

EFFECTS OF DIEL CYCLE AND TURBIDITY ON ANTIPREDATOR RESPONSE
TO MULTIPLE CUES OF PREDATORS BY *EURYCEA NANA* AND *EURYCEA*
SOSORUM

by

Kristina Zabierek, B.A.

A thesis submitted to the Graduate Council of
Texas State University in partial fulfillment
of the requirements for the degree of
Masters of Science
with a Major in Population and Conservation Biology
December 2014

Committee Members:

Caitlin R. Gabor, Chair

James R. Ott

Kristen J. Epp

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DEDICATION

I dedicate this work to the *Eurycea* salamanders and to all the people working to conserve our aquifers and rivers. My work would be impossible without you.

ACKNOWLEDGEMENTS

I would like to express extreme gratitude towards Caitlin Gabor for giving me the opportunity to pursue my interests, and for her continual guidance, patience, and support. I would also like to thank my committee members Kristen Epp and Jim Ott for constant moral support, for invaluable study design advice, and for statistical advice. Many thanks to Andrea Aspbury, Floyd Weckerly, and the EEB discussion group for extremely helpful advice and for support. I would also like to thank everyone at the San Marcos Aquatic Resources Center who has aided my research and cared so well for the salamanders, especially Ken Ostrand, Valentin Cantu, and Justin Crow. I could not imagine this experience without the constant support and friendship of my labmates past and present Diana Kim, Chelsea Blake, Laura Alberici da Barbiano, Sophia DeColo, Steven Nagy, Drew Davis, and my salamander sister, Megan Mondelli. Thank you to Mitchell Baker and Karyn Collie for introducing me to the world of research as an undergraduate and for pushing me to pursue my interests studying amphibian conservation. I am also indebted to Miranda Wait, Stephanie Miller, John Sistrunk, and Brendan Stanley for making my time here memorable and enjoyable. Thanks to Tj McAusland for his constant support of my endeavors and his faith in my abilities. Infinite thanks to my parents, Margaret and Dariusz Zabierek, for always encouraging me to pursue my interests and for their loving support. My brother, Matthew Mazewski, for introducing me to the fascinating world of science and discovery as a child. Finally, I would like to thank my grandparents and my eccentric aunts and uncles for their support and interest in my passions.

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ABSTRACT

Behavioral interactions between predators and prey are a significant component of balance in ecological communities. Prey must be able detect, recognize, and respond to predators to decrease threat of predation. However, investment in antipredator behavior diverts costly resources away from other behaviors such as foraging and mating. Therefore, it is expected that antipredator behavior is threat sensitive and that prey invest an optimal amount of time and energy in antipredator behavior, minimizing energetic costs of behavior. This optimum is not constant through time because it is dependent on threat of predation, which varies throughout the diel cycle of the predator. Therefore, we studied the effect of the diel cycle on the antipredator behavior of the San Marcos salamander, *Eurycea nana* to a nocturnal and a diurnal predator. We found that diel cycle of predators does not influence antipredator behavior, suggesting threat-sensitive behavior is uncoupled from the diel cycle of predators. The ability of prey to respond optimally to predators is dependent on the ability of prey to detect predators, and multimodal cues are often used to increase detection and recognition of predators. Use of multimodal cues can be affected by the sensory environment, and increased turbidity is an increasingly common problem in aquatic systems, which can have effects on predator-prey interactions. We studied the use of multimodal cues in the Barton Springs salamander, *Eurycea sosorum*, and the effects of turbidity on antipredator behavior in response to a predator and non-predator. We found that *E. sosorum* uses chemical cues, but not visual cues, to detect predators, and that turbidity decreases antipredator behavior.

CHAPTER I

PREDATOR-PREY INTERACTIONS IN FLUCTUATING ENVIRONMENTS

Predator Detection in Aquatic Habitats

Animal communication is defined as a transfer of signals or cues from a signaler that affect the sensory systems and behavior of the receiver (Johnstone 1997; Ruxton & Schaefer 2011). Signals and cues differ in that the former have been under selection as an intentional mode of communication granting a fitness advantage to the signaler, whereas cues have not undergone selection that is advantageous to the signaler (Higham & Hebets 2013). Communication often involves a direct and intentional interaction between signaler and receiver, although indirect communication is common, especially in predator-prey interactions. Prey must be able to detect predator cues, recognize those cues as a threat, and respond effectively as this would increase their ability of avoiding predation.

Depending on the spatiotemporal sensory environment and the receiver's sensory system, aquatic prey may use multiple sensory mechanisms, alone or in conjunction, to detect predator cues (Partan & Marler 1999; Stynoski & Noble 2012). Multi-modal communication can exist either as multiple cue types emanating from a single predator, or one cue type emanating from a predator that stimulates multiple sensory organs of the prey (Higham & Hebets 2013). For example, zebrafish, *Danio rerio*, can detect soundwaves using both their ear and specialized neuromasts (Dambly-Chaudière et al. 2003). Use of multi-modal cues by prey often increases detection of predators. Johnstone

(1996) proposed the backup signal hypothesis, which states that cues are often used in combination to provide greater accuracy for response to predators. For example, mosquitofish (*Gambusia holbrooki*) avoided predation more successfully when presented with both visual and chemical cues than when presented either cue alone (Ward & Mehner 2010). Similarly, wall lizards (*Podarcis muralis*) increased antipredator behavior when exposed to both visual and chemical predator cues simultaneously than either cue alone (Amo et al. 2004).

The use of multi-modal communication is particularly prevalent when one signaling modality is obstructed by environmental variables such as turbidity (Endler 1993; Leahy et al. 2011), or if the use of multiple sensory organs can increase chance of survival (Higham & Hebets 2013). The sensory compensation hypothesis states that response to a particular cue or combination of cues is mediated by both the level of risk associated with the cue and the availability of information (Hartman & Abrahams 2000). The concentration of chemical cues needed to elicit an antipredator response decreases with reduced visual information, suggesting prey are able to alter their response to cues depending on their sensory environment (Hartman & Abrahams 2000). Atlantic salmon (*Salmo salar*) exhibit heightened response to visual cues when exposed to low pH (which reduces chemical cue efficacy), as compared to neutral pH, showing their ability to compensate for environmental shifts in information through use of multi-modal communication (Elvidge et al. 2013).

Aquatic animals are exposed to many stimuli in their environment, only some of which are used for communication. This includes various communication modalities including chemical, visual, mechanical, and electric cues (Dodson et al. 1994; Bradbury

& Vehrencamp 1998). Mechanical signal sensing is used in response to hydrodynamic pressure waves or sound generated by other organisms. Although many aquatic amphibians have a well-developed mechanosensory lateral line system to detect vibrational signals, few studies have been conducted on their reliance on mechanical cues for predator recognition (Lannoo 1987; Fritsch & Neary 1998; Paoletti et al. 2011). Visual cues are commonly used as primary cues for antipredator detection and recognition in many aquatic organisms (Kelley & Magurran 2003; Brown et al. 2004; Hemmi 2005). However, visual cues are often less relevant in predator detection in amphibians than other sensory modalities (Petranka et al. 1987 Takahara et al. 2013). Tadpoles of *R. lessonae* and *R. esculenta* did not exhibit different antipredator response to a fish predator when exposed to visual cues alone or visual cues paired with mechanical signals (Stauffer & Semlitsch 1993). Tadpoles of *Hyla japonica* exhibited significant antipredator behavior when exposed to chemical cues, but not visual cues, of a predatory dragonfly nymph, *Anax parthenope julius* (Takahara et al. 2013). A few studies have shown that visual cues elicited antipredator behavior in amphibians, but did not give individuals the ability to discern level of predatory threat (Kiesecker et al. 1996; Mathis & Vincent 2000; Hickman et al. 2004). Therefore, the combination and singular use of chemical and visual cues in predator detection should be further explored.

The role of chemical cues in detection of predators of aquatic organisms has been studied extensively, including chemicals of not only aquatic predators, but piscivorous terrestrial predators (Ferrari 2010; Rosell et al. 2013). Chemical cues can disseminate information into the environment, such as size of predators (Kusch et al. 2004), proximity of predators (Ferrari et al. 2006a), and threat-level associated with predators (Wisenden

2000). The three main classes of cues common in predator-prey interactions are kairomones, disturbance cues, and alarm cues. These are all considered semiochemicals (chemicals that relay information among living organisms) and allelochemicals (specifically used in predator-prey interactions) (Dodson 1994). Kairomones are chemicals that are produced by a species, either as pheromones for conspecific interaction or as a secondary metabolite, and may be used for eavesdropping by predators on prey (Branco et al. 2011) or prey on predators (Mathis et al. 2003). Detection of kairomones can allow prey to detect and evade distant predators or predators waiting in ambush (Ferrari 2009a). Although kairomones are always present as background chemicals, prey are able to detect threshold concentration level that reflect significant threat (Dodson 1994). For example, coral reef damselfish (*Pomacentrus amboinensi*) had a marginal response to low concentrations of kairomones but increased their response at a threshold concentration (Holmes & McCormick 2011). Alarm cues are released by the skin of some prey when injured and often cause strong antipredator responses in conspecific or heterospecific prey (Chivers and Smith 1998; Wisenden 2000). Some studies have shown that amphibian prey respond to conspecific alarm cues, but not kairomones alone (Gonzalo et al. 2010; Gonzalo et al. 2012). A study by Wisenden (2008) found that redbelly dace (*Phoxinus eos*) had stronger antipredator response when exposed to alarm cues than when exposed to kairomones alone. It is thought that responding to alarm cues increases probability of survival because alarm cues signify threat level, unlike kairomones, which are more general (Smith 1992). McCarthy and Dickey (2002) found that although prey showed increased antipredator behavior in response to alarm cues, they did not confer any survival advantage for the prey or

disadvantage to the predator. Diet cues are a specific, and potentially more informative, form of alarm cue chemicals released after a predator digests prey and they are important in predator detection for many aquatic species, potentially because they allow prey to identify threat level (Pettersson et al. 2001; Brown & Dreier 2002; Roberts & Garcia de Leaniz 2011). One study found that diet cues, rather than kairomones alone, elicited a behavioral and morphological antipredator response in leopard frog tadpoles, *Rana pipiens* (Schoepner & Relyea 2009). Diet cues may be important for identifying congeneric predators because the magnitude of response to diet cues is higher when the prey is a more closely related species (Mathis & Smith 1993; Schoepner & Relyea 2005). The combination of alarm cues and kairomones may elicit behavioral antipredator response, aid in generalization of predators, and facilitate recognition of predators.

Learning and Innate Recognition in Predator-Prey Interactions

Despite the multitude of background cues, most organisms are able learn to respond to specific cues that represent a significant threat. Most studies achieved learning in prey when a naïve individual was exposed to a predatory cue paired with conspecific alarm cues, or in some instances, diet cues and heterospecific alarm cues (Mathis et al. 1996; Crane & Mathis 2013; Ferrari & Chivers 2013). Although alarm cues are most commonly paired with predator kairomones, a few studies have successfully paired alarm cues with visual and mechanical cues (Wildy & Blaustein 2001; Kelley & Magurran 2003). Some prey can learn to recognize multiple predators simultaneously. For example, Darwish et al. (2005) conditioned tetras (*Hemigrammus erythrozonus*) using a combination of cues from three predators and found that they subsequently exhibited

antipredator behavior to each predator individually. Learning can be threat sensitive; prey can learn to respond more strongly to certain predators based on concentration of alarm cues (Puttlitz et al. 1999; Ferrari et al. 2009a; Brown et al. 2014; but see Sullivan & Jensen 2013). Prey are also able to generalize antipredator behavior if the predators are closely related (Ferrari et al. 2009b; Davis et al. 2012). Learning is thought to allow prey to dynamically adjust their antipredator response in variable environments, which increase chances of survival (Ferrari et al. 2009a). However, multiple studies have found that latent inhibition, the event in which prey are unable to learn if previously exposed to predator cues without alarm cues, may impede the ability of prey to learn predators in a dynamic situation (Ferrari & Chivers 2009). Latent inhibition, however, may help prevent prey from associating alarm cues with non-predatory species (Hazlett 2003).

A few aquatic species respond innately to predators; they do not require learning or exposure to predators to elicit an antipredator response (Gallie et al. 2001; Epp & Gabor 2008; Dixson et al. 2012; Kempster et al. 2013). Innate recognition may be advantageous because it alleviates potential costs of learning by not requiring an initial naïve encounter with a predator. Therefore, innate recognition is expected in environments with high predation risk, or in environments with a static predator community (Brown & Chivers 2005). Innate antipredator response is expected to be less advantageous in changing environments with novel predators. Therefore, some prey possess innate predator recognition with the added ability to alter their response with experience (Gonzalo et al. 2007; Epp & Gabor 2008; Epp 2013). No studies in aquatic organisms to date have demonstrated true innate recognition by accounting for both embryonic learning and maternal effects. In a few aquatic species, embryos have the

ability to learn predator chemical cues (Ferrari & Chivers 2009; Mathis et al. 2009), which was not accounted for in some studies claiming innate predator recognition. Offspring of mothers exposed to predators exhibit morphological and behavioral changes resonant with antipredator defense (Storm & Lima 2010; Giesing et al. 2011; Coslovsky et al. 2012). Regardless of the mechanism, exploring the ability of prey to respond to predators without previous experience is important to understanding the complexities of predator-prey interactions.

Prey Response to Predators in Aquatic Systems

The way in which prey respond to predatory cues depends on the amount of exposure to cues (chronic or acute), the life history of an organism, and the spatiotemporal environment. The most commonly studied antipredator responses are behavioral, life history, and morphological changes. There is a wide range of behavioral responses to predatory cues, including increased use of refugia (Kats et al. 1988), aggregation (Holomuzki & Short 1990), defensive posturing (Shave et al. 1994), and change in activity (Kats & Dill 1988). In many amphibian studies, activity is reduced in response to predators because it reduces chance of attack by sit and wait predators (Skelly 1994; Kats & Dill 1998; Takahara et al. 2008; Epp & Gabor 2008; DeSantis et al. 2013). However, there is also evidence that reduced activity may reduce chance of survival if predators can detect immobile prey (Altwegg 2003). In many fish studies, increased escape behavior is more common (Kats & Dill 1998). However the strategy of antipredator defense depends on the ontogeny of an organism, the type of predator, and the level of threat. For example, Crane & Mathis (2012) found that juvenile hellbenders

(*Cryptobranchus alleganiensis*) decreased activity to learned predator cues, while adults increased activity. Additionally, the complexity of a habitat (DeBoom & Wahl 2013) and the predation strategy of a predator (i.e. ambush predator versus active predation) (Botham et al. 2006) may also influence the antipredator strategy of prey. It is therefore important to consider the biology of the prey and the predator in determining the expected antipredator response.

Many studies have examined the role of inducible morphological defenses in response to predators (Kishida et al. 2010). Tadpoles respond to chronic exposure to kairomones without alarm signals with increased tail length and brighter tail color (Schoeppner & Relyea 2005; Richardson 2006; El Bala & Blouin-Demers 2013). Having inducible defenses due to increased plasticity is more important in variable habitats than constant environments because it gives prey the flexibility to develop traits suited to the given predatory environment (LaFiandra & Babbitt 2004). Plasticity in life history characteristics in response to predator chemical cues in aquatic species are common but conflicting. Although studies have found delayed hatching, faster development, and earlier reproduction (Kiesecker et al. 2002; Pollock et al. 2005), others have found delayed development (Teplitsky et al. 2005; Mogali et al. 2011), and these conflicting findings may reflect differential spatiotemporal risk patterns (Oriazola et al. 2012).

Effects of Diel Cycle on Predation Threat and Antipredator Behavior

Risk of predation is not constant through time and is variable throughout seasons, diel cycles, and lunar cycles (Sih & Ziemba 2000). The risk allocation

hypothesis states that prey should alter their level of antipredator behavior in response to temporal variation in predator behavior (Lima & Bednekoff 1999). This hypothesis stems from the threat-sensitivity hypothesis, which predicts that antipredator behavior of prey is relative to perceived predatory threat (Helfman 1989). The model for the risk allocation hypothesis was designed by measuring foraging behavior of prey once conditioned to high and low risk environments, with the prediction that prey vigilance and foraging will depend on the amount of time predators are near and on risk of attack (Higginson et al. 2012). Since then, studies tested this hypothesis by examining foraging behavior under high and low risk of predation and found mixed results, though many studies may have not granted prey enough time to assess risk, and many did not work under the assumption that prey are energy limited (Ferrari et al. 2009a). The mixed support for the risk allocation hypothesis can also be explained by behavioral correlation, in which individuals possess behaviors independent of predatory threat, and that the behavior is correlated in the absence or presence of a predator (Sih et al. 2002; Slos & Stoks 2006). Sih et al. (2003) found that if an individual spends more time out of refuge in the absence of a predator, that individual will most likely spend more time out of refuge in the presence of a predator, regardless of risk. This behavioral correlation can reduce the effect that risk of predation has on the behavior of prey. Thus, the risk allocation hypothesis can be expanded by accounting for these issues and by assessing direct antipredator behaviors rather than focusing on just the foraging tradeoffs associated with predation risk (Bednekoff & Lima 2011).

The threat level of predators changes throughout the diel cycle and is dependent on whether predators follow a diurnal, nocturnal, or crepuscular cycle (Thorpe 1978).

Diel periodicity differs from circadian rhythm in that the latter is endogenous and often less flexible to alteration (Daan & Koene 1981). Circadian rhythm has been shown to be a factor in the antipredator response of some species; they continued to exhibit cyclical antipredator behavior despite constant exposure to either light or dark (Kronfeld-Schor & Dayan 2003; Fraker 2008; Jones et al. 2011). Since diel periodicity is more plastic, one would predict that prey would minimize their risk of predation by expressing more intense antipredator behavior when predators are most active. Presence of predators cause shifts in diel periodicity of prey (Holopainen et al. 1991; Fraser et al. 1993; Culp 1988; Jedrzejewska & Jedrzejewski 1990; Daly et al. 1992; Fenn & MacDonald 1995; Pettersson et al. 2000; Kronfeld & Dayan 2003; Stuart-Smith et al. 2008; Bool et al. 2011; Bosiger et al. 2012). Damselfly larvae, *Enallagma cyathigerum*, change their diel periodicity of foraging behavior in response to chemical and chemical/visual cues of a fish predator (Koperski 1997). Mayfly (*Baetis spp.*) nymphs primarily drift during the night, correlating with reduced visual predator activity at night and avoidance of benthic foragers that are often nocturnal (Huhta et al. 2000). Rahlfs & Fichtel (2010) found that the circadian activity of prey effected their antipredator strategies; crypsis, freezing, and use of visual detection versus alarm cues was more common in nocturnal species. Red-backed salamanders (*Plethodon cinereus*) only avoided garter snakes (*Thamnophis sirtalis*) fed conspecifics but avoided garter snakes fed conspecifics and snakes fed a neutral diet during the day, in both a lab and field setting, reflecting risk assessment based on diel patterns of predators (Madison et al. 1999a,b; Madison et al. 2002; Sullivan et al. 2002, 2005). *Plethodon cinereus* responded more significantly to cues of snakes during periods of peak foraging activity of snakes than non-peak periods, suggesting that

predator biorhythms are responsible for determining antipredator behavior (Maerz et al. 2001). These studies demonstrate that it is important to consider peak activity times of predators when examining antipredator response in prey.

The risk allocation hypothesis also predicts that the level of predation risk is dependent not only on predator activity, but also on prey susceptibility, which varies throughout the season and through ontogenetic development. Prey under chronically high predation are more energy limited than prey under low predation and show a greater increase foraging during times of low predator activity compared to prey under chronically low predation (Lima & Bednekoff 1999). Therefore, diel activity of prey can be state dependent: salmon (*Salmo salar*) exhibit more diurnal behavior (higher risk) when their risk of starvation was higher and if they were part of the migrating cohort that year (Metcalf et al. 1998). When Greylag geese (*Anser anser*) undergo molting, a stage which increases risk of predation, they shift to nocturnal foraging to reduce predation risk, reflecting state-dependent alterations in diel activity (Kahlert et al. 1996). Although much focus has been placed on the effect of predators on prey diel cycles, it is hypothesized that predator activity patterns depend on the cycles of their prey (Daan & Koene 1981). Worischka et al. (2012) found that the patterns of predator and prey behavior based on spatiotemporal factors result in variable predation risk, and that both predators and prey affect each other's foraging activity. Therefore, it is important to consider both predator and prey diel cycles in defining whether a predator-prey interaction is high or low risk.

Effects of Turbidity on Antipredator Behavior

Increased turbidity is the phenomenon in which water decreases in clarity due to suspended organic or inorganic solids, and can arise from natural or anthropogenic causes (Nixon 1995). Some anthropogenic activities that can increase turbidity are changes in nearby land use, pollution, and recreational activities (Davies-Colley & Smith 2001; Schwartz et al. 2008) Although turbidity is common in aquatic environments, its dynamics are variable in that it can be constant through space and time, or have dynamic shifts, primarily due to rainfall (Chow-Fraser 1999). The dynamics of turbidity in aquatic systems are expected to alter due to climate change because of increased precipitation events that contribute to erosion and run-off (Nelson et al. 2009). Turbidity affects the visual environment in aquatic systems by both decreasing penetration of light and increasing the scattering of light (Bruton 1985). This can decrease the transmission of visual information within a system, which has been shown to have impacts on community structure in streams (Davies-Colley & Smith 2001; VanLandeghem et al. 2011).

One way in which turbidity alters community structure is by affecting predator-prey dynamics; predators and prey may use different modalities to detect one another that may alter in sensitivity due to turbidity in a non-parallel way. For example, VanLandeghem et al. (2011) found that the proportion of prey caught by ambush predators and cruising predators did not differ in clear and intermediate levels of turbidity, but that cruising predators caught significantly more prey in high turbidity situations. This suggests that turbidity can change the ability of certain prey/predators to respond to cues accordingly, with potential to shift community dynamics. The negative effects of turbidity on a predator's ability include decreasing specificity of attack of

species and of size classes within a species (Crowl 1989; Jönsson 2011; Shoup & Wahl 2011). The negative effects of turbidity on prey include a reduction of the reaction distance of prey to predators (Miner & Stein 1996; Abrahams & Kattenfeld 1997), increased conspicuousness of clear-bodied prey (Rowe 1998), decreased foraging in prey in the presence of predator cues (Leahy et al. 2011), lack of antipredator behavior (Gregory 1993; Swanbrow-Becker & Gabor 2012), decreased ability to generalize learned recognition (Ferrari et al. 2010; Chivers et al. 2013), and increased mortality (Gadomski & Parsley 2005). However, turbidity may shield prey from predators by providing camouflage and reduced visibility, which may increase use of suitable habitat by prey (Abrahams & Kattenfeld 1997; Van de Meutter 2005; VanLandeghem et al. 2011), but this effect depends on the evolutionary history of the predator (Heimstra et al. 1969). For example, bluegill sunfish (*Lepomis macrochirus*) are commonly found in turbid habitats and have increased foraging efficiency in intermediate turbidity (Miner & Stein 1993). The effects of turbidity on prey survival depends on their ontogeny; larvae and small aquatic animals tend to benefit from less predation due to decreased conspicuousness in turbid conditions (Utne-Palm 2002; Lehtiniemi et al. 2005; Pekcan-Hekim & Lappalainen 2006; but see Ohata et al. 2011).

Study System and Habitat

The Edwards Plateau is a region of Central Texas that includes the Edwards Aquifer, an artesian aquifer composed primarily of karst, which creates localized springs and water-filled caves, many of which are thermostable (USFWS 1996). It is thought to be one of the most diverse aquifers in the world, supporting blind catfish, aquatic

crustaceans, fish, and terrestrial cave invertebrates (HCP 2012). The springs are also habitat for many species of *Eurycea*, a group of neotenic, fully-aquatic plethodontid salamanders, many of which are endangered (Chippindale et al. 2000). The phylogenetic and phylogeographic relationships of these *Eurycea* have been examined (Chippindale et al. 1998; Chippindale et al. 2000; Hillis et al. 2001; Bendik 2006; Lucas et al. 2009) and there is growing research on their antipredator behavior (Epp & Gabor 2008; Davis et al. 2012; Epp 2013). The Barton Springs salamander (*Eurycea sosorum*) is a federally endangered species, endemic to Barton Springs, Travis County, Texas (Chippindale et al., 1993). It is an epigean species, with largest population sizes in Eliza and Parthenia Spring (HCP 2013). Predators of *E. sosorum* include birds, aquatic invertebrates, and fish, with documented sightings of predation by crayfish, sunfish and bass (HCP 2013). Gillespie (2011) argued that *E. sosorum* responds to visual and bioelectric cues of bass (*Micropterus salmoides*) and crayfish (*Procambarus clarkii*), but not olfactory cues of either in the day time. However, using more standardized methods DeSantis et al. (2013) found that *E. sosorum* shows antipredator response (freezing) to chemical cues of three fish, with greater response towards the native *M. salmoides* than the introduced redbreasted sunfish (*Lepomis auritus*) and the non-predatory mosquitofish (*Gambusia affinis*), indicating that they exhibit a threat sensitive response to kairomones.

The San Marcos salamander (*Eurycea nana*) is a federally threatened species endemic to the San Marcos River, San Marcos, Texas (Nelson 1993). Water quality degradation, drought, recreational activity, and invasive predators threaten them. They are primarily found in rocky areas rich with vegetation and have not been found in silty, unvegetated areas (USFWS 1996). They are also primarily found in flowing water that is

stenothermal (Norris et al. 1963). Their main predators are sunfish (*Lepomis cyanellus*), the redbreast sunfish (*Lepomis auritus*), crayfish (*Procambarus clarkii*), and largemouth bass (*Micropterus salmoides*) (USFWS 1996). Wild-caught and first generation captive-bred *E. nana* respond to chemical cues of the native *M. salmoides*, and to the chemical cues of the non-native *L. auritus* (Epp & Gabor 2008; Davis et al. 2012; Epp 2013). Their antipredator response is a reduction of general activity. It was also found that captive-bred salamanders show risk-sensitive antipredator behavior in response to diet cues paired with cues of *L. auritus* and *M. salmoides*, but that wild-caught salamanders showed less response in general and exhibited no threat-sensitive response (Epp 2013). Davis et al. (2012) examined the antipredator response of *E. nana* to chemical cues of a native sunfish predator (*L. cyanellus*), a sympatric introduced sunfish (*L. auritus*), an allopatric sunfish (*L. gibbosus*), and a sympatric, non-native cichlid (*Herichthys cyanoguttatum*) and found that each cue caused a reduction in activity, indicating ability to generalize predator response. Additionally, *E. nana* exhibited similar antipredator behavior to *M. salmoides* as *L. auritus*, but stress hormone levels (CORT) were elevated in response to *M. salmoides* alone (Davis & Gabor in press). Both *E. sosorum* and *E. nana* are captive bred at the San Marcos Aquatic Resources Center for the purposes of potential reintroduction.

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CHAPTER II

ANTIPREDATOR RESPONSE OF *EURYCEA NANA* TO A NOCTURNAL AND A DIURNAL PREDATOR

Abstract

Predators and prey exhibit shifts in activity reflective of their diel cycle, which causes the risk posed by predators to vary through time. This represents a temporally variable threat for prey, and it is expected that prey would change their antipredator behavior based on the circadian foraging cycles of their predators. We examined whether *Eurycea nana*, a federally threatened neotenic salamander, exhibits a differential anti-predator response to kairomones of a diurnal predator (*Lepomis cyanellus*) and a nocturnal predator (*Procambarus clarkii*). In concordance with the threat sensitivity hypothesis, we predicted that *E. nana* would match their level of antipredator behavior with the perceived threat level. We predicted that *E. nana* would show increased antipredator behavior (reduced activity) to the diurnal predator during the day, and increased antipredator behavior to the nocturnal predator at night. We found that although there was significant antipredator behavior of *E. nana* toward *L. cyanellus*, there was no significant response to *P. clarkii* and no significant effect of time of day towards either predator. It is an important conservation initiative to study the effect of diel cycles on antipredator behavior for potential reintroduction purposes and to better understand the intricacies of behavior that can affect the vulnerability of a species.

Introduction

In natural habitats, the risk of predation often varies across space and time and between predator species. The threat sensitivity hypothesis (Helfman 1989) suggests that prey should alter the intensity of their antipredator behaviors to match the risk posed by a predator because it minimizes the costs associated with predator avoidance, such as missed foraging or mating opportunities (Sih 1992; Lima & Bednekoff 1999). Thus, when the patterns of variation in threat posed by predators are cyclic or predictable, prey may exhibit matching cyclic variations in the intensity of their antipredator behaviors. One cyclic and predictable pattern is the threat posed by most predators as they shift through diel cycles of foraging activity (Kronfeld-Schor & Dayan 2003). Because of this cycle of threat, selection should favor prey that respond more intensely to stimuli of a given predator during the peak foraging times of that species and less intensely at other times, thereby maximizing benefits and minimizing costs of predator avoidance.

Many studies have examined chemically-mediated shifts in foraging activity of prey as related to temporal shifts in predation threat, and found a decrease in prey foraging during diel periods with high predator activity (e.g. *Emallagma cyanthella*; Koperski 1997; *Salmo salar*; Metcalfe et al. 1998; *Vargula annecohenae*; Gerrish et al. 2009). Fewer studies, however, have examined directly the effect of diel cycles on antipredator behaviors. In those that did, heavy presence of diurnal predators caused prey to exhibit more nocturnal behavior and more intense antipredator responses during the day (Lima 1998; Kronfeld & Dayan 2003; Bosiger et al. 2012). Further, it has been demonstrated that some aquatic prey shift the intensity of their antipredator behavior after

experiencing diel fluctuations in predation threat (Jachner 1996; Koperski 1997; Sullivan et al. 2005; Ferrari 2008), demonstrating the importance of cyclic variation in predation threat for prey.

Learning provides an effective way for prey to exhibit threat-dependent antipredator behavior because prey can learn the spatio-temporal dynamics of their predators (Kelley & Magurran 2003). Therefore, most studies examining the role of diel cycles on antipredator behavior first conditioned prey using different concentrations of predation cues (chemical alarm cues) at differing times and tested subsequent antipredator response (Ferrari 2008; Ferrari & Chivers 2009; Bosiger et al. 2012). Field studies have examined how diel cycles of predator-experienced prey change due to the presence of introduced predators, differing migratory patterns of their predators, and environmental influences (Stuart-Smith et al. 2008; Nico 2010; Keefer et al. 2013). However, to our knowledge, no study has examined the effect of the diel cycle on threat-sensitive antipredator behavior using predator-naïve individuals and native prey. Flecker (1992) suggested that antipredator behavior could become hard-wired if prey face a high level of threat in natural habitats because that eliminates the need for potentially deadly encounters required for learning. It is therefore predicted that diel shifts in antipredator behavior, which minimize unnecessary energy costs of antipredator behavior, may be innate as well.

In this study we explored whether predator-naïve San Marcos salamanders (*Eurycea nana*) reflected a difference in perceived threat level by exhibiting differential antipredator response to a diurnal predator (green sunfish, *Lepomis cyanellus*) and a nocturnal predator (crayfish, *Procambarus clarkii*) during the day and at night. *Eurycea*

nana is a federally threatened (USDI 1980) and IUCN red-listed (IUCN 2011) neotenic salamander endemic to the headwaters of the San Marcos River, Hays Co., TX. *Eurycea nana* is typically found along the benthos and uses vegetation and rocks as refuge. Although *E. nana* is presumed to be nocturnal (Thaker 2006), the prey of *E. nana* consists mostly of diurnal aquatic invertebrates and it has been found that *E. nana* has significantly more prey in their gut during the day than at night, suggesting they may at least forage diurnally (Diaz 2010).

Predator-naïve individuals of *E. nana* exhibit antipredator responses (reduced activity) to the chemical cues of *L. cyanellus* during the night (Epp & Gabor 2008; Davis et al. 2012). While no study has examined the response of *E. nana* to crayfish, they are predators of *Eurycea* sp. and a congener, *Eurycea sosorum*, does respond to crayfish kairomones (Tupa & Davis 1976; Resetarits 1991; USFWS 1996; Davis et al. unpublished data). We predicted that the antipredator response of *E. nana* to a diurnal predator would be more intense (decreased ambulatory time) in the daytime as compared to nighttime, and conversely, that antipredator response to a nocturnal predator would be more intense at night, reflecting shifts in perceived predation threat.

Methods

We tested the antipredator response of predator-naïve, captive-bred *Eurycea nana* from the San Marcos Aquatic Resource Center (SMARC) to three treatments: (1) a diurnal predator, the green sunfish, (*Lepomis cyanellus*) (n=16) (2) a nocturnal predator, the crayfish (*Procambarus clarkii*) (n=16), and (3) a control, well water (n=16). We tested the antipredator response of *E. nana* to each of these three treatments during

daytime hours (2 hrs after sunrise - 2 hrs before sunset) and during nighttime hours (2 hrs past sunset - 2 hrs before sunrise).

Housing and Stimulus Acquisition

We acclimatized both male and female *E. nana* to their respective 12:12 light:dark diel schedule for 2 weeks prior to testing at the SMARC. Test subjects were the adult offspring of captive salamanders and thus, were naïve to predators, which controlled the confounding factor of habituation to or learning of predators. To acquire predator cues, we collected crayfish from the San Marcos River and fed them a controlled diet of Kyorin™ sinking wafers for two weeks to ensure absence of salamander cues in their diet. We collected green sunfish from pools devoid of salamanders at SMARC, and thus, we did not feed them a controlled diet. We collected crayfish and sunfish chemical cues by separately placing predator individuals in a volume of water proportional to their body volume by displacement (230 ml of water per 1 ml of stimulus animal) for 24 hours, as per Epp & Gabor (2008). After 24 hours, we stirred and collected water from the containers in 60 ml portions, randomized and coded the cues, and then froze them until immediately prior to testing.

Behavioral Assays

We observed salamanders under dim red light during nighttime trials and under full-spectrum fluorescent lighting during the day. We tested salamanders in 9.5 l glass aquaria, which were filled with 4.5 l of well water and covered by black plastic on three sides to reduce external disturbance. We acclimatized individuals for at least 20 min before testing. Following acclimatization, we recorded the amount of time spent ambulatory (walking or swimming) for 8 min to determine pre-stimulus activity levels

(Epp & Gabor 2008). We then administered a treatment stimulus (crayfish, sunfish, or control) by injecting 50 ml of cue followed by 50 ml of well water into the aquarium at a rate of 2 ml/s via a 60-ml syringe attached to a plastic tube. The tube was attached to the side of the aquarium and extended from 2 cm below the water surface on the interior to just below the aquarium base on the exterior. After introduction of the stimulus, we recorded the amount of time *E. nana* spent active for another 8 min to determine post-stimulus activity. After trial completion, we sexed subjects using a candling technique (Gillette and Peterson 2001) and measured their snout-vent length (SVL) before placing them into a separate tank for tested individuals. We did not use individuals more than once for any treatment. We cleaned all tanks and testing equipment with 3% hydrogen peroxide and fresh well water in between each trial to remove chemical stimuli (Epp & Gabor 2008).

Data Analysis

We calculated an activity index for each subject, defined as the difference between post-stimulus activity and pre-stimulus activity, such that positive values indicate increases in activity and negative values indicate decreases in activity in response to the stimulus. We compared activity indices using a two-factor Analysis of Variance and Tukey's HSD ($\alpha = 0.05$) with cue treatment and testing time (day vs. night) as factors. Additionally, to assess whether potential effects of testing time (day vs. night) were due to differences in general activity over the diel cycle, we compared pre-stimulus activity between day and night treatment groups. All analyses were performed in R 3.0.1 (R Project for Statistical Computing, <http://r-project.org/>).

Results

Two-factor ANOVA showed a significant effect of predator treatment ($F_{2,85}=23.98$, $P < 0.001$) but not time of day ($F_{1,85}=0.29$, $P=0.59$) and no interaction between predator treatment and time of day ($F_{2,85}=1.32$, $P=0.27$). (Fig. 1). Post-hoc comparisons between all treatments revealed a significant difference only between the control and the sunfish cue, and a significant difference between the crayfish cue and sunfish cue (Tukey's HSD, $P < 0.001$), but all other pairwise comparisons were non-significant. Finally, there was no difference in pre-stimulus activity levels of salamanders between day and night tested treatment groups ($t = -0.03$, $df = 89$, $P = 0.97$).

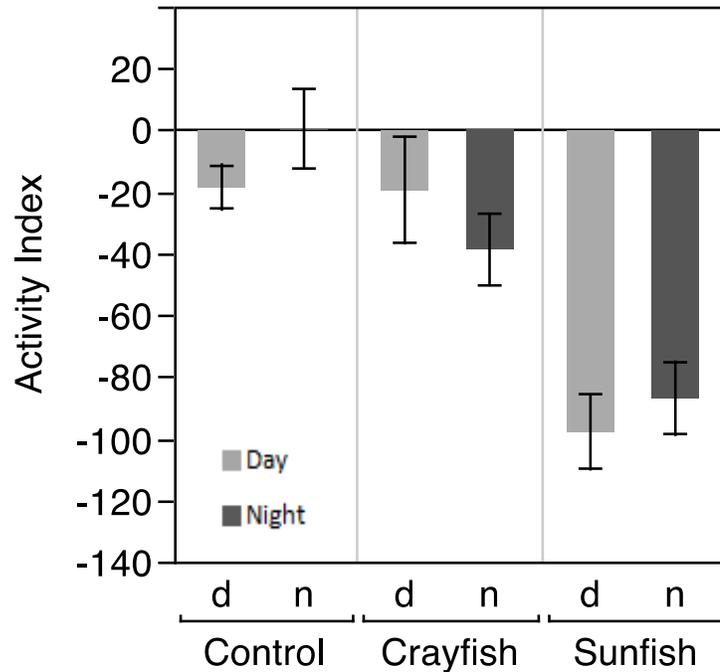


Figure 1. Activity Indices for *Eurycea nana* to a nocturnal (crayfish) and a diurnal (sunfish) predator, during the day and the night. Mean (\pm SE) activity index (difference between post-stimulus activity and pre-stimulus activity, in seconds) of *E. nana* in response to stimuli introduced in six treatments.

Discussion

There was no effect of day on the intensity of antipredator behavior of salamanders to either the nocturnal crayfish predator or diurnal sunfish predator. This is not consistent with the threat sensitivity hypothesis because *E. nana* did not exhibit a significant change in activity level based on temporally variable threat levels of predators. This lack of threat sensitivity to predation threat based on the time of day is consistent with a study of *Percids* (*Perca fluviatilis* and *Gymnocephalus cernuus*), which exhibited a lack of temporal difference in antipredator response to nocturnal and diurnal

piscivorous predators (Ylonen et al. 2007).

One hypothesis for our findings is that predator-naïve individuals are unable to associate predator threat levels with their circadian rhythm because they need experience with predators to assess diel changes in threat. For example, Madison et al. (2002) found that predator-experienced salamanders (*Plethodon cinereus*) had a threat-sensitive response towards predators related to shifts in diel activity. Thus, *E. nana* may first need experience coupling kairomone presence or other threat indicators (e.g. conspecific alarm cues) with time of day before exhibiting diel patterns of antipredator behavior (Ferrari & Chivers 2006). Epp (2013) demonstrated that predator-experienced (wild-caught) *E. nana* exhibited less intense responses to high-risk predators than predator-naïve (captive-bred) salamanders, suggesting that experience is important for risk assessment in this species. However, predator-experienced *E. nana* individuals still respond to diurnal predators during the night at low-risk times (Epp & Gabor 2008; Davis et al. 2012; Epp 2013).

Eurycea nana did not show antipredator response to crayfish chemical cues and it is a possibility that *E. nana* has not adapted an innate antipredator response to crayfish. Since it is unknown whether crayfish are native to the San Marcos River, a lack of innate response may be attributed to a shorter coevolutionary history than *E. nana* has with native sunfish. It has previously been demonstrated that prey may lack innate antipredator strategies in response to introduced species (Schlaepfer et al. 2005). Tadpoles of the western spadefoot toad (*Pelobates cultripes*) were unable to innately recognize kairomones of the invasive *P. clarkii*, but were able to learn to recognize cues with associative learning (Polo-Cavia & Gomez-Mestre 2014). Thus, *E. nana* may require learning in order to recognize crayfish as a threat, and our use predator-naïve

(captive-bred) salamanders may not reflect actual antipredator response to crayfish in the wild. For example, it was found that the antipredator response of tadpole prey (*Lithobates sylvaticus*) reflects the temporal variation in level of predator threat, but multiple conditioning stages are first needed for *L. sylvaticus* to exhibit a continuous response (Ferrari et al. 2009; Ferrari et al. 2013).

Fraker (2008) proposed that many species have circadian rhythms that correspond to predation risk, but because most prey have multiple predators which themselves vary in activity throughout the diel cycle, that prey should respond less strongly to threat during their period of high activity. However, *E. nana* does not have a clear circadian rhythm; there was no difference in pre-stimulus activity level of subjects between daytime and nighttime. Therefore, the lack of change in activity level of *E. nana* should be solely based on predatory threat, and not based on the diel periodicity of *E. nana*.

The strong cessation of activity to sunfish kairomones was consistent with previous work (Epp & Gabor 2008; Davis et al. 2012). The continuous response to sunfish throughout the diel cycle does not reflect a threat sensitive response to diel cycle, but corroborates the fact that predator-naïve *E. nana* have a strong response to sunfish. It is possible that *E. nana* is exhibiting threat sensitive behavior based on species, not diel cycle, and that sunfish are generally a greater predatory threat than are crayfish.

However, a hypothesis for a lack of response to crayfish is that *E. nana* may use other modalities, such as mechanical cues, to respond to crayfish, although other amphibians do respond to crayfish chemical cues (Nunes et al. 2013; Davis et al. unpublished data). *Eurycea nana* may require a combination of chemical cues, such as alarm or diet cues with predator kairomones in order to respond to crayfish, which would be consistent with

studies of other prey organisms (Ferrari et al. 2010; Nunes et al. 2013)

Since the foraging technique of crayfish and sunfish differs, it is possible that reduction of activity in *E. nana* is not an appropriate antipredator response to crayfish as it is to sunfish. The type of antipredator behavior exhibited by prey may vary based on both the foraging strategy of the predator (Rahlfis & Fitchel 2010) and the presence of refuge in the environment (Turner 1996). Our experiment may not have provided an adequate test for antipredator behavior towards crayfish, which may require an additional variable such as use of refuge.

Eurycea nana are currently being bred at the San Marcos Aquatic Resource Center for potential reintroduction purposes, so the lack of response of *E. nana* to crayfish may have significant conservation implications in that *E. nana* may have to undergo associative learning before reintroduction. Predator-naïve, hatchery-reared fish have been successfully trained to recognize predators by coupling alarm cues with predator kairomones (Olson et al. 2012). It is important to determine whether wild-caught *E. nana* respond to crayfish cues in order to create an effective reintroduction management plan.

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CHAPTER III

MULTIMODAL COMMUNICATION AND THE EFFECTS OF TURBIDITY ON THE ANTIPREDATOR BEHAVIOR OF THE BARTON SPRINGS SALAMANDER, *EURYCEA SOSORUM*

Abstract

Multimodal cue use can increase predator detection by prey, which decreases predation risk. Multimodal cue use is especially significant in habitats where a unimodal cue is altered by environmental noise. Turbidity can affect the behavior of aquatic organisms by degrading visual and chemical information in their environment, which may impair the ability of prey to appropriately recognize and respond to a predator. In such cases, prey may require a combination of cues to respond to predators. We examined multimodal cue use in the fully aquatic, endangered Barton Springs salamander, *Eurycea sosorum*, by determining whether they use chemical and visual cues to respond to a predator, the green sunfish (*Lepomis cyanellus*) and compared response to a non-predator control, the green-throat darter (*Ethostoma lepidum*). Further, we determined whether turbidity affected antipredator behavior. Our cue treatments included a fully crossed design including both chemical and visual cues of the predator and non-predator across two vision levels; clear (0 NTU) and low turbidity (~20 NTU). We found that *E. sosorum* significantly reduced activity and distance moved and increased latency to first move to predator chemical cues compared to non-predator chemical cues. There was no difference in antipredator behavior between predators and non-predators when *E. sosorum* had access to visual cues only. We found that *E. sosorum* had decreased antipredator behavior in the form of decreased latency to first move in turbid conditions.

Introduction

The use of multimodal cues by prey can improve localization, resolution, and amplification of predator cues (Partan & Marler 2005). Use of multimodal cues to recognize predators can either serve as a ‘back-up signal’ to increase accuracy of cue detection, or can function as ‘multiple messages’ to signify different aspects of predators (Johnstone 1996). Multimodal cue use as back-up signals is especially relevant in situations where one cue type compensates based on the sensory environment (Endler 1993). Use of multimodal cues by prey can increase fitness benefits by either decreasing risk of predation, thus increasing survival probability, or by decreasing the secondary costs associated with unnecessary antipredator behavior (Stynoski & Noble 2012). Aquatic prey may use multiple cues such as visual, chemical, tactile, and electric cues, to improve predator detection (Collin & Whitehead 2004; Park et al. 2008; Ward & Mehner 2010).

Amphibians can use visual cues alone to detect predators, however accuracy of detection is increased if visual cues are paired with other cues, suggesting that multimodal cues are important in predator detection (Mathis & Vincent 2000; Hettyey et al. 2012; Stynoski & Noble 2012). However, more studies found that amphibians generally do not use visual cues for predator recognition, instead relying on chemical and/or tactile cues (Stauffer & Semlitsch 1993; Kiesecker et al. 1996; Parris et al. 2006; Takahara et al. 2013). However, these results are based on terrestrial amphibians. It has generally been found that aquatic organisms use visual cues for predator recognition (Kelley & Magurran 2003; Brown et al. 2004; Hemmi 2005). The aquatic salamander, *Eurycea multiplicata griseogaster*, discriminates between predators and non-predators in

response to chemical cues, but exhibits antipredator behavior to both predator and non-predator in the presence of visual cues, suggesting the importance of multimodal cues in detection accuracy (Hickman et al. 2004). Thus, it is important to evaluate use of visual cues in amphibians to fully understand multimodal cue use in antipredator behavior.

Use of multimodal cues is linked to the signaling environment; increased environmental noise may contribute to use of multimodal cue use as backup signals (Higham & Hebets 2013; Smith & Evans 2013). Turbidity is a form of environmental noise that decreases penetrability of light within the water column, and can affect the visual and chemical environment for prey (Utne-Palm 2002). As well, turbidity can reduce antipredator behavior in aquatic prey (Gregory 1993; Miner & Stein 1996) and decrease generalized predator recognition (Ferrari 2009; Chivers et al. 2013), which can increase mortality (Horppila et al. 2012). If multimodal cues are used as ‘multiple messages’ to increase resolution of predator detection in clear conditions, then turbidity would likely decrease accuracy of predator recognition if one modality is obstructed (Ferrari et al. 2010). However, if multimodal cues function as back-up signals, then turbidity could increase antipredator response through sensory compensation by increasing use of chemical over visual cues (Hartman & Abrahams 2000; Leahy et al. 2011). Therefore, turbidity can cause an increase or decrease in antipredator behavior, depending on whether prey make use of multimodal cues in combination to improve detection accuracy, or if prey use multimodal cues to compensate for reduced perceptibility of a cue. If prey use unimodal cues for predator detection, turbidity is predicted to decrease antipredator behavior because turbidity affects predator detection in response to both visual cues and chemical cues (Lehtiniemi et al. 2005).

It is well known that anthropogenic factors such as land development, severe storms, and recreational activity have increased turbid conditions in waterways making turbidity an increasing issue for aquatic organisms (Henley et al. 2000; Davies-Colley & Smith 2001). Much of the work examining behavioral and life history impacts of turbidity has been on fish (Abrahams & Kattenfeld 1997; Gadomski & Parsley 2005; Ferrari et al. 2010; Swanbrow Becker & Gabor 2012; Chivers et al. 2013), but amphibians also suffer from increased turbidity (Secondi et al. 2007; Schmutzer et al. 2008). One reason turbidity is less studied in amphibians is because most amphibians leave the water after reproduction and metamorphosis. However, understanding the effects of turbidity on antipredator behavior is important for amphibians because alteration in predation regime is considered a significant threat to the class (Scoccianti 2004).

The Barton Springs salamander, *Eurycea sosorum*, is a federally endangered, IUCN red list species (IUCN 2013) that is neotenic, fully aquatic, and endemic to Barton Springs, Austin, Travis County, Texas. *Eurycea sosorum* is threatened by water quality degradation due to increased recreational use, increased land development, and reduced spring flow, which can increase turbidity (Feng et al. 2012; Dries et al. 2013). The turbidity levels of Barton Springs are typically <1 Nephelometric Turbidity Units (NTU), but can spike to ~40 NTU after rain events (White et al. 2003). NTU's measure how much light is reflected off of suspended solids, with higher reflectance (higher NTU's) corresponding to more suspended solids in a water column. *Eurycea sosorum* is often found in clear conditions and avoids silty turbid habitat (Dries et al. 2013). Therefore, increased turbidity might affect the ability of *E. sosorum* to detect and respond to

predators if they are unable to compensate for loss of sensory information, potentially decreasing their likelihood of survival in a predatory situation.

Eurycea sosorum shows antipredator response to kairomones, which are chemical cues emitted by a species that can be detected by prey, of redbreast sunfish (*Lepomis auritus*) in clear water (DeSantis et al. 2013). However, no study has determined whether *E. sosorum* can also use visual cues for antipredator response and whether turbidity has a significant effect on antipredator response. In this study, we examined the antipredator response of *E. sosorum* to the visual cues and chemical cues of two sympatric species, a green sunfish predator, *Lepomis cyanellus* and a greenthroat darter, *Etheostoma lepidum*, non-predatory control. We also determined whether turbidity had an impact on antipredator behavior and predator recognition.

Methods

Animal Maintenance

We conducted trials at the San Marcos Aquatic Resources Center (SMARC) in San Marcos, Texas, from June to August 2014 between 0900-1600 h. We tested 200 captive-bred, predator naïve, second-generation, adult *Eurycea sosorum* in response to a native predator, green sunfish (*Lepomis cyanellus*), and a non-predator control, greenthroat darter (*Etheostoma lepidum*). We housed salamanders in two flow-through tanks on a 12:12 h light/dark cycle and fed blackworms (*Lumbriculus variegatus*) and amphipods *ad libitum*. We collected three size-matched adult green sunfish on site at the SMARC, which were subsequently housed in a flow-through tank and fed a neutral diet of blackworms to control for diet cues. We collected fourteen size-matched adult

greenthroat darters from Comal Springs, TX, which we housed in three flow-through tanks and fed a neutral diet of blackworms and amphipods.

Study Design and Cue Acquisition

We examined the response of salamanders to cue treatments across two vision levels: (1) clear (0 NTU), and (2) turbid (20 NTU: mean \pm SE: 20 ± 0.4 NTU). We tested the following combination of cue treatments: (1) predator chemical cues paired with non-predator visual cues (n=25x2) (2) predator visual cues with non-predator chemical cues (25x2), (3) predator visual cues with predator chemical cues (25x2), (4) non-predator visual cues with non-predator chemical cues (n=25x2; Fig. 2). Individual salamanders were not used more than once for any treatment and were randomly assigned to their cue treatments using a random number generator. Chemical cues were collected by placing each fish in a volume of water proportional to their displacement (230 ml water per 1 ml displacement) for 24 hours, after which cues were pooled across individuals of the same species, and frozen until day of use (Mathis et al. 2003; Epp & Gabor 2008). The same individual recorded all behavior (KZ) and was blind to chemical cue treatment because chemical cues were coded.

Experimental Protocol

Our setup consisted of two adjacent 9.5 liter drip-flow tanks filled with 4.5 l of well water with an opaque divider between the tanks. Each tank had 12 3 x 3 cm squares drawn at the base. To quantify antipredator behavior in each trial we first allowed each salamander to acclimate for at least 20 min. Following acclimation, we recorded activity time (i.e. the amount of time of ambulatory activity (i.e. swimming or walking)) for 8 min to determine pre-stimulus activity levels. We also recorded total distance moved

(cm) by counting the number of squares through which each individual passed. We then introduced the chemical stimulus by injecting 50 ml of chemical cues with a 60-ml syringe attached to airline tubing 10 cm below the surface of the water at a rate of 2 ml/sec. We flushed the tubing with an additional 50 ml of well water at the same rate, following Epp & Gabor (2008). We introduced visual cues by removing the opaque divider and exposing the visual stimulus of either one adult green sunfish or four adult greenthroat darters (to provide similar size stimulus to a sunfish) in a clear tank adjacent to the focal salamander.

After introduction of the stimulus we recorded the amount of time *E. sosorum* spent active and the total distance moved for another 8 min to determine post-stimulus antipredator behavior. Following introduction of the cue we also recorded latency to first move, defined as the number of seconds before ambulatory activity was observed. We recorded these behaviors because decreased activity and decreased distance travelled are directly related to reduced activity levels, which decreases risk of predation in many aquatic organisms when exposed to sit and wait predators (Skelly 1994; Kats & Dill 1998). Latency to first move is also an important measure of antipredator behavior because the longer it takes for cryptic prey to resume activity; the more likely they are to avoid predation (Brown & Cowan 2000; Cooper et al. 2012). Following the experiment, individuals were sexed (Gillette and Peterson 2001) and had their snout-vent length (SVL) measured before being placed into a separate tank for tested individuals. We used 3% hydrogen peroxide and water to clean tanks of chemical cues between trials (Epp & Gabor 2008). For turbidity treatments, we added a measure of bentonite (Sturgis Rock Solid Solutions) to well water and stirred vigorously while using an aerator to maintain

suspension, prior to salamander acclimation. An aerator was placed in all treatment tanks to maintain consistency throughout the treatments. Turbidity levels were measured at the end of each trial using a calibrated turbidity meter (Hach 2100N Turbidimeter).

Data Analysis

Time spent moving (measured in seconds) in the pre- and post-stimulus trials was combined into an activity index for each individual, defined as the difference between post-stimulus activity and pre-stimulus activity, such that positive values indicate increases in activity. Distance index was calculated by taking the number of squares each individual moved pre- and post- stimulus and subtracting pre- from post-stimulus levels. We conducted information-theoretic model selection of linear models to assess the influence of turbidity, chemical cue, and visual cue treatments on activity index, distance index, and latency. We z-transformed the three variables in order to standardize the variables. To better-fit assumptions of normality, a constant was added to activity index (3.3) distance index (4.6) and latency (0.09) to make all values positive, then all three variables were ($x^{0.5}$) transformed. Removing outliers did not significantly affect results and were left in the analysis. We calculated Akaike Information Criterion corrected for small sample size (AICc) and Akaike weights. Model averaging was used to obtain parameter estimates by averaging candidate modes with Akaike weights within 10% of the highest ($\Delta < 4.5$) (Burnham & Anderson 2002; Symonds & Moussalli 2011). All tests were performed using R (3.0.1)

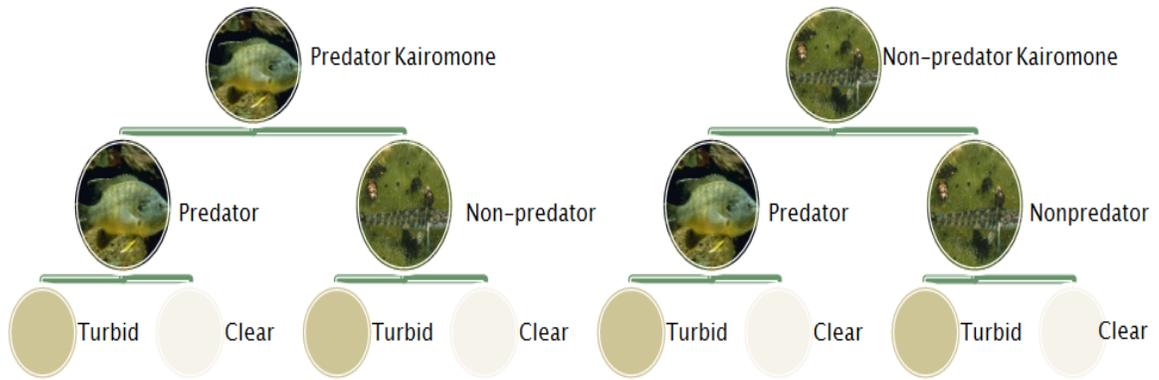


Figure 2: Schematic of experimental design involving chemical cues, visual cues, and turbidity. The predator treatment was in response to green sunfish (*Lepomis cyanellus*) while the green-throat darter (*Etheostoma lepidum*) was the non-predator. Turbidity treatments are either clear (0 NTU) or turbid (~20 NTU).

Results

We identified 8 plausible models to predict activity index in *E. sosorum* in response to treatments (Table 1). Chemical cue was the most plausible explanation for change in activity and was a consistent predictor across all models, being 2.89 times more plausible than use of visual cues and 1.5 times more plausible than turbidity affecting activity (Table 2). Salamanders reduced activity in response to predator chemical cues to a greater extent than non-predator chemical cues (Fig. 3). We identified 8 plausible models to explain distance index (Table 1). Chemical cue was also a consistent predictor in each model, and explained change in distance 1.56 times better than did visual cues (Table 2). Salamanders moved less distance when exposed to predatory sunfish chemical cues than to the non-predatory darter chemical cues (Fig. 4). Turbidity was included in six of the candidate models and explained change in distance 1.4 times better than did visual cues. Latency to first move was best predicted by chemical cue, which was present in all 9 candidate models (Table 1) and explained 1.56 times better than did visual cues.

Turbidity was a predictor in 8 models and explained latency to move after cue introduction 1.38 times better than did vision (Table 2). Latency to move increased in response to predator cues as compared to non-predator cues, and latency to move was less in turbid conditions as compared to clear conditions, regardless of cue type (Fig. 5).

Table 1: Values of AIC_c to determine the effects of turbidity, visual cues, and chemical cues on antipredator behavior. Values of the Akaike Information Criterion to determine the effect of chemical cue (Chem), visual cue (Vis), and turbidity (Turb) on antipredator behavior of *Eurycea sosorum*, for AIC < 4.5. We report the Akaike Information Criterion corrected for small sample size (AIC_c), number of parameter estimates (k), Akaike weight (w), and -2 log lik deviance (D) *'s indicate interactions between variables.

Model	AIC _c	Δ AIC _c	w	D	k
Activity Index					
Chem+Turb	71.8	0.00	0.27	-31.79	4
Chem	72.4	0.56	0.20	-33.11	3
Chem+Turb+(Chem*Turb)	73.2	1.37	0.14	-31.42	5
Chem+Vis+Turb	73.8	2.00	0.10	-31.74	5
Chem+Vis	74.3	2.55	0.08	-33.07	4
Chem+Vis+Turb+(Chem*Turb)+(Vis*Turb)	74.8	2.96	0.06	-30.08	7
Chem+Vis+Turb+(Chem*Vis)+(Vis*Turb)	75.0	3.16	0.06	-30.18	7
Chem+Vis+(Vis*Turb)	75.8	4.03	0.04	-32.75	5
Distance Index					
Chem+Vis+Turb	-10.3	0.00	0.25	10.31	5
Chem+Turb	-10.0	0.29	0.21	9.11	4
Chem+Vis+Turb+(Chem*Turb)+(Vis*Turb)	-8.8	1.47	0.12	11.72	7
Chem+Vis+Turb+(Chem*Vis)+(Vis*Turb)	-8.7	1.59	0.11	11.66	7
Chem+Turb+(Chem*Turb)	-8.1	2.26	0.08	9.19	5
Chem+Vis	-6.9	3.45	0.04	7.53	4
Chem+Vis+Turb+(Chem*Vis)+(Chem*Turb)+(Vis*Turb)	-6.7	3.62	0.04	11.73	8
Chem	-6.7	3.63	0.04	6.40	3
Latency					
Chem+Turb	247.7	0.00	0.22	-119.74	4
Chem+Vis+Turb	248.2	0.48	0.18	-118.92	5
Chem+Vis+Turb+(Chem*Vis)+(Vis*Turb)	248.6	0.86	0.15	-116.96	7
Chem+Vis+Turb+(Chem*Vis)+(Chem*Turb)	249.1	1.36	0.11	-117.20	7
Chem+Turb+(Chem*Turb)	249.7	1.94	0.09	-119.65	5
Chem+Vis+Turb+(Chem*Vis)+(Chem*Turb)+(Vis*Turb)	250.5	2.83	0.05	-116.84	8
Chem+Vis+(Chem*Vis)	250.6	2.92	0.05	-120.14	5
Chem	251.4	3.67	0.04	-122.63	3
Chem+Vis+Turb+(Chem*Turb)+(Vis*Turb)	251.7	3.95	0.03	-118.50	7

Table 2: Model-averaged coefficients for comparison of effects of turbidity, visual cues, and chemical cues on antipredator behavior. Model-averaged coefficients ($\Delta < 4.5$). Lower and Upper 95% CI reported.

Model	Estimate	Adjusted SE	z	P	Lower	Upper
Activity Index						
Chemical	-0.148	0.044	2.967	0.003*	-0.246	-0.050
Visual	0.028	0.056	1.187	0.395	-0.092	0.148
Turbidity	0.068	0.050	1.58	0.235	-0.044	0.179
Chemical*Visual	0.062	0.082	0.754	0.451	-0.100	0.225
Chemical*Turbidity	0.070	0.082	0.851	0.395	-0.092	0.232
Visual*Turbidity	-0.129	0.082	1.567	0.117	-0.291	0.032
Distance Index						
Chemical	-0.096	0.041	2.325	0.035*	-0.177	-0.015
Visual	0.074	0.051	1.437	0.161	-0.027	0.175
Turbidity	0.096	0.050	1.948	0.085	-0.0005	0.198
Chemical*Visual	0.010	0.067	0.150	0.597	-0.121	0.141
Chemical*Turbidity	-0.025	0.067	0.372	0.512	-0.156	0.106
Visual*Turbidity	-0.107	0.067	1.599	0.111	-0.237	0.024
Latency						
Chemical	0.237	0.113	2.102	0.036*	0.016	0.457
Visual	-0.201	0.116	0.365	0.715	-0.270	0.185
Turbidity	-0.201	0.097	2.083	0.037*	-0.391	0.012
Chemical*Visual	-0.261	0.146	1.777	0.076	-0.550	0.027
Chemical*Turbidity	0.067	0.146	0.455	0.649	-0.222	0.356
Visual*Turbidity	0.122	0.146	0.829	0.407	-0.166	0.410

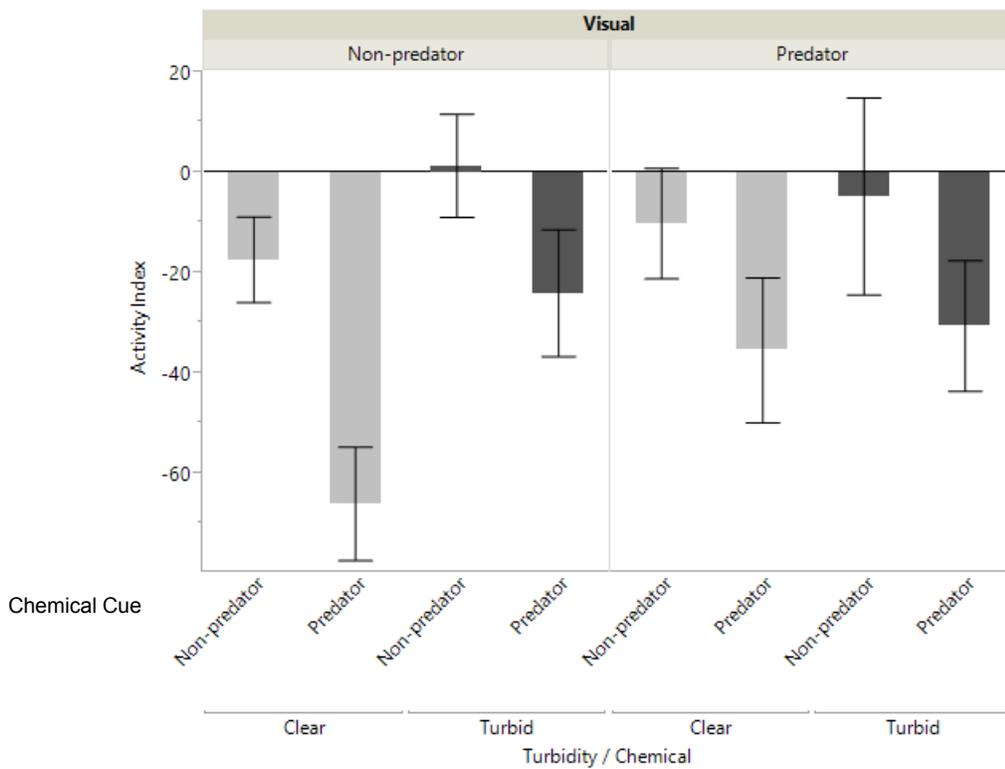


Figure 3: Activity index in response to predator and non-predator visual and chemical cues in clear and turbid conditions. Mean (\pm SE) untransformed activity index (difference between post-stimulus activity and pre-stimulus activity, in seconds) of *E. sosorum* in response to stimuli introduced in eight treatments. Light grey denotes clear conditions, dark grey denotes turbid conditions.

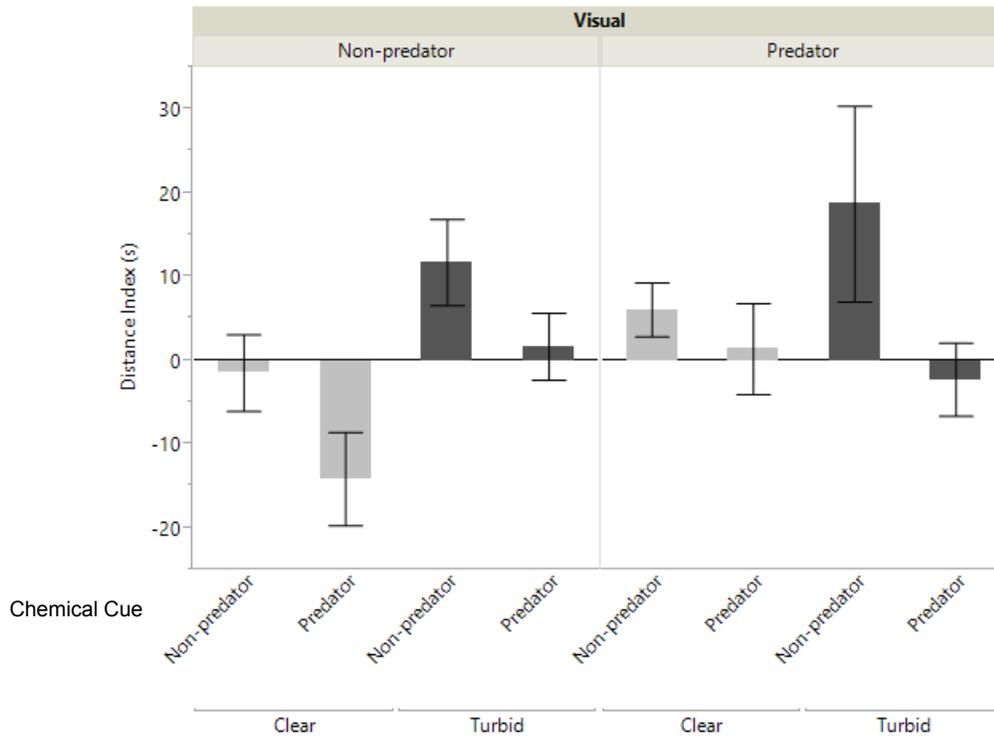


Figure 4: Distance index in response to predator and non-predator visual and chemical cues in clear and turbid conditions. Mean (\pm SE) untransformed distance index (difference between post-stimulus distance moved and pre-stimulus distance moved) of *E. sosorum* in response to stimuli introduced in eight treatments. Light grey denotes clear conditions, dark grey denotes turbid conditions.

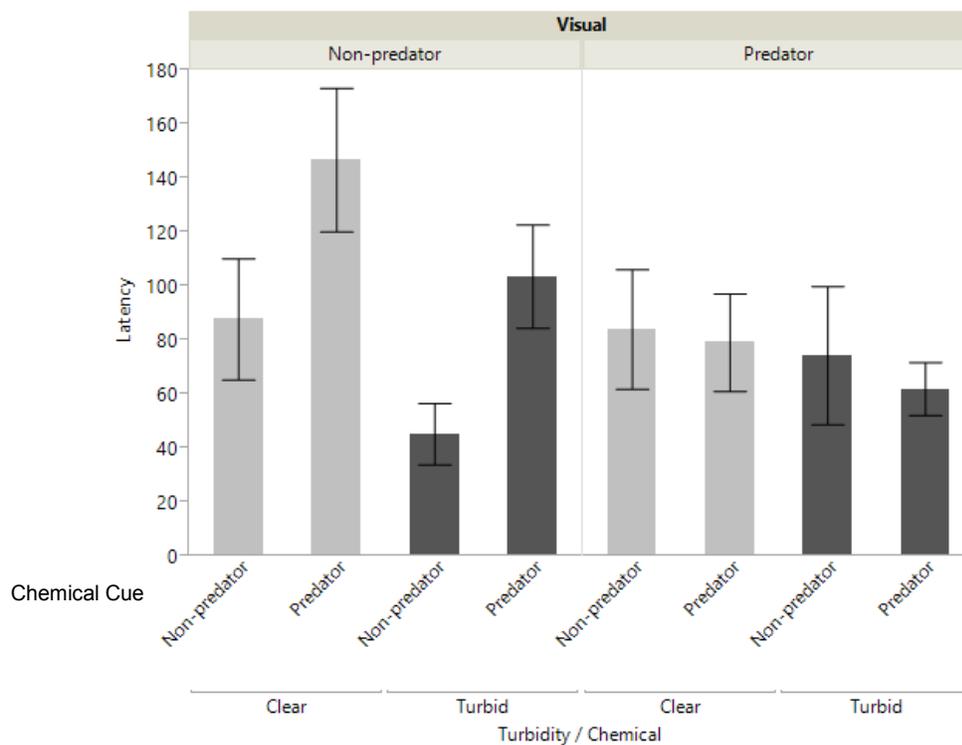


Figure 5: Latency to first move in response to predator and non-predator visual and chemical cues in clear and turbid conditions. Mean (\pm SE) untransformed latency to first show ambulatory activity (s) of *E. sosorum* in response to stimuli introduced in eight treatments. Light grey denotes clear conditions, dark grey denotes turbid conditions.

Discussion

Eurycea sosorum exhibited antipredator behavior in the form of decreased activity, decreased distance moved, and increased latency to first move, in response to predator chemical cues compared to non-predator chemical cues. The antipredator behavior of *E. sosorum* did not differ in response to visual cues of predator versus a non-predator. This result, as well as lack of any interactions between chemical and visual cues, suggests they do not use multimodal cues as ‘back-up cues’ or as ‘mixed message’ cues in their anti-predator detection. Therefore, we predict that any effects of turbidity act

on unimodal chemical cue detection. Under turbid conditions, salamanders showed decreased antipredator behavior, in the form of decreased latency to first move, as compared to clear conditions.

The lack of evidence of multimodal cue use in our experiment may be attributed to high neural cost of reducing uncertainty, which reduces reliance on multimodal cues in situations when unimodal cues can be used alone for predator detection (Bernays & Wcislo 1994; Santangelo et al. 2010; Munoz & Blumstein 2012). Another hypothesis is that visual cues do not contribute to antipredator detection due to the spatial range required for visual perception of predators (Utne-Palm 2002). It is also possible that our experimental design affected visual cue transmission, as previous experiments examining visual cues typically only have one pane of glass between stimulus and receiver rather than two (Hettyey et al. 2012; Swanbrow-Becker & Gabor 2012). Alternatively, the reliance on chemical cues alone could be phylogenetically constrained; reliance on chemical cues may be due to evolutionary persistence of specialized detection (Bernays & Wcislo 1994). Thaker & Gabor (2006) found that the closely related *E. nana* primarily uses chemical cues over visual to associate with conspecifics, suggesting a reliance on chemical cues, not visual cues, for detection. A fourth hypothesis for the lack of apparent multimodal cue use is that *E. solorum* may use modalities other than vision, such as tactile or electric cues, to detect predators. Gillespie (2011) found that *E. solorum* responded to visual and bioelectric cues crayfish predators, *Procambarus clarkii* and bass predators, *Micropterus salmoides* and did not respond to chemical cues of these predators. However, the study design had low sample size and lacked kairomone cue acquisition standardization. Further studies are required to determine whether other cues

are used for predator detection.

The lack of antipredator behavior in our experiment in response to predator visual cues is consistent with the data that show that many amphibians rely primarily on chemical rather than visual cues in predator detection, but is unexpected considering that other aquatic species tend to rely on vision (Brown et al. 2004; Takahara et al. 2013). However, our result of increased antipredator behavior in response to predator kairomones corroborates previous work showing that *E. sosorum* exhibits antipredator behavior towards chemical cues of the redbreast sunfish, *L. auritus* (DeSantis et al. 2013). The recognition of green sunfish as a predator is predicted given that Davis et al. (2012) found predator generalization using chemical cues to sunfish species in the closely related *Eurycea nana*. The reliance on chemical cues may be due to their ubiquitous nature and persistence in freshwater (Ward & Mehner 2010) although visual cues are perceived quicker than chemical signals (Endler 1993).

In response to predator and non-predator chemical cues, salamanders showed a decrease in latency to first move. This is consistent with our prediction that antipredator behavior would diminish in turbid conditions if unimodal cues were used. However, activity and distance indices were not significantly different in clear and turbid conditions, suggesting turbidity does not affect all behavioral antipredator responses. One hypothesis for reduced antipredator behavior is the ‘turbidity as cover’ hypothesis, which predicts that predation risk decreases in turbid conditions due to decreased conspicuousness, therefore reducing antipredator is adaptive because it reduces costs of unnecessary antipredator behavior (Gregory 1993). Although turbidity can affect foraging of predators (Turesson & Brönmark 2007), it is unlikely that risk of predation on *E.*

sosorum is decreased in turbid conditions. In fact, predation risk is greater in turbid conditions in systems where only predators are adapted to turbid conditions (Abrahams & Kattenfeld 1997). Green sunfish are tolerant of turbidity extremes: high turbidity does not affect the ability of *L. cyanellus* to forage or attack (Heimstra et al. 1969). In addition, juveniles of the related bluegill sunfish, *Lepomis macrochirus*, had greatest foraging efficiency at an intermediate turbidity level (~20 NTU) (Miner & Stein 1993). Since *E. sosorum* is more adapted to clear environments, turbidity may have a greater impact on the ability of *E. sosorum* to detect predators and less of an impact on the ability of green sunfish to capture prey. Effects of turbidity on other behaviors, such as foraging and mating, need to be assessed to determine the impact of turbidity on these endangered species.

The decreased antipredator behavior in turbid conditions has conservation implications for *E. sosorum*. We studied an intermediate turbidity range of 20 NTU, so our results are a conservative measure of changes in antipredator behavior because turbidity levels spike to double what we studied. Visual response to predators may be mediated by experience and turbidity could impact learning. For example, Chivers et al. (2013) found that prey were less able to learn and generalize predator recognition when raised in turbid conditions as compared to clear conditions. Van de Meutter (2005) found that antipredator behavior is unaffected if organisms are reared and subsequently tested in turbid conditions but antipredator behavior is decreased when reared in clear conditions and tested for response in turbid conditions. Munoz & Blumstein (2012) stress the importance of studying the interplay between learning and turbidity for reintroduction purposes, so determining the response of wild-caught salamanders that are predator-

experienced and turbid-experienced, is of great importance. Therefore, experience with learning in turbid conditions may be important, which can have significant implications for hatchery-raised species especially those that are being raised with the potential to return them to the field.

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