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Sensitivity of Two Salamander (*Ambystoma*) Species to Ultraviolet Radiation

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ABSTRACT.—**Increased ultraviolet-B (UV-B) radiation reaching the Earth's surface has been implicated in amphibian declines. Recent studies have shown that many amphibian species have differences in sensitivity depending on developmental stage. Embryos and larvae of *Ambystoma maculatum* (Spotted Salamander) and larvae of *Ambystoma talpoideum* (Mole Salamander) were exposed to five simulated UV-B treatments in controlled laboratory experiments to determine the relative sensitivity of different lifestages. Hatching success of the embryos exceeded 95% in all treatments; however, the larvae of both species exhibited greater sensitivity to UV-B exposure. Older larvae of *A. maculatum* that were not exposed to UV-B as embryos were more sensitive than larvae that had hatched during exposure to UV-B. Growth of surviving larvae of *A. maculatum* was significantly reduced as UV-B intensity increased, whereas growth of *A. talpoideum* was unaffected. These results were compared to ambient UV-B conditions in natural environments. It appears that the embryo stage is relatively unaffected by UV-B levels observed in natural habitats, probably because of protection from vegetation, organic matter in the water column, oviposition depth, and egg jelly. The larval stage of these species may be at greater risk, particularly if there is an increase in UV-B radiation exposure caused by increases in water clarity and/or decreases in dissolved organic carbon.**

Recently, documented global declines in amphibian species have been speculated to be caused by increased ultraviolet-b (UV-B) radiation exposure (Blaustein et al., 1998; Broomhall et al., 2000; Hakkinen et al., 2001). Although the extent to which UV-B radiation alone is responsible for amphibian declines is in contention (Licht, 2003; Blaustein et al., 2004; Corn and Muths, 2004; Palen et al., 2004), it is clear that the impact of UV-B radiation is often more severe when organisms are exposed simultaneously to other stressors (e.g., nutrients [Hatch and Blaustein, 2003], contaminants [Zaga et al., 1998, Hatch and Burton 1998], pathogens [Kiesecker et al., 2001]). Water characteristics such as clarity, color, and dissolved organic carbon (DOC) concentration can all affect UV-B transmission in the water column (Scully and Lean, 1994; Morris et al., 1995). Therefore, the degree of UV-B exposure is dependent on numerous factors including water chemistry, depth, egg laying behavior and specific sites of egg deposition (Marco et al., 2001; Palen et al., 2002). Other habitat characteristics such as woody debris, emergent and submergent vegetation can also shield organisms from exposure.

Although most of the studies involving amphibian sensitivity to UV-B have been conducted using anurans, effects on urodeles have also been observed. Salamander and newt species have demonstrated both lethal (Nagl and Hofer, 1997; Anzalone et al., 1998; Crump et al., 1999) and sublethal (Blaustein et al., 2000; Kats et al., 2000) responses to UV-B radiation alone. Specifically, the effects of UV-B on ambystomatid salamanders (such as those used in our study) include decreases in hatching success and growth (Blaustein et al., 1995, 1997; Belden et al., 2000) and increases in the rate of deformities (Blaustein et al., 1997).

Amphibian sensitivity to UV-B can vary with developmental stage (Blaustein et al., 1995; Grant and Licht, 1995; Crump et al., 1999; Pahkala et al., 2001). Free-swimming larvae have some behavioral capacity to minimize exposure by moving to areas of decreased UV-B exposure; given their immobility, eggs are thought to be more susceptible to exposure to UV-B radiation. However, amphibian eggs are encased within a protective jelly coat that sometimes forms an egg mass, which may mechanically protect developing larvae from exposure to UV-B radiation. In fact, some studies have shown that this surrounding jelly absorbs UV-B radiation (Grant and Licht, 1995; Ovaska et al., 1997; Licht, 2003). Amphibian eggs are also protected chemically by the presence of the UV-repair enzyme photolyase, which removes pyrimidine dimers, aiding in the repair of UV-B induced DNA damage. In some studies, the amount of photolyase in amphibian eggs has been found to be correlated with UV-B sensitivity (Blaustein et al., 1994, 1995; Smith et al., 2002). For instance, Blaustein et al. (1995)

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suggested that embryos of *Ambystoma gracile* are sensitive to ambient UV-B intensities because they have a low photolyase activity. In contrast, hatching success of embryos of the Boreal Toad (*Bufo boreas*) is unaffected by UV-B radiation even though they have low photolyase activity (Corn, 1998). Smith et al. (2002) observed an inverse relationship between photolyase concentration and jelly absorbance and demonstrated the mechanically and chemically protective properties of an egg mass.

Six species of *Ambystoma* occur in Missouri. The Spotted Salamander, *A. maculatum*, is found in the southern two-thirds of the state and breeds in early spring. Egg masses are usually laid in shallow, fishless, woodland ponds and may contain 12 to over 250 eggs (Johnson, 2000). The Mole Salamander, *A. talpoideum*, is restricted to southeastern Missouri and breeds in winter. The eggs are loosely attached to submerged vegetation in small clumps of 4–20 eggs (Johnson, 2000).

The primary objective of our study was to determine the sensitivity of embryo and larval stages of *A. maculatum* to ambient levels of UV-B radiation. Hatching success, mortality and growth were used as endpoints. We also determined whether exposure during embryonic development rendered individuals less sensitive to effects of UV-B radiation later in development. Finally, we determined the relative sensitivity of *A. maculatum* and *A. talpoideum* at similar larval stages. These studies were conducted to further evaluate the overall sensitivity of urodeles to UV-B radiation.

Materials and Methods

Collection of Egg Masses/Larvae.—On 10 May 2000, 20 egg masses of *A. maculatum* approximately 5–10 cm diameter were collected from a marshy area along the Eleven Point River just a few meters from the Riverton Access (off Hwy. 160) in Oregon County, Missouri. Egg masses were placed with pond water in coolers and transferred to the Columbia Environmental Research Center (CERC, Columbia, Missouri) and maintained in aquaria until testing.

In February 2003, adult *A. talpoideum* were collected at the Savannah River Ecology Laboratory in Aiken, South Carolina, and shipped to the University of Missouri, Columbia, Missouri. Breeding adults were placed in cattle tanks containing 1000 liters of well water with 1 kg of leaf litter inoculated with zooplankton. Resulting embryos were collected and brought into the laboratory where they were allowed to hatch. Resultant larvae were transported to CERC when they were approximately two weeks old. Both egg masses of *A. maculatum* and larvae of *A. talpoideum* were kept in separate aquaria where they were allowed to acclimate to fresh well water (pH 7.8, water hardness 283 mg/L $CaCO_3$, 17°C) for at least 24 h prior to initiation of tests.

Light Exposures and Test Conditions.—Simulated solar UV-B exposures were conducted to determine the sensitivity of eggs and larvae of *A. maculatum* to UV-B. The exposures applied during these studies were based on observed UV and visible irradiance values measured in the water column at various field sites supporting amphibian populations. A range of the observed intensities are reported in Table 1 for outside field measurements taken in water at 10 cm depth at various sites in central Missouri during the summer months of 2003 and 2004. Light intensity was measured with an Optronics OL-754 scanning spectroradiometer (Optronics Laboratories, Orlando, Florida) from the wavelength range of 280–700 nm. Thus, our laboratory exposures were designed to represent the maximum possible dose that the embryos and larvae would receive in the absence of shade and vegetation for protection from exposure. The light treatments included a range of UV-B irradiance values that bracketed observed field levels of UV-B (290–320 nm) and UVA (320–400 nm) wavebands and consisted of a control, low, medium, high and enhanced light treatment (Table 1). Visible radiation (400–800 nm) was applied at about 40% of ambient sunlight to ensure activation of endogenous cellular photorepair mechanisms for UV injury. The irradiance treatments were achieved by surrounding and covering the exposure chambers with various filters or filter combinations such as polycarbonate (10% UV transmission), Mylar-D (1% UV transmission), cellulose acetate (25% UV transmission), and shade cloth (15% light transmission; Table 1). All plastic filters were obtained from Cope Plastics, St. Louis, Missouri, and the shade cloth (Sun Guard 85 solar screening, New York Wire, Mt. Wolf, Pennsylvania) was purchased from a local hardware store.

All exposures were conducted in a 1×2 m solar simulator (Little and Fabacher, 1996) consisting of a light cap suspended over a water bath of similar dimensions enclosed with a highly UV-reflective specular aluminum (National Institute for Standards and Technology, Gaithersburg, Maryland). The light cap was equipped with cool white, UV-B and UVA fluorescent lamps, and halogen flood lamps. The cool white, halogen, and UVA fluorescent lamps were controlled by a timer to operate for 16 h daily. The UV-B lamps were activated with a second timer to operate for 5 h per day, starting five hours after the onset of the white light and UVA photoperiod, to simulate solar noon irradiance conditions during summer months. The simulator was checked daily for lamp function, water bath temperature, and photoperiod cycles.

TABLE 1. Comparison of simulated ultraviolet (UV) and visible irradiance provided by various filter treatments applied during the exposures of *Ambystoma maculatum* and *Ambystoma talpoideum* to natural solar radiation measured in a variety of amphibian habitats. All measurements were made at a water depth of 10 cm. Asterisks denote enhanced irradiance.

Light treatment	Intensity ($\mu W/cm^2$)			Filter combinations	Species
	UV-B	UV-A	Visible		
Control	0.002	14.9	2227	Top filter: 2 Polycarbonate (0.79 mm) layers + Mylar (0.13 mm) layer + shadecloth (85%) layer Side filter: Mylar (0.13 mm) wrap + shadecloth (85%) wrap	*A. maculatum*
Low	10.6	1680	3766	Top filter: Mylar (0.13 mm) layer Side filter: Mylar (0.13 mm) wrap	*A. maculatum*
Medium	37.6	1846	3255	Top filter: Mylar (0.13 mm) layer	*A. maculatum*
High	171	2255	3824	Top filter: Cellulose acetate (.015 mm)	*A. maculatum*
E High*	241	2385	3941	No filter combinations	*A. maculatum*
Control	0.001	9.2	1707	Top filter: 2 Polycarbonate (0.79 mm) layers + Mylar (0.13 mm) layer + shadecloth (85%) layer Side filter: Polycarbonate (0.79 mm) wrap + Mylar (0.13 mm) wrap + shadecloth (50%) wrap	*A. talpoideum*
Low	8.3	944	4045	Top filter: Frosted polystyrene (1 mm) layer	*A. talpoideum*
Medium	32	798	3773	Top filter: Polycarbonate (0.79 mm) layer	*A. talpoideum*
High	93	1913	4166	Top filter: Cellulose acetate (.015 mm) layer	*A. talpoideum*
E High*	156	2272	4057	No filter combinations	*A. talpoideum*
Field Site					
Missouri	1.9–60	298–1578	—	Measurements taken in water at various sites within central Missouri	

Exposure of Ambystoma maculatum.—Three replicate whole egg masses (approximately 60–100 embryos/egg mass) were singly placed in 3-liter glass square chambers and exposed to one of four UV intensities and a control (total $N = 15$; Table 1). Water temperature was held at 17°C. Embryos within the egg masses were approximately at the late neurula stage (Harrison stage 28; Harrison, 1969) at the onset of exposure. After seven days, hatching success was recorded. Ten of the surviving larvae were left in the chambers for an additional seven day exposure to reach the free-swimming stage at 14 days. At 14 days, mass and length of larvae were recorded.

To determine the sensitivity of older larvae to UV, larvae from several egg masses were pooled and reared under culture conditions (i.e., in the absence of ambient UV) and were exposed to UV at the age of two weeks posthatch. Three replicate groups of 10 larvae were placed into 3-liter glass square chambers and exposed to the same treatments as described above. Mortality was recorded every day throughout the duration of the 7-day exposure. At the termination of the exposure, mass and length of surviving larvae were recorded. Larvae were fed 1 mL of concentrated brine shrimp daily throughout the exposure. Feeding time was prior to the UV lights turning on.

Exposure of Ambystoma talpoideum.—Exposures of this species were initiated with larvae approximately two weeks posthatch. Groups of 10 larvae were placed in three-liter glass square chambers and exposed for seven days to approximately the same UV treatments used for the other species (see Table 1). Larvae were not previously exposed to UV radiation as embryos. Each treatment consisted of three replicates. Mortality was recorded daily, and weight and length were taken at the end of the exposure. Larvae were fed 1 mL of concentrated brine shrimp daily throughout the exposure. Feeding time was prior to the UV lights turning on.

Statistical Analyses.—For *A. maculatum*, the proportion of eggs hatching from each egg mass was angularly transformed and analyzed using analysis of variance (ANOVA; SAS®/STAT Users Guide, Vers. 8.02, Statistical Analysis Systems, Cary, NC, 2001). Length and mass were log transformed and also analyzed using ANOVA

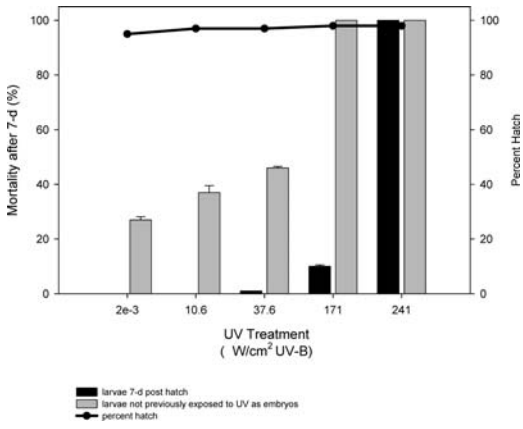

FIG. 1. Line represents percent *Ambystoma maculatum* eggs hatching. Shaded bars represent percent mortality of larvae after seven days (posthatch) of exposure (14 days total exposure) to UV during embryonic development. Light bars represent responses of larvae that had developed in the absence of ambient UV and were exposed for seven days (posthatch). Vertical bars represent ± SE; $N = 10$.

(SAS®/STAT Users Guide, Vers. 8.02, Statistical Analysis Systems, Cary, NC, 2001). For each variable, the number of eggs in each mass was used as a covariate to control for differences in density within the chambers.

The proportion of larvae of *A. talpoideum* surviving in each replicate, were pooled, angularly transformed and analyzed using ANOVA (SAS®/STAT Users Guide, Vers. 8.02, Statistical Analysis Systems, Cary, NC, 2001). Mass and length were similarly calculated and log transformed. When significant differences were detected in either species, LSD multiple comparison tests were used to determine statistical differences among treatments (SAS®/STAT Users Guide, Vers. 8.02, Statistical Analysis Systems, Cary, NC, 2001).

Probit analysis was used to determine the lethal or effective UV-B dose for the larval exposures for both species (Western EcoSystems Technology (WEST), TOXSTAT® Vers. 3.5, Cheyenne, WY, 1996).

RESULTS

Ambystoma maculatum.—There were no differences in hatching success for embryos of *A. maculatum* exposed to the five UV treatments ($F_{4,9} = 0.22$; $P < 0.9179$; Fig. 1). All egg masses were $\geq$ 95% viable with 285 of 299 embryos hatching in the controls, 215 of 222 in the low dose, 239 of 246 in the medium dose, 272 of 283 in the high dose, and 278 of 282 in the enhanced dose. The egg jelly encapsulating the embryos supported a growth of symbiotic green algae, which did not appear to affect hatching success across UV treatments.

After 14 days of exposure, surviving larvae exposed for seven days posthatch (exposed during both embryo and larval stage) experienced 100% mortality at the highest UV dose (60.7 J/cm^2 UV-B; Table 2, Fig. 1). Of the larvae that died, approximately 50% died after exposure three days posthatch, whereas mortality occurring in other treatments began after five days posthatch. Larvae exposed to the 10.6, 37.6 and 171 μW/cm^2 UV treatments and the control, incurred 10% or less mortality (Fig. 1) after 14 days of exposure. UV intensity significantly affected larval mass ($F_{4,9} = 4.24$; $P < 0.0335$). Larvae from the control and low-UV treatment were significantly larger than individuals in the three highest UV treatments, although the mass of larvae exposed to the low-UV and

TABLE 2. Exposure irradiances, daily doses and total doses for embryos and larvae of *Ambystoma maculatum* and for larvae of *Ambystoma talpoideum*.

Species	Light treatment	UV-B irradiance (μW/cm^2)	Daily Dose 5 hr/day (J/cm^2)	Number of days exposed		Total dose (J/cm^2)	
				Embryos	Larvae[a]	Embryos/Larvae (7-day)	Larvae (14-day)
A. maculatum							
	Control	0.002	0.000036	7	14 (7)	0.000252	0.000504
	Low	10.6	0.190800	7	14 (7)	1.3356	2.6712
	Medium	37.6	0.676800	7	14 (7)	4.7376	9.4752
	High	171	3.078000	7	14 (7)	21.546	43.092
	E High	241	4.338000	7	14 (7)	30.366	60.732
						Larvae (7-day)	
A. talpoideum							
	Control	0.001	0.000018	–	(7)	0.000126	–
	Low	8.3	0.149400	–	(7)	1.0458	–
	Medium	32	0.576000	–	(7)	4.032	–
	High	93	1.674000	–	(7)	11.718	–
	E High	156	2.808000	–	(7)	19.656	–

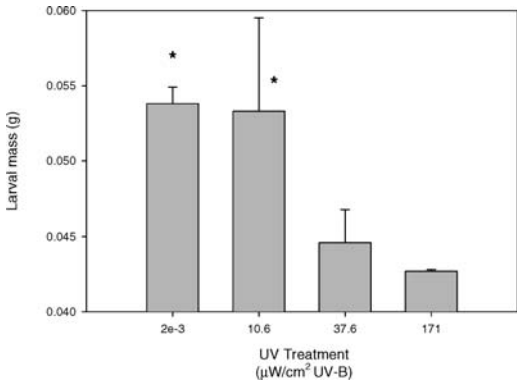

FIG. 2. Mass of *Ambystoma maculatum* larvae after seven days of UV exposure. Treatments with "*" are significantly different than treatments without the symbol. Vertical lines represent ± SE; $N = 10$.

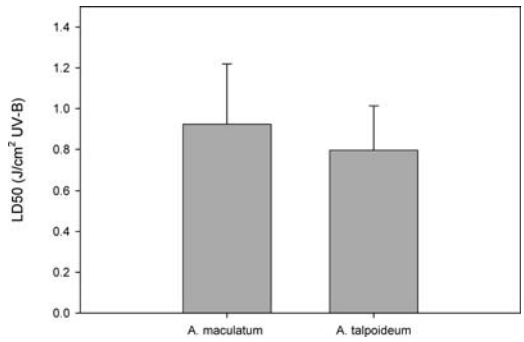

FIG. 3. LD_{50} estimates for *Ambystoma maculatum* and *Ambystoma talpoideum* larvae. Vertical lines represent ± SE; $N = 10$.

medium-UV treatments did not differ (Fig. 2). No significant effects of UV on larval length were observed ($F_{4,9} = 1.45$; $P < 0.2957$).

Hatching success was > 95% for embryos held in culture with no exposure to UV radiation which was similar to that of UV exposed egg masses. There were significant differences in larval survivorship among UV treatments ($F_{4,9} = 3.12$; $P < 0.0005$). No larvae survived at the two highest UV doses (60.7 J/cm^2 UV-B and 43.0 J/cm^2 UV-B; Fig. 1) after 96 h. In the two lowest UV treatments and the control, larvae experienced 27–46% mortality. An LD50 (lethal dose affecting 50% of population) was estimated to be 0.9234 J/cm^2 UV-B (95% C.I. 0.6264–1.219; Fig. 3). There were no significant effects of UV on larval length or mass of the surviving larvae in this group. The UV treatments tested did not illicit any deformities among the larvae.

Ambystoma talpoideum.—There were significant differences in survivorship of larvae among UV treatments ($F_{4,10} = 3.47$; $P < 0.0001$). Larvae suffered 100% and 93% mortality at the two highest UV doses, respectively (2.808 J/cm^2 UV-B and 1.674 J/cm^2 UV-B; Fig. 4) by 96 h. Although mortality at the control dose was 11%, there was no significant difference when compared to the lowest UV dose (i.e., 17%; $P > 0.05$). The LD50 was estimated to be 0.7956 J/cm^2 UV-B (C.I. 0.5652–1.028; Fig. 3). There were no significant effects of UV on the length ($F_{2,10} = 1.48$; $P < 0.2726$) or mass ($F_{2,10} = 0.09$; $P < 0.9168$) of the remaining surviving larvae. We noted no deformities among any larvae.

DISCUSSION

UV radiation can interact with other stressors, biotic and abiotic, to increase the injury to amphibians (Kiesecker and Blaustein, 1995; Long et al., 1995; Britson and Threlkeld, 2000; Little et al., 2000; Blaustein et al., 2003; Bridges and Boone, 2003). However, many studies suggest that ambient UV intensities alone have the potential to directly impact amphibians. Because natural intensities of UV radiation are increasing as a result of the thinning of the atmospheric ozone layer (Kerr and McElroy, 1993), examining the effects of UV radiation alone remains important.

Our experiments indicate that embryos of *A. maculatum* exposed to simulated UV radiation in the laboratory are relatively tolerant to this stressor. Similar to Crump et al. (1999), we found no effect of UV-B on the hatching success of *A. maculatum*. Spotted Salamander embryos are enclosed within egg capsules that are further imbedded in a thicker jelly layer surrounding all the eggs within a mass. The thick outer coat of these egg masses often contains symbiotic green algae, which is thought to provide oxygen to developing embryos (Goff and Sein 1978; Pinder 1994). Hatching success of embryos of *A. maculatum* exposed to enhanced and ambient

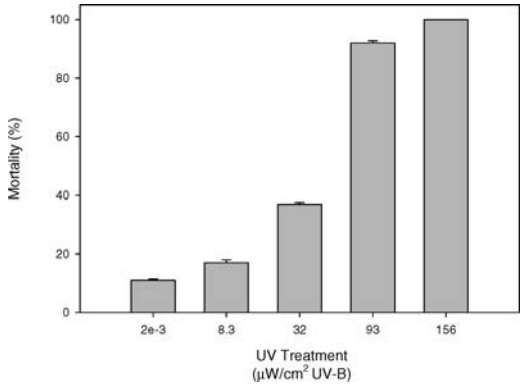

FIG. 4. Percent mortality of two-week-old *Ambystoma talpoideum* larvae after seven days of UV exposure. Vertical lines represent ± SE; $N = 10$.

UV-B has been found to be independent of whether the outer jelly coat is present or absent, clear, cloudy, or contains algae, suggesting the egg jelly does not play a role in photoprotection (Crump et al., 1999; Starnes et al., 2000). It is possible, though, that the thickness of this external jelly coat is in and of itself protective (Grant and Licht 1995), and Licht (2003) has shown that several species of *Ambystoma* have absorbance properties in their egg jelly envelopes. Contradictory to these studies and our study, Lesser et al. (2001) and Blaustein et al. (1995, 1997, 1998) found that salamander embryos exposed to UV-B radiation showed DNA damage, reduced hatching success, and increased mortality.

Blaustein et al. (1995, 1997) reported that embryos of the Long-Toed Salamander (*Ambystoma macrodactylum*) and Northwestern Salamander (*Ambystoma gracile*) shielded from UV-B exposure had significantly higher hatching success with fewer deformities than those exposed to UV-B radiation. This would suggest that species with low photolyase activity are negatively affected by UV-B exposure. Smith et al. (2002) found photolyase activity to be significantly different among seven species of amphibians. Furthermore, UV damage repair was correlated with expected ambient exposure, which included factors such as oviposition depth and the absorbance of the jelly layer. Although we did not measure photolyase activity in embryos of *A. maculatum*, Smith et al. (2002) reported moderate levels of activity for this species.

Larvae of *A. maculatum*, regardless of whether they were exposed or not exposed to UV radiation as developing embryos, were very sensitive to enhanced levels and exhibited 100% mortality within 96 h. At all but the highest (i.e., enhanced) UV dose, larvae that were exposed to UV during the egg stage had less than 10% mortality. In contrast, larvae that were not previously exposed to UV before hatching exhibited 100% mortality at the second highest dose, and nearly 50% mortality at the intermediate (i.e., naturally occurring) dose. This suggests that exposure during development within the egg may be an important factor in the UV tolerance of later developmental stages (e.g., initiation of melanin production, Belden and Blaustein, 2002). Also, selection for individual embryos that survive UV exposure might impart a higher UV tolerance of resultant free-swimming larvae. However, because hatching success was high (approximately 95%) and equal to the success of unexposed larvae, this seems unlikely.

Sublethal effects of UV radiation, such as the reduction of growth, have been previously documented in ambystomatid salamanders (Belden and Blaustein 2002). The size of larvae exposed to incident levels of UV in our experiment was significantly reduced in the three highest UV intensities. This suggests there may be carry-over effects from the embryo stage to the larval stage (Smith et al., 2000; Pahkala et al., 2001). Semlitsch et al. (1988) found that adult fitness in *A. talpoideum* was closely linked with size at metamorphosis and length of the larval period and that individuals growing more slowly may be less fit than larger animals. Thus, reductions in larval growth caused by UV radiation could ultimately have ramifications on fitness and, consequently, on amphibian population dynamics.

Larvae of *A. talpoideum* were also very sensitive to enhanced levels of UV radiation. The larvae were approximately two weeks old when they were exposed and had not been previously exposed to UV radiation as embryos. The response was similar for larvae of *A. maculatum* that were not exposed as embryos (Figs. 1, 3, 4) with an LD50 value of 0.9234 J/cm^2 UV-B, whereas the LD50 for *A. talpoideum* was 0.7956 J/cm^2 UV-B, just slightly more sensitive. Eggs of this species are laid singly or in small clusters and may not have UV protection during embryonic development offered by the thick jelly coat found on eggs of *A. maculatum*. However, although *A. maculatum* eggs are attached to submergent vegetation and are, therefore, likely to be near the surface of the water, *A. talpoideum* eggs are generally attached to leaves and debris on the bottom of the pond, which may limit the amount of UV reaching the embryos during development. We acknowledge that both species were collected at different stages of development and handled differently prior to their exposures. Although we used the same procedures for culture and UV exposure, any difference in larval sensitivity has to take handling into account along with population and species differences.

Relatively high UV intensities were applied in this study. However, the estimated LD50 values for both species reflect the range of doses that could potentially be found in nature. It is very unlikely that larvae would be subjected to such high doses in nature because of other environmental factors affecting UV exposure. Therefore, the lower UV-B intensities would realistically occur in natural habitats for this species and would likely be tolerated by them.

Many environmental and behavioral factors affect UV exposure of amphibian embryos and larvae. Embryos within egg masses are immobile so water characteristics (i.e., DOC content, vegetation, turbidity) play a big role in the amount of UV exposure individuals receive during development. The color of the embryos (i.e., dermal melanin content), egg jelly properties, and depth the eggs are oviposited can also

affect UV exposure during this life stage. Spotted Salamander embryos in our study had a high rate of hatching success, even in the highest UV intensities, perhaps because of these properties. Characteristics that protect developing embryos can also protect free-swimming larvae (water quality, melanin content), in addition to behavioral adaptations that may exist (e.g., UV avoidance). We found the larval stage was more sensitive than the embryo possibly because of decreased photolyase levels which are reported to be greater in the eggs (Smith et al., 2002); however, there is no supporting evidence to indicate that photolyase levels decline once embryos have hatched (Crump et al., 1999). Alternatively, there may be carry-over effects where lethal/sublethal effects may not occur until a certain developmental stage is reached (Pahkala et al., 2001). Regardless, it is important to take all of these factors into consideration when assessing UV sensitivity in salamanders.

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