



# Behavioral and physiological antipredator responses of the San Marcos salamander, *Eurycea nana*



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## HIGHLIGHTS

- Salamanders showed higher antipredator response to chemical cues of a fish predator with lower temporal encounter frequency.
- Salamanders only showed a corticosterone response to a fish predator with a lower temporal encounter frequency.
- Salamanders may mute behavioral and corticosterone responses to predators with high temporal encounter frequencies.

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## ABSTRACT

Exposure to predatory stimuli typically results in the elevation of circulating glucocorticoid levels and a behavioral response of freezing or escape behavior in many prey species. Corticosterone (CORT) is the main glucocorticoid in amphibians and is known to be important in modulating many behaviors and developmental functions. The federally threatened San Marcos salamander, *Eurycea nana*, decreases activity in response to both native and introduced predatory fish, however, experience may further influence these interactions. To better understand the indirect effects of fish predators on this salamander, we examined both the antipredator behavior and water-borne CORT release rates in response to chemical cues (kairomones) from two fish species that varied in temporal risk of predation: (1) a low encounter frequency predator (largemouth bass, *Micropterus salmoides*), (2) a high encounter frequency predator (redbreast sunfish, *Lepomis auritus*), and (3) a blank water control. Salamanders reduced activity (antipredator response) after exposure to both predator treatments, but not to the blank water control, and the response to *M. salmoides* was significantly stronger than that to *L. auritus*. The CORT response (post-stimulus/pre-stimulus release rates) did not differ between the blank water control and *L. auritus* treatments, and both were significantly less than the CORT response to *M. salmoides*. Overall, *E. nana* showed a decreased antipredator response and no CORT response towards the high encounter frequency *L. auritus* as compared to the low encounter frequency *M. salmoides*. *Eurycea nana* may mute antipredator and CORT responses to high temporal frequency predators. There was, however, no correlation between CORT release rates and antipredator behavior, which suggests that the presence of predators may be affecting CORT response and behavior independently.

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## 1. Introduction

Predators can influence prey fitness either directly, through the consumption of individuals, or indirectly, through costs accrued from antipredator behavior [1]. In aquatic systems, predator recognition may result in prey species forming groups [2], increasing refugia use [3,4], or reducing overall activity levels [5,6]. Although these behaviors may decrease the immediate threat of predation, predators can have lingering, nonlethal effects on prey species, which may persist through time [7]. Nonlethal effects are important considerations for the fitness of species, even though they may not be as apparent as direct predation

upon individuals. One such example includes the predatory influence on circulating stress hormone levels in prey species that may be involved in escape behaviors [8]. Additionally, stress hormones may also be important for responding appropriately in subsequent encounters with predators, as stress hormones may play a role in learned predator recognition [9–11].

Most vertebrates respond to stressors with a rapid elevation of glucocorticoid (GC) hormones, where the intensity of the stressor can affect the degree of the GC response [12]. Elevated GC levels trigger the metabolism of lipids, proteins, and carbohydrates, enhancing functions necessary for immediate survival of an individual [13,14]. However, over longer periods of time, chronically elevated GC levels can directly suppress immune responses, reproduction, growth, and decrease expression of androgen-mediated mating behaviors [13,15]. Acute stress responses are likely beneficial, but chronic activation of the stress

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system to prolonged predation pressure may have fitness consequences. Prior studies have assumed that as exposure to stressors increase, so do baseline levels of corticosterone (CORT; a major GC), and chronically higher levels of CORT are associated with reduced relative fitness of individuals or populations, also known as the Predation Stress Hypothesis [16] or the CORT-Fitness Hypothesis [17].

Direct predator exposure or indirect exposure through chemical cues (kairomones) can cause immediate increases in circulating GC levels in prey [18,19] but not always [16]. Elevated CORT levels can enhance antipredator response [11,20] and may modulate subsequent behavioral and morphological responses to predators. Hossie et al. [21] experimentally demonstrated that *Lithobates (Rana) pipiens* tadpoles, when exposed to a CORT receptor inhibitor, showed decreased behavioral and morphological responses to predators when compared to control groups. A second study by Middlemis Maher et al. [22] found that long-term exposure to CORT might enhance survivorship of *Lithobates sylvaticus (Rana sylvatica)* tadpoles through the induction of morphological changes in tail shape. Both Fraker et al. [23] and Middlemis Maher et al. [22] found lower levels of CORT immediately following predator exposure in *L. sylvaticus* tadpoles. This decrease in CORT levels is contrary to what has previously been seen in other vertebrate groups, even though the antipredator response (freezing behavior) is similar [24]. These differences across vertebrate groups suggest that the expression of CORT and the subsequent modulation of behaviors vary across taxa.

Another factor in antipredator (and possibly CORT response) to predators may be perceived risk levels. Lima and Bednekoff [25] developed the Risk Allocation Hypothesis (RAH) that suggests that prey foraging under temporal variation in risk of predation face problems in how to optimally display antipredator behavior. For example, if predators are encountered infrequently and periods of risk are brief, then foraging prey should exhibit heightened antipredator behavior; any costs to foraging or mating can be regained during periods of low or no risk. Alternatively, if predators are common and periods of predation risk are prolonged, prey should exhibit reduced antipredator behavior, and should continue to forage during these high-risk periods. At the same time, chronic CORT levels can have negative fitness consequences and therefore, blunted CORT responses may be expected when prey are exposed to common, abundant predators.

We examined the antipredator and CORT responses of the San Marcos salamander, *Eurycea nana*, to temporal variation in risk of predation by fish predators. *Eurycea nana* is a federally threatened, IUCN red-listed, neotenic (fully aquatic) salamander endemic to the headwaters of the San Marcos River, Hays County, Texas [26]. Previous studies have demonstrated the use of chemical stimuli in the detection of both conspecifics [27] and fish predators [6,28]. *Eurycea nana* shows the antipredator behavior of freezing in response to chemical cues emitted by a variety of fish predators (*Lepomis cyanellus*, *Lepomis auritus*, *Micropterus salmoides*, *Herichthys cyanoguttatum*) [6,28] including the allopatric species *Lepomis gibbosus* indicating a generalized antipredator response [6]. In this closed system, both *M. salmoides* and *L. auritus* are the most abundant species and they have significantly increased in abundance compared to other large, predatory fish species over the past three decades [29]. Because *E. nana* shows a generalized antipredator response to *Lepomis*, we propose that this species will be perceived as a higher temporal risk than *M. salmoides*. We examine the antipredator and CORT responses of wild-caught (predator experienced) *E. nana* to *M. salmoides* and *L. auritus*, and predict that *E. nana* will show antipredator and CORT responses to both species but will have a muted antipredator and CORT response to the temporally abundant *L. auritus*.

## 2. Materials and methods

### 2.1. Predator species

To further understand any differences in the effects that predatory fish have on *E. nana*, we collected chemical cues from two centrarchid

(Perciformes: Centrarchidae) fish: the redbreast sunfish (*L. auritus*) and the largemouth bass (*M. salmoides*). The diet of *L. auritus* within the San Marcos River is primarily aquatic invertebrates (Diptera, Ephemeroptera, and Trichoptera), suggesting that this species is a generalist benthic feeder [30]. Examination of the diet of *M. salmoides* suggests that it too is a generalist feeder, consuming roughly equal proportions of fish and aquatic invertebrates [31], though the specific diet has not been examined for individuals from the San Marcos River. Both species have been observed to consume *E. nana* [32] and spend large amounts of time foraging among benthic substrates where salamanders are generally found (D.R. Davis, C.R. Gabor, personal observation).

### 2.2. Stimulus acquisition

We collected adult *L. auritus* and *M. salmoides* from the headwaters of the San Marcos River, Hays County, Texas, USA (29.89321°N, 97.93148°W; WGS84). We only used adult fish to reduce any possible ontogenetic effects. Prior to the collection of chemical cues, we fed fish earthworms for 5 d to eliminate any effects of prior diet. We determined the volume of each stimulus animal through displacement. To maintain similar chemical cue concentrations between treatments, we used 230 ml of water per 1 ml of stimulus animal in the collecting chamber. We then placed stimulus animals into separate glass aquaria containing the appropriate volume of aerated, dechlorinated tap water for 24 h. Before acquisition of the chemical cues, we removed the stimulus animals from the tanks and stirred the water. We mixed equal proportions of water from two adult individuals to minimize any individual effects and froze 50-ml aliquots in a –20 °C freezer. This method has been used successfully in previous studies [6,33,34]. Samples were thawed immediately prior to testing.

### 2.3. Experimental protocol

We used wild-caught adult *E. nana* (N = 31 females, N = 29 males) collected from the headwaters of the San Marcos River, Hays County, Texas, USA (29.89401°N, 97.92997°W; WGS84) and allowed them to acclimate to laboratory conditions for a minimum of two weeks. All individuals were transported to the San Marcos Aquatic Resources Center (SMARC) and housed in large, 360-l fiberglass flow-through tanks, maintained on a 12:12 h light cycle, and fed blackworms (*Lumbriculus variegatus*) ad libitum. Following established water-borne hormone collection methods [35], we randomly selected a salamander and placed each individual into separate 250-ml Nalgene container (perforated at the bottom) that fit within a 250-ml glass beaker filled with 100 ml of well water for 1 h. We used well water because it is the same type of water in which salamanders are maintained. After collecting pre-stimulus CORT levels, we then gently lifted out the Nalgene insert (allowing the water to drain through the bottom) and transferred each individual salamander into a separate 9.5-l glass testing aquaria containing 4.5 l of well water. Each aquarium had three sides covered with black plastic to reduce background disturbance. We tested during peak activity times for *E. nana*, beginning 2 h after dark and lasting for up to 4 h. We used low-level red light (25 W) during observations to minimize disturbance from lights. After placement in the testing chamber, individuals were acclimated for 20 min. Following acclimation, we recorded the amount of time individuals spent moving for 8 min (following [6]). Active behavior included swimming or walking, but did not include buccal pumping or gill movement that was not accompanied by other movements of the body. These data constitute the pre-stimulus activity level for each individual. Following determination of pre-stimulus activity, we introduced 50 ml of chemical stimuli from one of the following treatments: (1) an introduced redbreast sunfish (*L. auritus*; N = 20), (2) a native largemouth bass (*M. salmoides*; N = 20), or (3) only water (a blank control; N = 20). Treatments were tested in random order and coded to control for observer bias

(all trials conducted by DRD). We introduced cues into the aquarium through a syringe attached to plastic tubing attached to the center of one side of the testing aquaria at a rate of 2 ml/s. The end of the introduction tube was positioned approximately 2 cm below the surface of the water to reduce disturbance during treatment introduction. After introduction of the stimulus, we recorded the activity level of the salamander for another 8 min as an indication of responsiveness. These data constitute the post-stimulus behavioral response for each individual. These methods follow closely those of previous studies [6,28,34]. Immediately after the post-stimulus observation, we transferred the salamander into a clean, 250-ml glass beaker with 100 ml of well water for 1 h. These data constitute the post-stimulus CORT levels. Afterwards, we measured the snout-vent length (SVL) and recorded the sex of each salamander. As a result, each trial yields information on both the behavioral and water-borne CORT levels of individual salamanders to chemical stimuli. Each individual was exposed to a single treatment to eliminate any effects of habituation to stimuli [36]. All testing was performed during the same time range each day to control for circadian variation in CORT levels and gloves were used during water-borne hormone collection. We washed all hormone collection equipment with 95% ethanol and DI water and all testing equipment with 3% hydrogen peroxide between each trial.

#### 2.4. Hormone analysis

Traditional methods of measuring circulating hormone concentration in aquatic vertebrates involve assaying blood plasma. Here, corticosterone (CORT) levels were measured using a recently developed, non-invasive process for measuring water-borne hormone levels [35]. This method allows for repeated sampling of individuals and does not require euthanizing salamanders to collect blood plasma. Gabor et al. [35] validated the use of water-borne hormones from *E. nana* as they found a significant positive correlation between plasma CORT levels and water-borne CORT release rates. They also demonstrated that serial dilution of CORT from the water-borne hormone collection shows parallelism to the standard curve and that expected recovery concentrations were relative to known amounts of CORT in the standards on enzyme-immunoassay (EIA) plates.

All hormone analysis methods follow that of Gabor et al. [35]. Water samples containing hormones were maintained at  $-20^{\circ}\text{C}$  until assays were performed. We primed C18 solid phase extraction columns (Sep-Pak, Waters Inc.) with 4 ml of HPLC-grade methanol and 4 ml of millipore water. Water samples were then passed through these primed columns using a vacuum manifold, thus extracting hormones from the water samples. Hormones were then eluted from the columns into borosilicate test tubes using HPLC-grade methanol, placed in a  $37^{\circ}\text{C}$  water bath, and dried under a stream of low flow nitrogen gas. Prior to assaying samples, we resuspended hormones in a solution of 5% ethanol and 95% EIA buffer for a final resuspension volume of 400  $\mu\text{l}$ . We used commercially available EIA plates (Cayman Chemicals Inc.) to measure CORT levels. We ran samples in duplicate on 96 well plates and read by a fluorescent plate reader set at 415 nm (BioTek Powerwave XS). We used a pooled control that was combined from ten non-experimental *E. nana* and run in duplicate to measure both intra-assay CV (0.74%, 1.47%, 3.40%, 5.77%, 8.37%, 8.46%) and inter-assay CV (17.59%) for the six plates.

#### 2.5. Statistical analysis

We combined behavioral data into a single activity index for each individual. We calculated the activity index as the difference between post-stimulus activity and pre-stimulus activity. Positive values indicate increases in activity and negative values indicate decreases in activity in response to the stimulus. Following Gabor et al. [34], we multiplied CORT release rates (pg/ml) by 0.4 ml (the volume of the resuspension solution) and standardized by dividing by the SVL of each individual, resulting in

the CORT release rate units being pg/SVL/h. However, there was no significant relationship between SVL and pre- ( $N = 51$ ,  $r = -0.012$ ,  $p = 0.45$ ) and post-stimulus ( $N = 51$ ,  $r = -0.005$ ,  $p = 0.97$ ) CORT release rates. All CORT data were Ln transformed. We measured CORT response as the ratio of post-stimulus to pre-stimulus CORT release rates, therefore responses greater than one indicate that CORT release rates increased in response to the stimuli. All data met the assumptions of parametric analyses and we analyzed both the behavioral and hormone data using an ANOVA followed by Tukey's HSD multiple comparisons test ( $\alpha = 0.05$ ). Additionally, we examined whether there was a difference in pre- and post-stimulus CORT release rates for each treatment using matched pairs t-tests. To explore whether behavior and CORT were related, we examined the relationship between CORT response and activity index and between pre-stimulus and post-stimulus CORT release rates and pre and post-stimulus behavior respectively using Pearson correlations. To aid in visualizing the data, we present non-transformed CORT response values in the figure.

### 3. Results

There were significant differences in the activity indices among the three treatments (ANOVA:  $F_{2,57} = 24.75$ ,  $p < 0.0001$ ; Fig. 1). The activity index from the blank water control was significantly greater than the mean activity index from both the introduced redbreast sunfish (*L. auritus*) treatment (Tukey's HSD:  $p = 0.0002$ ) and the native largemouth bass (*M. salmoides*) treatment ( $p < 0.0001$ ). Additionally, the activity index for the *M. salmoides* treatment was significantly lower than that of the *L. auritus* treatment ( $p = 0.028$ ).

There was no significant difference between pre-stimulus CORT release rates (pg/SVL/h) of male (mean pre-stimulus CORT [ $\pm 1$  S.E.M.]:  $5.22 \pm 0.78$ ) and female ( $4.19 \pm 0.44$ ) salamanders (Student's t-test:  $t_{49} = 1.170$ ,  $p = 0.25$ ), and therefore, we combined both males and females in our analyses. We found significant differences in CORT responses among the three treatments (ANOVA:  $F_{2,48} = 10.69$ ,  $p < 0.0001$ ; Fig. 2). The CORT response for the *M. salmoides* treatment was significantly greater than that of both the *L. auritus* (Tukey's HSD:  $p = 0.002$ ) and the blank water ( $p = 0.0002$ ) treatments. The CORT responses did not differ between the *L. auritus* and the blank water treatments ( $p = 0.764$ ). Additionally, we found that CORT release rates significantly increased after exposure to chemical cues of *M. salmoides* (matched pairs t-test:  $t_{16} = 4.64$ ,  $p = 0.0003$ ; Fig. 3) and *L. auritus* ( $t_{16} = 2.40$ ,  $p = 0.029$ ; Fig. 3) but not to the blank water control ( $t_{16} = -0.09$ ,  $p = 0.933$ ; Fig. 3).

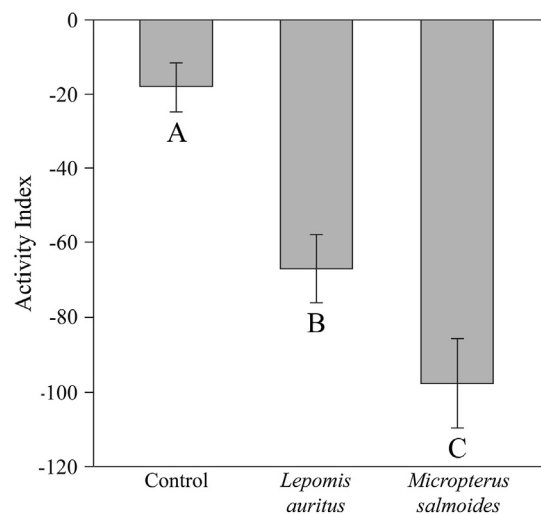
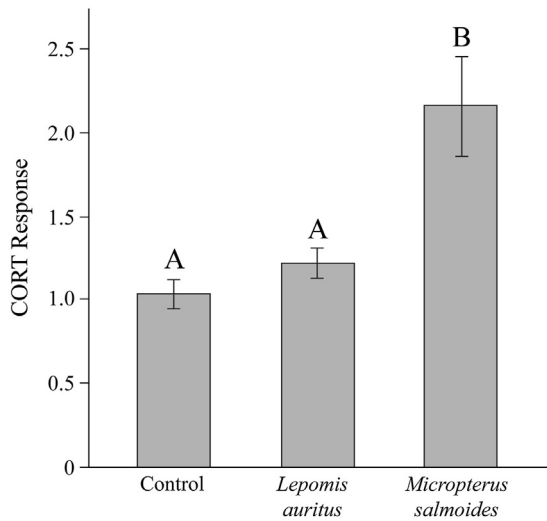


Fig. 1. Mean activity indices ( $\pm 1$  S.E.M.) of *Eurycea nana* in response to control, introduced *Lepomis auritus* (redbreast sunfish), and native *Micropterus salmoides* (largemouth bass) treatments. Letters indicate groupings from Tukey's HSD mean comparisons ( $\alpha = 0.05$ ).

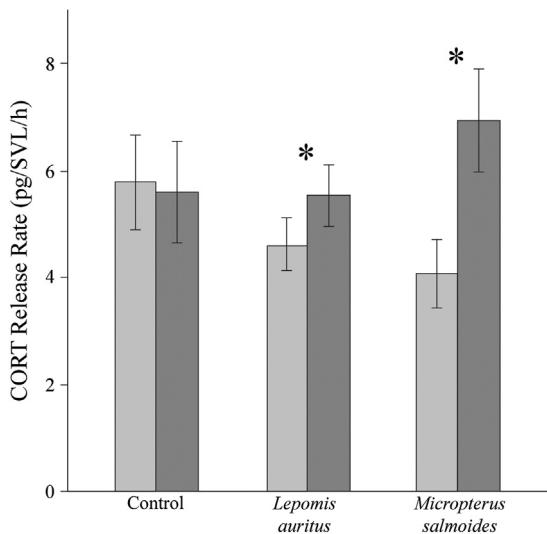


**Fig. 2.** Mean corticosterone (CORT) response levels ( $\pm 1$  S.E.M.) of *Eurycea nana* in response to control, introduced *Lepomis auritus* (redbreast sunfish), and native *Micropterus salmoides* (largemouth bass) treatments. Letters indicate groupings from Tukey's HSD mean comparisons ( $\alpha = 0.05$ ).

There was no significant relationship between pre-stimulus activity and pre-stimulus CORT release rates (Pearson correlation:  $N = 51$ ,  $r = -0.04$ ,  $p = 0.78$ ), nor was there a significant relationship between post-stimulus activity and post-stimulus CORT release rates in any of the treatments (control:  $N = 17$ ,  $r = -0.08$ ,  $p = 0.76$ ; *L. auritus*:  $N = 17$ ,  $r = 0.32$ ,  $p = 0.16$ ; *M. salmoides*:  $N = 17$ ,  $r = 0.15$ ,  $p = 0.54$ ). There was no significant relationship between CORT response and activity index in any of the treatments (control:  $N = 17$ ,  $r = -0.010$ ,  $p = 0.72$ ; *L. auritus*:  $N = 17$ ,  $r = 0.31$ ,  $p = 0.22$ ; *M. salmoides*:  $N = 17$ ,  $r = 0.01$ ,  $p = 0.97$ ).

#### 4. Discussion

*Eurycea nana* significantly reduced activity (antipredator behavior) in response to chemical cues from both the largemouth bass (*M. salmoides*) and the redbreast sunfish (*L. auritus*) when compared to the blank water treatment, and the response to *M. salmoides* was



**Fig. 3.** Mean pre-stimulus (light gray) and post-stimulus (dark gray) corticosterone (CORT) release rates ( $\pm 1$  S.E.M.) of *Eurycea nana* in response to control, introduced *Lepomis auritus* (redbreast sunfish), and native *Micropterus salmoides* (largemouth bass) treatments. Asterisks indicate significant differences from matched pairs t-tests ( $\alpha = 0.05$ ).

significantly stronger than the response to *L. auritus*. The CORT response to the blank water treatment and *L. auritus* did not differ statistically; however, the CORT response to *M. salmoides* was significantly greater than both the response to the blank water treatment and *L. auritus*. The differing behavioral response to both predators and the lower CORT response to *L. auritus* may reflect temporal variation in the risk of predation, thus supporting the RAH hypothesis [25]. *Lepomis auritus* is more abundant and frequently encountered compared to *M. salmoides*, and additionally, *E. nana* shows a generalized antipredator response to the chemical cues of the multiple *Lepomis* species found in this system [6]. The high encounter frequency and high abundance of *Lepomis* sp. may account for the muted antipredator and CORT responses of *E. nana* as compared to *M. salmoides*. Alternatively, differences in the way *E. nana* responds to predators may represent a shorter coevolutionary time with this introduced species of *Lepomis* (~60 years) [37]. Further experiments are necessary to tease apart these alternative hypotheses.

Even though *E. nana* clearly shows a behavioral and stress response to predators, when we examined the relationships between CORT release rates and behavior as well as the relationship between CORT response and behavior we found that CORT did not appear to directly modulate the behavioral response. The Predation Stress Hypothesis predicts a relationship between these two variables and support has been found in birds, rabbits and lizards (review, [16]). Wack et al. [38] found that CORT, however, was not correlated with changes in activity in a terrestrial salamander, *Plethodon shermani*. They suggested that the association between behavior and CORT is not necessarily causative but instead CORT may mediate transitions associated with a stressor. One hypothesis for why salamanders may not show the predicted relationship is because there are high energetic costs associated with increase CORT levels in salamanders [38], yet many plethodontid salamanders such as *Eurycea* are specialized for low energy lifestyles [39].

In contrast to our results, Epp and Gabor [28] found that predator-experienced *E. nana* did not decrease activity in response to *L. auritus*; however, predator-naïve *E. nana* did respond to *L. auritus* (indicating innate predator recognition), and therefore, this apparent lack of response may be muted by experience. Also, Epp and Gabor [28] did not control for diet of the predators and this may have resulted in the behavioral differences observed in our study versus theirs.

Few studies have directly examined the relationship between CORT levels and predation risk in amphibians. Fraker et al. [23] found that *L. sylvaticus* (*R. sylvatica*) tadpoles had significantly lower levels of CORT after exposure to high-risk predator diet-cues. Dahl et al. [40] found that tadpoles of *Rana temporaria* from low-latitude populations exhibited elevated CORT levels after exposure to dragonfly naiads while there was no response from individuals from high-latitude populations. Due to the negative influence of CORT on growth rates, this lack of mounting a CORT response to a common predator was attributed to selection maximizing growth rates in these high-latitude populations, which have short growing seasons [40]. Additionally, Middlemis Maher et al. [22] found that CORT of *L. sylvaticus* decreased immediately after exposure to dragonfly naiads. In tadpoles, increased CORT is associated with metamorphosis [41] and has been shown to increase locomotion and foraging behaviors and therefore, may also increase predation risk [42]. However, we found no relationship between CORT and activity in our study. Metamorphosis is driven by CORT in tadpoles and this relationship may drive the differences between the species because *E. nana* does not undergo metamorphosis. Unlike in the above studies where sacrificing the whole individual is required to obtain CORT values, we obtained both pre- and post-stimulus CORT levels for each individual using the non-invasive water-borne hormone collection method. Our method may allow us to gain a closer understanding of how predation exposure affects CORT values at an individual level.

Chronic increases in CORT may have negative fitness consequences including high energetic costs on salamanders, especially if cumulative effects of CORT are increasing due to highly abundant and diverse



assemblage of predatory fishes in the San Marcos River. In response to these numerous predators, and to mitigate the costs of responding, salamanders may have muted responses to *L. auritus*. Alternatively, antipredator behavior in plethodontid salamanders may not be linked to changes in CORT as suggested by our data. Future studies exploring the relationship between CORT levels (both chronically high and muted) and fitness in *E. nana* are needed to better understand these complex interactions between fish predators and *E. nana*. Additionally, experimental manipulation of CORT will aid in our understanding of the causal relationship between CORT and antipredator behaviors.

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### References

- [1] Lima SL, Dill LM. Behavioral decisions made under the risk of predation: a review and prospectus. *Can J Zool* 1990;68:619–40.
- [2] Rödel M-O, Linsenmair KE. Predator-induced swarms in the tadpoles of an African savanna frog, *Phrynomantis microps*. *Ethology* 1997;103:902–14.
- [3] Sih A, Kats LB, Moore RD. Effects of predatory sunfish on the density, drift, and refuge use of stream salamander larvae. *Ecology* 1992;73:1418–30.
- [4] Winandy L, Denoël M. Cues from introduced fish alter shelter use and feeding behaviour in adult alpine newts. *Ethology* 2013;119:121–9.
- [5] Semlitsch RD. Interactions between fish and salamander larvae. Costs of predator avoidance or competition? *Oecologia* 1987;72:481–6.
- [6] Davis DR, Epp KJ, Gabor CR. Predator generalization decreases the effect of introduced predators in the San Marcos salamander, *Eurycea nana*. *Ethology* 2012;118:1191–7.
- [7] Sih A. Prey uncertainty and the balancing of antipredator and feeding needs. *Am Nat* 1992;139:1052–69.
- [8] Wingfield JC, Maney DL, Breuner CW, Jacobs JD, Lynn S, Ramenofsky M, et al. Ecological bases of hormone-behavior interactions: the “emergency life history stage”. *Am Zool* 1998;38:191–206.
- [9] Dachir S, Kadar T, Robinzon B, Levy A. Cognitive deficits induced in young rats by long-term corticosterone administration. *Behav Neural Biol* 1993;60:103–9.
- [10] Mateo JM. Inverted-U shape relationship between cortisol and learning in ground squirrels. *Neurobiol Learn Mem* 2008;89:582–90.
- [11] Thaker M, Lima SL, Hews DK. Acute corticosterone elevation enhances antipredator behaviors in male tree lizard morphs. *Horm Behav* 2009;56:51–7.
- [12] McEwen BS, Wingfield JC. The concept of allostasis in biology and biomedicine. *Horm Behav* 2003;43:2–15.
- [13] Sapolsky RM, Romero LM, Munck AU. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocr Rev* 2000;21:55–89.
- [14] Moore IT, Jessop TS. Stress, reproduction, and adrenocortical modulation in amphibians and reptiles. *Horm Behav* 2003;43:39–47.
- [15] Greenberg GN, Wingfield JC. Stress and reproduction: reciprocal relationships. In: Norris DO, Jones RE, editors. *Reproductive endocrinology*. New York: Plenum Press; 1987. p. 461–503.
- [16] Creel S, Winnie Jr JA, Christianson D. Glucocorticoid stress hormones and the effect of predation risk on elk reproduction. *Proc Natl Acad Sci* 2009;106:12388–93.
- [17] Bonier F, Martin PR, Moore IT, Wingfield JC. Do baseline glucocorticoids predict fitness? *Trends Ecol Evol* 2009;24:634–42.
- [18] Thaker M, Lima SL, Hews DK. Alternative antipredator tactics in tree lizard morphs: hormonal and behavioural responses to a predator encounter. *Anim Behav* 2009;77:395–401.
- [19] Narayan EJ, Cockrem JF, Hero JM. Sight of a predator induces a corticosterone stress response and generates fear in an amphibian. *PLoS One* 2013;8:e73564.
- [20] Orchinik M. Glucocorticoids, stress, and behavior: shifting the timeframe. *Horm Behav* 1998;34:320–7.
- [21] Hossie TJ, Ferland-Raymond B, Burness G, Murray DL. Morphological and behavioural responses of frog tadpoles to perceived predation risk: a possible role for corticosterone mediation? *Ecoscience* 2010;17:100–8.
- [22] Middlemis Maher J, Werner EE, Denver RJ. Stress hormones mediate predator-induced phenotypic plasticity in amphibian tadpoles. *Proc R Soc B* 2013;280:20123075.
- [23] Fraker ME, Hu F, Cuddapah V, McCollum SA, Relyea RA, Hempel J, et al. Characterization of an alarm pheromone secreted by amphibian tadpoles that induces behavioral inhibition and suppression of the neuroendocrine stress axis. *Horm Behav* 2009;55:520–9.
- [24] Travers M, Clinchy M, Zanette L, Boonstra R, Williams TD. Indirect predator effects on clutch size and the cost of egg production. *Ecol Lett* 2010;13:980–8.
- [25] Lima SL, Bednekoff PA. Temporal variation in danger drives antipredator behavior: the predation risk allocation hypothesis. *Am Nat* 1999;153:649–59.
- [26] Nelson JM. Population size, distribution, and life history of *Eurycea nana* in the San Marcos River. [M.S. Thesis] Southwest Texas State University; 1993 [43 pp.].
- [27] Thaker M, Gabor CR, Fries JN. Sensory cues for conspecific associations in aquatic San Marcos salamanders. *Herpetologica* 2006;62:151–5.
- [28] Epp KJ, Gabor CR. Innate and learned predator recognition mediated by chemical signals in *Eurycea nana*. *Ethology* 2008;114:607–15.
- [29] Perkin JS, Bonner TH. Long-term changes in flow regime and fish assemblage composition in the Guadalupe and San Marcos Rivers of Texas. *River Res Appl* 2011;27:566–79.
- [30] Wallace SA. The food habits of the redbreast sunfish in the San Marcos River, Texas. [M.S. Thesis] Southwest Texas State University; 1984 [59 pp.].
- [31] Matthews VJ, Gelwick FP, Hoover JJ. Food of and habitat use by juveniles of species of *Micropterus* and *Morone* in a southwestern reservoir. *Trans Am Fish Soc* 1992;121:54–66.
- [32] Tupa DD, Davis WK. Population dynamics of the San Marcos salamander, *Eurycea nana* Bishop. *Tex J Sci* 1976;27:179–95.
- [33] Mathis A, Murray KL, Hickman CR. Do experience and body size play a role in responses of larval ringed salamanders, *Ambystoma annulatum*, to predator kairomones? Laboratory and field assays. *Ethology* 2003;109:159–70.
- [34] DeSantis DL, Davis DR, Gabor CR. Chemically mediated predator avoidance in the Barton Springs salamander. *Herpetologica* 2013;69:291–7.
- [35] Gabor CR, Bosch J, Fries JN, Davis DR. A non-invasive water-borne hormone assay for amphibians. *Amphibia-Reptilia* 2013;34:151–62.
- [36] Hazlett BA. Predator recognition and learned irrelevance in the crayfish *Orconectes virilis*. *Ethology* 2003;109:765–80.
- [37] Jurgens KC. The distribution and ecology of the fishes of the San Marcos River. [M.A. Thesis] University of Texas at Austin; 1951 [66 pp.].
- [38] Wack CL, Ratay MK, Woodley SK. Effects of corticosterone on locomotory activity in red-legged salamanders. *Herpetologica* 2013;69:118–26.
- [39] M.E. Feder, Integrating the ecology and physiology of plethodontid salamanders. *Herpetologica* 1983;39:291–310.
- [40] Dahl E, Orizaola G, Winberg S, Laurila A. Geographic variation in corticosterone response to chronic predator stress in tadpoles. *J Evol Biol* 2012;25:1066–76.
- [41] Denver RJ. Stress hormones mediate environment-genotype interactions during amphibian development. *Gen Comp Endocrinol* 2009;164:20–31.
- [42] Crespi EJ, Denver RJ. Ontogeny of corticotropin-releasing factor effects on locomotion and foraging in the Western spadefoot toad (*Spea hammondi*). *Horm Behav* 2004;46:399–410.