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INTERACTIVE EFFECTS OF TEMPERATURE AND GLYPHOSATE ON THE BEHAVIOR OF BLUE RIDGE TWO-LINED SALAMANDERS (*EURYCEA WILDERAE*)

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Abstract: The objective of the present study was to evaluate the potential interactive effects of stream temperatures and environmentally relevant glyphosate-based herbicide concentrations on movement and antipredator behaviors of larval *Eurycea wilderae* (Blue Ridge two-lined salamander). Larval salamanders were exposed to 1 of 4 environmentally relevant glyphosate concentrations (0.00 μg acid equivalent [a.e.]/L, 0.73 μg a.e./L, 1.46 μg a.e./L, and 2.92 μg a.e./L) at either ambient (12 °C) or elevated (23 °C) water temperature. Behaviors observed included the exploration of a novel habitat, use of refuge, habitat selection relative to a potential predator, and burst movement distance. In the absence of glyphosate, temperature consistently affected movement and refuge-use behavior, with individuals moving longer distances more frequently and using refuge less at warm temperatures; however, when glyphosate was added, the authors observed inconsistent effects of temperature that may have resulted from differential toxicity at various temperatures. Larval salamanders made shorter, more frequent movements and demonstrated reduced burst distance at higher glyphosate concentrations. The authors also found that lower glyphosate concentrations sometimes had stronger effects than higher concentrations (i.e., nonmonotonic dose responses), suggesting that standard safety tests conducted only at higher glyphosate concentrations might overlook important sublethal effects on salamander behavior. These data demonstrate that sublethal effects of glyphosate-based herbicides on natural behaviors of amphibians can occur with short-term exposure to environmentally relevant concentrations. *Environ Toxicol Chem* 2016;9999:1–7.

Keywords: Ecological context Ecotoxicology Nonmonotonic dose–response curve Pesticide Plethodontidae

INTRODUCTION

Current agricultural practices have impacted aquatic ecosystems in multiple ways [1]. Fertilizers, heavy metals, and pesticides are environmental contaminants that can impair biological communities through direct and indirect effects [2]. Amphibians represent 1 important vertebrate group sensitive to these types of contaminants [3]. Their responses include slowed growth and development [4], increased susceptibility to disease [5], decreased ability to avoid predation [6], physical abnormalities [3], and direct mortality [4]. Physiological, behavioral, and morphological responses that function properly are essential for individual survival and fitness in natural environments. Thus, exposure to these toxicants, even below their lethal doses, can lead to changes in amphibian ecology and future population persistence [7]. The widespread application of pesticides, surface runoff, and limited riparian buffers result in regular detection of agricultural contaminants in freshwater ecosystems [2].

Concurrently, removal of riparian forests for agriculture results in increased solar radiation and increased stream temperatures [8]. Riparian buffer requirements vary widely, are rarely enforced, and vary in efficacy depending on stream width, riparian forest maturity, and the mode and intensity of adjacent land use [8]. Stream temperature can still rise substantially after the removal of forests in the presence of riparian buffers [8]. Rishel et al. [9] found that average stream temperature increased 4.4 °C following riparian deforestation but could increase up to 10 °C relative to reference sites. Temperature often interacts with environmental contaminants to either intensify or decrease toxicity [10,11]. Environmental contaminants may become more toxic at high temperatures but also are degraded or metabolized more rapidly [10,11]. Consequently, it is necessary to evaluate the interaction of temperature and contaminant concentrations to understand the impact of contaminants on stream-dwelling organisms in agricultural regions.

Amphibians, the most threatened vertebrate group [12], are particularly susceptible to environmental contamination [7]. This might be because they use water for egg and larval development [13] and have highly permeable skin [13], resulting in higher epidermal diffusion rates than for other vertebrates [14]. Sublethal impacts include short-term and long-term changes to the physiology, development, and behavior of amphibians [7]. Specifically, pesticides have been demonstrated to inhibit acetylcholinesterase [15], induce tail deformities [16], and accelerate metamorphosis [17]. Studies on behavioral responses have reported conflicting results, but collectively, they suggest that physiological and behavioral responses to pesticides such as increased latency for predator avoidance responses may cause amphibians to be more susceptible to predation [18–20]. An additional study suggests that antipredator behaviors can also change the susceptibility of amphibians to environmental contaminants [21]. Because predators and prey are often tightly linked and predators may impact prey species to varying extents, toxicants in water that affect antipredator behaviors have the potential to disrupt entire aquatic communities [22]. Ultimately, the ecological effects resulting from these changes could alter amphibian success in agricultural regions [7].

Most of the effects described above have been documented exclusively within wetland-breeding anurans. Little is known of the effects of environmental contaminants on stream-dwelling caudates in agricultural areas [7]. Because of the flowing nature

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of streams where these animals live, exposure to pesticides is likely to be at low concentrations for short time intervals [2]. Riverine concentrations are also likely to be considerably lower than those used in prior behavioral studies of wetland-breeding amphibians because concentrations are diluted in lotic systems [7]. Many caudates occupying riverine systems are from the family Plethodontidae, which lack lungs, suggesting that their absorption of xenobiotics may be particularly high [14]. Finally, amphibian antipredator behaviors are consistently altered in response to a wide variety of environmental contaminants, including excess nitrogen [4], heavy metals [6,19], ultraviolet B [18], carbaryl [20], and atrazine [23]; and behavioral responses that impact survival require additional study [24].

The objective of the present study was to evaluate the potential interactive effects of stream temperatures and environmentally relevant pesticide concentrations on movement and antipredator behaviors of a larval stream salamander, *Eurycea wilderae* (Blue Ridge two-lined salamander, Dunn 1920) from the family Plethodontidae. We chose to observe a glyphosate-based herbicide because of its widespread use in the United States and known impacts on amphibian physiology and behavior [7]. We specifically observed the following behaviors: 1) exploration of a novel habitat, 2) use of refuge, 3) habitat selection relative to a potential predator, and 4) burst movement distance. Individuals exhibiting adaptive behavioral responses would move infrequently in a new environment, use cover frequently, locate themselves far from a potential predator, and move a considerable distance from a simulated predation attempt. We anticipated that amphibians as ectotherms would have higher burst movement distances at warm temperatures but that temperature would not impair any other antipredator behaviors. We hypothesized that, similar to prior studies on glyphosate-based herbicides [7], glyphosate would interfere with antipredator behavior, causing individuals to increase their movement frequency, decrease cover use and distance from a predator, and decrease burst movement distances. Because warm temperatures could increase amphibian metabolism and elimination of glyphosate, we hypothesized that high glyphosate concentrations at cooler temperatures would yield the most impaired antipredator behaviors.

MATERIALS AND METHODS

Glyphosate

Glyphosate is a widely used herbicide included in Roundup®, Spectracide, and Vision herbicides. Battaglin et al. [25] described the highest concentrations of glyphosate observed in streams to be 0.10 μg acid equivalent (a.e.)/L to 8.7 μg a.e./L, which are well below concentrations used in previous amphibian studies (e.g., 2.4 mg a.e./L [26], 5–500 μg a.e./L [27], 6.5 mg a.e./L [28], and 8 mg a.e./L [21]). Median lethal concentrations of glyphosate for amphibians range from 0.20 mg a.e./L to 494.00 mg a.e./L, with sublethal effects observed at concentrations as low as 10 μg a.e./L [7]. For the present study, we used the commercially sold Roundup at 4 concentrations: 0.0 mL/L, 0.5 mL/L, 1.0 mL/L, and 2.0 mL/L. We used the ready-to-use formulation with 2% glyphosate isopropylamine salt. Using the acid equivalent formula presented in Annett et al. [2] and the herbicide formula provided by Roundup, these concentrations were equivalent to 0.00 μg a.e./L, 0.73 μg a.e./L, 1.46 μg a.e./L, and 2.92 μg a.e./L. We refer to these concentrations as control, low, medium, and high, respectively. Solutions were created by adding the appropriate concentration of Roundup to 1 L of reverse osmosis water for each experimental enclosure.

Study species and area

We investigated the behavioral responses of *E. wilderae* that occur throughout high-elevation regions of the southeastern United States including the southern Appalachian Mountains and the Cumberland Plateau. *Eurycea wilderae* are the most abundantly detected species in many headwater streams and occur from high-elevation seeps to valley streams [29]. Although adults are often found considerable distances from streams, larval *E. wilderae* are aquatic and spend at least 12 mo in streams before metamorphosis [29]. Flat riparian zones in this region have long been used for farming, grazing, and settlement; and agriculture in this region has resulted in modification of riparian vegetation, increased sedimentation, and changes in flow [1,3].

Larval *E. wilderae* were collected using a dip-net from Tremlett Spring in Sewanee, Tennessee, USA, on the Cumberland Plateau. Collection occurred in June to July 2014. Mean stream temperature during this time was 19.2 ± 0.1 °C, with a minimum temperature of 18.4 °C and a maximum temperature of 20.2 °C. Individuals used in treatments had a mean snout–vent length of 22.8 ± 0.3 mm and a mean total length of 41.4 ± 0.5 mm (± 1 standard error). Although the Tremlett Spring watershed drains a residential area, water sampling has yielded nondetection of glyphosate, 2,4-dichlorophenoxyacetic acid, picloram, bifenthrin, malathion, triazicide, and devonicarbaryl (M. Knoll, University of the South, Sewanee, TN, USA, personal communication; quantification by ActLabs Agriculture). Crayfish were the most commonly observed predator in this system, but we did detect larval *Pseudotriton ruber* (red salamander, Latreille 1801), known predators of larval *E. wilderae* [29].

Experimental design

We used a completely randomized design with a full factorial combination of 4 Roundup concentrations and 2 temperatures, resulting in 8 treatments. We evaluated the response of salamanders to Roundup at an ambient temperature of 12 °C that represents spring temperatures in local streams and at 23 °C that represents maximum temperatures observed in local streams with deforested riparian zones and may represent temperatures in the future with climate change [8,30]. Individuals were allowed to acclimate to each temperature for 2 h prior to introducing individuals to the test enclosures.

We evaluated the behavior of 144 individuals with 18 individuals per treatment. After capture and before being introduced to the test enclosures, larval *E. wilderae* were held individually in plastic collection containers with native stream water and a paper towel refuge at 12 °C for less than 24 h prior to behavioral evaluation. Each individual was used in only 1 randomly assigned treatment before being released at the capture location within 24 h of completing behavioral observations. Although we cannot exclude the possibility that prior interactions with predators including crayfish may predetermine antipredator behaviors of our present study species, all individuals were collected from less than 50 m of stream reach, suggesting that their experience with predators should be similar among individuals.

The laboratory test enclosures were plastic Tupperware tubs and measured 30 cm × 15 cm. No substrate was added to the enclosures, to prevent binding of glyphosate to the substrate [2]. Experimental enclosures were bleached in between treatments because bleach has been recommended as an effective cleansing agent to remove glyphosate [31]. Glyphosate solutions were added to the enclosures at the beginning of the trials that lasted for 5 h. Individuals were randomly assigned to a treatment, but observations of behavior followed a consistent temporal sequence, observing exploratory movement frequency, distance, refuge use, distance from a potential predator, and finally burst distance after being exposed to a potential predator.

Exploratory movement frequency, distance, and refuge use. Antipredator behaviors of amphibians include freezing behavior because visual predators often use movement to find and successfully attack prey [13]. Refuge use is another way to avoid visual detection by a predator. To assess exploration in a novel habitat, we counted the number of movements and the distance of each movement in the 20 min following an individual's introduction to the enclosure. To be counted, each movement had to be at least 1 body length of the individual. To determine refuge use, the enclosure had a single piece of cobble approximately 25 cm^2. We recorded the length of time spent under the cover object. Movements were assumed to be absent after an individual located itself under the cover object.

Distance from a potential predator. Another mechanism to avoid predation is to avoid locations occupied by a predator [13]. Following the 20-min exploration period in the enclosure, we introduced a caged predatory crayfish (*Cambarus* sp.) and removed the rock refuge. Crayfish are documented predators of salamander larvae [29,32], and only crayfish larger than 70 mm in length were used. Crayfish were collected from the same stream as the salamanders and also held in native stream water at 12 °C for at least 48 h prior to evaluation to eliminate diet cues of the crayfish. Crayfish were returned to temporary enclosures between trials, fed bloodworms, and then starved for 48 h prior to the next trial. They were each used for a total of 8 trials. Mesh cages were used to prevent direct predation but allowed for the transmission of visual and chemical cues by the predator. We measured the distance the salamander located itself from the predator after 4 h of exposure to the crayfish.

Burst distance. Burst movement speed and distance during a predator attack are the final mechanisms for prey to avoid predation. After 4 h in the presence of a predator, we removed the predator and observed burst distance exhibited by larval *E. wilderae* in response to a simulated predation event. Predation attempts were simulated using forceps to pinch the tail of the salamander. We measured the distance between the start location and the stop location as the burst distance.

Data analysis

The impacts of temperature and Roundup on larval *E. wilderae* behavior were evaluated by a multivariate analysis of variance (MANOVA) using Pillai's trace critical value implemented in program R (R Development Core Team 2014). To evaluate differences in how glyphosate concentration and temperature affect each antipredator behavior, we used 2-factor analysis of variance for each response variable. We assessed the significance of our 2 fixed factors, temperature and glyphosate concentration, to affect our 5 behavioral responses: movement frequency, movement distance, frequency of cover use, distance from a predator, and burst distance. For movement distance, we used an individual's mean movement distance to prevent pseudoreplication. All other response variables had only a single measurement per individual. Individual effects were not assessed because each individual was only used in a single treatment. Tukey's post hoc tests were used to distinguish differences among treatments.

RESULTS

We observed 144 individuals in 8 different trials and recorded no *E. wilderae* mortality as a result of exposure to glyphosate. Similarly, crayfish predators also did not experience any mortality during the present study despite repeated exposure to environmentally relevant concentrations of glyphosate. Overall, our MANOVA indicated that glyphosate concentration ($F_{df=5,132} = 6.14$, $p < 0.001$), temperature ($F_{df=15,402} = 9.87$, $p < 0.001$), and their interaction ($F_{df=15,402} = 2.45$, $p = 0.002$) significantly altered antipredator behaviors of larval salamanders. None of the conclusions changed if we treated glyphosate concentration as a continuous predictor, indicating that the present study's results were robust to analytical methodology.

Exploratory movement, frequency, distance, and refuge use

The frequency of movements was influenced significantly by both of our main effects as well as an interaction between temperature and glyphosate concentration (Table 1 and Figure 1A). The significance of concentration was primarily driven by significant differences between the lowest concentration of glyphosate and all other concentrations, whereas the effect of temperature was primarily driven by differences between temperature treatments in the control and high concentration (Table 2). In the absence of glyphosate and at the highest concentration, we observed that individuals moved more frequently at elevated temperatures. Low and medium doses of glyphosate minimized the difference between temperatures with movement frequency similar to the control, ambient temperature movements at the low concentration but more similar to the elevated temperature control at the medium concentration (Table 2).

Mean movement distance was significantly influenced by temperature and concentration but not by an interaction between them (Table 1 and Figure 1B). In the absence of glyphosate,

Table 1. Results of 2-factor analyses of variance evaluating the effects of glyphosate concentration and temperature on behavioral responses

	df	*F*	*p*
Movement frequency			
Concentration	3	14.475	<0.001
Temperature	1	29.636	<0.001
Interaction	3	7.362	<0.001
Residuals	136		
Movement distance			
Temperature	1	30.841	<0.001
Concentration	3	2.718	0.047
Interaction	3	2.517	0.061
Residuals	136		
Refuge use frequency			
Concentration	3	4.89	0.003
Temperature	1	16.612	<0.001
Interaction	3	4.252	0.007
Residuals	136		
Distance from a predator			
Concentration	3	1.963	0.123
Temperature	1	9.125	0.003
Interaction	3	2.019	0.114
Residuals	136		
Burst distance			
Concentration	3	6.935	<0.001
Temperature	1	0.256	0.614
Interaction	3	0.045	0.987
Residuals	136		

df = degrees of freedom.

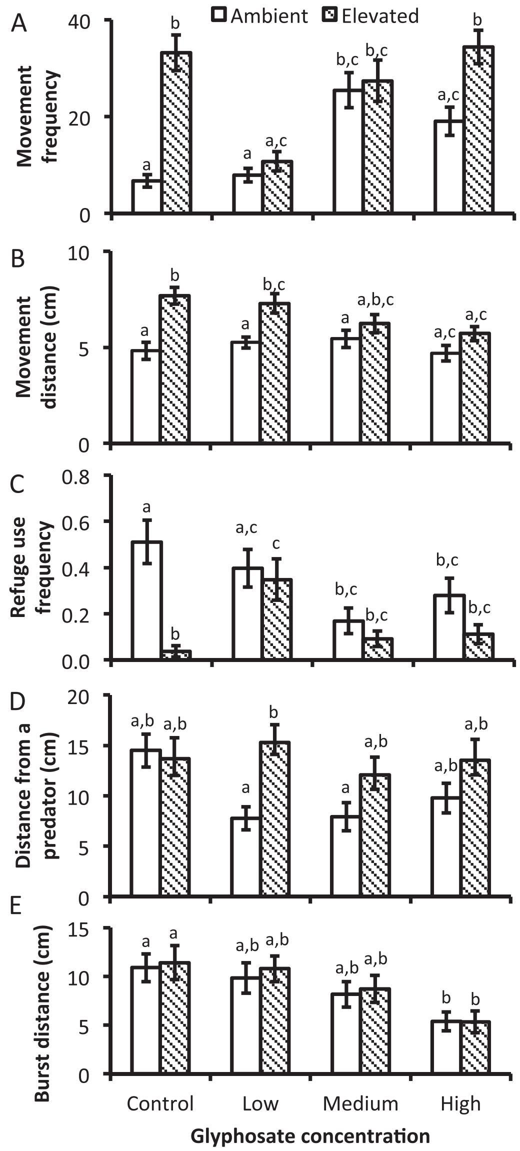

Figure 1. Results of behavioral trials investigating the interactive impacts of glyphosate (control, 0.00 acid equivalent [a.e.] μg/L; low, 0.73 a.e. μg/L; medium, 1.46 a.e. μg/L; and high, 2.92 a.e. μg/L) and temperature (ambient, 12 °C; elevated, 23 °C) on (**A**) mean movement frequency, (**B**) mean movement distance, (**C**) mean refuge-use frequency, (**D**) mean distance from a predator, and (**E**) mean burst distance. Error bars represent mean ±1 standard error. Letters denote significance of Tukey's post hoc tests among values. Letters indicate groups with no significant difference.

individuals moved shorter distances at ambient temperatures. Although not significant at the $\alpha = 0.05$ level, as the concentration of glyphosate increased, individuals exposed to elevated temperatures tended to move shorter distances and became more similar to individuals at ambient temperatures ($p = 0.061$, interaction between temperature and concentration; Table 2). High and medium concentrations of glyphosate minimized the temperature effect relative to low and control concentrations of glyphosate (Table 2).

Frequency of refuge use was influenced significantly by temperature, glyphosate concentration, and their interaction (Table 1 and Figure 1C). Similar to movement frequency, low glyphosate concentrations minimized the effect of temperature. In the absence of glyphosate and at the highest concentration, individuals in ambient temperatures used refuge more. At ambient temperatures, frequency of refuge use declined with increasing glyphosate concentration (Table 2). With the exception of the lowest glyphosate concentration, frequency of cover use remained similar to the control when glyphosate was present (Table 2).

Distance from a potential predator

Distance from a predator was significantly affected by temperature but not glyphosate concentration (Table 1 and Figure 1D). This relationship was primarily driven by temperature differences observed at the low concentration (Table 2).

Burst distance

Glyphosate concentration significantly reduced burst distance, but neither temperature nor the interaction significantly affected burst distance (Table 1 and Figure 1E). Burst distances were significantly shorter at the high concentration of glyphosate relative to the control (Table 2).

DISCUSSION

Complex relationships exist between glyphosate and temperature effects on larval salamander behaviors. Temperature specifically affected movement and refuge use but did not affect burst movement distances. Alterations to movement and antipredator behaviors in the presence of glyphosate particularly at elevated temperatures may increase the likelihood that an individual is predated or swept downstream. Stream salamanders demonstrate reduced survival and occupancy in downstream habitats, suggesting that salamander populations are larger in upstream habitats and that individuals swept into them may experience reduced success [33]. Consequently, glyphosate use may have indirect effects on stream salamander survival even at low, environmentally relevant concentrations for short temporal periods.

In the absence of glyphosate, temperature consistently affected movement and refuge-use behavior, with individuals moving longer distances more frequently and using refuge less at warmer temperatures. The present study's results correspond to the prediction of metabolic theory, which suggests that ectothermic organismal activity should increase with temperature within the organism's normal temperature range [34]. The exception to this pattern was burst distance, which was not affected by temperature. When glyphosate was added, we observed inconsistent effects of temperature on toxicity. Previous studies suggest that some contaminants exhibit greater toxicity at elevated temperatures because of higher metabolic rates or higher movement rates that increase contaminant absorption [10,11,35]. Conversely, elevated temperatures in the present study minimized differences for trials with and without glyphosate while evaluating the distance to a predator. Individuals located themselves closer to a predator only at ambient temperature when glyphosate was present, whereas no difference occurred at elevated temperatures when glyphosate was added. Previous research suggests that predator presence can interfere with predicted responses of amphibians to glyphosate when glyphosate stratifies in the water column, but it is unlikely that stratification occurred within the shallow water depth used in the present study [21,36]. Low temperatures constrain ectothermic metabolic rates, limiting metabolism of contaminants, movement capabilities, and reaction times [34]. Low metabolic rates may maintain glyphosate within the body for longer time periods, interfering with natural behaviors.

Table 2. Significance (*p* values) of Tukey's post hoc tests for all interactions of glyphosate concentration (control, low, medium, high) and temperature (ambient, elevated) on behavioral response variables

		Control		Low		Medium		High	
		Ambient	Elevated	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated
Movement frequency									
Control	Ambient	—	<0.001	0.999	0.982	0.001	<0.001	0.087	<0.001
	Elevated		—	<0.001	<0.001	0.619	0.878	0.026	0.999
Low	Ambient			—	0.998	0.002	<0.001	0.167	<0.001
	Elevated				—	0.017	0.004	<0.001	<0.001
Medium	Ambient					—	0.998	0.513	0.427
	Elevated						—	0.513	0.728
High	Ambient							—	0.010
	Elevated								—
Movement distance									
Control	Ambient	—	<0.001	0.996	0.002	0.967	0.273	0.999	0.812
	Elevated		—	0.002	0.998	0.007	0.255	<0.001	0.030
Low	Ambient			—	0.022	0.999	0.732	0.983	0.995
	Elevated				—	0.050	0.664	0.001	0.165
Medium	Ambient					—	0.893	0.918	0.999
	Elevated						—	0.183	0.989
High	Ambient							—	0.695
	Elevated								—
Refuge use frequency									
Control	Ambient	—	<0.001	0.928	0.668	0.010	<0.001	0.225	0.001
	Elevated		—	0.005	0.027	0.855	0.999	0.176	0.994
Low	Ambient			—	0.999	0.244	0.032	0.917	0.051
	Elevated				—	0.558	0.126	0.996	0.201
Medium	Ambient					—	0.991	0.939	0.999
	Elevated						—	0.490	0.999
High	Ambient							—	0.632
	Elevated								—
Distance from a predator									
Control	Ambient	—	0.999	0.106	0.999	0.125	0.972	0.513	0.999
	Elevated		—	0.229	0.998	0.262	0.998	0.741	0.998
Low	Ambient			—	0.045	0.999	0.638	0.991	0.251
	Elevated				—	0.049	0.883	0.311	0.996
Medium	Ambient					—	0.683	0.995	0.286
	Elevated						—	0.981	0.999
High	Ambient							—	0.768
	Elevated								—
Burst distance									
Control	Ambient	—	0.999	0.999	0.999	0.857	0.953	0.098	0.091
	Elevated		—	0.992	0.999	0.708	0.864	0.048	0.044
Low	Ambient			—	0.999	0.989	0.999	0.312	0.296
	Elevated				—	0.882	0.965	0.113	0.105
Medium	Ambient					—	0.999	0.844	0.830
	Elevated						—	0.681	0.662
High	Ambient							—	0.999
	Elevated								—

Glyphosate's impact on amphibian physiology may explain why we observed differences in movement capabilities. Similar to a previous study on the effects of the insecticide carbaryl on salamander movement [37], larval salamanders in the present study made shorter but more frequent movements at higher glyphosate concentrations, suggesting that individuals may experience fatigue more quickly in the presence of glyphosate. This response was context-dependent, with larvae exhibiting this response only at ambient temperatures. Previous studies have also documented hyperactivity, increased energy expenditures, and reduced refuge use in the presence of higher doses of glyphosate-based herbicides [7]. Altered larval activity and movement in short temporal periods may suggest underlying nervous system malfunction as a result of inhibited acetylcholinesterase [2,15]. Other physiological or genotoxic effects of glyphosate are likely to require greater time to demonstrate altered function.

Altered movement capabilities demonstrated in the present study suggest that short-term exposure to low concentrations of glyphosate could negatively affect the maintenance of individuals within headwater stream habitat. Increased frequency of movements can result in higher predation rates through enhanced detection by visually oriented predators [7]. Decreased robustness of responses to simulated predation attempts also suggests that individuals exposed to glyphosate may be unable to evade predation attempts. These results combined with the potential for individuals to fatigue more quickly when exposed to glyphosate also suggest that individuals may have difficulty avoiding downstream displacement particularly at high stream flows [33].

Studies using low chemical concentrations that display nonlinear effects challenge the concept that a substance can produce harmful effects only at high concentrations [38]. The present study's results suggest that contaminants such as glyphosate can have behavioral effects at low doses that are not predicted by effects at higher doses. Nonmonotonic dose–response curves or nonlinear relationships between dose and effect [38] can be problematic for assessing potential impacts of exposure when nonmonotonicity is evident at levels below those that are typically used in toxicological assessments. Current herbicide and pesticide exposure standards employed by government agencies have been developed using the assumption of monotonicity [39], with low-dose ranges rarely tested directly. Safety testing at high concentrations may not indicate safety at untested, environmentally relevant concentrations, particularly for chemicals that exhibit nonlinear effects. Further studies using concentrations lower than those typically used in standard testing protocols give valuable insight into the field of environmental toxicity because these doses occur within the typical range of environmental concentrations [39]. We recommend additional testing to evaluate environmentally relevant concentrations for no-observed-effect and lowest-observed-effect concentrations because they may be far lower than previous studies suggest.

The present study demonstrates that environmentally relevant concentrations of glyphosate-based herbicides can affect natural behaviors of amphibians with short-term exposure to low, environmentally relevant concentrations. These altered behaviors may make larval salamanders more susceptible to predation and downstream displacement if salamanders exhibit fatigue [37]. During salamander development prior to metamorphosis, stream salamanders are likely to experience repeated exposure to glyphosate-based herbicides and other environmental contaminants that ultimately may change the recruitment rates of salamander populations inhabiting agricultural and residential areas. Overspray and runoff following precipitation may both result in introduction of environmental contaminants into headwater streams [2]. The maintenance of riparian buffers is essential to minimize thermal shifts and exposure of headwater streams to terrestrial environmental contaminants.

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Conflict of interest—The authors have no conflict of interest to declare.

Data availability—All data are available on request to the corresponding author (kkcecala@sewanee.edu).

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