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Animal behaviour

Making the dead talk: alarm cue-mediated antipredator behaviour and learning are enhanced when injured conspecifics experience high predation risk

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Due to the costs of antipredator behaviour, prey have the ability to finely modulate their response according to the risk they have experienced, and adjust it over different scales of ecological time. Information on which to base their responses can be obtained from direct experience, but also indirectly from nearby conspecifics. In aquatic environments, alarm cues from injured conspecifics are an important and reliable source of information about current predation risk. We used wood frog tadpoles, *Lithobates sylvaticus*, to investigate whether prey responses to alarm cues match the level of background predation risk experienced by injured conspecifics. We found that tadpoles exposed to alarm cues from conspecifics raised in a high-risk environment showed a stronger antipredator response and an enhanced learned response to novel predators, when compared with tadpoles exposed to alarm cues from conspecifics raised in a low-risk environment. Alarm cues not only allow prey to cope with an ongoing predation event, but also to adjust their behaviour to match background risk in the environment.

1. Introduction

Predation is a major force affecting fitness and has led to sophisticated, phenotypically plastic responses in prey. In high-risk environments, some species may develop morphological defences [1,2] or show a variety of behavioural responses to predators [3–5]. These responses are often costly and may cause, for instance, decreased reproductive outputs [1], reduced activity and decreased feeding success and growth [4,5].

Because predation risk fluctuates widely across space and time, prey are expected to continually update their perception of risk and finely modulate their behaviour accordingly [6]. Exposure to a single predation event does not necessarily inform prey about the risk of being attacked in the future. Predation risk can be better be estimated based on the frequency of predation events experienced over a longer period of time (background risk). Perhaps for this reason, background level of risk often has a large effect in determining future antipredator decisions [7–9].

Accurate assessment of background predation risk through direct experience requires considerable sampling through time. A faster and safer strategy is to supplement individual knowledge with publicly available information [10]. Many aquatic species rely on chemicals released from injured conspecifics (alarm cues) as an important source of public information regarding predation risk. These cues are only released through the mechanical damage of a

conspecific and, as such, represent a reliable risk indicator for nearby conspecifics. Our test species, wood frog tadpoles, *Lithobates sylvaticus*, respond innately to conspecific alarm cues with a marked decrease in activity [11] and, as with many species, use alarm cues to mediate learning of novel predator cues [11–13]. A single pairing of alarm cues and a novel predator cue (sight, sound or smell) is enough for the naive prey to subsequently learn to respond to the predator cue with an antipredator response [12].

Here, we tested whether prey responses to alarm cues can be modulated by the level of background predation risk experienced by the alarm cue donors. In experiment 1, we compared the behavioural response of tadpoles raised under high- or low-risk conditions, and exposed them to alarm cues from conspecific donors raised in a high- or low-risk environment. In experiment 2, we compared the intensity of predator odour learning when tadpoles learned to recognize a novel predator via a pairing of alarm cues obtained from high- versus low-risk donors. If background risk alters alarm cue production and prey can respond differentially, we predict that alarm cues from high-risk conspecifics should evoke a greater behavioural response in receivers and stronger learning compared with alarm cues from low-risk conspecifics.

2. Material and methods

Wood frogs were collected as eggs from six clutches and raised outdoors, until they reached Gosner stage 25 (see [11,12] for details). They were fed alfalfa pellets and algae. Background risk was manipulated following established protocols [3]: we exposed 12 3-l pails with 30 tadpoles each for 4 days to low-risk (water exposure 3× per day) and 12 pails to high-risk (injured conspecific cues exposure 3× per day—concentration: 1 tadpole l^{-1}) conditions. Cues were introduced at a random time of the day, with a minimum of a 2 h interval between successive injections, and a 100% water change occurred at the end of each day. Alarm cues were prepared fresh from tadpoles euthanized with a blow to the head and homogenized with a mortar and pestle. The solution was filtered and diluted appropriately.

(a) Experiment 1—does background risk of donor and receiver influence the behavioural response to alarm cues?

This experiment followed a 2 × 3 fully randomized design, where tadpoles raised under low- or high-risk conditions were exposed to alarm cues made from low- versus high-risk conspecifics, or a water control. Following a well-established protocol [11], the day after the conclusion of the background risk treatment each tadpole was acclimated to its test arena (0.5 l cup filled with water) for 30 min prior to being tested. We first measured the baseline activity of the subject for 4 min by counting the number of times the tadpole crossed the median line of the cup (pre-injection activity). We then injected either 5 ml of alarm cue (equal to $\frac{1}{4}$ tadpole per subject) from low-risk ($n = 46$) or high-risk ($n = 44$) tadpoles, or 5 ml of control water ($n = 50$) into each of the cups and we immediately quantified post-injection activity for 4 min. Behavioural response was measured as percentage decrease in activity from the pre-injection baseline.

(b) Experiment 2—does a donor's background risk influence predator recognition learning?

Here, we tested whether tadpoles would learn to respond stronger to a novel predator odour if they were conditioned using alarm
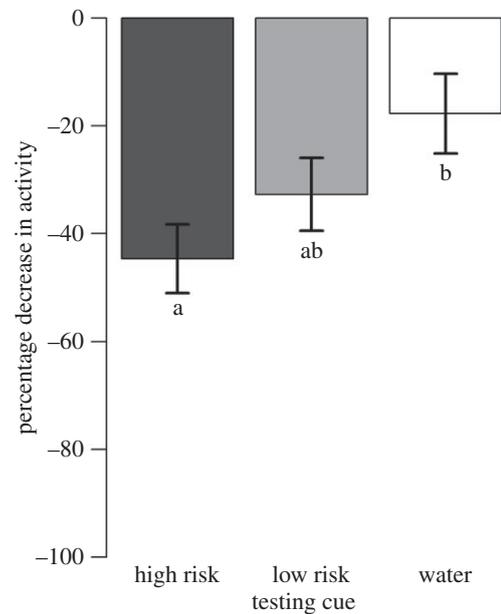

Figure 1. Behavioural response of tadpoles (mean ± s.e.m. percentage decrease in activity) to control water or alarm cues from donors raised in high and low predation risk environments. Different letters represent significant differences (Student–Newman–Keuls test).

cues from high-risk versus low-risk tadpoles. Predator odour was obtained by soaking two salamanders (*Ambystoma tigrinum*) in 2 l of water for 24 h with no food. We used a well-established protocol to test learning [11,12]. After a 1 h acclimation period, we conditioned individual low-risk tadpoles in a 0.5 l arena, by exposing them to 5 ml of salamander odour paired with either 5 ml of alarm cue (equal to $\frac{1}{2}$ tadpole l^{-1}) from low-risk ($n = 25$) or high-risk ($n = 29$) donors or a water control ($n = 27$). As subject risk background did not affect response in experiment 1, high-risk tadpoles were not tested. After 1 h, the tadpoles were placed in large holding tanks with food overnight. The next day, they were tested for their recognition of the salamander odour, using a similar testing protocol as described above. We exposed all tadpoles to 5 ml of salamander odour. A reduction in activity between pre- and post-injection periods of tadpoles conditioned with alarm cues indicates that they learned to identify the predator as a threat [11]. See the electronic supplementary material, S1 for additional details on methods.

3. Results

(a) Experiment 1

The two-way ANOVA on the percentage decrease in activity revealed a significant effect of testing cue ($F_{2,129} = 3.8$, $p = 0.025$; figure 1), no effect of subject risk background ($F_{1,129} = 0.5$, $p = 0.463$) and no interaction between the two factors ($F_{2,129} = 0.9$, $p = 0.405$). The response decreased linearly from high-risk cues to low-risk cues to water (linear trend: $p = 0.006$), but a Student–Newman–Keuls test (summarized in figure 1) revealed no significant difference between the response elicited by alarm cues from low-risk and high-risk.

(b) Experiment 2

Conditioning cue had a significant effect on tadpoles' change in activity (one-way ANOVA, $F_{2,75} = 9.3$, $p < 0.001$; figure 2). Again, the response linearly decreased from high-risk cues to
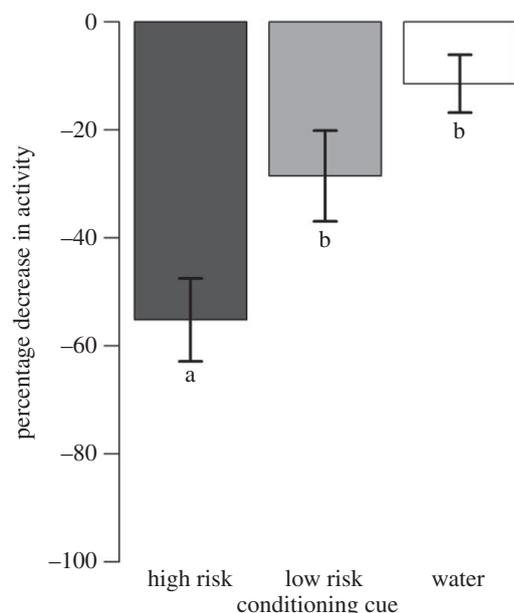
Figure 2. Response of tadpoles (mean ± s.e.m. percentage decrease in activity) to predator odour after conditioning with alarm cues of donors from a high and low predation risk environment and false conditioning with control water. Different letters represent significant differences (Student – Newman – Keuls test).

low-risk cues to water ($p < 0.001$). A Student–Newman–Keuls test (figure 2) revealed that tadpoles conditioned with high-risk donors responded the strongest to the predator.

4. Discussion

Our results provide evidence that the background level of risk experienced by an individual alters how conspecifics respond to its alarm cues. Both behavioural response to alarm cues and learning via alarm cues were greater when the donors experienced high-predation risk. Thus, prey can use conspecific alarm cues to finely tune their antipredator responses according to the background risk of their environment.

While the trend was similar in the two experiments, in experiment 1 we did not detect a significant difference in the responses of tadpoles to alarm cues from low- versus high-risk donors. This might be because of a threshold for the behavioural response to alarm cues that usually is lower than the threshold for learning [14]. However, we cannot exclude that background risk experienced by the donor strengthens learned antipredator responses more than direct responses to alarm cues. Alarm cues released into the water represent an ongoing predation event in real time; therefore, by reducing activity, tadpoles should minimize their immediate risk of

being captured independently from the background risk experienced by the donor [15]. By contrast, learning provides protection against future predation risk; thus, if the likelihood of suffering predation attacks is high, as in high-background risk environments, it is important to enhance learning to adequately cope with future predation events.

The proximate mechanisms involved in differential responses to alarm cues are not clear, partly because the identity of alarm cues is unknown [16]. Tadpoles might have evolved the ability to discriminate between the composition of alarm cues of conspecifics from low- versus high-risk environments. An alternative explanation does not necessarily imply the evolution of such adaptive ability. As both behavioural responses and learning strength increase with alarm cues concentration [11,17], it is possible that high-predation risk leads tadpoles to produce a higher amount of the alarm cue 'active' compound, even if the alarm cues were not selected for a signalling function [18]. Studies in other taxa have failed to find support for this scenario. In ostariophysan fishes, alarm cues are produced in epidermal club cells located in the epidermis. Background level of risk does not alter the number of club cells produced [18]. Another explanation would be that tadpoles exposed to prolonged predation risk have increased levels of circulating corticosterone [19] and tadpoles may detect and respond to corticosterone dissolved in water with phenotypic changes similar to what occurs during predator exposure [20].

Whatever the mechanism, the ecological outcome remains the same. By increasing antipredator responses to alarm cues produced by injured conspecifics that experienced high-predation risk, prey can match the levels of risk in their environment following the first predation event that they experienced without extensive direct sampling. This would reduce latency to develop appropriate responses and increase individual fitness.

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