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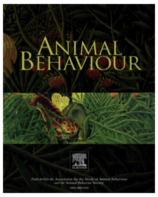

Understanding the effect of uncertainty on the development of neophobic antipredator phenotypes

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Exposure of prey animals to high background risk environments generates high-risk behavioural phenotypes characterized by prey that often display transient neophobic responses to novel stimuli. To manipulate background risk, researchers have most often used chemical alarm cues from injured conspecifics because these cues provide a general indication of a high-risk environment but no information regarding the identity of the source of the threat. Here, we hypothesized that the expression of neophobia (fear of unknown stimuli) may not be the result of elevated background risk per se, but rather the result of high uncertainty associated with the predation environment. Here we showed that woodfrog, *Lithobates sylvaticus,* tadpoles exposed to alarm cues alone for several days subsequently displayed neophobic phenotypes. The same was true for tadpoles exposed to alarm cues paired with the odour of a new novel predator each day for several days. However, tadpoles exposed to alarm cues paired with the same predator cue everyday did not develop the neophobic phenotype. This suggests that if the predator environment is certain (i.e. the alarm cue and predator cues always coincide), then the ratio of costs to benefits of neophobia is high and limits its expression. However, if the prey's uncertainty regarding the predator environment is high (i.e. the alarm cues are often associated with new unknown predators), then the potential survival benefits of expressing neophobia likely override the costs. The prey's perception of uncertainty may be a key driver of the expression of neophobic phenotypes.

The risk of predation is highly variable over space and time, hence accurately predicting risk is a substantive challenge for most animals (Ferrari, Sih, & Chivers, 2009; Lima & Dill, 1990; Sih, 1992). Risk assessment influences all aspects of an individual's life, including many short- and long-term habitat, foraging and mating decisions (Lima, 1998). Furthermore, accurate risk assessment often allows individuals to display particular morphotypes or life-history strategies that maximize fitness in particular environments (Benard, 2004; Chivers, Zhao, Brown, Marchant, & Ferrari, 2008). Because such responses are often costly, selection favours individuals that carefully balance the costs and benefits of these induced defences. Successful risk assessment rests on the prey having enough information about the risk to which they are exposed. Ideally, information used to detect and assess risk should be devoid of uncertainty. However, in reality, complete information about predator-related information is rarely achieved. Therefore, mechanisms should be in place for prey to cope with their lack of information (Dall & Johnstone, 2002).

Prey are equipped with efficient learning mechanisms that allows them to identify quickly and respond appropriately to predators according to the threat they pose (Brown, Ferrari, & Chivers, 2011; Crane & Ferrari, 2013). However, we do not have a good sense of how naïve prey survive in a novel environment where threats are unknown. The complete absence of information is arguably the most risky situation for prey. Beside juvenile naïvety, such situations are quite common, especially when we consider the number of ontogenetic or life-history habitat shifts associated with migration or short-term movements from one habitat to another. This exposes prey to a novel array of threats. Fortunately for prey, the odds of surviving a first predator encounter are much improved when prey exhibit a neophobic phenotype. Neophobia, considered the avoidance of unknown stimuli, can be viewed as a plastic, antipredator strategy displayed by prey exposed to high-risk situations (Brown, Ferrari, Elvidge, Ramnarine, & Chivers, 2013). For instance, larval woodfrog, *Lithobates sylvaticus*, and damselfish

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(*Pomacentrus* spp.), as well guppies, *Poecilia reticulata*, cichlids (*Amatitlania nigrofasciata*) and minnows (*Pimephales promelas*) maintained for a few days in a high-risk environment displayed antipredator responses after detecting novel smells, whereas their low-risk counterparts did not (Brown, Chivers, Elvidge, Jackson, & Ferrari, 2014; Brown et al., 2013; Chivers, McCormick, Mitchell, Ramasamy, & Ferrari, 2014; Crane, Mathiron, & Ferrari, 2015). Such phenotypes have been shown to increase survival of predator-naïve prey in the wild (Ferrari, McCormick, Meekan, & Chivers, 2015) or in mesocosm studies (Ferrari, Crane, Brown, & Chivers, 2015). The obvious cost, however, is the repeated waste of foraging or mating opportunities associated with responding to novel, but nonthreatening, cues. Hence, mechanisms should be in place to optimize the expression of this neophobic phenotype. Guppies and cichlids, for instance, lose their neophobic response to the odour of tilapia (*Oreochromis niloticus*) or trout (*Oncorhynchus mykiss*), respectively, after four or five exposures to the odour without negative reinforcement (Brown, Demers, Joyce, Ferrari, & Chivers, 2015; Brown, Elvidge, Ramnarine, Ferrari, & Chivers, 2015).

Our present understanding of neophobia assumes that its onset is linked to exposure to risky environments. Risk in previously published literature was created by exposing fish or larval amphibians to cues from injured conspecifics several times per day for 4–7 days (Brown et al., 2013; Crane et al., 2015; Ferrari, Crane et al., 2015; Ferrari, McCormick et al, 2015; Ferrari, McCormick, Meekan et al, 2015). Cues from injured conspecifics were chosen as they provide, by their very nature, a reliable indicator of risk, but lack predator-specific information that may inform the prey about the identity of the threat (Ferrari, Wisenden, & Chivers, 2010). While we know how to turn on this neophobic phenotype, we still lack an understanding of the link between the detection of risk and the expression of neophobia. What controls the onset and the loss of this phenotype? Our first hypothesis is that the onset of neophobia comes from repeated exposure to any risk cues, allowing prey to cope with the general stress related to high-risk situations (the environment is risky, so most novel cues are likely to be risky). If this hypothesis is supported, then neophobia would be expressed after exposure to cues mediating risk, regardless of the information content of the cues. Our second hypothesis is that neophobia is not a response to risk per se, but rather a response to uncertainty. Specifically, the lack of information provided by the injured conspecific cues is a coping mechanism against the unknown nature of the specific threat. Something unknown is responsible for the release of injured conspecific cues, so everything novel will be treated as risky until the threat is identified. Here, we predicted that neophobia would only be expressed if prey lacked clear information about the source of the risk (i.e. the identity of the predator causing the release of injured conspecific cues).

METHODS

Experimental Overview

To test the above-mentioned hypotheses, we conducted two experiments. In the first, we exposed predator-naïve woodfrog tadpoles to four environmental conditions for 4 days: a safe environment (water exposure), an environment containing an unknown salamander (*Ambystoma tigrinum*) (salamander odour exposure), an environment containing injured tadpole cues, or an environment containing a previously unknown predator paired with injured tadpole cues. At the end of the period, tadpoles were exposed to the odour of a novel crayfish (*Orconectes virilis*) or a water control to assess their neophobic tendencies. If neophobia is a temporary response to risky environments, then we expected the tadpoles from the two risky environments (injured tadpoles cues and tadpole cues paired with salamander odour) to display neophobic responses to the crayfish. However, if neophobia is a response to uncertain threats only, then we expected only tadpoles in the injured tadpole cue group to be neophobic to crayfish cues; tadpoles from the injured tadpole + salamander group should not be neophobic, as the source of the threat was identified (i.e. the salamander).

In a second experiment, we wanted to further tease apart the role of uncertainty in the onset of neophobia. We exposed tadpoles to one of three environmental conditions for 4 days: an unidentified, risky environment (injured tadpole cues exposure), a constant-threat environment (injured tadpole cues paired with salamander odour), or a variable-threat environment (injured tadpole cues paired with a novel odour every day). Tadpoles were tested the following day for their response to a novel crayfish or a water control. In this experiment all of the tadpoles had the same background level of risk (daily exposure to alarm cues), but their certainty regarding the cause of the risk was variable. Certainty regarding the cause of the risk was either completely unknown (i.e. injured tadpole group), associated with the salamander, or associated with a new predator each day. If tadpoles experiencing alarm cues with the same predator every day do not exhibit neophobic tendencies, we would conclude that the certainty of the association between the risk cues and the predator would be the main driver preventing the neophobic phenotype. However, if pairing alarm cues with variable predators leads to neophobic responses, then we would conclude that, despite the pairing, uncertainty with regards to other unknown animals would likely be driving the expression of the neophobic phenotype.

Experimental Subjects

Tadpoles used in this experiment were raised from six wild-caught clutches laid in early May 2014 in central Alberta and maintained in pools floating on their pond of origin, so that they would experience natural water temperature and light exposure. However, they were maintained in aged well water (hereafter water), to ensure they would not be exposed to any odours from potential aquatic predators, effectively keeping them predator-naïve. After hatching, tadpoles were fed alfalfa pellets to complement the algae already present in the pools.

Salamanders were collected from a local pond on the University of Saskatchewan campus 4 years prior and fed earthworms. Water tigers (*Dytiscus alaskensis*) were collected from a nearby pond using dip-nets, a few days prior to being used. Crayfish were trapped from Blackstrap Lake, Saskatchewan, 3 weeks prior to being used and maintained on a diet of vegetables (leaves, carrots). Forty juvenile rainbow trout were obtained from the Cold Lake Fish Hatchery, Alberta, 2 weeks before the start of the experiment. Goldfish, *Carassius auratus*, were obtained from a local pet store. Both fish species were fed fish pellets. At our field site, trout and crayfish were housed separately in 100-gallon (378.54-litre) black troughs filled with well water, while the water tigers were housed individually in 0.5-litre cups, due to their high aggression and cannibalistic tendencies.

Cue Collection

Predator odours were made from soaking predators in water for 24 h. Some predators were kept individually to decrease aggression and stress. Four salamanders (snout–vent length: 12–14 cm) and four crayfish (rostrum–telson length: 11–14 cm) were placed individually in plastic containers filled with 1 litre of water while eight water tigers (body length: 3 cm) were placed individually in 0.5-litre cup; the water from each species was combined after the