Thus, the low-risk period may have allowed them to display higher levels of vigilance at the time of testing, as predicted by the risk allocation hypothesis. While this is a reasonable explanation, the risk allocation hypothesis posits that context-dependent behavioural responses could simply be due to decisions based on immediate trade-offs. Responding less does not necessarily mean that the situation is perceived to be less risky. That high-risk tadpoles retained information about predator risk significantly longer suggests that the cue itself was encoded as more risky and that their behavioural response was not just a product of foraging trade-offs. This result is similar to what would be observed if the high-risk tadpoles had received a higher concentration of injured conspecific cues (Ferrari, Brown, et al., 2010). One explanation for this finding is that the increased background level of risk caused a sensitization-type response to injured conspecific cues (Shettleworth, 2001). In such cases, the amplified signal causes learned responses to be stronger than they would have been in the absence of risk exposure.

There is a wealth of literature on the influence of stress on cognitive functions, and in this case, predation risk can be viewed as a stressor. But, contrary to the original idea that all stressors are bad for learning, some research suggests that stressors that are in line, temporally and contextually, with a learning event may in fact enhance cognitive function, while those that are countercontextual may impede cognitive functions (Berger, Swenson, & Persson,


References


