



Threat sensitivity in the San Marcos salamander: effects of predator diet and prey experience

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Abstract

Prey must constantly balance foraging and predator avoidance demands. Avoidance response efficiency may be improved when prey match the intensity of their avoidance behaviours to a perceived level of predatory threat (threat sensitivity). Additionally, experience with predators may influence the intensity of avoidance responses. I examined the possibility that experience with predators in the natural habitat would influence threat sensitive avoidance behaviours of an aquatic salamander, *Eurycea nana*, by comparing the intensity of avoidance responses to predators that had been fed a neutral diet (low-risk) or a diet of conspecifics (high-risk) between laboratory-reared and recently-collected adult salamanders. I found that laboratory-reared salamanders exhibited graded responses to low- and high-risk predators consistent with threat-sensitive predator avoidance. Predator-experienced salamanders (recently-collected), however, responded less intensely to all predators and their responses showed little evidence of threat sensitivity. These less intense responses observed in experienced salamanders may result from mechanisms of adaptive forgetting, which allow prey to respond to environmental variation. I discuss implications of these results for *E. nana* and other prey as well as highlighting the need for researchers to consider the longer-term experiences of prey used in studies of predation risk.

Keywords

risk assessment, predator avoidance, amphibian, diet cues, chemical cues, predator recognition.

1. Introduction

The most beneficial predator avoidance tactics consist of complex interactions between effectiveness and efficiency. Effective avoidance behaviours such as decreased activity (Anholt et al., 2000; Gonzalo et al., 2007), increased use of refuge (Kiesecker & Blaustein, 1997; Orizaola & Brana,

2003), or spatial or temporal habitat shifts (Petranka, 1983; Sih & Moore, 1993) reduce the risk of predation. However, there are time allocation trade-offs between predator avoidance tactics and other beneficial behaviours such as foraging or mating (Sih, 1992; Werner & Anholt, 1996). Because individual predators can vary in the risk they pose, prey may match the intensity of antipredator behaviours with the perceived level of threat posed by the predator, exhibiting stronger responses to those predators that it identifies as more dangerous (threat sensitivity hypothesis; Helfman, 1989). Threat sensitivity can result in more efficient predator avoidance by reducing associated costs.

In aquatic prey, threat sensitivity is often mediated by chemical stimuli (Kats & Dill, 1998; Wisenden, 2003) such as those associated with predator species (kairomones) (Watson et al., 2004) or secondary cues from predator diet (Laurila et al., 1997; Wisenden, 2003). Predator kairomones can mediate recognition of predatory species by prey (Kats & Dill, 1998; Mathis et al., 2003), while secondary threat-indicators may provide more complete information about predation risk. For example, predator diet cues (stimuli associated with recent foraging activity) can aid in allowing prey to discern low- from high-risk predators. Often prey respond more intensely to the chemical stimuli of predators that have recently consumed conspecifics as opposed to those that have not (Wilson & Lefcort, 1993; Laurila et al., 1997; Mathis, 2003; Murray et al., 2004), although this more intense response is not observed in all species (Bryer et al., 2001; Wirsing et al., 2005). Predator diet cues are thought to result from the mixing of predator kairomones with damaged-released alarm cues of conspecific (Mathis & Smith, 1993; Chivers & Mirza, 2001) or heterospecific (Mirza & Chivers, 2004; Schoeppner & Relyea, 2009) prey. Some research suggests that the digestive process results in chemical stimuli that prey perceive as higher risk than undigested alarm cues and kairomones presented simultaneously (Schoeppner & Relyea, 2005, 2009). Especially in environments where prey experience chronic, high levels of predation, the ability to assess predation risk and exhibit threat sensitive-predator avoidance responses should result in increased effectiveness and reduced costs associated with antipredator behaviours.

The experiences that prey individuals have with predators can also affect a prey's perception of predation risk and, thus, the intensity with which it responds to predators. Experience-mediated threat sensitivity (learning) has been demonstrated for a variety of aquatic prey including fishes (reviewed

in Brown, 2003; Kelley & Magurran, 2003b), amphibians (Gonzalo et al., 2007; Ferrari & Chivers, 2010), and invertebrates (Kesavaraju et al., 2007). Threat-sensitive learning is expected to be favoured in habitats with diverse or fluctuating predatory communities (Brown & Chivers, 2005) because it allows prey to adjust to variations in predation risk over time, thereby optimizing time allocated to foraging or mating (Helfman, 1989; Sih, 1992). Much of the literature examining experience-mediated (learned) threat-sensitivity examines the effects of variation in predation risk over relatively short periods of time (one exposure to a few weeks) (see Wisenden, 2003 for reviews; Brown & Chivers, 2005; Ferrari et al., 2009). What is less well understood is how ambient predation pressure over longer time scales influences threat-sensitive avoidance (Brown et al., 2009). In the natural habitat, prey are likely to encounter predators repeatedly over the course of their lifetime and this can influence the intensity with which they respond to predators. Repetitive interactions with predators can reinforce avoidance responses of prey, resulting in more intense antipredator behaviours (Kelley & Magurran, 2003a, b), or habituation to predator stimuli, which results in reduced response intensity to predation risk over time (Magurran & Pitcher, 1987; Jackson & Semlitsch, 1993). Given the effect of prolonged exposure, it is possible that ambient levels of predation risk also influence a prey individual's assessment of predation threat and, therefore, its threat-sensitive avoidance responses.

To examine the possibility that prolonged exposure to natural predation pressures can affect threat-sensitive avoidance, I compared responses of salamander prey to high-risk and low-risk predators between predator-naïve (captive) and predator-experienced (recently-collected) individuals. Predator experience in captive vs. wild populations may not be the only factor contributing to behavioural differences amongst these groups; however, comparison between them can provide valuable insights regarding the plasticity of predator avoidance. While recognizing differences beyond predation between captive and wild populations, the purpose of this study was to examine differences in antipredator behaviour; thus, 'predator-naïve' and 'predator-experienced' will be used interchangeably to refer to captive-reared and wild-caught salamanders respectively.

The San Marcos salamander, *Eurycea nana*, is a federally threatened (USDI, 1980) paedomorphic salamander (obligatorily aquatic) endemic to the headwaters of the San Marcos River, Hays County, TX, USA (Bishop,

1941; Nelson, 1993; Chippindale et al., 1998). It experiences consistent predation throughout life from a diverse and densely populated community of both native and non-native fish predators (Kelsey, 1997; Bowles & Bowles, 2001). Epp & Gabor (2008) showed that adult, predator-naïve salamanders reduced activity in response to the chemical stimuli of both a native predator (largemouth bass, *Micropterus salmoides*) and a non-native predator (redbreast sunfish, *Lepomis auritus*) and not to a blank control or a native non-predator (largespring gambusia, *Gambusia geiseri*), indicating innate antipredator behaviour in this species. Conversely, predator-experienced salamanders responded similarly to the native predator, but showed no significant response to sunfish suggesting that experience with predators can alter the intensity of avoidance responses (Epp & Gabor, 2008). I predicted that predator-naïve adult salamanders (captive-reared) might exhibit threat sensitivity (Helfman, 1989) by responding more intensely to predators that had recently consumed conspecific prey as compared to predators that had not. I predicted that salamanders collected from the natural habitat (predator-experienced) might exhibit differences in response intensity or threat sensitivity as compared to naïve salamanders in that they might respond less intensely to predators.

2. Materials and methods

2.1. Test subjects

Predator-naïve *E. nana* salamanders were adult (snout–vent length (SVL) > 20 mm; Tupa & Davis, 1976), first-generation offspring of salamanders collected from the natural habitat. The breeding population of the parental generation was composed of at least 200 adult salamanders accumulated over 2–3 years in multiple collection events. It was not possible to track familial relationships because adults were housed in large, mixed-sex tanks with 20–40 individuals/tank, and females lay eggs singly instead of in clutches. However, most eggs do not survive to adulthood and it is unlikely that subjects were related. Predator-experienced salamanders were individuals collected from the natural habitat as adults and tested within six months of collection. The SVL of captive (mean \pm SE 36.2 \pm 0.3 mm) and wild-caught (mean \pm SE 27.3 \pm 0.5 mm) salamanders significantly differed (Student's *t*-test; $t = 15.75$; $df = 148$; $p < 0.01$); however, all subjects were adults and the size of wild-caught salamanders was representative of adult size in the

natural population (pers. obs.). All test subjects were housed on a 12L:12D light cycle at the San Marcos National Fish Hatchery and Technology Center and fed commercially raised annelids (*Lumbriculus variegatus*) and copepod mixtures *ad libitum*. Housing tanks were large, recirculating, fibreglass raceways partitioned by screen into sections of about 61 cm D × 81 cm W × 90 cm L holding about 30 salamanders per section. Water was sourced from and maintained at the same temperature (21–23°C) as the aquifer that sources the natural river habitat of *E. nana*.

2.2. Stimulus acquisition

I collected the predatory species, largemouth bass (*M. salmoides*, $N = 4$) and redbreast sunfish (*L. auritus*, $N = 4$), from the natural habitat of *E. nana* in October of 2007. I chose these species for three reasons: (1) previous work showed that *E. nana* exhibits innate avoidance responses (reduced activity) to both of these species (Epp & Gabor, 2008), (2) these species appear to be among the most abundant predatory species in this system and share similar opportunistic foraging habits (Day, 1981; Wallace, 1984) and (3) individuals of both of these species have been observed preying on *E. nana* in the wild (pers. obs.; E. Chappel, pers. commun.) and consume them readily in captivity (Epp & Gabor, 2008). Stimulus fish were size-matched within 5 cm standard length (SL; mean \pm SE 18.15 ± 1.03 cm). Fish were then housed in flow-through fibreglass housing tanks for at least 5 days and fed a neutral diet consisting of earthworms (*Lumbricus terrestris*) to allow time to flush the gut of chemical stimuli from previous foraging. Then, I fed half the stimulus fish of each species (largemouth bass and redbreast sunfish) one of two treatments: (1) two mature *E. nana* salamanders along with earthworms (high-risk diet) or (2) earthworms only (low-risk, neutral diet). For both of the species, the other half of the stimulus fish were fed the alternate treatment. After feeding, stimulus fish remained in housing tanks for 12 h to allow time for ingestion and the start of digestion. Then, following established methods (Epp & Gabor, 2008), I placed stimulus fish individually into aerated and unfiltered stimulus acquisition tanks with 230 ml of dechlorinated tap water/ml of stimulus fish by volume for 24 h. After 24 h, I removed and released stimulus fish, stirred stimulus water, and mixed water from the two stimulus fish of the same species with the same diet (neutral or salamanders) to eliminate the potential for individual effects from stimulus

fish. I then collected stimulus water in 50-ml portions and froze it until immediately prior to testing. For the control stimulus (see below), I froze 50-ml portions of dechlorinated tap water until testing (Epp & Gabor, 2008).

2.3. Behavioural trials

I tested the motor activity of two experience groups of *E. nana*, predator-naïve and predator-experienced, from November 2007–April 2009 in response to five treatments: (1) redbreast sunfish, neutral diet (low risk); (2) redbreast sunfish, salamander diet (high risk); (3) largemouth bass, neutral diet (low risk); (4) largemouth bass, salamander diet (high risk); or (5) blank control (no risk); $N = 15/\text{treatment}$. I tested all salamanders under a dim red light at night (Gillette et al., 2000) when they are most active. I placed subjects individually into a 9.5-l testing aquarium with 5.5 l of fresh well water and allowed them to acclimatize for at least 15 min until consistent activity was observed for at least 120 s. The testing aquarium had an attached stimulus introduction tube that extended 5 cm below the surface of the water on the interior and 2 cm below the base on the exterior. After acclimatization, I recorded the amount of time(s) that salamanders spent moving in an 8-min period (pre-stimulus activity). Then I slowly injected 50 ml of one of the five treatment stimuli into the aquarium through the stimulus introduction tube followed by 50 ml of fresh well water at about 2 ml/s so as not to disturb test subjects. After stimulus introduction, I recorded the amount of time salamanders spent moving in the subsequent 8-min period (post-stimulus activity). The stimuli were randomly assigned and coded so that I was blind to the treatment, and all treatments were administered in a randomized block design within experience groups. I scrubbed the testing aquarium and testing supplies with 3% hydrogen peroxide and rinsed it with well water to remove chemical stimuli and maintain independence between trials.

2.4. Statistical analyses

I calculated a response index (relative activity) by dividing post-stimulus time by pre-stimulus time (Epp & Gabor, 2008), which accounts for each individual's change in activity from pre-stimulus (normal) to post-stimulus (response). In this way, relative activity = 1 would indicate no response, <1 reduced activity and >1 increased activity.

I first tested whether salamanders in each experience group exhibited detectable avoidance behaviours by comparing relative activity of individuals exposed to predator treatments with relative activity of those exposed to the blank control within salamander experience groups (predator-naïve and -experienced) using Dunnett's tests ($\alpha = 0.05$). I next tested whether salamanders exhibited threat sensitivity by responding more intensely to high-risk (salamander diet) than to low-risk predators (neutral diet) and whether these responses differed between naïve and experienced salamanders by examining interactions between prey experience and predator risk (diet). I removed the control treatment responses from the analysis and performed a three-factor ANOVA with subsequent multiple comparisons (Tukey's; $\alpha = 0.05$) using predator species, predator risk, and experience of prey as factors. All data met the assumptions for parametric analyses. All analyses were performed using JMP[®] 8.0 (SAS Institute, Cary, NC, USA) software.

3. Results

Differences in relative activity were not caused by differences in pre-stimulus activity between captive-reared (mean \pm SE 248.79 \pm 6.86 s) and wild-caught (mean \pm SE 260.73 \pm 7.59 s) salamanders (unpaired *t*-test for unequal variances: $t = 1.167$, $df = 145.48$, $p = 0.245$). For predator-naïve individuals, relative activity of salamanders exposed to chemical stimuli of all predator treatments (1–4) was significantly lower (reduced activity) than that of salamanders exposed to the blank control treatment (5). For predator-experienced individuals, only relative activity of salamanders exposed to chemical stimuli of high-risk sunfish (salamander diet; treatment 2) was significantly lower than responses of salamanders exposed to the blank control (Figure 1). Three-factor ANOVA of predator species, predator risk (diet), and prey experience indicated a significant second-order interaction between the factors of predator risk and prey experience (Table 1). To elucidate the nature of the interaction I compared relative activity of predator-naïve salamanders to the relative activity of predator-experienced salamanders within predator species and predator risk treatments using Tukey's multiple comparisons procedures. I found that predator-naïve salamanders exposed to the chemical stimuli of high-risk (salamander diet) predator treatments (2 and 4) had significantly lower relative activity than naïve salamanders exposed

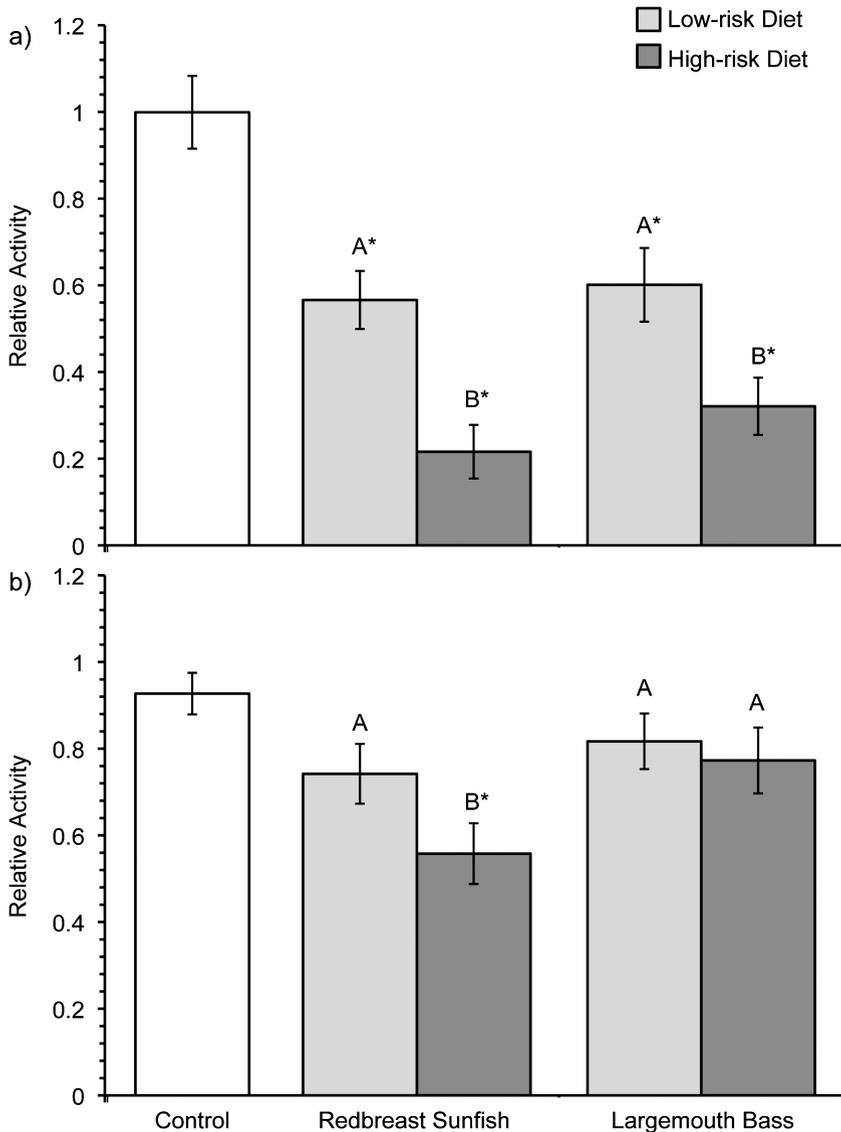


Figure 1. Responses of experienced and naïve *E. nana* to low- and high-risk predators. Mean \pm SE responses (relative activity) of (a) predator-naïve and (b) predator-experienced *Eurycea nana* to the chemical stimuli of low-risk (neutral diet) or high-risk (conspecific salamander diet) predators. Relative activity = 1 indicate no response, <1 reduced activity, and >1 increased activity. Responses not connected by the same letter significantly differ ($\alpha = 0.05$). *Responses significantly differed from responses to the blank water control (Dunnett's tests; $\alpha = 0.05$).

Table 1.

Three-factor ANOVA of risk, experience and predator species.

Factor	<i>F</i>	df	<i>p</i>
Prey experience	35.506	1, 112	<0.0001
Predator species	4.651	1, 112	0.0332
Predator risk	18.555	1, 112	<0.0001
Prey experience × Predator species	0.555	1, 112	0.4581
Prey experience × Predator risk	4.068	1, 112	0.0461
Predator risk × Predator species	1.105	1, 112	0.2955
Prey experience × Predator risk × Predator species	0.125	1, 112	0.7246

Results of 3-factor ANOVA assessing the impacts of predator species (native bass or non-native sunfish), predator risk (neutral diet or conspecific diet), and prey experience (captive or collected) on avoidance responses of *E. nana*.

to low-risk (neutral diet) predator treatments (1 and 3). Relative activity of naïve salamanders to high-risk predators was also significantly lower than that of predator-experienced salamanders to both low- and high-risk predator treatments (1–4). Additionally, the responses of predator-experienced salamanders to both low- and high-risk predators did not differ from responses of predator-naïve salamanders to the chemical stimuli of low-risk (neutral diet) predators (Figure 1).

4. Discussion

I found that predator-naïve *E. nana* salamanders reduced activity significantly more in response to predators that had recently consumed conspecific prey as compared to those that had not, which is probably reflective of predator risk; however, for predator-experienced salamanders, this threat-sensitive response was observed for only the high-risk sunfish treatment, but not the high-risk bass treatment. Further, these results demonstrate that predator-naïve salamanders significantly reduced activity in response to all predatory treatments as compared to predator-naïve salamanders exposed to a blank control. In contrast, predator-experienced salamanders only decreased activity in response to the high-risk sunfish treatment as compared to responses to the control treatment.

The balancing of predator avoidance behaviours and the demands of foraging or mating can be difficult for prey (Sih, 1992). By matching the

intensity of responses to the level of threat posed by predators, prey can minimize the time-allocation costs associated with predator avoidance (Helfman, 1989). Predator-naïve salamanders responded more intensely to predators that had recently consumed conspecific prey than to those that had been fed a neutral diet, indicating that *E. nana* identifies predators that have recently consumed conspecifics as more dangerous. Threat sensitivity mediated by predator diet cues has been observed in amphibian prey (Schoeppner & Relyea, 2005), and conspecific cues in the diet of predators is likely a reliable indicator of the risk posed by individuals. My data suggest that these salamanders are able to assess variation in predatory threat and exhibit threat-sensitive responses appropriate for the risk posed.

As predicted, results of this study indicate that exposure to ambient predatory threat in the natural habitat affected the intensity of avoidance behaviours exhibited by prey. Behavioural plasticity in avoidance responses is often influenced by prior experiences of prey with predators (Ferrari et al., 2010b), even during embryonic life stages (Mathis et al., 2008). Much research on the mechanisms underlying this plasticity has focused on the ability of prey to acquire recognition of novel stimuli as either threatening, through associative learning (Chivers & Smith, 1998), or as non-threatening, through latent inhibition (Ferrari & Chivers, 2009; Mitchell et al., 2011); however, less work has focused on behavioural plasticity in prey that have preformed associations, such as those exhibiting innate avoidance of predators. In this study, experienced salamanders from the natural habitat responded less intensely to all predators than did naïve salamanders. Two plausible hypotheses may explain the disparity in responses of experienced as compared to naïve salamanders: (1) habituation to predators or (2) learned irrelevance.

Habituation to predators can occur when prey are exposed to predatory stimuli for prolonged time periods or repeatedly without experiences that reinforce the risk of the predator for the prey. Prey habituation to predators has been demonstrated in invertebrates (isopods, Holomuzki & Hatchett, 1994; crabs, Hemmi & Merkle, 2009), amphibians (salamanders, Jackson & Semlitsch, 1993; Madison et al., 2005) and fish (minnows, Kelley & Magurran, 2003a; perch, Oosten et al., 2010). The propensity of prey to habituate to stimuli is considered a form of non-associative learning that allows organisms to cope with environmental variability. When considering responses to predators, habituation to cues that are relevant to the risk posed may result in

ineffective avoidance responses if prey overly reduce responses to dangerous predators. Habituation is expected to be especially prevalent in habitats with high predator densities because prey would be exposed to predator stimuli more frequently or even continuously. Given the diversity and abundance of predatory species in the habitat of *E. nana*, it seems possible that habituation to predator odours could occur. This possibility is especially plausible given that prey may be more likely to habituate when adequate refuges are available for prey (Holomuzki & Hatchett, 1994). In its natural habitat, *E. nana* is commonly found under rocks and logs along the substrate of the river, and fish predators frequently hover over these refuges while foraging (pers. obs.). It seems probable that chemical stimuli of fish predators are almost continuously available to salamanders while they are in refuge and relatively safe. The exact mechanisms by which habituation occurs remain unclear, so it is not understood how this occurs in *E. nana*.

A related hypothesis that may explain the less intense responses exhibited by experienced salamanders in this study is the phenomenon of learned irrelevance (Mackintosh, 1973). In the natural habitat prey are repeatedly and continuously exposed to predatory stimuli. Prey typically exhibit threat-sensitive learning or acquired (learned) recognition of novel predators through associative learning when they encounter stimuli associated with a predator species (e.g., kairomones) simultaneously to cues associated with risk (e.g., diet cues of digested conspecifics or damage-released conspecific alarm cues), which causes them to respond more intensely to that predator's kairomones (Kats & Dill, 1998; Brown & Chivers, 2005). However, especially when predator diversity and density is high and predators are opportunistic foragers, prey may detect predator kairomones and threat-indicative cues at random times, not necessarily in association with each other. Similarly to latent inhibition, the hypothesis of learned irrelevance suggests that prey presented with predator and risk stimuli independently of paired presentations, may be inhibited from forming future associations of the predator's stimulus with a threat (Bennet et al., 2000). In predator-prey systems, learned irrelevance can be beneficial because it may reduce the probability that prey associate stimuli of less dangerous predators or non-predators with high-risk threats because that would result in excessive antipredator behaviours.

Studies examining the phenomenon of learned irrelevance have typically addressed outcomes for associative learning of novel stimuli (latent inhibition), but prey exhibiting innate recognition have preformed associations

of predators with threat. In crayfish (*Orconectes virilis*), Hazlett & Schoolmaster (1998) showed that a single simultaneous exposure to a novel predator kairomone with crayfish alarm cues resulted in associative learning of the kairomone as a predatory threat. However, when the novel kairomone and alarm cue were presented independently either prior to (novel association) or subsequent to (preformed association) a simultaneous exposure, crayfish exhibited reduced responses to the kairomone at subsequent exposures as compared to crayfish that had experienced only a paired exposure of alarm cue and goldfish odour (Hazlett, 2003). This result suggests that prey with preformed associations, such as prey exhibiting innate recognition and avoidance of predators, may reduce the intensity of their responses to predators if they encounter predator kairomones and risk indicators independently in the natural habitat. If learned irrelevance reduces costs of excessive predator avoidance, then salamanders that respond less intensely to predator kairomones that they frequently encounter independently of risk indicators should be at a selective advantage in this system.

Both the hypotheses of habituation and learned irrelevance could be considered mechanisms for 'adaptive forgetting' in prey animals (Kraemer & Golding, 1997). Until recently, a lack of response to a previously recognized stimulus was considered a memory failure that could negatively affect fitness. However, because behavioural plasticity allows organisms to better respond to environmental heterogeneity, prolonged retention of information without reinforcement could result in maladaptive behaviours in organisms (Stephens, 1991). The environmental factors that can affect the retention of information, or memory, have been studied in other contexts, but remain less explored in how prey recognize and respond to predators (Ferrari et al., 2010a; Mitchell et al., 2011). In the habitat of *E. nana*, numerous predator species are likely to move in and out of the immediate, detectable range of prey individuals throughout the course of a day. Additionally, because of continuous water flow in the habitat, detection of each individual predator cue is probably short-lived and the probability of threat-sensitive reinforcement through simultaneous exposure to risk cues with predator kairomones is limited. Adaptive forgetting may be a mechanism by which salamanders are able to respond to this continual variation in predation risk.

These results also indicated that experienced salamanders exhibited a low-intensity response to the chemical stimuli of the high-risk sunfish treatment and no detectable response to the high-risk bass treatment. However,

naïve salamanders exhibited responses to low-risk and to high-risk predator treatments that were similar between the predators. The differences in responses of predator-experienced individuals to bass and sunfish are opposite to those observed in a previous study (Epp & Gabor, 2008); however, predator diet was not controlled in that study. Experienced salamanders may have responded differently to these species in each study because of digestive differences between the species (Masagounder et al., 2009). For example, digestive differences may result in differing concentrations of the secondary threat indicator of diet cues. Experience with predators can affect the threshold cue concentration required to elicit behavioural responses in prey (Mirza et al., 2006). Given the threshold effect, it may be that the response threshold of naïve and experienced salamanders differed and sub-threshold concentrations of diet cue were present in the high-risk bass stimulus, while concentrations above that required to elicit a response from experienced individuals were present in the sunfish cue (Mirza et al., 2006). These digestive differences may also explain differences in responses of experienced *E. nana* to these predators in previous work (Epp & Gabor, 2008); however, this remains to be determined.

Aside from experience with predators, other differences between wild-caught and laboratory-reared salamanders might exist. One notable difference is that food availability is probably more limited in the natural habitat as compared to the laboratory. However, salamanders collected from the wild were housed in the laboratory with identical food supplies to those of captive salamanders for a minimum of two weeks prior to testing. This time should have allowed wild-caught salamanders to recoup potential energy deficits as compared to captive salamanders. A second potential difference between the treatment groups is that salamanders raised in captivity may have been more acclimatized to laboratory conditions than wild-caught salamanders; however, comparison of mean pre-stimulus activity times indicated no significant difference. Because reduced activity is a response to stress or risk, the lack of difference in pre-stimulus activity between experience groups suggests that wild-caught salamanders had sufficiently acclimatized to laboratory conditions.

This study highlights the need for research aimed at understanding how experience in natural habitats can alter the intensity of predator avoidance behaviours of prey. I found that prey collected from natural habitats respond

differently to predatory threats than predator-naïve prey and that the influence of past experiences is retained for at least six months in captivity. This finding further emphasizes the need for researchers to consider the past experiences of prey when interpreting the results of studies aimed at understanding avoidance behaviours, especially as it pertains to variations in predation risk. Studying prey that have experienced predation risk in their natural environment in conjunction with their naïve counterparts provides more complete information about when plasticity and threat sensitivity should be favoured.

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