

PREDATOR RECOGNITION AND AVOIDANCE IN
THE SAN MARCOS SALAMANDER

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PREDATOR RECOGNITION AND AVOIDANCE IN THE SAN MARCOS
SALAMANDER

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DEDICATION

This dissertation is dedicated to my grandfather, Allen Epp (1927-2009). A laborer by birth, a teacher by nature, and a philosopher by choice, he taught us the value of working tirelessly, encouraged our intellectual curiosity, and exemplified open-mindedness and enlightenment. His actions and words have touched many lives, and his ineffaceable influence has inspired me in every way.

“Often, it is when my hands are their dirtiest that my mind is clearest, and my heart is at peace.” ~ Allen Epp

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ABSTRACT

PREDATOR RECOGNITION AND AVOIDANCE IN THE SAN MARCOS SALAMANDER

by

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Selection by predators is among the most important forces acting on prey populations. To persist with predators, prey must be able to recognize and respond to predatory threats. Despite the benefits of avoidance, there are time allocation tradeoffs with foraging and mating that make excessive responses to predators costly. Thus, antipredator behaviors of prey are often the result of complex interactions of effectiveness and efficiency. The San Marcos salamander (*Eurycea nana*) is a fully aquatic salamander endemic to the headwaters of the San Marcos River. *Eurycea nana* is subject to predation by a diverse community of native and nonnative fish predators. Despite the importance of predation pressures on prey populations and the protected status of *E. nana* and other regional congeners, no studies had examined predator-prey interactions in these species. This research was aimed at elucidating the mechanisms by which *E. nana* recognizes

and responds to predators. Specifically, I examined recognition of native and nonnative predators, predation risk assessment via predator diet cues and cues of damaged conspecifics, and how experience with predators affects avoidance behaviors. I found that *E. nana* use chemical stimuli to detect predators and respond to predators by decreasing activity levels. These salamanders innately recognize some predators, including nonnative species that are closely related to native predators, but experience with predators can alter the intensity of avoidance responses. These studies provided insight into the mechanisms by which *E. nana* recognizes predators. Additionally, I conclude that repeated exposures to predators can enhance avoidance behaviors exhibited by prey. In sum, these studies highlight the need for research exploring the plasticity of avoidance behaviors in prey that innately recognize and respond to predators.

CHAPTER I

THE COMPLEXITIES OF PREDATOR RECOGNITION AND AVOIDANCE

The most basic requirement for the persistence of predator-prey systems is that a sufficient proportion of the prey population is able to successfully avoid predation and reproduce. Because of this, selection has favored a variety of predator recognition mechanisms and avoidance behaviors in prey (see references later in this chapter). Antipredator behaviors in prey can be shaped by a number of factors, including genetic predispositions for innate and learned recognition, the suite of information available to prey about predators at a given point in time, the past experiences of prey with predators, and the level of environmental risk experienced by prey over prolonged periods. Given the merciless nature of predation, prey populations are under intense selective pressures that have resulted in highly complex and sophisticated avoidance strategies. Characteristics of the prey, the predatory community, and the habitat can all shape antipredator behaviors, therefore, exploring the mechanisms by which prey recognize and respond to predators is essential for understanding the ecology of prey species.

Study Species and System

The San Marcos salamander, *Eurycea nana*, is a neotenic (obligatorily aquatic and perennibranchiate) salamander endemic to the thermostable headwaters of the San Marcos River, Hays Co., Texas. It is one of more than nine described species that make up a monophyletic clade of Hemidactyliine plethodontid salamanders in the Edward's Plateau region of Texas (Chippindale 2000). As with other plethodontids, they are

completely lungless. The Edward's Plateau *Eurycea* are mostly neotenic and restricted to localized spring outflows or the water-filled caves of underground aquifers. Given the high level of endemism, species boundaries are typically restricted to one or a few populations and human encroachment in the region has resulted in federal protection of six species of *Eurycea* in the region, including *E. nana* (USDI 1980). Currently, a captive breeding population of *E. nana* is maintained at the San Marcos National Fish Hatchery and Technology Center (SMNFHTC) for the purposes of species recovery or reintroduction in the event that the natural population is extirpated. There is a growing body of literature addressing the phylogenetic and phylogeographic relationships of the Edward's Plateau *Eurycea* (Chippindale et al. 1998; Chippindale 2000; Chippindale et al. 2000; Hillis et al. 2001; Lucas et al. 2009), but, unfortunately, little is known about their ecology, and, prior to this dissertation, nothing was known of the predator-prey relationships of these species.

In its native habitat (Spring Lake; UTM 14R 602880.63 E, 3307083.52 N), *E. nana* is subject to predation by a diverse community of both native and introduced predatory species (Kelsey 1997; Petranka 1998; Bowles and Bowles 2001). In particular, fishes of the family Centrarchidae can be voracious opportunistic foragers (Hodgson and Kitchell 1987; Aday et al. 2009), and may present the greatest predatory threat to *E. nana*. Among these are native centrarchid species such as the largemouth bass (*Micropterus salmoides*), the green sunfish (*Lepomis cyanellus*), and the bluegill (*Lepomis macrochirus*) and a nonnative species, the redbreast sunfish (*Lepomis auritus*). The introduction of nonnative predators has been shown to have detrimental impacts on native amphibian populations when the prey species lacks mechanisms that allow it to successfully evade novel predators or to cope with increased predation pressures (Knapp and Matthews 2000; Blaustein and Bancroft 2007). Thus, understanding the mechanisms by which *E. nana* recognizes and responds to predators is important for understanding the ecology of *E. nana* and assessing the potential impacts of species introductions in the

system.

Predator Avoidance and Risk Assessment

Effective predator recognition and avoidance is crucial for the survival of prey. To escape predation, prey must detect predatory stimuli, identify the stimulus as a threat, and exhibit antipredator strategies that reduce the probability of capture by predators. Predators can induce a suite of behavioral (Petranka 1983; Anholt et al. 2000; Kesavaraju et al. 2007), morphological (Brönmark and Miner 1992; Brönmark and Pettersson 1994; Relyea 2001; Domenici et al. 2008), and life history (Crowl and Covich 1990; Sih and Moore 1993; Werner and Anholt 1996) responses in their prey. Of these antipredator strategies, behavioral responses are among the most documented. This difference may be because behavioral changes are easier to detect or because morphological and life history strategies can be relatively more costly for prey (Schoeppner and Relyea 2009a). Decreased activity levels (Anholt et al. 2000; Laurila 2000; Hervant et al. 2001; Marquis et al. 2004) or increased shelter use (Kats et al. 1988; Sih et al. 1992; Ferrer and Zimmer 2007) are common predator avoidance behaviors observed in amphibian prey. Reduced activity has been shown to increase survival of prey with predators (Skelly 1994) making it an effective avoidance strategy for many prey species in response to visually-oriented predators.

The obvious benefit of effective predator avoidance is that prey survive a predator encounter; however, because of time allocation trade-offs with beneficial activities like foraging (Sih 1992) or mating, excessive predator avoidance can be costly. The threat sensitivity hypothesis (Helfman 1989) predicts that prey should exhibit responses to predators that are reflective of predatory threat by responding more intensely to riskier predators and less intensely to less dangerous predators because it reduces the costs associated with antipredator strategies. Threat-sensitive avoidance responses have been shown across a wide range of aquatic prey including amphibians (Laurila et al. 1997;

Ferrari et al. 2008b; Fraker 2009), fish (Brown 2003; McCormick and Manassa 2008), and invertebrates (Sih 1982; Kesavaraju et al. 2007; Ferrari et al. 2008c). Given the tradeoffs of antipredator behaviors and foraging (Sih 1992; Lima 1998), prey that exhibit both effective and efficient avoidance responses should be at a selective advantage in most systems.

Predator Detection in Aquatic Environments

In aquatic environments, prey are exposed to a complex suite of stimuli including tactile, visual, and chemical cues which may allow them to detect predators and assess predation risk. Visual cues appear to be important for risk assessment by many prey fishes (Murphy and Pitcher 1997; Smith and Belk 2001). For example, damselfish (*Stegastes planifrons*) responded more intensely to visual cues of a predator model (trumpetfish, *Aulostomus maculatus*) that was larger, closer, or in a feeding position (Helfman 1989). In amphibian prey, primary reliance on chemical over visual cues has been demonstrated repeatedly (Petranka et al. 1987; Stauffer and Semlitsch 1993; Kiesecker et al. 1996; Mathis and Vincent 2000; Thaker et al. 2006). Chemical cues are useful when other cues can be limited such as at night or in murky waters or highly vegetated habitats. However, amphibians from high visibility habitats still rely primarily on chemical stimuli for predator detection (Hickman et al. 2004) and this may be due to limited visual capacity in amphibian prey (Mathis and Vincent 2000).

Chemical cues can convey large amounts of information to prey about predators and predation risk. Prey may use chemical stimuli emitted by the predator such as odors associated with predatory species (kairomones; Kats and Dill 1998; Turner et al. 1999; Relyea 2001; Mathis et al. 2003) or cues associated with recent foraging activity (diet cues; Chivers and Mirza 2001; Sullivan et al. 2005). Predator kairomones can convey a high degree of specificity as prey are able to discern predators from non-predators based solely on kairomones (Mathis 2003; Mathis et al. 2003) and prey respond differently

to kairomones of different predators (Turner et al. 1999; Relyea 2001). For minnows, kairomones can further convey threat-indicative information about the size (Kusch et al. 2004), density, or proximity (Ferrari et al. 2006) of predators. Despite the specificity of kairomones, research suggests that the chemical stimuli of closely related predators may share similar characteristics because prey that respond to the kairomones of a recognized predator will also respond to those of a related novel predator (Mathis and Smith 1993b; Ferrari et al. 2007; Ferrari et al. 2008a; Ferrari et al. 2009; Ferrari and Chivers 2009). This generalization of predator recognition appears to be correlated with the phylogenetic relationship of predators because response intensity declines as phylogenetic distance increases (Ferrari et al. 2007; Schoeppner and Relyea 2009b).

In addition to kairomones alone, prey may respond more intensely to the chemical stimuli of predators that have recently consumed conspecific (Wilson and Lefcort 1993; Laurila et al. 1997; Schoeppner and Relyea 2005) or related heterospecific (Mathis and Smith 1993a; Schoeppner and Relyea 2005) prey as compared to predators that were fed neutral diets or starved. It is thought that predator diet cues result from the mixing of chemicals emitted by damaged prey (e.g. alarm cues) with predator kairomones, which then 'labels' the predator as higher risk (Crowl and Covich 1990; Mathis and Smith 1993a; Jacobsen and Stabell 2004). Interestingly, recent work has shown that prey respond most intensely to predators that have consumed conspecifics as compared to a combination of predator kairomones mixed with conspecific alarm cues or the cues presented independently (Jacobsen and Stabell 2004; Schoeppner and Relyea 2009a). This suggests that cues released by the predator during digestion are an important component to risk assessment based on diet cues.

Chemical cues released by prey (reviewed in Chivers and Smith 1998; Brown and Chivers 2005) can also provide valuable information about the risk that predators pose. Chemical alarm cues are compounds released when prey are damaged, such as would occur during a predator attack, and should provide prey with reliable information

about foraging predators. Chemical alarm cues are thought to be localized in the integument of fish (Chivers et al. 2007) and amphibians (Fraker et al. 2009), although not all species possess or respond to alarm cues (Crowl and Covich 1990; Wilson and Lefcort 1993; Magurran et al. 1996; Summey and Mathis 1998). For prey that do use chemical alarm cues, these stimuli may mediate learning of predator odors (Chivers et al. 1995; Woody and Mathis 1998; Hazlett 2003) or they can provide information about the risk of predators to prey (Puttlitz et al. 1999; Brown 2003). Like the ability of prey to generalize recognition of predator odors, prey may also respond to the alarm cues of heterospecific prey in the same guild (Mathis and Smith 1993b; Mirza et al. 2003; Golub et al. 2005; Vance-Chalcraft et al. 2007), although the importance of phylogenetic relatedness of prey has received less attention than with predators (Sullivan et al. 2003; Schoeppner and Relyea 2005). Additionally, prey may also detect disturbance cues from other prey. The release of disturbance cues does not require damage to prey individuals (Fraker et al. 2009) and some are thought to be stress-related urinary secretions (Hazlett 1990; Kiesecker et al. 1999). Similar to alarm cues, disturbance cues can be important in mediating learning and assessing predation risk (Wisenden 2003; Brown and Chivers 2005). The suite of chemical information available to prey can provide information important for predator detection and predation risk assessment and these cues are accessible to prey regardless of habitat structure or timing. These characteristics make chemical cues among the most important stimuli used by prey.

Innate and Learned Predator Recognition Mechanisms

Studies examining predator recognition in aquatic prey typically consider recognition mechanisms as being either innate or learned (reviewed in Brown 2003; Wisenden 2003). Innate recognition is defined as the ability of prey to detect and identify predators without prior experience. Innate recognition mechanisms are expected to be favored in habitats with high predation pressures because learning could be overly costly

or in habitats with low variation in predation risk (Brown and Chivers 2005) because this recognition mechanism often considered to be 'genetically fixed' (Wisenden 2003). Innate predator recognition has been demonstrated for numerous aquatic prey including fishes (Breden et al. 1987; Utne-Palm 2001; Berejikian et al. 2003) and amphibians (Gallie et al. 2001; Mathis et al. 2003; Orizaola and Brana 2003; Murray et al. 2004) and is beneficial because a potentially costly predator encounter is not required for prey to respond adaptively.

Some prey lack innate predator recognition and must instead learn to recognize predators. Predator recognition is typically acquired through associative learning when a novel predatory stimulus is paired with a stimulus indicative of predatory threat. If learning has been successful, prey exhibit adaptive avoidance responses to subsequent exposures to the predator stimulus alone. Primary reliance on learned recognition mechanisms is expected for prey in habitats with diverse or fluctuating predatory communities (Brown and Chivers 2005) because prey that learn to recognize predators may be better able to respond to variations in the predatory community or predation risk over time (Chivers and Smith 1998). Studies examining learning in aquatic prey are numerous and clearly demonstrate the intricacies and adaptive value of updating information about the identity of predator species (e.g. Woody and Mathis 1998; Darwish et al. 2005; Gonzalo et al. 2007), the relative risk that predators pose (Brown 2003; Ferrari and Chivers 2006; Ferrari et al. 2008c), and the temporal patterns that can affect risk (Ferrari et al. 2008d). Despite the benefits of learning, prey lacking innate recognition of predators that are unable to learn from social cues of conspecific or heterospecific prey (Clark and Dukas 1994; Griffin 2004; Galef and Laland 2005), must instead experience a potentially costly predator encounter to respond adaptively to predators.

Behavioral Plasticity and Predator Avoidance

The risk of predation can vary through space and time and prey that exhibit

behavioral plasticity in avoidance responses may be better able to cope with this ecological heterogeneity. This ability of prey to update or adjust avoidance behaviors can result in more effective avoidance of more dangerous predators and more efficient avoidance of less dangerous predators. Despite these benefits, phenotypic plasticity can be costly. Costs of plasticity are defined as negative fitness effects that result from expressing a phenotype via plastic as compared to fixed or canalized mechanisms (van Kleunen and Fischer 2005; Auld et al. 2010). DeWitt et al. (1998) described five possible sources for the costs of phenotypic plasticity including maintenance, production, information acquisition, development instability, and genetic costs; however, given that prey, regardless of whether they exhibit fixed or plastic avoidance responses, must detect, identify, and respond to predators, the costs of plastic avoidance behaviors are probably limited to mechanisms of risk assessment and threat-sensitive antipredator behaviors. Further, when considered in the context of predator avoidance responses, behavioral plasticity may be relatively less costly than other types of plasticity such as morphological or developmental plasticity because it is energetically less costly to produce and maintain and is typically reversible (West-Eberhard 1989; but see Relyea 2003). Thus, unless the expression of plasticity is otherwise limited, the relatively low costs of plasticity in avoidance behaviors and the potential benefits of more effective and efficient antipredator responses conferred through experience-mediated threat sensitivity, plasticity in avoidance behaviors are expected to be favored in most prey.

Many amphibian prey innately recognize and respond to predators, although learning can also be important for predator detection (Woody and Mathis 1998; Ferrari et al. 2008b). Despite this, it remains unclear the extent to which amphibians that innately recognize predators also exhibit experience-mediated plasticity in avoidance responses. If studies of amphibians only examine innate responses of naïve prey, they may inaccurately predict the actual behaviors of prey in natural habitats. Conversely, by examining behaviors of only experienced prey, researchers may fail to detect the extent of innate

recognition abilities. Examining avoidance behaviors of both naïve and experienced prey is important for understanding both the daily interactions and the selective forces that shape antipredator behaviors and lend insight into the extent of plasticity in avoidance behaviors in amphibians.

The goals of this dissertation were to: (1) determine whether *E. nana* recognized and responded to a common nonnative predator similarly to a native predator, (2) examine mechanisms of predator recognition and predation risk assessment, (3) determine the extent of threat-sensitive avoidance behaviors, and (4) determine the effects of experience with predators on avoidance behaviors. The results of these studies lend valuable insight into the predator-prey ecology of *E. nana* and other similar species. This information will be useful for management of both the captive and natural population.

Literature Cited

- Aday DD, Parkos III JJ, Wahl DH. 2009. Population and community ecology of Centrarchidae. In: Cooke S, Philip DP, editors. Centrarchid Fishes: Diversity, Biology and Conservation. West Sussex, UK: Wiley-Blackwell. p. 134-164.
- Anholt BR, Werner E, Skelly DK. 2000. Effect of food and predators on the activity of four larval ranid frogs. *Ecology* 81:3509-3521.
- Auld JR, Agrawal AA, Relyea RA. 2010. Re-evaluating the costs and limits of adaptive phenotypic plasticity. *Proceedings of the Royal Society B-Biological Sciences* 277:503-511.
- Berejikian BA, Tezaka EP, LaRaeb AL. 2003. Innate and enhanced predator recognition in hatchery-reared Chinook salmon. *Environmental Biology of Fishes* 67:241-251.
- Blaustein AR, Bancroft BA. 2007. Amphibian population declines: Evolutionary considerations. *Bioscience* 57:437-444.
- Bowles D, Bowles B. 2001. A review of the exotic species inhabiting the upper San Marcos River, Texas, U.S.A.: Texas Parks and Wildlife Department, Austin, TX.

- Breden F, Scott M, Michel E. 1987. Genetic differentiation for anti-predator behavior in the Trinidadian guppy, *Poecilia reticulata*. *Animal Behaviour* 35:618-620.
- Brönmark C, Miner JG. 1992. Predator-induced phenotypical change in body morphology in Crucian carp. *Science* 258:1348-1350.
- Brönmark C, Pettersson LB. 1994. Chemical cues from piscivores induce a change in morphology in cruscian carp. *Oikos* 70:396-402.
- Brown GE. 2003. Learning about danger: chemical alarm cues and local risk assessment in prey fishes. *Fish and Fisheries* 4:227-234.
- Brown GE, Chivers DP. 2005. Learning as an adaptive response to predation. In: Barbosa P, Castellanos P, editors. *Ecology of Predator-Prey Interactions*. New York: Oxford University. p. 34-53.
- Chippindale PT. 2000. Species boundaries and species diversity in the central Texas Hemidactyliine Plethodontid salamanders, Genus *Eurycea*. In: Bruce RC, Jaeger R, Houck LD, editors. *The Biology of Plethodontid Salamanders*. New York: Kluwer Academic / Plenum. p. 149-165.
- Chippindale PT, Price AH, Hillis DM. 1998. Systematic status of the San Marcos salamander, *Eurycea nana* (Caudata: Plethodontidae). *Copeia* 1998:1046-1049.
- Chippindale PT, Price AH, Weins JJ, Hillis DM. 2000. Phylogenetic relationships and systematic revision of central Texas Hemidactyliine Plethodontid salamanders. *Herpetological Monographs* 14:1-80.
- Chivers DP, Brown GE, Smith RJF. 1995. Acquired recognition of chemical stimuli from pike, *Esox lucius*, by brook sticklebacks, *Culaea inconstans* (Osteichthyes, Gasterosteidae). *Journal of Chemical Ecology* 25:2455-2463.
- Chivers DP, Mirza RS. 2001. Importance of predator diet cues in responses of larval wood frogs to fish and invertebrate predators. *Journal of Chemical Ecology* 27:45-51.
- Chivers DP, Smith RJF. 1998. Chemical alarm signaling in aquatic predator-prey systems: a review and prospectus. *Écoscience* 5:338-352.

- Chivers DP, Wisenden BD, Hindman CJ, Michalak TA, Kusch RC, Kaminskyj SGW, Jack KL, Ferrari MCO, Pollock RJ, Halbgewachs CF, Pollock MS, Alemadi S, James CT, Savaloja RK, Goater CP, Corwin A, Mirza RS, Kiesecker JM, Brown GE, Adrian JC, Krone PH, Blaustein AR, Mathis A. 2007. Epidermal 'alarm substance' cells of fishes are maintained by non-alarm functions: possible defence against pathogens, parasites, and UVB radiation. *Proceedings of the Royal Society B-Biological Sciences* 274:2611-2619.
- Clark CW, Dukas R. 1994. Balancing foraging and antipredator demands: an advantage of sociality. *American Naturalist* 144:542-548.
- Crowl TA, Covich AP. 1990. Predator-induced life-history shifts in a freshwater snail. *Science* 247:949-951.
- Darwish TL, Mirza RS, Leduc A, Brown GE. 2005. Acquired recognition of novel predator odour cocktails by juvenile glowlight tetras. *Animal Behaviour* 70:83-89.
- DeWitt TJ, Sih A, Wilson DS. 1998. Costs and limits of phenotypic plasticity. *Trends in Ecology & Evolution* 13:77-81.
- Domenici P, Turesson H, Brodersen J, Brönmark C. 2008. Predator-induced morphology enhances escape locomotion in crucian carp. *Proceedings of the Royal Society B-Biological Sciences* 275:195-201.
- Ferrari MCO, Brown GE, Messier F, Chivers DP. 2009. Threat-sensitive generalization of predator recognition by larval amphibians. *Behavioral Ecology and Sociobiology* 63:1369-1375.
- Ferrari MCO, Chivers DP. 2006. Learning threat-sensitive predator avoidance: how do fathead minnows incorporate conflicting information? *Animal Behaviour* 71:19-26.
- Ferrari MCO, Chivers DP. 2009. Sophisticated early life lessons: threat-sensitive generalization of predator recognition by embryonic amphibians. *Behavioral Ecology* 20:1295-1298.
- Ferrari MCO, Gonzalo A, Messier F, Chivers DP. 2007. Generalization of learned predator recognition: an experimental test and framework for future studies. *Proceedings of the Royal Society B-Biological Sciences* 274:1853-1859.

- Ferrari MCO, Messier F, Chivers DP. 2006. The nose knows: minnows determine predator proximity and density through detection of predator odours. *Animal Behaviour* 72:927-932.
- Ferrari MCO, Messier F, Chivers DP. 2008a. Can prey exhibit threat-sensitive generalization of predator recognition? Extending the Predator Recognition Continuum Hypothesis. *Proceedings of the Royal Society B-Biological Sciences* 275:1811-1816.
- Ferrari MCO, Messier F, Chivers DP. 2008b. Larval amphibians learn to match antipredator response intensity to temporal patterns of risk. *Behavioral Ecology* 19:980-983.
- Ferrari MCO, Messier F, Chivers DP. 2008c. Threat-sensitive learning of predators by larval mosquitoes *Culex restuans*. *Behavioral Ecology and Sociobiology* 62:1079-1083.
- Ferrari MCO, Rive AC, MacNaughton CJ, Brown GE, Chivers DP. 2008d. Fixed vs. random temporal predictability of predation risk: an extension of the risk allocation hypothesis. *Ethology* 114:238-244.
- Ferrer RP, Zimmer RK. 2007. The scent of danger: arginine as an olfactory cue of reduced predation risk. *Journal of Experimental Biology* 210:1768-1775.
- Fraker ME. 2009. Predation risk assessment by green frog (*Rana clamitans*) tadpoles through chemical cues produced by multiple prey. *Behavioral Ecology and Sociobiology* 63:1397-1402.
- Fraker ME, Hu F, Cuddapah V, McCollum SA, Relyea RA, Hempel J, Denver RJ. 2009. Characterization of an alarm pheromone secreted by amphibian tadpoles that induces behavioral inhibition and suppression of the neuroendocrine stress axis. *Hormones and Behavior* 55:520-529.
- Galef BG, Laland KN. 2005. Social learning in animals: empirical studies and theoretical models. *Bioscience* 55:489-499.
- Gallie JA, Mumme RL, Wissinger SA. 2001. Experience has no effect on the development of chemosensory recognition of predators by tadpoles of the American toad, *Bufo americanus*. *Herpetologica* 57:376-383.

- Golub JL, Vermette V, Brown GE. 2005. Response to conspecific and heterospecific alarm cues by pumpkinseeds in simple and complex habitats: field verification of an ontogenetic shift. *Journal of Fish Biology* 66:1073-1081.
- Gonzalo A, Lopez P, Martin J. 2007. Iberian green frog tadpoles may learn to recognize novel predators from chemical alarm cues of conspecifics. *Animal Behaviour* 74:447-453.
- Griffin AS. 2004. Social learning about predators: a review and prospectus. *Learning & Behavior* 32:131-140.
- Hazlett BA. 1990. Source and nature of disturbance-chemical system in crayfish. *Journal of Chemical Ecology* 15:381-385.
- Hazlett BA. 2003. Predator recognition and learned irrelevance in the crayfish *Orconectes virilis*. *Ethology* 109:765-780.
- Helfman GS. 1989. Threat-sensitive predator avoidance in damselfish-trumpetfish interactions. *Behavioral Ecology and Sociobiology* 24:47-58.
- Hervant F, Mathieu J, Durand J. 2001. Behavioural, physiological and metabolic responses to long-term starvation and refeeding in a blind cave-dwelling (*Proteus anguinus*) and a surface-dwelling (*Euproctus asper*) salamander. *Journal of Experimental Biology* 204:269-281.
- Hickman CR, Stone MD, Mathis A. 2004. Priority use of chemical over visual cues for detection of predators by graybelly salamanders, *Eurycea multiplicata griseogaster*. *Herpetologica* 60:203-210.
- Hillis DM, Chamberlain DA, Wilcox TP, Chippindale PT. 2001. A new species of subterranean blind salamander (Plethodontidae : Hemidactyliini : Eurycea : Typhlomolge) from Austin, Texas, and a systematic revision of central Texas paedomorphic salamanders. *Herpetologica* 57:266-280.
- Hodgson JR, Kitchell JF. 1987. Opportunistic foraging by largemouth bass (*Micropterus salmoides*). *American Midland Naturalist* 118:323-336.

- Jacobsen HP, Stabell OB. 2004. Antipredator behaviour mediated by chemical cues: the role of conspecific alarm signaling and predator labeling in the avoidance response of a marine gastropod. *Oikos* 104:43-50.
- Kats LB, Dill LM. 1998. The scent of death: chemosensory assessment of predation risk by prey animals. *Écoscience* 5:361-394.
- Kats LB, Petranka JW, Sih A. 1988. Antipredator defenses and the persistence of amphibian larvae with fishes. *Ecology* 69:1865-1870.
- Kelsey T. 1997. Fish community structure and water quality assessment (Index of Biotic Integrity) of the San Marcos River, Texas [M.S. Thesis]. [San Marcos, TX]: Southwest Texas State University. 45 pp.
- Kesavaraju B, Damal K, Juliano SA. 2007. Threat-sensitive behavioral responses to concentrations of water-borne cues from predation. *Ethology* 113:199-206.
- Kiesecker JM, Chivers DP, Blaustein AR. 1996. The use of chemical cues in predator recognition by western toad tadpoles. *Animal Behaviour* 52:1237-1245.
- Kiesecker JM, Chivers DP, Marco A, Quilchano C, Anderson MT, Blaustein AR. 1999. Identification of a disturbance signal in larval red-legged frogs (*Rana aurora*). *Animal Behaviour* 57:1295-1300.
- Knapp RA, Matthews KR. 2000. Non-native fish introductions and the decline of the mountain yellow-legged frog from within protected areas. *Conservation Biology* 14:428-438.
- Kusch RC, Mirza RS, Chivers DP. 2004. Making sense of predator scents: investigating the sophistication of predator assessment abilities of fathead minnows. *Behavioral Ecology and Sociobiology* 55:551-555.
- Laurila A. 2000. Behavioral responses to predator chemical cues and local variation in antipredator performance in *Rana temporaria* tadpoles. *Oikos* 88:159-168.
- Laurila A, Kujasalo J, Ranta E. 1997. Different antipredator behaviour in two anuran tadpoles: Effects of predator diet. *Behavioral Ecology and Sociobiology* 40:329-336.

- Lima SL. 1998. Nonlethal effects in the ecology of predator-prey interactions: what are the ecological effects of anti-predator decision-making? *Bioscience* 48:25-34.
- Lucas LK, Gompert Z, Ott JR, Nice CC. 2009. Geographic and genetic isolation in spring-associated Eurycea salamanders endemic to the Edwards Plateau region of Texas. *Conservation Genetics* 10:1309-1319.
- Magurran AE, Irving PW, Henderson PA. 1996. Is there a fish alarm pheromone? A wild study and critique. *Proceedings of the Royal Society B-Biological Sciences* 263:1551-1556.
- Marquis O, Saglio P, Neveu A. 2004. Effects of predators and conspecific chemical cues on the swimming activity of *Rana temporaria* and *Bufo bufo* tadpoles. *Archiv Fur Hydrobiologie* 160:153-170.
- Mathis A. 2003. Use of chemical cues in detection of conspecific predators and prey by newts, *Notophthalmus viridescens*. *Chemoecology* 13:193-197.
- Mathis A, Murray KL, Hickman CR. 2003. Do experience and body size play a role in responses of larval ringed salamanders, *Ambystoma annulatum*, to predator kairomones? Laboratory and field assays. *Ethology* 109:159-170.
- Mathis A, Smith RJF. 1993a. Chemical labeling of northern pike (*Esox lucius*) by the alarm pheromone of fathead minnows (*Pimephales promelas*). *Journal of Chemical Ecology* 19:1967-1979.
- Mathis A, Smith RJF. 1993b. Intraspecific and cross-superorder responses to chemical alarm signals by brook stickleback. *Ecology* 74:2395-2404.
- Mathis A, Vincent F. 2000. Differential use of visual and chemical cues in predator recognition and threat-sensitive predator-avoidance responses by larval newts (*Notophthalmus viridescens*). *Canadian Journal of Zoology* 78:1646-1652.
- McCormick MI, Manassa R. 2008. Predation risk assessment by olfactory and visual cues in a coral reef fish. *Coral Reefs* 27:105-113.

- Mirza RS, Fisher SA, Chivers DP. 2003. Assessment of predation risk by juvenile yellow perch, *Perca flavescens*: responses to alarm cues from conspecifics and prey guild members. *Environmental Biology of Fishes* 66:321-327.
- Murphy KE, Pitcher TJ. 1997. Predator attack motivation influences the inspection behaviour of European minnows. *Journal of Fish Biology* 50:407-417.
- Murray DL, Roth JD, Wirsing AJ. 2004. Predation risk avoidance by terrestrial amphibians: The role of prey experience and vulnerability to native and exotic predators. *Ethology* 110:635-647.
- Orizaola G, Brana F. 2003. Response of predator-naive newt larvae to food and predator presence. *Canadian Journal of Zoology* 81:1845-1850.
- Petranka JW. 1983. Fish predation: a factor affecting the spatial distribution of a stream-breeding salamander. *Copeia* 1983:624-628.
- Petranka JW. 1998. *Salamanders of the United States and Canada*. Washington, D.C.: Smithsonian Institution Press.
- Petranka JW, Kats LB, Sih A. 1987. Predator-prey interactions between fish and larval amphibians: use of chemical cues to detect predatory fish. *Animal Behaviour* 35:420-425.
- Puttlitz MH, Chivers DP, Kiesecker JM, Blaustein AR. 1999. Threat-sensitive predator avoidance by larval Pacific treefrogs (*Amphibia*, *Hylidae*). *Ethology* 105:449-456.
- Relyea RA. 2001. Morphological and behavioral plasticity of larval anurans in response to different predators. *Ecology* 82:523-540.
- Relyea RA. 2003. Predators come and predators go: the reversibility of predator-induced traits. *Ecology* 84:1840-1848.
- Schoeppner NM, Relyea RA. 2005. Damage, digestion, and defence: the roles of alarm cues and kairomones for inducing prey defences. *Ecology Letters* 8:505-512.

- Schoeppner NM, Relyea RA. 2009a. Interpreting the smells of predation: how alarm cues and kairomones induce different prey defences. *Functional Ecology* 23:1114-1121.
- Schoeppner NM, Relyea RA. 2009b. When should prey respond to consumed heterospecifics? Testing hypotheses of perceived risk. *Copeia* 2009:190-194.
- Sih A. 1982. Foraging strategies and the avoidance of predation by an aquatic insect. *Ecology* 63:786-796.
- Sih A. 1992. Prey uncertainty and the balancing of antipredator and feeding needs. *American Naturalist* 139:1052-1069.
- Sih A, Kats LB, Moore RD. 1992. Effects of predatory sunfish on the density, drift, and refuge use of stream salamander larvae. *Ecology* 73:1418-1430.
- Sih A, Moore RD. 1993. Delayed hatching of salamander eggs in response to enhanced larval predation risk. *American Naturalist* 142:947-960.
- Skelly DK. 1994. Activity level and the susceptibility of anuran larvae to predation. *Animal Behaviour* 47:465-468.
- Smith ME, Belk MC. 2001. Risk assessment in western mosquitofish (*Gambusia affinis*): do multiple cues have additive effects? *Behavioral Ecology and Sociobiology* 51:101-107.
- Stauffer HP, Semlitsch RD. 1993. Effects of visual, chemical and tactile cues on the behavioural responses of tadpoles. *Animal Behaviour* 46:355-364.
- Sullivan AM, Madison DM, Rohr JR. 2003. Behavioural responses by red-backed salamanders to conspecific and heterospecific cues. *Behaviour* 140:553-564.
- Sullivan AM, Picard AL, Madison DM. 2005. To avoid or not to avoid? Factors influencing the discrimination of predator diet cues by a terrestrial salamander. *Animal Behaviour* 69:1425-1433.

- Summey MR, Mathis A. 1998. Alarm responses to chemical stimuli from damaged conspecifics by larval anurans: Tests of three neotropical species. *Herpetologica* 54:402-408.
- Thaker M, Gabor CR, Fries JN. 2006. Sensory cues for conspecific associations in aquatic San Marcos salamanders. *Herpetologica* 62:151-155.
- Turner AM, Fetterolf SA, Bernot RJ. 1999. Predator identity and consumer behavior: differential effects of fish and crayfish on the habitat use of a freshwater snail. *Oecologia* 118:242-247.
- [USDI] United States Department of the Interior. 1980. Endangered and threatened wildlife and plants: listing of San Marcos salamander as threatened, the San Marcos gambusia as endangered, and the listing of critical habitat for Texas wild rice, San Marcos gambusia, and fountain darter. *Federal Register*. Pp. 47355-47364.
- Utne-Palm AC. 2001. Response of naïve two-spotted gobies *Gobiusculus flavescens* to visual and chemical stimuli of their natural predator, cod *Gadus marhua*. *Marine Ecology - Progress Series* 218:267-274.
- van Kleunen M, Fischer M. 2005. Constraints on the evolution of adaptive phenotypic plasticity in plants. *New Phytologist* 166:49-60.
- Vance-Chalcraft HD, Rosenheim JA, Vonesh JR, Osenberg CW, Sih A. 2007. The influence of intraguild predation on prey suppression and prey release: A meta-analysis. *Ecology* 88:2689-2696.
- Werner EE, Anholt BR. 1996. Predator-induced behavioral indirect effects: consequences to competitive interactions anuran larvae. *Ecology* 77:157-169.
- West-Eberhard MJ. 1989. Phenotypic plasticity and the origins of diversity. *Annual Review of Ecology and Systematics* 20:249-278.
- Wilson DJ, Lefcort H. 1993. The effect of predator diet on the alarm response of red-legged frog, *Rana aurora*, tadpoles. *Animal Behaviour* 46:1017-1019.

Wisenden BD. 2003. Chemically mediated strategies to counter predation. In: Collin SP, Marshall NJ, editors. *Sensory Processing in Aquatic Environments*. New York: Springer-Verlag. p. 236-251.

Woody DR, Mathis A. 1998. Acquired recognition of chemical stimuli from an unfamiliar predator: Associative learning by adult newts, *Notophthalmus viridescens*. *Copeia* 1988:1027-1031.

CHAPTER II

INNATE AND LEARNED PREDATOR RECOGNITION MEDIATED BY CHEMICAL SIGNALS IN *EURYCEA NANA*¹

Abstract

Effective and efficient predator recognition and avoidance are essential to the persistence of prey populations, especially in habitats where nonnative predators have been introduced. Predator recognition studies are commonly couched within a learned or innate dichotomous framework; however, characteristics of some systems or species could favor innate recognition combined with the ability to alter avoidance responses based on experience with predators. *Eurycea nana* is a fully aquatic salamander inhabiting a system with a diverse, yet temporally stable, community of native and nonnative opportunistically foraging fish predators. To examine predator recognition, we examined avoidance responses (decreased activity) of predator-naïve (first generation, captive-reared) and predator-experienced (recently collected) *E. nana* to the chemical cues of a native predator, a nonnative predator, a non-predator, and a blank control. Both predator-naïve and predator-experienced *E. nana* significantly lowered activity in response to the native fish predator as compared to a blank control. Interestingly, predator-naïve *E. nana* decreased activity in response to the nonnative fish predator while predator-experienced *E. nana* did not. These results indicate that while there is an innate component to predator recognition in *E. nana*, experience and risk assessment may also be important.

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Introduction

Predation affects prey populations through removal of prey individuals and the induction of antipredator behaviors (Lima 1998; Werner and Peacor 2003). To persist with predators, prey in diverse systems must be able to recognize and avoid a variety of predatory threats. Predator recognition can be especially challenging for native prey in systems where predators have been introduced. Here, we define predator recognition as the detection and identification of predatory stimuli that elicit avoidance responses in prey. Prey may be able to detect and identify a number of predatory stimuli in aquatic habitats including visual (Brown et al. 1997; Miklosi et al. 1997; Utne-Palm 2001) and chemical (as reviewed by Kats and Dill 1998) cues, although chemical stimuli appear to be the most commonly used cue by amphibian prey (Kats and Dill 1998; Mathis and Vincent 2000; Ferrer and Zimmer 2007). Recognition of predators may have an innate basis (Griffiths et al. 1998; Laurila 2000) or be learned (Chivers and Smith 1998; reviewed in Wisenden 2003). Prey exhibiting learned predator recognition require experience with predators to identify predatory threats while prey exhibiting innate predator recognition do not.

Innate predator recognition confers different costs and benefits than does learned predator recognition. Innate predator recognition can result from the co-evolution of prey and predator and is advantageous because a predator encounter is not required for naïve prey to respond adaptively to predatory threats (Laurila 2000). However, innate recognition alone can be costly as it may limit the number of recognized predatory species (Wisenden 2003). Prey that must learn to recognize predatory threats typically do so through a potentially costly naïve encounter with predators. Despite this cost, the ability of prey to acquire recognition of novel predators can be beneficial, especially within diverse or fluctuating predatory communities (Wisenden 2003). Additionally, prey may use experience with predators to assess predation risk and adjust the intensity of their avoidance responses accordingly (Brown 2003; Ferrari et al. 2005; Gonzalo et al.

2007), which reduces the costs associated with predator avoidance such as time allocation trade-offs between avoidance and foraging (Sih 1992; Lima and Bednekoff 1999).

Studies exploring learned and innate predator recognition by vertebrate aquatic prey have found that, in general, fish exhibit learned predator recognition (Brown 2003; Kelley and Magurran 2003) while amphibians rely on innate recognition (Kats and Dill 1998), though notable exceptions exist (Suboski 1992; Miklosi et al. 1997; Woody and Mathis 1998; Wildy and Blaustein 2001; Mandrillon and Saglio 2005). This evidence, in many cases, has led to a conceptual dichotomy when considering how prey recognize predators. However, it is important to consider that some systems may favor prey species that use both methods in concert for more efficient predator recognition and avoidance. Interestingly, while some studies have explored the possibility (Sih and Kats 1994; Laurila et al. 1997; Gallie et al. 2001), we know of only one study which clearly demonstrated experience-mediated, risk sensitive adjustments to innate avoidance responses of an amphibian species, *Rana perezi* (Gonzalo et al. 2007).

Determining the role of experience in predator recognition is especially important for native prey in habitats where predators have been introduced. If prey exhibiting innate predator recognition are unable to acquire recognition of novel predatory stimuli, they may not respond adaptively to nonnative predatory threats (Kiesecker and Blaustein 1997; Pearl et al. 2003; Anthony et al. 2007). This failure has been named as a primary cause of native amphibian population declines and extinctions in some habitats where nonnative predators have been introduced (Knapp and Matthews 2000; Adams et al. 2001; Pilliod and Peterson 2001; Kats and Ferrer 2003). However, prey that are able to recognize some predators at birth and also exhibit the capacity to use experience to acquire recognition of or alter the intensity of avoidance responses to predators would be at a selective advantage in these systems.

It is important to explore the role of experience on predator recognition and avoidance in systems where both innate and acquired responses are expected to be

important. We explored the role of experience on recognition of fish predators using the San Marcos salamander, *Eurycea nana*, is a federally threatened (USDI 1980) paedomorphic species (obligately aquatic throughout life). The thermostable (21.0°C – 21.5°C, Groeger et al. 1997) habitat of *E. nana* has a diverse and temporally stable predatory community composed of many native and nonnative opportunistically foraging predators (Bowles and Bowles 2001). Because *E. nana* must avoid fish predation as reproductive adults as well as juveniles, the propensity for adverse impacts of predatory fish on the population may be relatively greater, or at least different, than on most amphibian prey that exhibit both aquatic and terrestrial life stages. As with other amphibian species, we predicted that *E. nana* would exhibit innate recognition of native predators and that they might not recognize nonnative predators as threats. However, given that *E. nana* face life-long predation pressures from fish due to being paedomorphic, learning may also be an important factor in this system. To examine this possibility we explored the responses of both predator-experienced (recently collected) and predator-naïve (first generation, captive-reared) adult *E. nana* to the chemical cues of native and nonnative syntopic predatory fish species.

Materials and Methods

Study Species

Eurycea nana is endemic to and found only in the headwaters of the San Marcos River, Hays County, Texas (Bishop 1941; Nelson 1993; Chippindale et al. 1998). A captive population is also maintained at the San Marcos National Fish Hatchery and Technology Center (SMNFHTC). In the wild, *E. nana* are typically found along the substrate under refuges such as rocks and vegetation (Tupa and Davis 1976). Gravid females and juveniles of *E. nana* are present throughout the year (Bogart 1967; Tupa and Davis 1976) indicating year-round reproduction and activity for this species. Thaker et

al. (2006) showed that *E. nana* relies primarily on chemical rather than visual cues for conspecific association preference.

Stimulus Species Selection

We used the native species *Micropterus salmoides* (largemouth bass) and the nonnative species *Lepomis auritus* (redbreast sunfish) for our predatory stimuli based on available literature (Kelsey 1997) and personal communications (E. Chappel). We used heterogenic species because prey may recognize congeners across species boundaries and respond similarly between native and nonnative species (Mirza et al. 2003; Ferrari et al. 2007a). Additionally, these species appear to be among the most abundant predatory species in the San Marcos river headwaters and share similar opportunistic foraging habits (Day 1981; Wallace 1984). Analysis of stomach contents obtained through stomach pumping of the predatory species (*M. salmoides* n = 10; *L. auritus* n = 10) revealed similar digestive remains including benthic organisms which indicates that both species are likely to encounter *E. nana* during regular foraging activity (K. Epp and C. Gabor unpub. data). Additionally, a diet study on *L. auritus* collected from the San Marcos River 4 – 6.5 km downstream from the habitat of *E. nana* found that benthic invertebrates composed a substantial portion of the diet (Wallace 1984), indicating that in the headwaters, *L. auritus* are benthic foragers and thus are likely to encounter *E. nana* while foraging. In the headwaters, both species have been observed preying on *E. nana* (K. Epp, pers. obs.; pers. com., E. Chappel) and *Lepomis* spp. and *Micropterus salmoides* are considered predatory threats to *E. nana* in this system (Tupa and Davis 1976; Petranka 1998). We further demonstrated in captivity that both species will prey on *E. nana* when given the opportunity by placing an individual of each predatory species (n = 5) in individual holding tanks with one *E. nana* /tank. After 24 h, presence/absence of *E. nana* was recorded. Absent *E. nana* were assumed to have been consumed, as no alternative escape was available. All individuals of *M. salmoides* and *L. auritus* consumed *E. nana* within 24 h of offering.

To control for response of *E. nana* to predator cues as opposed to fish cues in general, we chose to expose *E. nana* to a native, non-predatory fish species, *Gambusia geiseri*. While *Gambusia* spp. are potential predators of amphibian larvae or eggs in other systems (Hamer et al. 2002; Lane and Mahony 2002; Baber and Babbitt 2003), because of their size, they are not expected to pose a predatory threat to adult *E. nana* used in this study. Additionally, the microhabitat use of *G. geiseri* (Hubbs and Peden 1969) and *E. nana* (Tupa and Davis 1976) differ drastically in this system because *G. geiseri* is typically located in shallow waters along the edges of the lake while *E. nana* is typically located along the substrate in deeper waters near spring openings. These differences make predation by *G. geiseri* unlikely in this system.

Stimulus Acquisition

Stimulus animals (*M. salmoides* $n = 28$, *L. auritus* $n = 16$, *Gambusia geiseri* $n = 237$) were collected from the San Marcos River headwaters, Hays County, Texas, every two weeks during the same time as testing occurred. They were placed in species-specific aerated tanks for 24 h with 230 ml of de-chlorinated tap water for every 1 cm³ of stimulus animal by volume. Standard length (SL; $\bar{x} \pm SE$; *M. salmoides*: 193.68 mm \pm 12.48; *L. auritus*: 183.44 mm \pm 9.82) and volume (*M. salmoides*: 498.81 cc \pm 39.73; *L. auritus*: 442.88 cc \pm 31.98) of predatory individuals did not differ between species (unpaired t-test: SL: $t = -0.645$, $df = 41.886$, $p = 0.5225$; volume: $t = 1.097$, $df = 41.886$, $p = 0.281$). To control for the response of *E. nana* to individual fish as opposed to species kairomones, *M. salmoides* tanks contained two individuals per stimulus tank. Because of difficulties with tank size, *L. auritus* were housed individually for 24 h and tank water from two individual tanks was evenly mixed prior to stimulus collection. *Gambusia geiseri* were housed in tanks with 27 – 33 individuals per collection tank with 230 ml of water per 1 cm³ of stimulus animal by volume. Each stimulus tank contributed no more than five stimulus samples for use in testing. Tank water was not filtered and stimulus animals were not fed during this time. After 24 h, stimulus animals were released and

water from the tanks was stirred, collected in 50 ml plastic falconer tubes, and frozen at -20°C for at least 24 h prior to testing. While freezing may alter the chemical composition of stimuli, this method has been used successfully in many studies (Woody and Mathis 1998; Mathis 2003; Hickman et al. 2004). No visible particulates (e.g. feces) were included in collected stimulus samples. For control stimuli, falconer tubes were filled with de-chlorinated tap water and then frozen. Stimuli were thawed using a circulating well water bath immediately prior to testing.

Predator Avoidance in Predator-Experienced *E. nana*

We collected adult *E. nana* ($n = 62$) from the San Marcos River headwaters, Hays County, Texas from March – June 2005. These salamanders were assumed to have had experience with predators or predator cues in their natural habitat. Individuals with snout-vent lengths (SVL) greater than 20 mm were considered adults as *E. nana* have been determined to be sexually mature at this size (Tupa and Davis 1976). Individuals of *E. nana* were housed in flow-through fiberglass tanks at the SMNFHTC on a 12:12 h light:dark cycle with 40-W fluorescent lights during daylight hours for at least two weeks prior to testing. The tanks were filled with well water and maintained at 22°C - 23°C . We fed *E. nana* commercially-raised annelids (*Lumbriculus variegatus*) and copepod mixtures ad libitum. Testing occurred from April – July 2005. Thus, all *E. nana* were collected relatively recently from the wild and were likely to have maintained their original predatory responses (Mirza and Chivers 2000). Because decreased activity is a common antipredator behavior in amphibians (Wisenden 2003), we used changes in activity to determine responses of salamanders to predatory stimuli. Salamanders were selected haphazardly from the housing tanks and placed individually into 9.5-l glass aquaria filled with 4.5 l of well water. A 50-ml syringe attached to airline tubing was used for stimulus introduction. The tubing was attached to one corner of the testing chamber and extended 5 cm below the surface of the water on the interior and 10 cm below the base of the testing chamber on the exterior. Using established methods (Jaeger 1981;

Thaker et al. 2006), *E. nana* were tested under dim 25-W red lighting at night when they are most active (pers. obs.). After 15 min of acclimation the amount of time (sec) that *E. nana* spent actively moving was recorded for 10 min (pre-stimulus activity). Individuals of *Eurycea nana* were then exposed to one of four treatments: (1) native predator, largemouth bass, *M. salmoides* ($n = 15$), (2) nonnative predator, redbreast sunfish, *L. auritus* ($n = 15$), (3) native non-predator, largespring Gambusia, *Gambusia geiseri* ($n = 17$), or (4) only water ($n = 15$). Fifty ml of one of the four treatments was injected at about 1 ml/sec into the testing chamber. Pilot tests using food dye showed that when injected at this rate, stimulus water had spread through the entire chamber by the completion of stimulus introduction. Treatment stimuli were randomly assigned and coded so the observer was blind to the treatment. After stimulus introduction, the time salamanders spent actively moving (sec) was recorded for another 10 min (post-stimulus activity). Relative activity scores of *E. nana* to stimuli were calculated as an index ratio of post-stimulus activity to pre-stimulus activity. In this way the response variable is representative of each individual's change in activity from pre-stimulus (normal) activity to post-stimulus activity. A relative activity score ≥ 1 indicates that individuals were equally or more active after stimulus exposure as before, while relative activity scores < 1 indicate reduced activity after stimulus exposure. Each individual was used only once for these studies.

Predator Avoidance in Predator-Naïve *E. nana*

Similar methods were followed for testing and stimulus acquisition in this experiment; however, first-generation captive-born (predator-naïve) adult *E. nana* (SVL ≥ 20 mm; $n = 60$) were used as test subjects. Testing occurred from December 2005 – May 2006.

Statistical Analyses

Using residual plots and Shapiro-Wilks' tests ($\alpha = 0.05$) we determined that the data met the assumption of normality. We examined residual plots of relative activity

scores and determined that the data met the assumption of homoscedasticity. We used a boxplot outlier test to determine statistical outliers (Barnett and Lewis 1984). We determined that one data point in the predator-experienced, native predator treatment was an extreme outlier. When all other data were combined this point fell 4.5 standard deviations above the mean ($n = 121$, $\bar{x} = 0.704$, $SD = 0.375$). Outliers may bias analyses when sample sizes are small (Iglewicz and Hoaglin 1993) and this one was removed from further analyses. We combined predator-experienced and predator-naïve data and performed a two-factor analysis of variance (ANOVA) with predator experience and stimulus treatment as factors. All analyses were performed using JMP® 6.0 (SAS Institute Inc. 2005) software.

Results

Complete Model

Two-factor ANOVA indicated a significant interaction between the factors of stimulus treatment and predator experience ($F_{3,113} = 2.713$, $p = 0.048$). To elucidate the nature of this interaction we used single-factor ANOVA's with subsequent Fisher's least significant difference (LSD) multiple comparisons procedures to compare relative activity scores among treatments within predator experience groups. We then compared responses of predator-experienced and predator-naïve *E. nana* for each treatment using Tukey's honestly significant difference (HSD) multiple comparison procedure ($\alpha = 0.05$).

Predator Avoidance in Predator-Experienced *E. nana*

We found significant differences in the relative activity score between stimulus treatments (ANOVA: $F_{3,57} = 2.880$, $p = 0.044$). Mean relative activity score for the native predator treatment was significantly lower than the control (Fisher's LSD: $p = 0.021$) and the nonnative predator treatments (Fisher's LSD: $p = 0.011$), but did not differ from the non-predator treatment (Fisher's LSD: $p = 0.191$). Relative activity scores did not differ between the control and non-predator (Fisher's LSD: $p = 0.258$) and nonnative predator

($p = 0.791$) treatments or the non-predator and nonnative predator ($p = 0.162$) treatments (Fig. 1a).

Predator Avoidance in Predator-Naïve *E. nana*

We found significant differences in relative activity scores between treatments for predator-naïve *E. nana* (ANOVA: $F_{3,56} = 6.495$, $p < 0.001$). Mean relative activity score for the control significantly differed from the native predator (Fisher's LSD: $p < 0.001$)

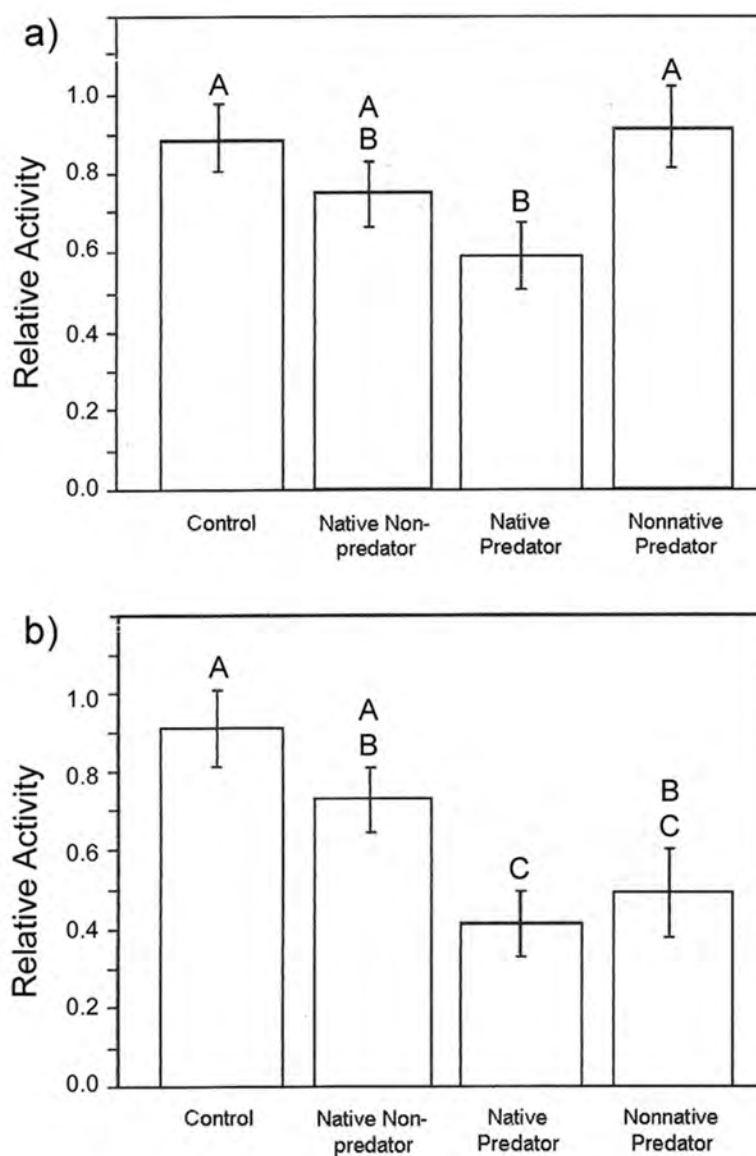


Figure 1. Avoidance Responses of Naïve and Experienced *Eurycea nana* to Native and Nonnative Predators. Mean (\pm SE) relative activity after exposure to the chemical cues of one of four fish stimuli for: (a) predator-experienced and (b) predator-naïve *Eurycea nana*. Responses not connected by the same letter are different ($\alpha = 0.05$).

and nonnative predator (Fisher's LSD: $p = 0.002$) treatments and did not differ from the non-predator treatment (Fisher's LSD: $p = 0.147$). Relative activity scores for the non-predator treatment differed significantly from the native predator treatment (Fisher's LSD: $p = 0.016$), but did not differ from the nonnative predator treatment (Fisher's LSD: $p = 0.069$). Relative activity scores for the native predator and nonnative predator treatments were not different (Fisher's LSD: $p = 0.526$; Fig. 1b).

Comparisons of Predator-Naïve Versus Predator-Experienced Responses

Predator-naïve and predator-experienced *E. nana* did not respond differently to the water control, non-predator, and native predator treatments (Tukey's HSD: $p > 0.05$). For the nonnative predator treatment, the relative activity score was lower for predator-naïve than predator-experienced *E. nana* (Tukey's HSD: $p < 0.05$).

Discussion

Both predator-naïve and predator-experienced *E. nana* exhibited reductions in activity when presented with the chemical cues of a native fish predator as compared to a blank control. This response is consistent with predator avoidance behaviors exhibited by other amphibian prey species (e.g. Griffiths et al. 1998; Mathis and Vincent 2000; Mathis et al. 2003; Mandrillon and Saglio 2005). Because predator-naïve *E. nana* exhibited avoidance responses when presented with chemical cues of the native predator, there is good evidence that there is an innate component to predator recognition in *E. nana*. Innate predator recognition has been documented for numerous amphibian species (reviewed in Wisenden 2003). Interestingly, predator-experienced *E. nana* did not exhibit significant decreases in activity when presented with the chemical cues of the syntopic nonnative predator; however, predator-naïve *E. nana* significantly decreased activity levels when presented with the chemical cues of the nonnative fish predator. Because congeners of the nonnative predator (e.g. *L. cyanellus*) are native to the study habitat, *E. nana* may innately recognize *Lepomis auritus* as a predatory threat. For example, Ferrari

et al. (2007a) demonstrated that prey may recognize congeneric predators across species boundaries because the kairomones produced are expected to be similar. Experienced *E. nana* exhibited diminished responses, however, as compared to naïve *E. nana* suggesting that wild caught *E. nana* may have refined responses to predators given experience.

Certain characteristics of both *E. nana* and the predatory community may encourage the development of both innate and experience-mediated avoidance responses. Because *E. nana* inhabits a flowing river system and females lay eggs individually as opposed to in clutches, the opportunities that predator-naïve juveniles have to acquire recognition of predators from conspecifics prior to an attack may be limited. This would promote the development of innate recognition of those predators posing the most significant predatory threats to *E. nana*. However, because *E. nana* experiences fish predation throughout life, the ability to refine responses to predators based on experience may also be important for this species. This is especially true when considering the highly diverse yet temporally stable predator community which preys on *E. nana*. These characteristics imply that experience-mediated modifications to innate responses would be favored in this system.

While *E. nana* may inherently recognize the predatory species used in this experiment as a threat, the intensity of their avoidance responses may be altered based on their perception of predation risk. The ability of prey to alter the intensity of their avoidance responses based on their perception of risk (Ferrer and Zimmer 2007) is beneficial in that it allows prey to minimize the costs associated with predator avoidance (Sih et al. 1992; Anholt et al. 2000). Prey may assess predation risk in many ways including detection of predatory diet cues (reviewed by Chivers and Smith 1998; Wisenden 2003) or through experience with species-specific predator kairomones (reviewed in Kats and Dill 1998). Although primary reliance on predatory diet cues for risk assessment has been shown for some amphibian prey (Wilson and Lefcort 1993; Lefcort 1996; Chivers et al. 1999; Murray et al. 2004), stimulus individuals in this study

were collected using consistent methods across studies and multiple individuals of each species provided stimulus for each study. Thus, we would expect greater variation in responses of *E. nana* within predator treatment groups as well as more similar responses between experienced and naïve *E. nana* than was recorded if predator diet cues alone accounted for the differences observed. Additionally, primary reliance on predator diet cues for identification of risky predators is not expected to be favored in systems composed primarily of opportunistic foragers as opposed to specialist predators because diet cues alone may not be reliable indicators predatory threat (Ferrari et al. 2007b). Therefore, predation risk assessment based on detection of and experience with species-specific predator kairomones is the most probable explanation for the differences in responses to *L. auritus* observed between predator-experienced and predator-naïve *E. nana*.

If the difference in response between experienced and naïve *E. nana* to the chemical cues of *L. auritus* and *M. salmoides* are reflective of their perception of the risk posed by these species based on their experience, this result would indicate that *M. salmoides* poses a more significant predatory threat than the nonnative predator *L. auritus*. This result would also suggest that costs associated with avoidance of less risky species are greater than the benefits of avoiding all detected predatory threats in this system. Studies on the foraging behavior of these two species indicate that *M. salmoides* tend to feed on column-dwelling vertebrate prey (e.g. fish) as well as benthic invertebrates (Peterson et al. 2006) while *L. auritus* tend to forage primarily on benthic invertebrate prey (Wallace 1984); however, diet studies with *L. auritus* and *M. salmoides* have not been conducted where vertebrate prey such as *E. nana* are available in the benthos. Thus, to understand the relative risk posed to *E. nana* by these species, diet studies comparing feeding habits of *L. auritus* and *M. salmoides* in this habitat are needed. Through accurate assessment of predation risk, *E. nana* should be able to more efficiently allocate time to other beneficial behaviors such as foraging or mating (Sih

1992; Anholt et al. 2000).

Prior studies examining innate and learned responses to the chemical cues of predators suggest that amphibians and fish differ in their responses. In general, studies indicate that fishes are more likely to learn which chemical stimuli are dangerous (Brown 2003; Kelley and Magurran 2003) while amphibians more often exhibit innate responses (Kats and Dill 1998). Our data go further, as they indicate that *E. nana* exhibit innate recognition of predators and, with experience, are also able to learn about the predatory threat posed by predators. While studies demonstrating co-reliance on both innate and learned responses are limited in amphibians, Gonzalo et al. (2007) demonstrated that *R. perezii* also alter innate antipredator responses in a threat-sensitive manner after experiencing predator kairomones in association with damage-released conspecific alarm pheromones. Like *E. nana*, some amphibian species for which associative learning has been demonstrated (e.g. *Notophthalmus viridescens*, Woody and Mathis 1998; *R. perezii*, Gonzalo et al. 2007) have relatively lengthy aquatic stages as compared to other metamorphic amphibians. This life history may make experience-mediated plasticity in antipredator behavior more useful for these species (Gonzalo et al. 2007). Obligate paedomorphic salamanders pose interesting prey subjects as they must avoid fish predation throughout the entirety of their life cycle and thus, both acquired and innate responses can be beneficial for these species.

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Literature Cited

- Adams MJ, Schindler DE, Bury RB. 2001. Association of amphibians with attenuation of ultraviolet-b radiation in montane ponds. *Oecologia* 128:519-525.
- Anholt BR, Werner E, Skelly DK. 2000. Effect of food and predators on the activity of four larval ranid frogs. *Ecology* 81:3509-3521.
- Anthony CD, Hickerson CAM, Venesky MD. 2007. Responses of juvenile terrestrial salamanders to introduced (*Lithobius forficatus*) and native centipedes (*Scolopocryptops sexspinosus*). *Journal of Zoology* 271:54-62.
- Baber MJ, Babbitt KJ. 2003. The relative impacts of native and introduced predatory fish on a temporary wetland tadpole assemblage. *Oecologia* 136:289-295.
- Barnett V, Lewis T. 1984. *Outliers in Statistical Data*. Chichester [West Sussex], New York: John Wiley & Sons.
- Bishop SC. 1941. Notes on the salamanders with descriptions of several new forms. *Occasional Papers from the Museum of Zoology, University of Michigan* 451:1-21.
- Bogart JP. 1967. *Life History and Chromosomes of the Neotenic Salamanders of the Edwards Plateau* [M.S. Thesis]. [Austin, Texas, U.S.A.]: University of Texas.
- Bowles D, Bowles B. 2001. A review of the exotic species inhabiting the upper San Marcos River, Texas, U.S.A.: Texas Parks and Wildlife Department, Austin, TX.
- Brown GE. 2003. Learning about danger: chemical alarm cues and local risk assessment in prey fishes. *Fish and Fisheries* 4:227-234.

- Brown GE, Chivers DP, Smith RJF. 1997. Differential learning rates of chemical versus visual cues of a northern pike by fathead minnows in a natural habitat. *Environmental Biology of Fishes* 49:89-96.
- Chippindale PT, Price AH, Hillis DM. 1998. Systematic status of the San Marcos salamander, *Eurycea nana* (Caudata: Plethodontidae). *Copeia* 1998:1046-1049.
- Chivers DP, Kiesecker JM, Marco A, Wildy EL, Blaustein AR. 1999. Shifts in life history as a response to predation in western toads (*Bufo boreas*). *Journal of Chemical Ecology* 25:2455-2464.
- Chivers DP, Smith RJF. 1998. Chemical alarm signaling in aquatic predator-prey systems: a review and prospectus. *Écoscience* 5:338-352.
- Day HP. 1981. Food habit study of the largemouth bass, Guadalupe bass, and white bass X striped bass hybrid in a Central Texas reservoir receiving heated effluent [M.S. Thesis]. [San Marcos, TX]: Southwest Texas State University. 103 pp.
- Ferrari MCO, Gonzalo A, Messier F, Chivers DP. 2007a. Generalization of learned predator recognition: an experimental test and framework for future studies. *Proceedings of the Royal Society B-Biological Sciences* 274:1853-1859.
- Ferrari MCO, Myles RB, Pollock MS, Chivers DP. 2007b. The paradox of risk assessment: comparing responses of fathead minnows to capture-released and diet-released alarm cues from two different predators. *Chemoecology* 17:157-161.
- Ferrari MCO, Trowell JJ, Brown GE, Chivers DP. 2005. The role of learning in the development of threat-sensitive predator avoidance by flathead minnows. *Animal Behaviour* 70:777-784.
- Ferrer RP, Zimmer RK. 2007. The scent of danger: arginine as an olfactory cue of reduced predation risk. *Journal of Experimental Biology* 210:1768-1775.
- Gallie JA, Mumme RL, Wissinger SA. 2001. Experience has no effect on the development of chemosensory recognition of predators by tadpoles of the American toad, *Bufo americanus*. *Herpetologica* 57:376-383.

- Gonzalo A, Lopez P, Martin J. 2007. Iberian green frog tadpoles may learn to recognize novel predators from chemical alarm cues of conspecifics. *Animal Behaviour* 74:447-453.
- Griffiths RA, Schley L, Sharp PE, Dennis JL, Roman A. 1998. Behavioral responses of Mallorcan midwife toad tadpoles to natural and unnatural snake predators. *Animal Behaviour* 55:207-214.
- Groeger AW, Brown PF, Tietjen TE, Kelsey TC. 1997. Water quality of the San Marcos River. *Texas Journal of Science* 49:279-294.
- Hamer AJ, Lane SJ, Mahony MJ. 2002. The role of introduced mosquitofish (*Gambusia holbrooki*) in excluding the native green and golden bell frog (*Litoria aurea*) from original habitats in south-eastern Australia. *Oecologia* 132:445-452.
- Hickman CR, Stone MD, Mathis A. 2004. Priority use of chemical over visual cues for detection of predators by graybelly salamanders, *Eurycea multiplicata griseogaster*. *Herpetologica* 60:203-210.
- Hubbs C, Peden AE. 1969. *Gambusia georgei* sp. nov. from San Marcos, Texas. *Copeia* 1969:357-364.
- Iglewicz B, Hoaglin DC. 1993. How to Detect and Handle Outliers. Milwaukee, Wisconsin: ASQC Quality Press.
- Jaeger RG. 1981. Dear enemy recognition and the costs of aggression between salamanders. *American Naturalist* 117:962-974.
- Kats LB, Dill LM. 1998. The scent of death: chemosensory assessment of predation risk by prey animals. *Écoscience* 5:361-394.
- Kats LB, Ferrer RP. 2003. Alien predators and amphibian declines: review of two decades of science and the transition to conservation. *Diversity and Distributions* 9:99-110.
- Kelley JL, Magurran AE. 2003. Learned predator recognition and antipredator responses in fishes. *Fish and Fisheries* 4:216-226.

- Kelsey T. 1997. Fish community structure and water quality assessment (Index of Biotic Integrity) of the San Marcos River, Texas [M.S. Thesis]. [San Marcos, TX]: Southwest Texas State University. 45 pp.
- Kiesecker JM, Blaustein AR. 1997. Population differences in responses of red-legged frogs (*Rana aurora*) to introduced bullfrogs. *Ecology* 78:1752-1760.
- Knapp RA, Matthews KR. 2000. Non-native fish introductions and the decline of the mountain yellow-legged frog from within protected areas. *Conservation Biology* 14:428-438.
- Lane SJ, Mahony MJ. 2002. Larval anurans with synchronous and asynchronous development periods: contrasting responses to water reduction and predator presence. *Journal of Animal Ecology* 71:780-792.
- Laurila A. 2000. Behavioral responses to predator chemical cues and local variation in antipredator performance in *Rana temporaria* tadpoles. *Oikos* 88:159-168.
- Laurila A, Kujasalo J, Ranta E. 1997. Different antipredator behaviour in two anuran tadpoles: Effects of predator diet. *Behavioral Ecology and Sociobiology* 40:329-336.
- Lefcort H. 1996. Adaptive, chemically mediated fright response in tadpoles of the southern leopard frog, *Rana utricularia*. *Copeia* 1996:455-459.
- Lima SL. 1998. Nonlethal effects in the ecology of predator-prey interactions: what are the ecological effects of anti-predator decision-making? *Bioscience* 48:25-34.
- Lima SL, Bednekoff PA. 1999. Temporal variation in danger drives antipredator behavior: The predation risk allocation hypothesis. *American Naturalist* 153:649-659.
- Mandrillon AL, Saglio P. 2005. Prior exposure to conspecific chemical cues affects predator recognition in larval common toad (*Bufo bufo*). *Archiv Fur Hydrobiologie* 164:1-12.
- Mathis A. 2003. Use of chemical cues in detection of conspecific predators and prey by newts, *Notophthalmus viridescens*. *Chemoecology* 13:193-197.

- Mathis A, Murray KL, Hickman CR. 2003. Do experience and body size play a role in responses of larval ringed salamanders, *Ambystoma annulatum*, to predator kairomones? Laboratory and field assays. *Ethology* 109:159-170.
- Mathis A, Vincent F. 2000. Differential use of visual and chemical cues in predator recognition and threat-sensitive predator-avoidance responses by larval newts (*Notophthalmus viridescens*). *Canadian Journal of Zoology* 78:1646-1652.
- Miklosi A, Csanyi V, Gerlai R. 1997. Antipredator behavior in paradise fish (*Macropodus opercularis*) larvae: the role of genetic factors and paternal influence. *Behavioral Genetics* 27:191-2000.
- Mirza RS, Chivers DP. 2000. Predator-recognition training enhances survival of brook trout: evidence from laboratory and field-enclosure studies. *Canadian Journal of Zoology* 78:2198-2208.
- Mirza RS, Fisher SA, Chivers DP. 2003. Assessment of predation risk by juvenile yellow perch, *Perca flavescens*: responses to alarm cues from conspecifics and prey guild members. *Environmental Biology of Fishes* 66:321-327.
- Murray DL, Roth JD, Wirsing AJ. 2004. Predation risk avoidance by terrestrial amphibians: The role of prey experience and vulnerability to native and exotic predators. *Ethology* 110:635-647.
- Nelson JM. 1993. Population size, distribution, and life history of *Eurycea nana* in the San Marcos River [M.S. Thesis]. [San Marcos, TX]: Southwest Texas State University. 30 pp.
- Pearl CA, Adams MJ, Schuytema GS, Nebeker AV. 2003. Behavioral responses of anuran larvae to chemical cues of native and introduced predators in the Pacific Northwestern United States. *Journal of Herpetology* 37:572-576.
- Peterson MS, Slack WT, Waggy GL, Finley J, Woodley CM, Partyka ML. 2006. Foraging in non-native environments: Comparison of Nile tilapia and three co-occurring native centrarchids in invaded coastal Mississippi watersheds. *Environmental Biology of Fishes* 76:283-301.
- Petranka JW. 1998. Salamanders of the United States and Canada. Washington, D.C.: Smithsonian Institution Press.

- Pilliod DS, Peterson CR. 2001. Local and landscape effects of introduced trout on amphibians in historically fishless watersheds. *Ecosystems* 4:322-333.
- Sih A. 1992. Prey uncertainty and the balancing of antipredator and feeding needs. *American Naturalist* 139:1052-1069.
- Sih A, Kats LB. 1994. Age, experience, and the response of streamside salamander hatchlings to chemical cues from predatory sunfish. *Ethology* 96:253-259.
- Sih A, Kats LB, Moore RD. 1992. Effects of predatory sunfish on the density, drift, and refuge use of stream salamander larvae. *Ecology* 73:1418-1430.
- Suboski MD. 1992. Releaser-induced recognition learning by amphibians and reptiles. *Animal Learning & Behavior* 20:63-82.
- Thaker M, Gabor CR, Fries JN. 2006. Sensory cues for conspecific associations in aquatic San Marcos salamanders. *Herpetologica* 62:151-155.
- Tupa D, Davis W. 1976. Population dynamics of the San Marcos salamander, *Eurycea nana* Bishop. *Texas Journal of Science* 27:179-195.
- [USDI] United States Department of the Interior. 1980. Endangered and threatened wildlife and plants: listing of San Marcos salamander as threatened, the San Marcos gambusia as endangered, and the listing of critical habitat for Texas wild rice, San Marcos gambusia, and fountain darter. *Federal Register*. Pp. 47355-47364.
- Utne-Palm AC. 2001. Response of naïve two-spotted gobies *Gobiusculus flavescens* to visual and chemical stimuli of their natural predator, cod *Gadus marhua*. *Marine Ecology - Progress Series* 218:267-274.
- Wallace SA. 1984. The food habits of redbreast sunfish in the San Marcos River, Texas [M. S. Thesis]. [San Marcos, TX]: Southwest Texas State University. 59 pp.
- Werner EE, Peacor SD. 2003. A review of trait-mediated indirect interactions in ecological communities. *Ecology* 84:1083-1100.

- Wildy NJ, Blaustein AR. 2001. Learned recognition of intraspecific predators in larval long-toed salamanders *Ambystoma macrodactylum*. *Ethology* 107:479-493.
- Wilson DJ, Lefcort H. 1993. The effect of predator diet on the alarm response of red-legged frog, *Rana aurora*, tadpoles. *Animal Behaviour* 46:1017-1019.
- Wisenden BD. 2003. Chemically mediated strategies to counter predation. In: Collin SP, Marshall NJ, editors. *Sensory Processing in Aquatic Environments*. New York: Springer-Verlag. p. 236-251.
- Woody DR, Mathis A. 1998. Acquired recognition of chemical stimuli from an unfamiliar predator: associative learning by adult newts, *Notophthalmus viridescens*. *Copeia* 1988:1027-1031.

CHAPTER III

THREAT SENSITIVITY IN THE SAN MARCOS SALAMANDER: PREDATOR DIET AND PREY EXPERIENCE¹

Abstract

Prey must constantly balance foraging and predator avoidance demands. Avoidance response efficiency may be improved when prey match the intensity of their avoidance behaviors 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 behaviors 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 *E. nana* 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.

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Introduction

The most beneficial predator avoidance tactics consist of complex interactions between effectiveness and efficiency. Effective avoidance behaviors such as decreased activity (Anholt et al. 2000; Gonzalo et al. 2007), increased use of refuge (Kiesecker and Blaustein 1997; Orizaola and Brana 2003), or spatial or temporal habitat shifts (Petranka 1983; Sih and Moore 1993) reduce the risk of predation. However, there are time allocation trade-offs between predator avoidance tactics and other beneficial behaviors such as foraging or mating (Sih 1992; Werner and Anholt 1996). Because individual predators can vary in the risk they pose, prey may match the intensity of antipredator behaviors 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). This 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 and 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 and Dill 1998; Mathis et al. 2003) and may further convey threat-indicative information. For example, fathead minnows, *Pimephales promelas*, respond more intensely to pike (*Esox lucius*) kairomones when the density of predators that produce the cue is increased (Ferrari et al. 2006). Detecting kairomones can allow prey to discern predators from non-predators (Mathis 2003; Mathis et al. 2003; Epp and Gabor 2008) and to differentiate between predatory species (Turner et al. 1999; Relyea 2001). While kairomones may be important for predator detection and identification in some species (e.g. invertebrates, Hazlett and Schoolmaster 1998; McCarthy and Fisher 2000; fish, Pettersson et al. 2000), not all prey exhibit avoidance behaviors in response to the kairomones of starved

predators (freshwater snails, Crowl and Covich 1990; amphibians, McCollum and Leimberger 1997; Schoeppner and Relyea 2005; 2009a). Thus, the ability to identify predatory threats based solely on predator odor does not appear to be ubiquitous across systems.

In some systems, secondary threat-indicators may provide more complete information to prey 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 and 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 and Smith 1993; Chivers and Mirza 2001) or heterospecific (Mirza and Chivers 2004; Schoeppner and Relyea 2009b) prey. Interestingly, recent work showed that frog tadpoles (*Rana pipiens* and *Hyla versicolor*) exhibited more intense avoidance behaviors in response to chemical cues of predators that had consumed conspecifics as compared to responses to predator kairomones and damage-released alarm cues presented simultaneously or independently (Schoeppner and Relyea 2005; 2009b). This indicates that the digestive process results in chemical stimuli that prey perceive as relatively high risk, although the mechanism for this is not well understood. 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 reduced costs associated with antipredator behaviors.

The experiences that prey 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 and Magurran 2003b), amphibians (Gonzalo et al. 2007; Ferrari and Chivers 2010), and invertebrates (Kesavaraju et al. 2007). Threat-sensitive learning is expected to be favored in habitats with diverse or fluctuating predatory communities (Brown and 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 and 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 result in reinforcement of avoidance responses of prey. For example, Kelley and Magurran (2003a; 2003b) showed that guppies (*Poecilia reticulata*) collected from high-risk populations responded more intensely to predators than did those from low-risk populations, but offspring reared in the lab did not differ in their responses. This finding indicated that the experiences of guppies with predators in the natural habitat had reinforced avoidance behaviors in this species. Brown et al. (2009) found a similar pattern in guppies from a different set of high- and low-risk populations, but the mechanism for the difference (heritable or learned) was not clear. Alternatively, some prey that experience prolonged exposures to predators habituate to predator stimuli which results in reduced response intensity to predation risk over time. Jackson and Semlitsch (1993) found that tadpoles reared in artificial ponds for three months with caged predators steadily increased activity levels throughout the study. Given that predators were unable to attack prey in this scenario, the lack of reinforcement may have resulted in prey perceiving those predator stimuli

as lower risk. Similarly, Magurran and Pitcher (1987) showed that minnows (*Phoxinus phoxinus*) increased inspection behaviors towards predators after repeated exposures to predator stimulus. Whether through reinforcement or habituation, these studies indicate that prolonged exposure to predators, such as would occur in natural habitats, can affect avoidance behaviors of prey. Given the effect of prolonged exposure, it is possible that ambient levels of predation risk could also influence a prey's assessment of predation risk, and therefore, threat-sensitive avoidance responses exhibited by prey.

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. The San Marcos salamander, *Eurycea nana*, is a federally threatened (1980) paedomorphic salamander (obligatorily aquatic) endemic to the headwaters of the San Marcos River, Hays County, Texas (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 nonnative fish predators (Kelsey 1997; Bowles and Bowles 2001). Epp and Gabor (2008) showed that adult, predator-naïve *E. nana* reduced activity in response to the chemical stimuli of both a native predator (largemouth bass, *Micropterus salmoides*) and a nonnative predator (redbreast sunfish, *Lepomis auritus*) and not to a blank control or a native non-predator (largespring gambusia, *Gambusia geiseri*) indicating innate antipredator behavior in this species. Interestingly, predator-experienced *E. nana* 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 and Gabor 2008). I predicted that, like other amphibian prey (Laurila et al. 1997; Mathis 2003; Schoeppner and Relyea 2005), predator-naïve adult *E. nana* (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. Based on previous

work with *E. nana* (Epp and Gabor 2008), I predicted that *E. nana* 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 more less intensely to predators than naïve salamanders. I further predicted that responses of experienced *E. nana* to *M. salmoides* might be more intense than responses to *L. auritus*.

Materials and Methods

Test Subjects

Predator-naïve *E. nana* were adult (SVL>20 mm; Tupa and Davis 1976), first-generation offspring of salamanders collected from the natural habitat. Predator-experienced *E. nana* were individuals collected from the natural habitat as adults and tested within six months of collection. 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 aquifer-fed, recirculating, temperature-controlled (21-23°C) tanks with water sourced from the same aquifer system as that feeding the natural habitat of *E. nana*.

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 and 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. comm) and consume them readily in captivity (Epp and 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 fiberglass 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* 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 and Gabor 2008), I placed stimulus fish individually into aerated and unfiltered stimulus acquisition tanks with 230 ml of dechlorinated tap water/1 cm³ 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 *E. nana*) 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 and Gabor 2008).

Testing

I tested the 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 – *E. nana* diet (high risk), (3) largemouth bass – neutral diet (low risk), (4) largemouth bass – *E. nana* diet (high risk), or (5) blank control (no risk); n = 15/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 9.5-l testing aquaria with 5.5 l of fresh well water and allowed them to acclimate for at least 15 min until consistent activity was observed for at least 120 s. Testing aquaria 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 acclimation, 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 administered in a block design within experience groups. I scrubbed all testing aquaria and testing supplies with 3% hydrogen peroxide and rinsed them with well water to remove chemical stimuli and maintain independence between trials.

Statistical Analyses

I calculated a response index (relative activity) by dividing post-stimulus time by pre-stimulus time (Epp and 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 *E. nana* in each experience group exhibited detectible avoidance behaviors 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 (diet of *E. nana*) 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 blank 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.

Results

For predator-naïve *E. nana*, 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 *E. nana*, only relative activity of salamanders exposed to chemical stimuli of high-risk sunfish (diet of *E. nana*; treatment 2) was significantly lower than responses of salamanders exposed to the blank control treatment (Fig. 2). Three-factor ANOVA of predator species, predator risk (diet), and prey experience indicated a significant 2nd 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 *E. nana* within predator species and predator risk treatments using Tukey's multiple comparisons procedures. I found that predator-naïve *E. nana* exposed to the chemical stimuli of high-risk (diet of *E. nana*) predator treatments (2 and 4) had significantly lower relative activity than naïve salamanders exposed to low-risk (neutral diet) predator treatments (1 and 3). These responses of naïve *E. nana* to high-risk predators were also significantly lower than responses of predator-experienced *E. nana* to both low- and high-risk predator treatments

Table 1. Three-Factor ANOVA of Risk, Experience, and Predator Species. Results of 3-factor ANOVA assessing the impacts of predator species (native bass or nonnative sunfish), predator risk (neutral diet or conspecific diet), and prey experience (captive or collected) on avoidance responses of *E. nana*.

Factor	<i>F</i>	<i>df</i>	<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

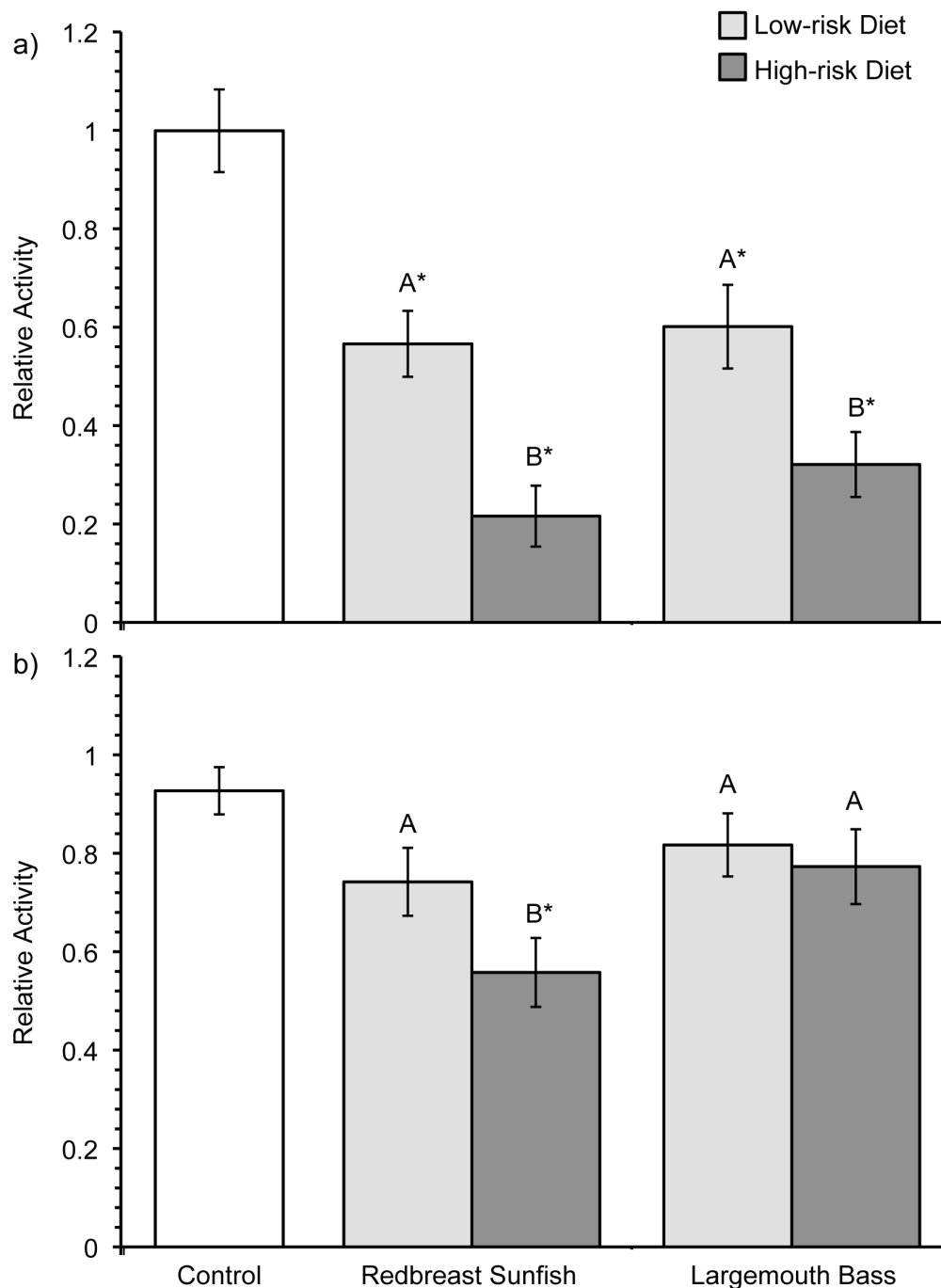


Figure 2. Responses of Experienced and Naïve *E. nana* to Low- and High-Risk Predators. Mean (\pm SE) responses (relative activity) of (a) predator-naïve and (b) predator-experienced *Eurycea nana* to the chemical stimuli of low-risk (neutral diet) or high-risk (conspecific salamander diet) predators. 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; $\alpha = 0.05$).

(1-4). Additionally, the responses of predator-experienced *E. nana* to both low- and high-risk predators did not differ from responses of predator-naïve *E. nana* to the chemical stimuli of low-risk (neutral diet) predators (Fig. 2).

Discussion

I found that predator-naïve *E. nana* reduced activity significantly more in response to predators that had recently consumed conspecifics as compared to those that had not which is probably reflective of predator risk; however, for predator-experienced *E. nana*, 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 *E. nana* 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 behaviors 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 *E. nana* 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 and Relyea 2005) and conspecific cues in the diet of predators is likely a reliable indicator of the risk posed by individuals. My data suggests that *E. nana* is able to assess variation in predatory threat and exhibits threat-sensitive responses appropriate for the risk posed.

As predicted, results of this study indicate that prolonged exposure to ambient predatory threat in the natural habitat affected the intensity of avoidance behaviors

exhibited by prey. Experienced *E. nana* from the natural habitat responded less intensely to all predators than did naïve salamanders that had been raised in captivity. There are two plausible hypotheses that may explain the less intense responses of experienced *E. nana* as compared to naïve salamanders in this study: (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 and Hatchett 1994; crabs, Hemmi and Merkle 2009), amphibians (salamanders, Jackson and Semlitsch 1993; Madison et al. 2005), and fish (minnows, Kelley and 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 odors could occur. This possibility is especially plausible given that prey may be more likely to habituate when adequate refuges are available for prey (Holomuzki and 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 *E. nana* 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*.

An alternative, but related hypothesis that may explain the less intense responses

exhibited by experienced *E. nana* 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 and Dill 1998; Brown and 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. The hypothesis of learned irrelevance suggests that if prey are presented with predator and risk stimuli independently prior to paired presentations, the ability of prey to acquire recognition of and response to the predator stimulus when it is presented simultaneously with risk-indicative cues is inhibited (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 as that would result in excessive antipredator behaviors.

Studies examining the phenomenon of learned irrelevance have typically addressed outcomes for associative learning of novel stimuli (e.g. learned recognition), but prey exhibiting innate recognition have preformed associations of predators with threat. In crayfish (*Orconectes virilis*), Hazlett and 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 a paired exposure of alarm cue and goldfish odor (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 individuals of *E. nana* 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 and 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 behavioral plasticity allows organisms to better respond to environmental heterogeneity, prolonged retention of information without reinforcement could result in maladaptive behaviors in organisms (Stephens 1991). The environmental factors that can affect the retention of information or memory have been studied in other contexts, but remain virtually unexplored in how prey recognize and respond to predators (Ferrari et al. 2010). 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 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 *E. nana* is able to respond to this continual variation in predation risk.

These results also indicated that experienced *E. nana* 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 *E. nana* exhibited responses to low-risk and to high-risk predator treatments that were similar between the species. The differences in responses of predator-experienced *E. nana* to bass and sunfish are opposite

to those observed in a previous study (Epp and Gabor 2008); however, diet of predators was not controlled in that study. Experienced *E. nana* 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 behavioral responses in prey (Mirza et al. 2006). Given the threshold effect, it may be that the response threshold of naïve and experienced *E. nana* 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 *E. nana* 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 and Gabor 2008) as diet of predators was not controlled in that study. 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 any potential energy demand 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 levels between experienced and naïve salamanders showed that activity prior to stimulus introduction did not differ between captive-reared (mean \pm SE; 248.79 ± 6.86) and wild-caught (mean \pm SE; 260.73 ± 7.59) salamanders (unpaired t-test for unequal variances: $t = 1.167$, $DF = 145.48$, $p = 0.245$). 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 acclimated 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 behaviors 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 behaviors, 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 learning and threat sensitivity should be favored.

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Literature Cited

- Anholt BR, Werner E, Skelly DK. 2000. Effect of food and predators on the activity of four larval ranid frogs. *Ecology* 81:3509-3521.
- Bennet CH, Wills SJ, Oaakeshott SM, Mackintosh NJ. 2000. Is the context specificity of latent inhibition a sufficient explanation of learned irrelevance? *Quarterly Journal of Experimental Psychology. B, Comparative and Physiological Psychology* 53B:239-253.
- Bishop SC. 1941. Notes on the salamanders with descriptions of several new forms. *Occasional Papers from the Museum of Zoology, University of Michigan* 451:1-21.
- Bowles D, Bowles B. 2001. A review of the exotic species inhabiting the upper San Marcos River, Texas, U.S.A.: Texas Parks and Wildlife Department, Austin, TX.
- Brown GE. 2003. Learning about danger: chemical alarm cues and local risk assessment in prey fishes. *Fish and Fisheries* 4:227-234.
- Brown GE, Chivers DP. 2005. Learning as an adaptive response to predation. In: Barbosa P, Castellanos P, editors. *Ecology of Predator-Prey Interactions*. New York: Oxford University. p. 34-53.
- Brown GE, Macnaughton CJ, Elvidge CK, Ramnarine I, Godin JJ. 2009. Provenance and threat-sensitive predator avoidance patterns in wild-caught Trinidadian guppies. *Behavioral Ecology and Sociobiology* 63:699-706.
- Bryer PJ, Mirza RS, Chivers DP. 2001. Chemosensory assessment of predation risk by slimy sculpins (*Cottus cognatus*): Responses to alarm, disturbance, and predator cues. *Journal of Chemical Ecology* 27:533-546.
- Chippindale PT, Price AH, Hillis DM. 1998. Systematic status of the San Marcos salamander, *Eurycea nana* (Caudata: Plethodontidae). *Copeia* 1998:1046-1049.
- Chivers DP, Mirza RS. 2001. Importance of predator diet cues in responses of larval wood frogs to fish and invertebrate predators. *Journal of Chemical Ecology* 27:45-51.

- Crowl TA, Covich AP. 1990. Predator-induced life-history shifts in a freshwater snail. *Science* 247:949-951.
- Day HP. 1981. Food habit study of the largemouth bass, Guadalupe bass, and white bass X striped bass hybrid in a Central Texas reservoir receiving heated effluent [M.S. Thesis]. [San Marcos, TX]: Southwest Texas State University. 103 pp.
- Epp KJ, Gabor CR. 2008. Innate and learned predator recognition mediated by chemical signals in *Eurycea nana*. *Ethology* 114:607-615.
- Ferrari MCO, Brown GE, Bortolotti GR, Chivers DP. 2010. Linking predator risk and uncertainty to adaptive forgetting: a theoretical framework and empirical test using tadpoles. *Proceedings of the Royal Society B-Biological Sciences*.
- Ferrari MCO, Chivers DP. 2010. The ghost of predation future: threat-sensitive and temporal assessment of risk by embryonic woodfrogs. *Behavioral Ecology and Sociobiology* 64:549-555.
- Ferrari MCO, Messier F, Chivers DP. 2006. The nose knows: minnows determine predator proximity and density through detection of predator odours. *Animal Behaviour* 72:927-932.
- Ferrari MCO, Sih A, Chivers DP. 2009. The paradox of risk allocation: a review and prospectus. *Animal Behaviour* 78:579-585.
- Gillette JR, Jaeger RG, Peterson MG. 2000. Social monogamy in a territorial salamander. *Animal Behaviour* 59:1241-1250.
- Gonzalo A, Lopez P, Martin J. 2007. Iberian green frog tadpoles may learn to recognize novel predators from chemical alarm cues of conspecifics. *Animal Behaviour* 74:447-453.
- Hazlett BA. 2003. Predator recognition and learned irrelevance in the crayfish *Orconectes virilis*. *Ethology* 109:765-780.
- Hazlett BA, Schoolmaster DR. 1998. Responses of cambarid crayfish to predator odor. *Journal of Chemical Ecology* 24:1757-1770.

- Helfman GS. 1989. Threat-sensitive predator avoidance in damselfish-trumpetfish interactions. *Behavioral Ecology and Sociobiology* 24:47-58.
- Hemmi JM, Merkle T. 2009. High stimulus specificity characterizes anti-predator habituation under natural conditions. *Proceedings of the Royal Society B-Biological Sciences* 276:4381-4388.
- Holomuzki JR, Hatchett L. 1994. Predator avoidance costs and habituation to fish chemicals by a stream isopod. *Freshwater Biology* 32:585-592.
- Jackson ME, Semlitsch RD. 1993. Paedomorphosis in the salamander *Ambystoma talpoideum*: effects of a fish predator. *Ecology* 74:342-350.
- Kats LB, Dill LM. 1998. The scent of death: chemosensory assessment of predation risk by prey animals. *Écoscience* 5:361-394.
- Kelley JL, Magurran AE. 2003a. Effects of relaxed predation pressure on visual predator recognition in the guppy. *Behavioral Ecology and Sociobiology* 54:225-232.
- Kelley JL, Magurran AE. 2003b. Learned predator recognition and antipredator responses in fishes. *Fish and Fisheries* 4:216-226.
- Kelsey T. 1997. Fish community structure and water quality assessment (Index of Biotic Integrity) of the San Marcos River, Texas [M.S. Thesis]. [San Marcos, TX]: Southwest Texas State University. 45 pp.
- Kesavaraju B, Damal K, Juliano SA. 2007. Threat-sensitive behavioral responses to concentrations of water-borne cues from predation. *Ethology* 113:199-206.
- Kiesecker JM, Blaustein AR. 1997. Population differences in responses of red-legged frogs (*Rana aurora*) to introduced bullfrogs. *Ecology* 78:1752-1760.
- Kraemer PJ, Golding JM. 1997. Adaptive forgetting in animals. *Psychonomic Bulletin and Review* 4:480-491.
- Laurila A, Kujasalo J, Ranta E. 1997. Different antipredator behaviour in two anuran tadpoles: Effects of predator diet. *Behavioral Ecology and Sociobiology* 40:329-336.

- Mackintosh NJ. 1973. Stimulus selection: learning to ignore stimuli that predict no change in reinforcement. In: Hinde RA, Hinde LS, editors. *Constraints of Learning*. London: Academic Press. p. 75-96.
- Madison DM, Maerz JC, Sullivan AM. 2005. Decline in avoidance of predator chemical cues: habituation or biorhythm shift? In: Mason RT, LeMaster MP, Muller-Schwarze D, editors. *Chemical Signals in Vertebrates*. Springer. p. 365-372.
- Magurran AE, Pitcher TJ. 1987. Provenance, shoal size, and the sociobiology of predator evasion in minnow shoals. *Proceedings of the Royal Society B-Biological Sciences* 229:439-365.
- Masagounder K, Firman JD, Hayward RS, Sun S, Brown PB. 2009. Apparent digestibilities of common feedstuffs for bluegill *Lepomis macrochirus* and largemouth bass *Micropterus salmoides* using individual test ingredients. *Aquaculture Nutrition* 15:29-37.
- Mathis A. 2003. Use of chemical cues in detection of conspecific predators and prey by newts, *Notophthalmus viridescens*. *Chemoecology* 13:193-197.
- Mathis A, Murray KL, Hickman CR. 2003. Do experience and body size play a role in responses of larval ringed salamanders, *Ambystoma annulatum*, to predator kairomones? Laboratory and field assays. *Ethology* 109:159-170.
- Mathis A, Smith RJF. 1993. Fathead minnows, *Pimephales promelas*, learn to recognize northern pike, *Esox lucius*, as predators on the basis of chemical stimuli from minnows in the pike's diet. *Animal Behaviour* 46:645-656.
- McCarthy TM, Fisher WA. 2000. Multiple predator-avoidance behaviours of the freshwater snail *Physella heterostropha pomila*: responses vary with risk. *Freshwater Biology* 44:387-397.
- McCollum SA, Leimberger JD. 1997. Predator-induced morphological changes in an amphibian: predation by dragonflies affects tadpole shape and color. *Oecologia* 109:615-621.
- Mirza RS, Chivers DP. 2004. Fathead minnows learn to recognize heterospecific alarm cues they detect in the diet of a known predator. *Behaviour* 140:1359-1369.

- Mirza RS, Ferrari MCO, Kiesecker JM, Chivers DP. 2006. Responses of American toad tadpoles to predation cues: behavioral response thresholds, threat-sensitivity and acquired predation recognition. *Behaviour* 143:877-889.
- Murray DL, Roth JD, Wirsing AJ. 2004. Predation risk avoidance by terrestrial amphibians: The role of prey experience and vulnerability to native and exotic predators. *Ethology* 110:635-647.
- Nelson JM. 1993. Population size, distribution, and life history of *Eurycea nana* in the San Marcos River [M.S. Thesis]. [San Marcos, TX]: Southwest Texas State University. 30 pp.
- Oosten JE, Magnhagen C, Hemelrijk CK. 2010. Boldness by habituation and social interactions: a model. *Behavioral Ecology and Sociobiology* 64:793-802.
- Orizaola G, Brana F. 2003. Response of predator-naive newt larvae to food and predator presence. *Canadian Journal of Zoology* 81:1845-1850.
- Petranka JW. 1983. Fish predation: a factor affecting the spatial distribution of a stream-breeding salamander. *Copeia* 1983:624-628.
- Pettersson LB, Nilsson PA, Brönmark C. 2000. Predator recognition and defence strategies in crucian carp, *Carassius carassius*. *Oikos* 88:200-212.
- Relyea RA. 2001. Morphological and behavioral plasticity of larval anurans in response to different predators. *Ecology* 82:523-540.
- Schoeppner NM, Relyea RA. 2005. Damage, digestion, and defence: the roles of alarm cues and kairomones for inducing prey defences. *Ecology Letters* 8:505-512.
- Schoeppner NM, Relyea RA. 2009a. Interpreting the smells of predation: how alarm cues and kairomones induce different prey defences. *Functional Ecology* 23:1114-1121.
- Schoeppner NM, Relyea RA. 2009b. When should prey respond to consumed heterospecifics? Testing hypotheses of perceived risk. *Copeia* 2009:190-194.

- Sih A. 1992. Prey uncertainty and the balancing of antipredator and feeding needs. *American Naturalist* 139:1052-1069.
- Sih A, Moore RD. 1993. Delayed hatching of salamander eggs in response to enhanced larval predation risk. *American Naturalist* 142:947-960.
- Stephens DW. 1991. Change, regularity, and value in the evolution of animal learning. *Behavioral Ecology* 2:77-89.
- Tupa D, Davis W. 1976. Population dynamics of the San Marcos salamander, *Eurycea nana* Bishop. *Texas Journal of Science* 27:179-195.
- Turner AM, Fetterolf SA, Bernot RJ. 1999. Predator identity and consumer behavior: differential effects of fish and crayfish on the habitat use of a freshwater snail. *Oecologia* 118:242-247.
- [USDI] United States Department of the Interior. 1980. Endangered and threatened wildlife and plants: listing of San Marcos salamander as threatened, the San Marcos gambusia as endangered, and the listing of critical habitat for Texas wild rice, San Marcos gambusia, and fountain darter. *Federal Register*. Pp. 47355-47364.
- Wallace SA. 1984. The food habits of redbreast sunfish in the San Marcos River, Texas [M. S. Thesis]. [San Marcos, TX]: Southwest Texas State University. 59 pp.
- Watson RT, Mathis A, Thompson R. 2004. Influence of physical stress, distress, and predator kairomones on the foraging behavior of Ozark zigzag salamanders, *Plethodon angusticlavius*. *Behavioural Processes* 65:201-209.
- Werner EE, Anholt BR. 1996. Predator-induced behavioral indirect effects: consequences to competitive interactions anuran larvae. *Ecology* 77:157-169.
- Wilson DJ, Lefcort H. 1993. The effect of predator diet on the alarm response of red-legged frog, *Rana aurora*, tadpoles. *Animal Behaviour* 46:1017-1019.
- Wirsing AJ, Roth JD, Murray DL. 2005. Can prey use dietary cues to distinguish predators? A test involving three terrestrial amphibians. *Herpetologica* 61:104-110.

Wisenden BD. 2003. Chemically mediated strategies to counter predation. In: Collin SP, Marshall NJ, editors. *Sensory Processing in Aquatic Environments*. New York: Springer-Verlag. p. 236-251.

CHAPTER IV

THE INFLUENCE OF DAMAGED CONSPECIFICS, ENCOUNTER FREQUENCY, AND EXPERIENCE ON PREDATOR AVOIDANCE IN THE SAN MARCOS SALAMANDER, *EURYCEA NANA*¹

Abstract

Predator avoidance can be costly and prey should match the intensity of their avoidance responses to the level of threat posed by a predator. Because of this cost, predator avoidance behaviors often result from complex interactions of current perceived predation risk and past experiences of prey with predators. Aquatic prey may gain information about predation risk from chemical stimuli associated with a predator (kairomones) or conspecific prey (damage-released cues). Additionally, the frequency with which prey encounter predators can affect the intensity of avoidance behaviors. In this study, I examined whether an aquatic salamander (*Eurycea nana*) uses chemical stimuli from damaged conspecifics as an indicator of immediate predatory threat. I then assessed whether prolonged exposure to treatments varying in predator encounter frequency and predator risk affected avoidance behaviors, activity, and shelter use patterns. *Eurycea nana* significantly reduced activity in response to cues of damaged conspecifics; however, I detected no effects of encounter frequency or predator risk on activity or response intensity in this study. Conversely, shelter use patterns were impacted by predatory stimuli as salamanders moved to shelter within 15 min of stimulus exposure and *E. nana* in high risk treatments were more likely to be out of shelters

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during safe periods than *E. nana* in low risk treatments. Additionally, salamanders exhibited decreased activity and increased response intensity after prolonged exposure to predatory stimuli as compared to the initial exposure. These results indicate that avoidance behaviors of *E. nana* can be plastic. I propose that this behavioral plasticity can be beneficial for prey in high-risk habitats, especially those with diverse predatory communities.

Introduction

Prey species must constantly balance the demands of predator avoidance and foraging or mating (Sih 1992). Excessive antipredator responses can be costly as they reduce the time allotted for other beneficial activities. Thus, prey should respond to predators with an intensity that matches the level of threat posed (threat sensitivity hypothesis; Helfman 1989) as this minimizes avoidance costs. The risk of predators can vary between individuals and species (Lima and Dill 1990), or through space and time (Lima and Bednekoff 1999), and prey that exhibit experience-mediated plasticity in risk-sensitive avoidance should be at a selective advantage in most systems.

A plethora of studies have demonstrated the use of chemical stimuli by amphibian prey to detect predators and assess predation risk. Prey may use kairomones (predator-specific chemical cues) to detect and identify predators (Kats and Dill 1998; Mathis et al. 2003) and secondary cues such as damage-released conspecific alarm cues (Chivers and Smith 1998) to assess predation risk. When paired with predator kairomones, the presence of chemical alarm cues often elicits more intense antipredator behaviors in prey than when kairomones are presented alone (reviewed in Chivers and Smith 1998; but see Schoeppner and Relyea 2009). In response to alarm cues alone, some prey exhibit avoidance responses (reviewed in Wisenden 2003) while others exhibit relatively weak or undetectable responses (Wilson and Lefcort 1993; Summey and Mathis 1998; Schoeppner and Relyea 2005; 2009). Given the discrepancy in responses of amphibians

to conspecific alarm cues, their use for risk assessment does not appear ubiquitous.

Experiences with predators can also influence a prey individual's perception of predation risk (Ferrari and Chivers 2009; Fraker 2009). Experience-mediated (learned) threat sensitivity has been shown in many amphibian prey (Mirza et al. 2006a; Ferrari et al. 2009a) and usually occurs through associative learning. For example, prey that detect predator kairomones simultaneously with conspecific alarm cues may perceive that predator as higher risk and subsequently respond more intensely to those kairomones. The ambient level of predation risk experienced by prey can also influence the intensity of avoidance responses. The predation risk allocation hypothesis (RAH; Lima and Bednekoff 1999) addresses the tradeoffs between predator vigilance and foraging in environments that vary in overall predation risk. Specifically, the RAH predicts that prey that experience frequent, high risk predation may compensate for reduced foraging opportunities by foraging more during safe periods and responding less intensely to predators during periods of risk as compared to prey from safer environments. While tests of the RAH offer mixed support (Ferrari et al. 2009b), some studies that altered the frequency of predator encounters showed that prey from environments in which predator encounters were more frequent exhibited less intense responses to predators than prey from lower encounter frequency treatments (Sih and McCarthy 2002; Brown et al. 2006; Mirza et al. 2006b). Therefore, variation in predator encounter frequency over time can affect the intensity of avoidance behaviors of prey.

The ability of prey to update information about predation risk or exhibit plasticity in avoidance behaviors over time has been well studied in prey species that exhibit learned predator recognition and avoidance behaviors (Brown 2003; Griffin 2004). Experience-mediated plasticity in antipredator responses remains relatively unexplored in prey species that innately recognize and respond to predators. Innate predator recognition is defined as the ability of prey to detect and respond to predator stimuli without prior experience, and is common in amphibians (Griffiths et al. 1998; Laurila 2000; Mathis

et al. 2003; Epp and Gabor 2008; Gall and Mathis 2010). The lack of studies examining experience-mediated alterations to innate avoidance behaviors may result from an assumption that these responses are relatively fixed or canalized (Brown and Chivers 2005). However, given the benefits of experience-mediated plasticity for reducing excessive antipredator behaviors, selection should favor prey that exhibit plasticity in their avoidance behaviors.

The goal of this study was to explore mechanisms of experience-mediated predation risk assessment in *Eurycea nana*, an obligatorily aquatic salamander endemic to the headwaters of the San Marcos River (Spring Lake), Texas. Previous work with *E. nana* demonstrated that while it innately recognizes and responds to predators (*Lepomis auritus* and *Micropterus salmoides*), predator-experienced (recently collected) individuals responded less intensely to *L. auritus* than predator-naïve (captive-reared) salamanders (Epp and Gabor 2008), indicating experience-mediated plasticity of innate antipredator behaviour. Spring Lake is habitat for a diverse community of fish predators (Kelsey 1997) and threat-sensitive learning may allow *E. nana* to adjust to variations in predation risk (Brown and Chivers 2005). First, I determined whether *E. nana* uses cues of damaged conspecifics as an indicator of predation risk by comparing responses of naïve *E. nana* to a blank control or chemical stimuli of injured *E. nana*. In a second experiment, I assessed whether prolonged exposure to environments varying in predator encounter frequency and predator risk influenced shelter use patterns, activity level, and avoidance response intensity of salamanders. I predicted that if threat-sensitive learning affected avoidance behaviors, then salamanders in high-risk treatments would show greater antipredator response (decreased activity) to predatory stimuli than salamanders in low risk treatments. However, if ambient levels of predation risk affected response intensity as suggested by the RAH, I predicted that salamanders in high encounter frequency, high risk treatments may be more active during safe periods and exhibit less intense responses to predators than salamanders in low encounter frequency treatments.

Materials and Methods

Experiment 1: Cues of Damaged Conspecifics

Stimulus Acquisition

Adult *E. nana* used to obtain damage-released cues were collected from the natural habitat (Spring Lake; UTM 14R 602880.63 E, 3307083.52 N) and were sacrificed via pithing. After thorough rinsing, the stimulus was prepared by immediately placing the individual in a dish with 200 ml of deionized water and raking the skin with a blade in 3 places: (1) along the dorsal surface, (2) on each side of the body, and (3) along the ventral surface. This method was used instead of maceration (e.g. Gonzalo et al. 2007) because the digestive tract and tissues of these federally protected salamanders were needed for another study. Research with amphibian tadpoles illustrated that the chemicals that elicit alarm responses are localized in the skin (Fraker et al. 2009). After soaking for 5 minutes, individuals were removed and the water was stirred, filtered, collected in 10 ml portions, and frozen until immediately prior to testing. Control stimuli were 10 ml portions of deionized water.

Behavioral Trials

To determine whether *E. nana* exhibit avoidance behaviors in response to cues of damaged conspecifics, I exposed adult predator-naïve *E. nana* ($n = 20$) to either a control (only water) or stimuli from injured conspecifics. Subjects were placed individually into 9.5 l aquaria with 5.5 l of fresh well water and allowed to acclimate for 15 min. After acclimation, I recorded the amount of time (sec) that salamanders spent moving in an 8-min period (pre-stimulus activity). I then injected 10 ml of stimulus (conspecific alarm cue or control) through a stimulus introduction tube that extended 5 cm below the surface water on the interior. Next, I recorded the time salamanders moved in the subsequent

8-min period (post-stimulus activity). All testing supplies were rinsed with 3% hydrogen peroxide and well water to remove chemical cues between trials. Testing occurred at night when *E. nana* is most active from July-August 2009 and stimuli were randomly assigned and coded so that I was blind to the treatment.

Statistical Analysis

To determine whether *E. nana* exhibited avoidance behaviour (reduced activity) in response to damage-released conspecific chemical alarm cues, I calculated their response as an index of avoidance (post-stimulus activity - pre-stimulus activity; Woody and Mathis 1998). I determined that the data met the assumptions for parametric analyses and compared the avoidance indices between control and alarm cue treatment groups using a 2-sample, 2-tailed t-test for unequal variances.

Experiment 2: Encounter Frequency and Risk

Stimulus Acquisition

Cues of damaged conspecifics were obtained as described in experiment 1. Predator chemical cues were obtained from adult *L. auritus* (n = 4) collected from the natural habitat of *E. nana*. Stimulus individuals were housed for 5 days in flow-through fiberglass tanks filled with water from the habitat and fed a diet of earthworms (*Lumbricus terrestris*) to flush chemical stimuli of previous meals from the system. After 5 days, following established methods (Epp and Gabor 2008), stimulus fish were placed individually into aerated, unfiltered stimulus collection tanks with 230 ml of dechlorinated tap water per 1 cm³ of stimulus fish by volume. After 24 hours, fish were removed and released, and water from the tanks was stirred, collected, and frozen until immediately prior to testing.

Behavioral Trials

To examine how different experiences with the predator *L. auritus* affect avoidance responses of *E. nana*, I maintained predator-naïve adult *E. nana* for 10 days in treatments varying in predator encounter frequency (high and low) and risk (high and low). Predator encounters were simulated by introducing predator stimulus into each tank; low risk (LR) encounters were 50 ml of diet-neutral predator cues and high risk (HR) encounters were diet-neutral predator cues with 10 ml of cues from damaged conspecifics. Stimuli were injected into each aquarium twice daily: once in the morning within 2 hours of sunrise and once at night within 2 hours of sunset. In high encounter frequency (HF) treatments, all stimulus injections consisted of predatory stimuli. In low encounter frequency (LF) treatments, predator stimulus was injected once every two days at night with all other injections consisting of dechlorinated tap water to control for disturbance. Testing occurred from September-December 2009. I first tested initial activity and avoidance responses to diet neutral predator cues at night on day 0 (pre-predator exposure). Methods for behavioral trials were the same as experiment 1 except that I injected 50 ml of *L. auritus* stimulus. I then randomly assigned test subjects (n = 60) to one of the 4 treatments: (1) HF/HR, (2) HF/LR, (3) LF/HR, or (4) LF/LR. Subjects were maintained in treatments for 10 days under a natural light regime in individual 9.5-l housing aquaria with two cover objects (½ tube; 0.75 cm tall X 1.25 cm wide X 8 cm long) and fed ad libitum throughout the study. Housing aquaria were recirculating tanks with 7.5 l of well water maintained at 21-23°C. I recorded whether salamanders were under shelter or not 5 min prior to and post stimulus introduction. After 10 days of treatment, I repeated the behavioral trials at night on day 11 (post-predator exposure).

Statistical Analyses

I first assessed whether activity levels (pre-stimulus activity) and avoidance response intensity (activity indices) of *E. nana* were different before and after the

treatment period and whether these differences were related to the treatment conditions by comparing pre-stimulus activity of subjects on day 0 (pre-exposure) and day 11 (post-exposure) between treatment groups using 3-factor repeated measures Analyses of Variance (ANOVA) with encounter frequency and predator risk as fully crossed factors and predator exposure (pre-exposure, day 0; post-exposure, day 11) as a repeated factor. All data met the assumptions for the analyses. To determine if treatment influenced the amount of time that *E. nana* spent in shelters I compared the mean number of observations out of 10 in which salamanders were under shelter prior to stimulus introduction during the day and the night using 2-factor ANOVA's with encounter frequency and predator risk as crossed factors. I used Tukey's Highly Significant Difference (HSD) procedures for multiple comparisons.

To determine if treatment influenced the amount of time that *E. nana* spent in shelters, I compared the mean number of observations out of 10 in which salamanders were under shelter prior to stimulus introduction during the day and the night using a repeated measures ANOVA with encounter frequency and predator risk as crossed factors and observation time (day or night) as the repeated factor. I used Tukey's Highly Significant Difference (HSD) procedures for multiple comparisons where appropriate. I then assessed whether the introduction of chemical cues caused salamanders to move into shelters on day 1 (beginning), day 5 (middle), and day 9 (end) using McNemar's tests.

Results

Experiment 1: Cues of Damaged Conspecifics

Avoidance indices of *E. nana* exposed to conspecific damage-released alarm cues were significantly lower than activity indices of *E. nana* exposed to the control treatment ($t_{16} = 2.650, P = 0.017$; Fig. 3).

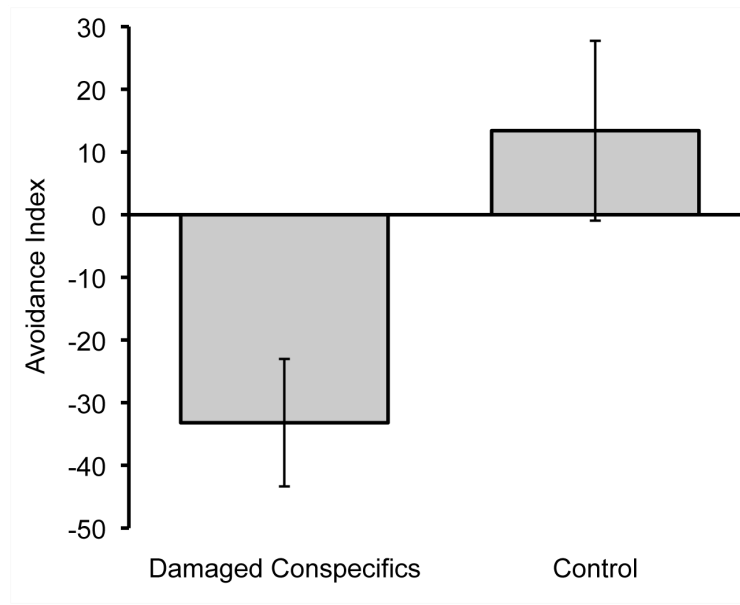


Figure 3. Responses of *E. nana* to Cues of Damaged Conspecifics. Mean (\pm SE) avoidance indices for *Eurycea nana* that were exposed to either a blank control or chemical cues obtained from damaged conspecifics. Salamanders reduced activity significantly more in response to damage-released cues than the control.

Experiment 2: Encounter Frequency and Risk

For activity and avoidance indices, there were no significant effects of encounter frequency, risk, or their interaction, but there was a significant effect of predator exposure (day 0 vs. day 11; Table 2), such that mean activity was greater (Fig. 4a) and avoidance indices were higher (Fig. 4b) before repeated exposures to predators on day 0 than after exposure to predators on day 11. For shelter use over the 10 days of observation, *E. nana* was in shelter more during the day than at night (Fig. 5). There were no differences between treatments in shelter use during the day, but there were treatment effects at night (Table 3). Subjects in low risk treatments were in shelters more prior to stimulus introduction than were *E. nana* in high risk treatments ($\alpha = 0.05$; Fig. 5). When determining whether exposure to predator stimulus caused *E. nana* to move to shelter, McNemar's test indicated that the number of salamanders that moved to shelter after stimulus exposure significantly differed from random on day 1 (beginning; $\chi^2 = 4.00$, $p = 0.04$) and day 5 (middle; $\chi^2 = 5.88$, $p = 0.01$), but not on day 9 (end; $\chi^2 = 1.56$, $p = 0.21$).

Table 2. Results of Repeated Measures ANOVA on Activity and Avoidance. ANOVA on activity and avoidance of *Eurycea nana* pre-predator exposure (day 0) and again after 10 days of repeated exposures to predatory stimulus (day 11).

	<i>df</i>	<i>F</i>	<i>P</i>
Activity			
Between Subjects	3	0.807	0.495
Encounter Frequency	1	0.853	0.360
Predator Risk	1	1.288	0.261
Frequency*Risk	1	0.279	0.599
Residuals	56		
Within Subjects	3	0.907	0.444
Predator Exposure	1	17.605	<0.001
Exposure*Frequency	1	0.047	0.829
Exposure*Risk	1	2.431	0.125
Exposure*Frequency*Risk	1	0.243	0.624
Residuals	56		
Avoidance Indices			
Between Subjects	3	0.725	0.542
Encounter Frequency	1	0.691	0.410
Predator Risk	1	1.071	0.305
Frequency*Risk	1	0.412	0.523
Residuals	56		
Within Subjects	3	0.562	0.642
Predator Exposure	1	5.840	0.019
Exposure*Frequency	1	0.044	0.835
Exposure*Risk	1	0.889	0.350
Exposure*Frequency*Risk	1	0.754	0.389
Residuals	56		

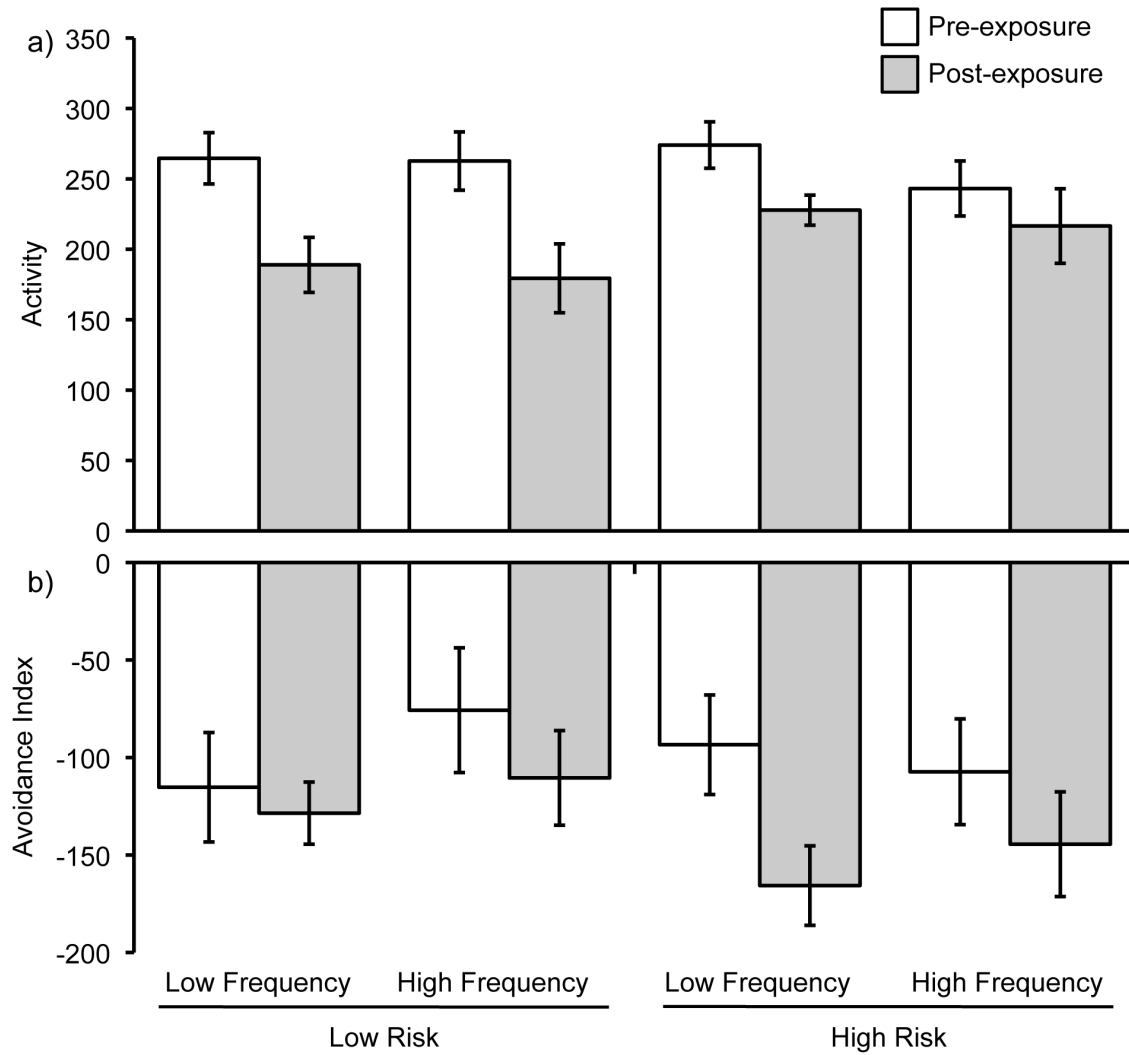


Figure 4. Effects of Variation in Predation Risk on Activity and Avoidance of *E. nana*. Comparison of mean (\pm SE) activity (a) and avoidance responses (b) of *Eurycea nana* before (Day 0) and after (Day 11) 10 days of exposure to predator treatments varying in predation risk and predator encounter frequency.

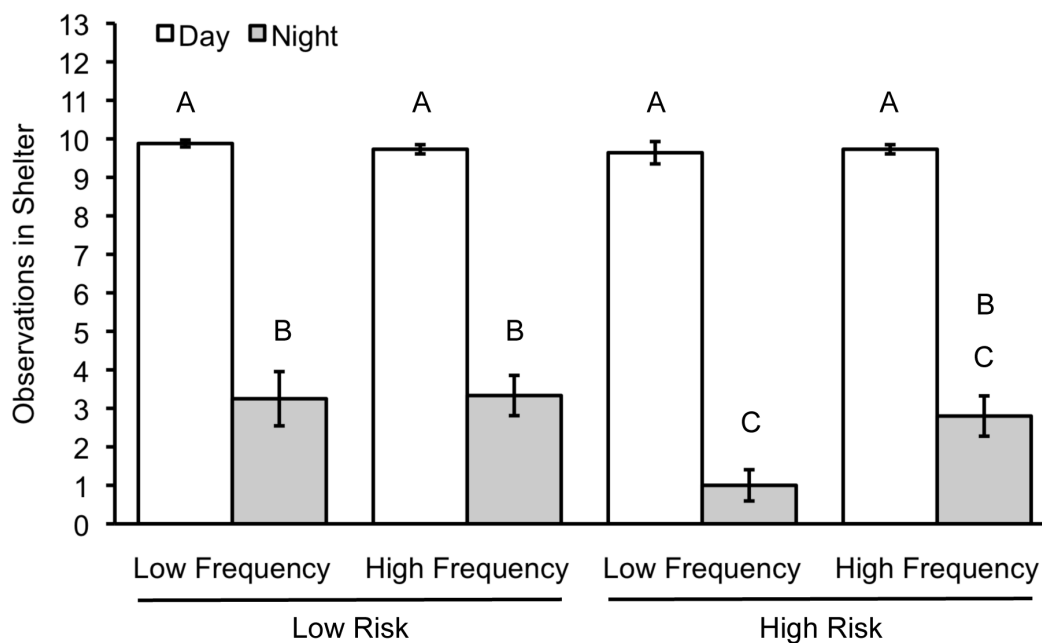


Figure 5. Shelter Use in *E. nana* During Prolonged Exposure to Predator Stimuli.

Mean (\pm SE) number of observations out of ten consecutive mornings and nights in which individuals of *Eurycea nana* were observed under shelter prior to predator encounters. Low risk treatments were diet neutral chemical stimuli of the fish predator *Lepomis auritus*. High risk treatments were predator cues combined with cues of injured *E. nana*. Salamanders were exposed to predatory stimulus once every two days (low frequency) or twice daily (high frequency). Means not connected by the same letter significantly differ.

Table 3. Results of Repeated Measures ANOVA on Shelter Use in *E. nana*. ANOVA on shelter use of *Eurycea nana* across 10 days of treatments varying in predator risk and predator encounter frequency.

	<i>df</i>	<i>F</i>	<i>P</i>
Shelter Use			
Morning	3	0.343	0.795
Encounter Frequency	1	0.024	0.877
Predator Risk	1	0.495	0.485
Frequency*Risk	1	0.495	0.485
Residuals	56		
Night	3	3.404	0.024
Encounter Frequency	1	2.647	0.109
Predator Risk	1	5.782	0.019
Frequency*Risk	1	2.199	0.144
Residuals	56		

Discussion

Salamanders exposed to cues of damaged conspecifics reduced activity more so than salamanders exposed to a blank control. This result suggests that *E. nana* may use these cues as an indicator of immediate predation risk. This response to damaged conspecifics is consistent with responses of other amphibian prey to conspecific alarm cues (Summey and Mathis 1998; Gonzalo et al. 2007; Fraker et al. 2009). Marvin et al. (2004) defined alarm cues as any chemical released by an injured individual that is detected by and benefits a receiver. The methods used in this study did not allow me to conclude definitively whether *E. nana* was responding to an alarm cue *per se* or whether salamanders were simply responding to the odor of damaged tissues or blood. However, responses to conspecific alarm cues have been documented in other species of *Eurycea* (Marvin et al. 2004) and plethodontids (Lutterschmidt et al. 1994; Sullivan et al. 2003) and, like alarm cues, the odors associated with damaged tissues should be a reliable indicator of imminent predatory threat for prey. Thus, regardless of the source, prey that detect and respond to the cues of damaged conspecifics should be at a selective advantage.

Reductions in activity can result in increased survival of prey, especially with visually-oriented predators (Skelly 1994). Despite the responses of *E. nana* to a single exposure of damage-released conspecific cue, I detected no difference in activity levels or avoidance response intensity between high risk and low risk treatments. Thus, cues of damaged conspecifics may not be important for experience-mediated (learned) risk assessment in *E. nana*. In housing aquaria during treatment conditioning, salamanders typically had to leave shelters to forage so time spent out of shelters may be reflective of foraging activity. In all treatments prey would have experienced the safest periods immediately prior to predator encounters (5 min prior to stimulus exposure) with the dangerous periods occurring during and immediately after encounters. Salamanders in

low risk treatments (LR) were in shelters prior to stimulus introduction (safe period) more than salamanders in high risk treatments (HR). This is consistent with increased activity during safer periods for salamanders in high risk treatments. The RAH (Lima and Bednekoff 1999) predicts that prey in riskier environments should increase foraging more during safe periods than prey in lower risk environments in compensation for more time allocation to predator avoidance. Thus, the reduced time spent in shelters by *E. nana* in high risk treatments may be reflective of increased activity, and potentially foraging, during safe, pre-stimulus exposure periods as compared to *E. nana* in low risk treatments.

I also found no significant effect of predator encounter frequency on shelter use, activity level, or response intensity in *E. nana* (Table 1). Other studies that manipulated the frequency of exposure of prey to predatory chemical stimuli found partial support for the RAH in that prey in high frequency treatments responded less intensely to predator cues than prey in lower frequency treatments (Sih and McCarthy 2002; Brown et al. 2006; Mirza et al. 2006b). In these studies, prey in high encounter frequency treatments were exposed to predatory stimuli three times each day and it is possible that the twice daily exposure used in this study was not frequent enough to simulate a high risk environment. One assumption of the RAH is that predator vigilance in high-risk environments hampers the ability of prey to obtain a minimum required amount of energy, which results in reduced predator vigilance over time (Sih 1992; Lima and Bednekoff 1999; Ferrari et al. 2009b). However, *E. nana* is a federally protected species (USDI 1980) so I was not able to limit food availability to test subjects. It is possible that treatments resulting in suboptimal levels of food intake may have yielded differences in response intensities of *E. nana*.

Since it was proposed, the predation risk allocation hypothesis (Lima and Bednekoff 1999) has received much attention. Despite having been cited more than 250 times (Web of Science® 2010), empirical tests of the predictions of the RAH are limited and offer mixed support (Ferrari et al. 2009b). In a recent review, Ferrari et al. (2009b)

suggested that the inconsistent results of these studies may be attributed to violations of the model assumptions about food availability or conditioning period because prey may not experience food stress over short time frames or when food is abundant throughout. In this study, I found no effects of predator encounter frequency or predator risk on the intensity of avoidance responses or activity levels of *E. nana*; however, food supplies were ample and my ability to detect treatment effects was limited (Pillai $V = 0.096$; $1-\beta = 0.512$). Given its impact in the literature and the mixed results of the few available empirical tests, more tests of the predictions of the RAH are needed to understand its applicability to predator-prey ecology and the extent to which it may explain variation in antipredator behaviors of prey between systems.

The results of this study clearly show that activity levels during safe periods decreased and the intensity of avoidance responses increased from before predator exposure (pre-exposure, day 0) to after exposure (post-exposure, day 11). These results are consistent with increased predator vigilance as a result of repeated exposure to predatory stimuli. This mechanism of plasticity should be favored in habitats where repetitious exposure to predators is a reliable indicator of imminent predatory threat (Fraker 2009). For example, three-spined stickleback, *Gasterosteus aculeatus*, from high predation risk populations exhibit increased predator vigilance after repeated exposures to predatory stimuli while sticklebacks from low predation risk populations are more likely to habituate to predator odor (Huntingford and Wright 1992). Habituation to predator odor in high-risk environments would be maladaptive because it could increase the susceptibility of prey to predators while increased vigilance in low risk environments would increase the costs of predator avoidance.

Previous work with *E. nana* illustrated that individuals collected from the natural habitat (presumably predator-experienced) exhibited less intense avoidance behaviors to *L. auritus* than naïve individuals (Epp and Gabor 2008). Conversely, the results of this study showed that *E. nana* exhibited more intense avoidance behaviors in response to

the chemical stimuli of sunfish after repeated exposures than they did on their primary exposure. There are two alternative hypotheses for this discrepancy in results: (1) In the natural system sunfish are less risky predators or are encountered infrequently and innate avoidance behaviors of *E. nana* diminish without reinforcement, or (2) increased predation risk in the natural habitat combined with longer exposure periods and lower food availability as compared to captivity have resulted in reduced predator vigilance as a means of coping with the costs of predator avoidance (RAH). Because I was unable to detect effects of predator encounter frequency or predator risk in this study, it is not possible to determine which hypothesis provides the most accurate explanation for behaviors of prey from the natural habitat in this system.

Few other studies have specifically examined experience-mediated behavioral plasticity in prey species that exhibit innate avoidance behaviors. While treatment effects of encounter frequency and predator risk on avoidance and activity were not detected here, these results, as well as those from previous work, clearly illustrate that *E. nana* exhibits experience-mediated plasticity of innate avoidance behaviors because activity and responses of salamanders differed between pre-exposure and post-exposure trials. This shows that innate avoidance responses are not necessarily 'genetically fixed' and that behavioral plasticity in antipredator behaviors need not be associated only with prey exhibiting learned predator recognition mechanisms. Future research addressing the conditions under which plasticity of antipredator behaviors is favored for prey exhibiting innate avoidance would be useful.

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and methods used to sacrifice salamanders were approved by the Texas State University-San Marcos Institutional Animal Care and Use Committee (#0731-1101-07 and #0821-0429-22 respectively). Salamanders were collected and housed under permits from the U. S. Fish and Wildlife Service (TE676811-9) and Texas Parks and Wildlife (SPR-0390-045).

Literature Cited

- Brown GE. 2003. Learning about danger: chemical alarm cues and local risk assessment in prey fishes. *Fish and Fisheries* 4:227-234.
- Brown GE, Chivers DP. 2005. Learning as an adaptive response to predation. In: Barbosa P, Castellanos P, editors. *Ecology of Predator-Prey Interactions*. New York: Oxford University. p. 34-53.
- Brown GE, Rive AC, Ferrari MCO, Chivers DP. 2006. The dynamic nature of antipredator behavior: prey fish integrate threat-sensitive antipredator responses within background levels of predation risk. *Behavioral Ecology and Sociobiology* 61:9-16.
- Chivers DP, Smith RJF. 1998. Chemical alarm signaling in aquatic predator-prey systems: a review and prospectus. *Écoscience* 5:338-352.
- Epp KJ, Gabor CR. 2008. Innate and learned predator recognition mediated by chemical signals in *Eurycea nana*. *Ethology* 114:607-615.
- Ferrari MCO, Brown GE, Messier F, Chivers DP. 2009a. Threat-sensitive generalization of predator recognition by larval amphibians. *Behavioral Ecology and Sociobiology* 63:1369-1375.
- Ferrari MCO, Chivers DP. 2009. Temporal variability, threat sensitivity and conflicting information about the nature of risk: understanding the dynamics of tadpole antipredator behaviour. *Animal Behaviour* 78:11-16.
- Ferrari MCO, Sih A, Chivers DP. 2009b. The paradox of risk allocation: a review and prospectus. *Animal Behaviour* 78:579-585.

- Fraker M. 2009. The effect of prior experience on a prey's current perceived risk. *Oecologia* 158:765-774.
- Fraker ME, Hu F, Cuddapah V, McCollum SA, Relyea RA, Hempel J, Denver RJ. 2009. Characterization of an alarm pheromone secreted by amphibian tadpoles that induces behavioral inhibition and suppression of the neuroendocrine stress axis. *Hormones and Behavior* 55:520-529.
- Gall BG, Mathis A. 2010. Innate predator recognition and the problem of introduced trout. *Ethology* 116:47-58.
- Gonzalo A, Lopez P, Martin J. 2007. Iberian green frog tadpoles may learn to recognize novel predators from chemical alarm cues of conspecifics. *Animal Behaviour* 74:447-453.
- Griffin AS. 2004. Social learning about predators: a review and prospectus. *Learning & Behavior* 32:131-140.
- Griffiths RA, Schley L, Sharp PE, Dennis JL, Roman A. 1998. Behavioral responses of Mallorcan midwife toad tadpoles to natural and unnatural snake predators. *Animal Behaviour* 55:207-214.
- Helfman GS. 1989. Threat-sensitive predator avoidance in damselfish-trumpetfish interactions. *Behavioral Ecology and Sociobiology* 24:47-58.
- Huntingford FA, Wright PJ. 1992. Inherited population differences in avoidance of conditioning in three-spined sticklebacks, *Gasterosteus aculeatus*. *Behaviour* 122:164-173.
- Kats LB, Dill LM. 1998. The scent of death: chemosensory assessment of predation risk by prey animals. *Écoscience* 5:361-394.
- Kelsey T. 1997. Fish community structure and water quality assessment (Index of Biotic Integrity) of the San Marcos River, Texas [M.S. Thesis]. [San Marcos, TX]: Southwest Texas State University. 45 pp.
- Laurila A. 2000. Behavioral responses to predator chemical cues and local variation in antipredator performance in *Rana temporaria* tadpoles. *Oikos* 88:159-168.

- Lima SL, Bednekoff PA. 1999. Temporal variation in danger drives antipredator behavior: The predation risk allocation hypothesis. *American Naturalist* 153:649-659.
- Lima SL, Dill LM. 1990. Behavioral decisions made under the risk of predation: a review and prospectus. *Canadian Journal of Zoology* 68:619-640.
- Lutterschmidt WI, Maerz JC, McDarby JH. 1994. Alarm response by a plethodontid salamander (*Desmognathus ochrophaeus*): conspecific and heterospecific "Schreckstoff". *Journal of Chemical Ecology* 20:2471-2479.
- Marvin GA, Whitekiller RR, Hutchison VH. 2004. Avoidance of alarm chemicals by plethodontid salamanders (genus *Eurycea*): Importance of phylogeny, ecology, and methodology. *Herpetologica* 60:24-33.
- Mathis A, Murray KL, Hickman CR. 2003. Do experience and body size play a role in responses of larval ringed salamanders, *Ambystoma annulatum*, to predator kairomones? Laboratory and field assays. *Ethology* 109:159-170.
- Mirza RS, Ferrari MCO, Kiesecker JM, Chivers DP. 2006a. Responses of American toad tadpoles to predation cues: behavioral response thresholds, threat-sensitivity and acquired predation recognition. *Behaviour* 143:877-889.
- Mirza RS, Mathis A, Chivers DP. 2006b. Does temporal variation in predation risk influence the intensity of antipredator responses? A test of the risk allocation hypothesis. *Ethology* 112:44-51.
- Schoeppner NM, Relyea RA. 2005. Damage, digestion, and defence: the roles of alarm cues and kairomones for inducing prey defences. *Ecology Letters* 8:505-512.
- Schoeppner NM, Relyea RA. 2009. When should prey respond to consumed heterospecifics? Testing hypotheses of perceived risk. *Copeia* 2009:190-194.
- Sih A. 1992. Prey uncertainty and the balancing of antipredator and feeding needs. *American Naturalist* 139:1052-1069.
- Sih A, McCarthy TM. 2002. Prey responses to pulses of risk and safety: testing the risk allocation hypothesis. *Animal Behaviour* 63:437-443.

- Skelly DK. 1994. Activity level and the susceptibility of anuran larvae to predation. *Animal Behaviour* 47:465-468.
- Sullivan AM, Madison DM, Rohr JR. 2003. Behavioural responses by red-backed salamanders to conspecific and heterospecific cues. *Behaviour* 140:553-564.
- Summey MR, Mathis A. 1998. Alarm responses to chemical stimuli from damaged conspecifics by larval anurans: Tests of three neotropical species. *Herpetologica* 54:402-408.
- [USDI] United States Department of the Interior. 1980. Endangered and threatened wildlife and plants: listing of San Marcos salamander as threatened, the San Marcos gambusia as endangered, and the listing of critical habitat for Texas wild rice, San Marcos gambusia, and fountain darter. *Federal Register*. Pp. 47355-47364.
- Wilson DJ, Lefcort H. 1993. The effect of predator diet on the alarm response of red-legged frog, *Rana aurora*, tadpoles. *Animal Behaviour* 46:1017-1019.
- Wisenden BD. 2003. Chemically mediated strategies to counter predation. In: Collin SP, Marshall NJ, editors. *Sensory Processing in Aquatic Environments*. New York: Springer-Verlag. p. 236-251.
- Woody DR, Mathis A. 1998. Acquired recognition of chemical stimuli from an unfamiliar predator: associative learning by adult newts, *Notophthalmus viridescens*. *Copeia* 1988:1027-1031.

CHAPTER V

DISCUSSION AND CONCLUSIONS

Predation is an intense selective pressure acting on prey populations. For prey, the result of ineffective predator recognition or avoidance is death. Despite the high cost of avoidance mistakes, the strategies used by prey to avoid predation can also result in lowered fitness. Especially for prey that experience chronic, high predation pressures, efficiency in avoidance responses is important for reducing the costs associated with antipredator strategies. Given the diverse effects of predators on prey, it is important to understand the mechanisms by which prey recognize and respond to predators to better understand the ecology of the prey species. This need is especially true for prey in habitats where predators have been introduced. Previously, nothing was known of the predator-prey ecology of aquatic *Eurycea* in the Edwards Plateau of Texas. The results of this research have elucidated some predator recognition and avoidance mechanisms of *E. nana*, although other questions remain unanswered. Studies examining responses of amphibians to predators usually use either the aquatic tadpole stage or adult terrestrial stage of metamorphosing frogs, toads, or salamanders. Few studies have examined predator recognition and avoidance in adults of fully aquatic amphibian species (Gall and Mathis 2010) and, given that aquatic species must persist with the same or similar predators throughout life, the selective pressures from predation acting on these species may differ.

Responses of laboratory-reared (predator-naïve) *Eurycea nana* to nonnative

sunfish and native bass were consistent between the studies in chapters 2 and 3 in that salamanders reduced activity in response to chemical stimuli of both species and their responses to bass and sunfish did not differ. However, responses of wild-caught (predator-experienced) salamanders were not consistent between studies. In the study from chapter 2, experienced *E. nana* exhibited less intense responses to sunfish stimulus, but not to bass while the opposite was true in chapter 3. Diet of predators was not controlled in the first study, but it was controlled in the second. In the second study, naïve *E. nana* responded much more intensely to the chemical stimuli of predators that had recently consumed prey as compared to fish that had eaten only earthworms indicating that diet cues released by predators may be important for threat sensitivity in this species. In the first study differences in responses of experienced salamanders may have resulted from differences in recent foraging activity between individuals used to collect stimulus; however, two fish were used for each cue and multiple stimulus fish were used across the studies so it seems unlikely that diet cues are the primary source for the difference in the first study. Additionally, we would not expect similar variation and means between responses of naïve *E. nana* to both predators in the first study if diet cues of predators were the primary source the difference in responses of experienced *E. nana*. In the second study, it may be that metabolic or digestive differences exist between the two species which altered the amount of conspecific diet cue released into the stimulus water by bass as compared to sunfish.

Chemical stimuli can be an important source of information about predators and predation risk for prey (Kats and Dill 1998; Wisenden 2003). The results of these studies clearly show that chemical cues mediate predator avoidance behaviors in *E. nana*. I found that *E. nana* could discriminate between chemical stimuli of predatory and non-predatory species and that, at least for predator-experienced individuals, responses to chemical stimuli of different species of predators can differ. This result indicates that *E. nana* can identify species based solely on chemical information. In previous work with *E. nana*,

Thaker et al. (2006) determined that individuals preferred to associate with conspecifics of the opposite sex when chemical cues were present, but not when visual cues were presented alone. This reliance on chemical over visual cues has been found in numerous amphibian species (Stauffer and Semlitsch 1993; Kiesecker et al. 1996; Mathis and Vincent 2000; Hickman et al. 2004) and could result from limitations in the visual acuity of amphibians (Mathis and Vincent 2000).

Prey may acquire information about predation risk from chemical cues specific to predatory species (kairomones), those associated with recent foraging activity of predators (diet cues), or cues released by conspecific and heterospecific prey (alarm and disturbance cues) (Marvin et al. 2004). For some species, the presence of conspecific cues in the diet of predators or damage-released alarm cues indicates higher predatory threat resulting in more intense predator avoidance responses. This research showed that naïve *E. nana* responded more intensely to the chemical stimuli of predators that had recently consumed conspecific prey than predators that had been fed a neutral diet. This is consistent with threat-sensitive predator avoidance observed in other amphibians (Wilson and Lefcort 1993; Laurila et al. 1997; Mathis 2003). However, unlike some amphibians (Schoeppner and Relyea 2005; 2009a), the presence of diet cues was not required to elicit antipredator behaviors because *E. nana* exposed to the kairomones of predators fed a neutral diet reduced activity more than those exposed to a blank control. These results clearly illustrate the importance of predator diet cues in mediating threat sensitivity for *E. nana*; however, the importance of cues of damaged conspecifics for risk assessment remains unclear. I found that *E. nana* exposed to chemical stimuli from injured conspecifics exhibited a weak, but detectable decrease in activity as compared to those exposed to a blank control. However, simultaneous exposure to cues of injured conspecifics and predators did not result in more intense responses of *E. nana* to subsequent exposure to predator kairomones alone. This suggests that the presence of stimuli from damaged *E. nana* may indicate greater risk at a given time, but might not

be important for threat-sensitive learning. Conspecific alarm cues have been shown to mediate threat-sensitivity (Summey and Mathis 1998; Marvin et al. 2004) and learning in other amphibians (Gonzalo et al. 2007; Ferrari and Chivers 2009b), although recent work has shown that tadpoles respond more intensely to chemical stimuli produced when predators consume conspecific prey as compared to responses of tadpoles exposed to alarm cues and predator kairomones simultaneously or either cue independently (Schoeppner and Relyea 2005; 2009c). It may be that predator diet cues are a more reliable indicator of predatory threat for *E. nana* or they may be easier to detect in the natural habitat, although this remains to be determined.

It has been suggested that differences in the capacity to exhibit behavioral plasticity in antipredator behaviors exist between prey that exhibit innate and learned predator avoidance mechanisms (Wisenden 2003; Brown and Chivers 2005; Ferrari et al. 2007). This assumption; however, can create a bias in expectations when examining the role of experience with predators on avoidance responses of prey that innately recognize predators. I found that predator-naïve *E. nana* from a captive population exhibited avoidance behaviors to the chemical stimuli of predators indicating that there is an innate component to predator avoidance in this species. However, *E. nana* from the natural population exhibited generally less intense responses to the same predatory cues indicating plasticity in avoidance response intensity of this species. Additionally, repeated exposures to predator stimulus in the laboratory resulted in more intense avoidance behaviors and reduced overall activity of *E. nana*. These results suggest that *E. nana* may refine innate avoidance behaviors based on prior experience with predators, which can allow them to respond more efficiently to predatory threats. Similarly, Gonzalo et al. (2007) found that the simultaneous exposure of frog tadpoles to chemical stimuli from a native snake predator and injured conspecifics resulted in more intense responses to snake stimulus at subsequent exposures. Like *E. nana*, the tadpoles exhibited a weak, but innate response to the snake stimulus that was enhanced through learning. Learning is adaptive

for many prey because it allows them to update information about new or changing predatory threats and this results in improved effectiveness and efficiency of avoidance behaviors (Brown and Chivers 2005).

Experience-mediated behavioral plasticity can confer benefits to prey in the form of improved effectiveness and efficiency in avoidance responses; however, there are costs and limitations associated with phenotypic plasticity as compared to more canalized traits (Auld et al. 2010). Plasticity in avoidance behaviors is expected to be favored when predator communities fluctuate or change through time (Relyea 2003) because it would allow prey to adjust to changes in predation risk. However, in habitats where the predator community is more stable (e.g. lakes vs. temporary pools), the benefits of plasticity over canalized responses may be less and could result in traits with very little plasticity or that are fixed. In chapters 2 and 3, I found that laboratory-reared (predator-naïve) *E. nana*, in general, responded more intensely to predators than did adult salamanders collected from the natural habitat indicating that *E. nana* exhibits at least some level of plasticity in avoidance behaviors. However, in these studies, wild-caught salamanders were maintained under laboratory conditions for between two weeks and six months prior to testing, but did not revert to avoidance behaviors that were similar to laboratory-reared salamanders. This suggests there may be limits to the extent of plasticity for avoidance responses in this species. Prey that experience fitness costs as a result of an antipredator phenotype should be at a selective advantage if they are able to reverse the trait expression at a later time (Gabriel et al. 2005). In the habitat of *E. nana*, the predatory community of fish is relatively constant through time. In other words, there is a high probability that if fish predators were present in the past, they will likely be present in the future. Because of this consistency, reverting to phenotypes expressed in safer habitats may result in a avoidance responses that are not well-matched to the environment (Relyea 2003). Retention or reversibility of avoidance behavior phenotypes has not been well-studied in amphibians other than metamorphosing tadpoles (Relyea 2003;

Schoeppner and Relyea 2009b), but it seems possible that the affects of past experiences with predators may impact prey behaviors longer than expected in some systems.

Global amphibian declines have been well-documented over the last few decades (reviewed in Collins and Storer 2003; Blaustein and Bancroft 2007), and research suggests that the introduction of nonnative predators may contribute to local extinctions in some systems (Knapp and Matthews 2000; Pilliod and Peterson 2001; Kats and Ferrer 2003). Nonnative predators may negatively impact prey that lack evolutionary experience with introduced species because the prey may not recognize the predator as a threat or may exhibit ineffective avoidance behaviors (Gillespie 2001; Gall and Mathis 2010). This research showed that predator-naïve *E. nana* exhibited avoidance behaviors consistent with those to native predators in response to the chemical stimuli of the nonnative predator *Lepomis auritus*. Despite the lack of evolutionary history between *E. nana* and *L. auritus*, it is possible that the chemical stimuli of *L. auritus* share similar characteristics to the kairomones of native congeneric predators like *L. cyanellus* or *L. macrochirus*. Thus, *E. nana* may be able to generalize predator recognition from innately recognized native predators to novel, but similar nonnative predators. Other research has shown that aquatic prey can generalize learned predator recognition to closely related predatory species (Ferrari et al. 2008; Ferrari et al. 2009; Ferrari and Chivers 2009a); however, Ferrari et al. (2007) assert that prey that innately recognize predators lack this capacity. One study using a stream-adapted salamander (*Cryptobranchus alleganiensis*) showed that larvae from populations with and without a native trout predator differed in their responses to an introduced trout species in that those sympatric with a related native predator exhibited behaviors consistent antipredator responses while those from the populations without a native trout species did not (Mathis and Crane 2009). While results of this study are preliminary given relatedness of test subjects in each treatment group, they suggest that prey exhibiting innate recognition are able to generalize responses to novel predators provided there is a native related predator

in the habitat. My results suggest that *E. nana* is able to generalize predator recognition to some species, although the extent of this capacity remains to be explored.

The results of these studies have elucidated some mechanisms by which *E. nana* recognize and respond to predatory threats. This information is essential to understanding the predator-prey interactions of this and other similar species. Human encroachment and predator introductions in the Edwards Plateau of Texas have exacerbated the need for a more complete grasp of the ecology of *E. nana* and other protected regional congeners. This research is important for understanding the impacts that predators have on prey populations and for more effective management of both captive and natural populations of *E. nana*. Additionally, this research provides insights into management of other closely related endemic *Eurycea*.

Literature Cited

- Auld JR, Agrawal AA, Relyea RA. 2010. Re-evaluating the costs and limits of adaptive phenotypic plasticity. *Proceedings of the Royal Society B-Biological Sciences* 277:503-511.
- Blaustein AR, Bancroft BA. 2007. Amphibian population declines: Evolutionary considerations. *Bioscience* 57:437-444.
- Brown GE, Chivers DP. 2005. Learning as an adaptive response to predation. In: Barbosa P, Castellanos P, editors. *Ecology of Predator-Prey Interactions*. New York: Oxford University. p. 34-53.
- Collins JP, Storfer A. 2003. Global amphibian declines: sorting the hypotheses. *Diversity and Distributions* 9:89-98.
- Ferrari MCO, Brown GE, Messier F, Chivers DP. 2009. Threat-sensitive generalization of predator recognition by larval amphibians. *Behavioral Ecology and Sociobiology* 63:1369-1375.

- Ferrari MCO, Chivers DP. 2009a. Sophisticated early life lessons: threat-sensitive generalization of predator recognition by embryonic amphibians. *Behavioral Ecology* 20:1295-1298.
- Ferrari MCO, Chivers DP. 2009b. Temporal variability, threat sensitivity and conflicting information about the nature of risk: understanding the dynamics of tadpole antipredator behaviour. *Animal Behaviour* 78:11-16.
- Ferrari MCO, Gonzalo A, Messier F, Chivers DP. 2007. Generalization of learned predator recognition: an experimental test and framework for future studies. *Proceedings of the Royal Society B-Biological Sciences* 274:1853-1859.
- Ferrari MCO, Messier F, Chivers DP. 2008. Can prey exhibit threat-sensitive generalization of predator recognition? Extending the Predator Recognition Continuum Hypothesis. *Proceedings of the Royal Society B-Biological Sciences* 275:1811-1816.
- Gabriel W, Luttbeg B, Sih A, Tollrian R. 2005. Environmental tolerance, heterogeneity, and the evolution of reversible plastic responses. *American Naturalist* 166:339-353.
- Gall BG, Mathis A. 2010. Innate predator recognition and the problem of introduced trout. *Ethology* 116:47-58.
- Gillespie GR. 2001. The role of introduced trout in the decline of the spotted tree frog (*Litoria spenceri*) in south-eastern Australia. *Biological Conservation* 100:187-198.
- Gonzalo A, Lopez P, Martin J. 2007. Iberian green frog tadpoles may learn to recognize novel predators from chemical alarm cues of conspecifics. *Animal Behaviour* 74:447-453.
- Hickman CR, Stone MD, Mathis A. 2004. Priority use of chemical over visual cues for detection of predators by graybelly salamanders, *Eurycea multiplicata griseogaster*. *Herpetologica* 60:203-210.
- Kats LB, Dill LM. 1998. The scent of death: chemosensory assessment of predation risk by prey animals. *Écoscience* 5:361-394.

- Kats LB, Ferrer RP. 2003. Alien predators and amphibian declines: review of two decades of science and the transition to conservation. *Diversity and Distributions* 9:99-110.
- Kiesecker JM, Chivers DP, Blaustein AR. 1996. The use of chemical cues in predator recognition by western toad tadpoles. *Animal Behaviour* 52:1237-1245.
- Knapp RA, Matthews KR. 2000. Non-native fish introductions and the decline of the mountain yellow-legged frog from within protected areas. *Conservation Biology* 14:428-438.
- Laurila A, Kujasalo J, Ranta E. 1997. Different antipredator behaviour in two anuran tadpoles: Effects of predator diet. *Behavioral Ecology and Sociobiology* 40:329-336.
- Marvin GA, Whitekiller RR, Hutchison VH. 2004. Avoidance of alarm chemicals by plethodontid salamanders (genus *Eurycea*): Importance of phylogeny, ecology, and methodology. *Herpetologica* 60:24-33.
- Mathis A. 2003. Use of chemical cues in detection of conspecific predators and prey by newts, *Notophthalmus viridescens*. *Chemoecology* 13:193-197.
- Mathis A, Crane A. 2009. Saving a giant salamander. *IRCF Reptiles & Amphibians* 16:18-23.
- Mathis A, Vincent F. 2000. Differential use of visual and chemical cues in predator recognition and threat-sensitive predator-avoidance responses by larval newts (*Notophthalmus viridescens*). *Canadian Journal of Zoology* 78:1646-1652.
- Pilliod DS, Peterson CR. 2001. Local and landscape effects of introduced trout on amphibians in historically fishless watersheds. *Ecosystems* 4:322-333.
- Relyea RA. 2003. Predators come and predators go: the reversibility of predator-induced traits. *Ecology* 84:1840-1848.
- Schoeppner NM, Relyea RA. 2005. Damage, digestion, and defence: the roles of alarm cues and kairomones for inducing prey defences. *Ecology Letters* 8:505-512.

- Schoeppner NM, Relyea RA. 2009a. Interpreting the smells of predation: how alarm cues and kairomones induce different prey defences. *Functional Ecology* 23:1114-1121.
- Schoeppner NM, Relyea RA. 2009b. Phenotypic plasticity in response to fine-grained environmental variation in predation. *Functional Ecology* 23:587-594.
- Schoeppner NM, Relyea RA. 2009c. When should prey respond to consumed heterospecifics? Testing hypotheses of perceived risk. *Copeia* 2009:190-194.
- Stauffer HP, Semlitsch RD. 1993. Effects of visual, chemical and tactile cues on the behavioural responses of tadpoles. *Animal Behaviour* 46:355-364.
- Summey MR, Mathis A. 1998. Alarm responses to chemical stimuli from damaged conspecifics by larval anurans: Tests of three neotropical species. *Herpetologica* 54:402-408.
- Thaker M, Gabor CR, Fries JN. 2006. Sensory cues for conspecific associations in aquatic San Marcos salamanders. *Herpetologica* 62:151-155.
- Wilson DJ, Lefcort H. 1993. The effect of predator diet on the alarm response of red-legged frog, *Rana aurora*, tadpoles. *Animal Behaviour* 46:1017-1019.
- Wisenden BD. 2003. Chemically mediated strategies to counter predation. In: Collin SP, Marshall NJ, editors. *Sensory Processing in Aquatic Environments*. New York: Springer-Verlag. p. 236-251.

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