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Possible Behavioral Avoidance of UV-B Radiation and Sunlight in Wood Frog (*Lithobates sylvaticus*) Tadpoles

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Abstract: Little is known of the ability of amphibian larvae to behaviorally avoid exposure to UV radiation. We examined whether wood frog (*Lithobates sylvaticus*) tadpoles avoid UV-B radiation and, more generally, sunlight. We found that wood frog tadpoles were located more often under a UV-B blocking filter (only after 60 min) or under shade than would be expected if their behavior was random. Our observations suggest that wood frog tadpoles may minimize exposure to UV-B, either by directly avoiding UV-B or coincidentally by choosing shade.

Key words: Amphibian; Behavioral avoidance; *Lithobates sylvaticus*; Sunlight; UV-B radiation

INTRODUCTION

Throughout the last century, there has been an increase in the amount of UV-B radiation reaching the surface of the Earth (McKenzie et al., 2003). Some have argued that such increases in UV-B radiation could negatively affect amphibians and other aquatic organisms (Blaustein et al., 2003; Bancroft et al., 2007, 2008a; Croteau et al., 2008). However, some studies have suggested that while UV-B can negatively affect individual amphibians under some conditions, the actual effect on natural amphibian populations is less clear and could be moderated by a variety of factors (e.g., dissolved organic content, shading, behavioral responses, phenology; Corn and Muths, 2002; Palen et al., 2002, 2005).

Much of the focus on the potential effects of UV-B on amphibians has centered on exposure of egg or embryo stages (Blaustein et al., 1998, 2003; Blaustein and Belden, 2005; Bancroft et al., 2008a). Fewer studies have examined the larval stage directly, with some studies finding elevated mortality rates when exposed to UV-B radiation (e.g., Belden et al., 2000, 2003; Belden and Blaustein, 2002; Weyrauch and Grubb, 2006) and others finding no effect on mortality (e.g., Pahkala et al., 2003). In addition, decreased growth and the development of deformities due to UV-B exposure can potentially lead to an increased susceptibility to predation (Romansic et al., 2009).

Amphibian larvae may be able to behaviorally avoid harmful UV-B radiation (see Blaustein and Belden, 2003). Thus, larvae that can avoid exposure to UV-B radiation might have increased fitness. Several studies have shown that some larval amphibians can avoid UV-B

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radiation (van de Mortel and Buttemer, 1998; Garcia et al., 2004), whereas others alter aspects of their behavior (e.g., activity levels; Nagl and Hofer, 1997; Garcia et al., 2009a, b) in ways that are likely to reduce exposure to UV-B radiation. However, not all larval amphibians appear able to detect or avoid UV-B radiation (e.g., van de Mortel and Buttemer, 1998; Belden et al., 2000, 2003; Pahkala et al., 2003; Bancroft et al., 2008b).

We examined whether wood frog (*Lithobates sylvaticus*) tadpoles behaviorally avoid UV-B and, more generally, sunlight. Wood frogs occur in ponds across a gradient of canopy cover (Skelly et al., 2002, 2005; Halverson et al., 2003). However, wood frog tadpoles tend to grow and survive better in low canopy cover ponds (e.g., Halverson et al., 2003; Stevens et al., 2006) and at cooler water temperatures (Castano et al., 2010). Wood frog tadpoles exposed to UV-B radiation had increased mortality compared to those not exposed to UV-B (Weyrauch and Grubb, 2006). Based on these observations, it would appear that it would be advantageous for wood frog tadpoles to avoid UV-B radiation, especially since they often occur in ponds with little canopy cover. We therefore conducted this experiment to assess whether wood frog tadpoles do show any indications of behaviorally avoiding UV-B radiation.

Materials and Methods

We conducted two choice experiments on 6 April 2010 (Mylar experiment; air temperature ≈ 26 C) and on 20 April 2010 (Shaded experiment; air temperature ≈ 16 C). Both experiments were conducted in a fenced field on the Denison University Biological Reserve in Granville, Licking Co., Ohio (40°05' N, 82°31' W). Each experiment was conducted in the early afternoon from 1200 h to 1400 h during full sunlight. Tadpoles for this experiment were obtained from five field-collected wood frog egg masses from a local pond and represented a random sample from these five clutches. Prior to the experiment eggs and tadpoles were maintained in a laboratory with *ad libitum* food and without direct exposure to UV-B radiation or sunlight (i.e., under indoor lighting).

Both experiments were conducted in clear, plastic containers (31 cm L×18.5 cm W× 11 cm H) filled with well water to a depth of 10 cm. The orientation of the containers was systematically varied to prevent external cues from influencing tadpole behavior by having the long axis of containers aligned so that the filter side of the container rotated among the four cardinal directions among replicates. For the first experiment, half of each container was covered with a filter, placed directly on top of the container, that blocks UV-B (measured transmission: UV [300–400 nm]=81%, UV-A [315–400 nm]=83%, UV-B [300–315 nm]= 0.5%) while the other half was exposed to full sunlight. We performed 40 replicates, each of which contained 5 randomly selected tadpoles in each container. The five tadpoles were introduced into the container simultaneously to the middle of the container. We recorded the tadpoles' position (open side or filter side) in the container every 15 min for one hour. The second experiment used the same experimental protocol, except that instead of a UV-B filter, we used manila folder material to block sunlight (i.e., create a shaded half) and with 20 replicates.

We did not measure water temperature in either experiment, but given the small size of the containers and short duration of the experiment, it seems unlikely that a temperature gradient would have developed in the containers. We also did not measure dissolved organic content, but given the shallowness of the water in our experiment, we feel any attenuation of UV would be relatively minor (see Häder et al., 2007; Croteau et al., 2008).

After arcsine-square root transforming proportional data, we used one sample t-tests for each 15 min observation period to compare the mean proportion of tadpoles using the filter or shaded side to the 50% expected if tadpoles were using each side of the container randomly (i.e., for each observation, we tested

TABLE 1. Mean±SE proportion of wood frog (*Lithobates sylvaticus*) tadpoles found under UV-B filter (n=40) and under shade (n=20) at 15 min intervals. NS: not significant; *: P≤0.05; **: P≤0.01; ***: P≤0.0001.

	Time from Start of Experiment			
Treatment	15 min	30 min	45 min	60 min
UV-B filter	0.49 ± 0.04^{NS}	0.52 ± 0.04^{NS}	0.55 ± 0.04^{NS}	0.62±0.04**
Shade	0.84±0.04***	0.84±0.04***	0.87±0.03***	0.88±0.04***

to see if tadpoles randomly used the two sides). Since we are comparing mean proportional use of the filter or shaded side of the container to an expected value, we could not use a repeated measures analysis. We used an α-value of 0.05.

RESULTS

For the first 45 min of the first experiment, the proportion of tadpoles using the filter side of the container did not significantly differ from 0.5 (Table 1). However, at one hour there were significantly more tadpoles using the UV-B filter side of the container than would be expected by chance (Table 1). For the second experiment, more tadpoles were found on the shaded side of the container than would be expected if the tadpoles were distributed at random at every 15 min interval (Table 1).

DISCUSSION

Our results clearly indicate that wood frog tadpoles select areas that block direct sunlight, and that this choice occurs rapidly (<15 min). Our results for the ability of wood frog tadpoles to avoid UV-B are more ambiguous. Avoidance was only significant at the final observation point (i.e., after an hour), and the proportion of tadpoles choosing the filtered side was only 61.5%. Thus our results suggest that Wood Frog tadpoles may detect and avoid UV-B radiation, but the response takes time and is not very strong. However, the dramatic and rapid response to shade likely allows these tadpoles to avoid UV-B radiation at the same time they are avoiding cues of potentially high temperatures (i.e., shade might provide a visual cue that the water might be cooler) or it may reflect the tadpoles seeking refuge from predation under the shade. In results similar to ours, Belden et al. (2000) found that larval *Ambystoma macrodactylum* chose shaded areas more than unshaded areas, but did not differ in their use of low and high UV-B areas. Since UV-B exposure increases mortality of wood frog tadpoles (Weyrauch and Grubb, 2006), our observations suggest that they may be able to minimize their exposure to potentially lethal UV-B, either by directly avoiding UV-B exposure or coincidentally by seeking shade.

Our results for the use of shade and the avoidance of direct sunlight are consistent with some aspects of the ecology of wood frogs. Although wood frogs are considered canopy generalists (i.e. they use ponds that range from no canopy to ponds that have a great deal of canopy cover; Skelly et al., 2002, 2005; Halverson et al., 2003) and tend to grow and survive better in low canopy cover ponds (e.g., Halverson et al., 2003; Stevens et al., 2006), they also grow and survive better at cooler water temperatures (Castano et al., 2010). Thus, selection of shaded habitats may help wood frog tadpoles maintain a beneficial thermal environment that is most conducive to their growth and survival or alternatively, they may select the shaded habitats as a means to reduce predation risk if shade is indicative of shelter or refuge.

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