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Red-backed Salamander, *Plethodon cinereus* (Green, 1818): avoidance of urea

Carolyn M. Gaglione, Elizabeth M. O'Meara, Kelsey L. Pence, Anna C. Petterson, Geoffrey R. Smith* and Jessica E. Rettig

Abstract. Although nitrogenous fertilizers are used in forested landscapes, few studies have examined their impacts on terrestrial amphibians. Unlike their aquatic counterparts, terrestrial amphibians may temporarily avoid contaminated areas. We examined whether red-backed salamanders (*Plethodon cinereus*) can avoid urea contaminated substrates. Red-backed salamanders significantly avoided urea treated substrates (100 mg L^{-1} and 300 mg L^{-1}). Our results suggest avoidance can occur on a relatively short time frame (< 2 hours) and on natural soil substrates as well as artificial, filter paper substrates. By exhibiting avoidance behaviours in response to urea, red-backed salamanders may minimize their exposure to potentially lethal concentrations of urea.

Keywords. Avoidance behaviour, Nitrogenous fertilizers, *Plethodon cinereus*, Urea

Introduction

Fertilizer use in both agricultural and industrial areas has been increasing rapidly (Vitousek et al., 1997; Tilman, 1999; Galloway et al., 2003). In particular, the use of urea as a fertilizer worldwide has doubled in the past decade alone (Tilman, 1999; Glibert et al., 2006). Many studies have found that nitrogenous fertilizers can have a negative impact on the survival and growth of aquatic amphibian larvae (e.g., Burgett et al., 2007; Shinn, Marco and Serrano, 2008; Earl and Whiteman, 2009; Oromí, Sanuy and Vilches, 2009). Fewer studies have examined their potential impact on terrestrial amphibians (see Mann et al., 2009), even though nitrogenous fertilizers, especially urea, are commonly used in forested habitats (e.g., Albaugh, Allen and Fox, 2007; Footen, Harrison and Strahm, 2009; Vance, Maguire and Zalesny, 2010), and such exposure can cause increased mortality in terrestrial amphibians at field application rates (e.g., *Plethodon vehiculum* and *Rhyacotriton variegatus*; Marco et al., 2001).

Unlike many of their aquatic counterparts, terrestrial amphibians may be able to behaviourally evade toxic compounds by temporarily avoiding a contaminated area. For example, several salamanders and anurans have been shown to avoid substrates contaminated with nitrogenous fertilizers (Hatch et al., 2001; Marco et al., 2001; Ortiz-Santaliestra, Marco and Lizana, 2005), whereas other terrestrial amphibians are not able to either detect or avoid nitrogenous fertilizers (Hatch et al., 2001). We examined the ability of red-backed salamanders (*Plethodon cinereus*) to avoid different substrates, including filter paper and soil, contaminated with urea.

Department of Biology, Denison University, Granville, OH 43023; e-mail: smithg@denison.edu
* Corresponding author.

Materials and Methods

We collected salamanders from under cover objects and boards on the Denison University Biological Reserve, Granville, Licking Co., Ohio (40°05'00N, 82°31'05"W) and transported them back to the lab. Prior to our two experiments, salamanders were stored in rectangular plastic containers (21.4cm × 14cm) with leaf litter and a moist paper towel for 24 h. We conducted both experiments in a laboratory (21 °C) with window shades drawn. Each salamander was used only once in an experiment.

For each trial of Experiment 1, we placed a single salamander in a 15 cm diameter petri dish containing two separate halves of filter paper saturated in two different solutions (Marco et al., 2001). One half was saturated in urea fertilizer [$(NH_2)_2CO$] while the other half was saturated with aged tap water. Two different treatments of urea were prepared to represent high (300mg/L) and low (100mg/L) concentrations. Thirty high treatment trials and 29 low treatment trials were conducted. At the start of each trial, we placed a salamander in the centre of the petri dish (several trials were run simultaneously over two separate days). The treatment halves (water vs. urea) of the petri dishes were oriented randomly. During the experiment, we rotated the petri dishes 180 degrees every 30 minutes to minimize bias from external cues. Each set of trials ran for a total of two hours. We recorded at which side of the dish the salamander's head was located after 15 minutes, 60 minutes, and 120 minutes. We used chi-squared tests to examine for avoidance responses at each observation period.

For Experiment 2, we divided plastic containers (31 cm x 18.5 cm x 11 cm) into two equal-sized sections using waterproof tape (across the short axis of the container), which provided a water-tight divide between the two sides. We added 1 L of 100 mg L^{-1} urea solution (made with aged tap water) to 9 kg of commercial top soil to make low urea concentration soil, and 1 L of 300 mg L^{-1} urea solution to 9 kg of commercial top soil to make high urea concentration soil. We added 2 L of untreated, aged tap water to 18 kg of commercial top soil to make control soil. As with experiment 1, we ran 29 trials comparing low urea concentration with control and 30 trials high urea concentration with control. For each trial, 0.47 L of urea treated soil was placed onto one side of the container and 0.47 L of control soil was placed on the other side of the container. At the start of each trial, we placed a single salamander on the centre divider of each container. We covered each container with a lid to prevent salamanders from escaping. Salamanders were left in the containers for 24 hours, at which time we recorded whether each salamander was on the control soil or the urea treated soil side of the container. We used chi-squared tests to examine for avoidance responses.

Results

For the low urea concentration trials of Experiment 1 there was no significant tendency for salamanders to be found on either the treated or control side of the petri dish at 15 minutes (Table 1; $\chi_1^2 = 1.69$; $P = 0.194$). After 60 minutes, there were significantly more salamanders on the control side than the low urea side (Table 1; $\chi_1^2 = 9.96$; $P = 0.002$). There were significantly fewer salamanders on the filter paper with low urea than on the control filter paper at 120 minutes (Table 1; $\chi_1^2 = 15.21$; $P < 0.0001$).

After the first 15 minutes, there were similar numbers of salamanders on the filter paper with the high concentration of urea and the control filter paper (Table 1; $\chi_1^2 = 0.133$; $P = 0.715$). After 60 minutes, there was still no significant tendency of the salamanders to be found on the control or high urea side of the petri dish (Table 1; $\chi_1^2 = 0.533$; $P = 0.465$). However, after 120 minutes, there were significantly more salamanders on the control sides than on the high urea sides of the petri dishes (Table 1; $\chi_1^2 = 4.8$; $P = 0.028$).

Table 1. The number of red-backed salamanders (*Plethodon cinereus*) found on the control or urea treated sides of petri dishes at 15 minutes, 60 minutes, or 120 minutes.

	15 minutes	60 minutes	120 minutes
Control	11	23	25
Low urea	18	6	4
Control	14	17	21
High urea	16	13	9

In experiment 2, more salamanders were found on the control soil than the low urea soil (25 vs. 4; $\chi_1^2 = 15.2$; $P < 0.0001$). We also found more salamanders on the control soil than on the high urea soil (21 vs. 9; $\chi_1^2 = 4.8$; $P = 0.028$).

Discussion

Our results indicate that red-backed salamanders will behaviourally avoid urea treated substrates, at least at the concentrations used in this study. In addition, our results suggest this avoidance can take place on a relatively short time frame (< 2 hours) and on natural soil substrates as well as artificial, filter paper substrates. Other amphibians also show the ability to avoid substrates contaminated with nitrogenous fertilizers. For example, Iberian newts (*Lissotriton boscai*) can avoid ammonium nitrate contaminated substrates (Ortiz-Santaliestra, Marco and Lizana, 2005) and *Rhyacotriton variegatus* and *Taricha granulosa* can avoid urea contaminated substrates (Marco et al., 2001). Juvenile *Anaxyrus boreas* and *Rana cascadae* avoid lethal levels of urea on paper towel substrates, but not on soil substrates (Hatch et al., 2001). *Plethodon vehiculum* did not show a strong avoidance response to urea in a choice experiment using a paper towel substrate, although the subjects frequently climbed the walls when exposed to paper towels with urea, which was interpreted as avoidance behaviour (Marco et al., 2001). However, Hatch et al. (2001) found that *Ambystoma macrodactylum* did not avoid soil substrates contaminated with lethal levels of urea. In addition, *P. cinereus* have also been shown to avoid soils contaminated with high levels of lead (Bazar et al., 2010) and to choose substrates based on pH (Mushinsky and Brodie, 1975), displaying a capability for detecting and responding to a variety of contaminants.

Our results suggest that by exhibiting avoidance behaviours in response to urea, red-backed salamanders, and perhaps other terrestrial salamanders, including other *Plethodon* (see Marco et al., 2001), may be able to minimize their exposure to potentially lethal concentrations of urea. Marco et al. (2001) showed that field application rates of urea can be lethal to some terrestrial salamanders, including *P. vehiculum*. While it appears the salamanders can detect and avoid urea contamination, it is not clear if this would be possible in a field setting. For example, if urea is used over a large area of a forest there may be few refugia from the urea or the refugia may be spread over a larger area than the salamanders could travel or detect. We need further studies to examine how salamanders may respond behaviourally to larger scale urea applications, as well as experiments that can examine a three-dimensional behavioural avoidance response (i.e., salamanders escaping below ground or under cover objects). Salamanders may also be able to avoid urea contamination by reducing their activity on the forest floor, but it is not clear if this actually happens. In addition, there may be sub lethal consequences for avoidance responses, such as changes in the levels of interactions among salamanders, decreased feeding, etc., that should be considered in future experiments.

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