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Sensitivity of Spotted Salamander (Ambystoma maculatum) Embryos to UV-B Radiation in Central Virginia

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data and locations of large populations can lead to ways to protect turtles from being killed on roads. More documentation and data are needed in this effort to reduce the effects of road mortality on Virginia's native turtle fauna.

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Sensitivity of Spotted Salamander (*Ambystoma maculatum*) Embryos to UV-B Radiation in Central Virginia

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Worldwide, many amphibian populations have been declining and undergoing range reductions (Blaustein et al., 1998; Houlahan et al., 2000; Alford et al., 2001). Causes for the declines are often difficult to determine but fungal infections, habitat destruction, changes in local climate, pollution and increased ultraviolet (UV-B) radiation have been listed as potential causes (Blaustein and Wake, 1995; Houlahan et al., 2000). Most studies for assessing effects of UV-B radiation on amphibians occurred in Australia, Europe or northwest United States (Blaustein et al., 1998) and only one has been conducted in the eastern United States (Starnes et al., 2000). Several of these studies have included ambystomatid salamanders (*Ambystoma macrodactylum* and *A. gracile*) and an adverse UV-B effect on embryonic survival has been demonstrated (Blaustein et al., 1995, 1998). In contrast, Starnes et al. (2000) did not show a statistically significant UV-B effect on *A. maculatum* embryonic survival although decreased incidence of deformities was noted for embryos shielded from UV-B. Our goal was to determine if the results from Starnes et al. (2000) apply to *A. maculatum* populations in Virginia.

Materials and Methods

Ten egg masses of the clear jelly type (Hardy and Lucas, 1991) were collected from a ditch in a dirt road near Riverville, Amherst County, Virginia on 24 February 2001. The egg masses were returned to the laboratory and kept at 4 C for 3 days until placement in the experimental units on 27 February. Counts were made of the number of embryos per egg mass and the embryonic developmental stage was determined using the scale by Harrison (1969). Egg masses were randomly assigned to numbered plastic cases that were either shielded (n = 5) with Luminar/Plexiglas shields (shields completely block wavelengths below 380 nm and therefore UV-B radiation [280-320 nm], Lizana and Pedraza, 1998) or left exposed to sunlight using plastic mesh covers (n = 5). Each egg masses and a styrofoam 'skirt' to keep the case afloat. A completely randomized design was used to place the cases in a linear array in an exposed location in a lake near Liberty University, Lynchburg, Virginia.

During the field experiment, one egg mass was lost when a storm blew off the Luminar/Plexiglas shield and two other cases lost their styrofoam skirts at the end of the experiment; thus, some larvae were able to escape from these cases. After hatching, all egg masses and live larvae were removed to the laboratory. Mortality was assessed as eggs that did not hatch. Live larvae were anesthetized, preserved in formalin and then examined for deformities.

Percent survival and deformities for shielded and unshielded egg masses were analyzed using t-tests. Because of the small sample size, when the null hypothesis was not rejected a power analysis was done to see how large a sample size would be needed to conclude the effect was significant at $\alpha = 0.05$ and power = 0.8. If the sample size needed was large, we concluded the effect was not significant. If the sample size was small, then the failure to reject the null hypothesis (H_0: average for unshielded egg masses = average for shielded egg masses) would likely be attributable to small sample size (Gregory, 2001). Methods used for calculating power and sample size were from Zar (1996).

Results and Discussion

The number of embryos per egg mass ranged from 17 to 106 with an average of 51 embryos. The embryos were at Harrison stages 6-8 (cleavage stages to early blastula or approximately 20-30 hours old) when the egg masses were placed in the field (27 February). Hatching was completed by 17 April 2001. Percent survival averaged 96.5 and 97.8 for shielded and unshielded egg masses, respectively, and these were not significantly different ($t = 0.4$, d.f.= 7, $P = 0.7$, Table 1). All unshielded egg masses produced deformities, whereas only one of the shielded egg masses did so. The average percent deformities of 0.4 and 1.2 for shielded and unshielded egg masses, respectively, were not significantly different ($t = 1.4$, d.f.= 5, $P = 0.2$, Table 1).

Table 1. Percent survival and deformity for spotted salamander (*Ambystoma* maculatum) embryos exposed to and shielded from UV-B radiation. Means are followed by one standard deviation.

Case Types	% Survival	% Deformities
Shielded	100	0
	100	1.7
	100	0
	86.1	0
	----[A]	----[A]
	96.5 ± 7.0	0.4 ± 0.9
Unshielded	100	0.9
	94.3	0.9
	94.6	1.8
	100	----[B]
	100	----[B]
	97.8 ± 3.0	1.2 ± 0.5

A) Egg mass lost during storm.
B) Percent deformities could not be assessed due to missing larvae since cases which lost styrofoam skirts allowed for newly hatched larvae to escape.

Power analysis of the survival data indicated that a sample size of 235 for each treatment would have been required to reject the null hypothesis. Taking into consideration the large sample size that would have been needed in addition to the higher survivorship being with the unshielded treatment, we would conclude that the UV-B effect on survivorship was really not significant. In contrast, for percent deformities, the sample size needed would have only been 14 egg masses per treatment. It is likely that our lack of rejecting the null hypothesis was due to small sample size.

Our results follow those of Starnes et al. (2000) indicating there is no statistically significant evidence that UV-B increases either mortality or deformities in developing spotted salamander embryos. For percent deformities, Starnes et al. (2000, Figure 1) showed a decline from approximately 6% to 3% for clear egg masses (n=10/treatment) unshielded and shielded (98% UV-B blocking treatment), respectively. Perhaps if they had used a larger sample size, the null hypothesis for percent deformities would have been rejected. The question of biological significance would, though, still need to be addressed since the overall deformity rates were below 10% for all treatments both in this study and in Starnes et al. (2000).

The high survivorship and low rates of deformity may be due to various factors which reduce the risk of developing embryos to UV-B such as DNA repair mechanisms, reduced transmission of UV-B due to the jelly surrounding the embryo, cloud cover, and water turbidity (Hatch and Burton, 1998; Starnes et al., 2000). Even though the risk of UV-B to *A. maculatum* embryos appears minimal at this time, we consider it prudent that Virginia amphibian species that lay eggs in exposed sites be monitored for UV-B effects. There may be species-specific and/or locational differences in risk to UV-B. For example, UV-B increased *Hyla chrysoscelis* and *Pseudacris triseriata* embryonic deformity rates (Starnes et al., 2000). In addition, pollutants in some locations might work in a synergistic fashion with UV-B similar to that noted by Hatch and Burton (1998). They found photoinduced toxicity of fluoranthene (a polycyclic aromatic hydrocarbon) on spotted salamander larvae. Therefore, research on the effects of UV-B on amphibian reproductive success in a variety of locations in Virginia is encouraged.

Acknowledgments

We would like to thank the students of the 1999 Environmental Biology class at Liberty University for the construction of the cases and the 2001 class for their aid in the setup of the cases at the site on Lake Hydaway. We would also like to thank the staff at Thomas Road Baptist Church for the use of their property for the performance of this study.

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Guidelines for VHS Field-Study Grants

The purpose of Field-study Grants from the Virginia Herpetological Society is to stimulate and encourage herpetological research in Virginia. These Grants will be in variable amounts up to $200.00 and are available to VHS members who do not have access to other sources of funding, such as institutions of higher learning and government grants.

Grant requests should include a description of the proposed research, or in the case of surveys the extent of the geographic area to be surveyed, and the methods which are to be used. A rough budget would be helpful. A brief justification of the importance of the work in contributing to the knowledge of Virginia's herpetofauna, citing standard works (e.g., Mitchell, J. C. 1994. The Reptiles of Virginia. Smithsonian Institution Press, Washington, D.C. 352 pp.; Mitchell, J. C. and K. K. Reay. 1999. Atlas of Amphibians and Reptiles in Virginia. Virginia Department of Game and Inland Fisheries, Special Publication No. 1, Richmond, Virginia. 122 pp.; and Tobey, F. J. 1985. Virginia's Amphibians and Reptiles: A Distributional Survey. Virginia Herpetological Society, Privately Printed, Purcellville, Virginia. 114 pp.) should be included. The results of all funded surveys must be submitted in manuscript form for publication in *Catesbeiana*.

Grant requests will be received by the current President until March 15 of each year. The President will then send copies to Executive Committee members by the end of March, and a Committee vote will be scheduled sometime during the annual Spring meeting. The Executive Committee will first determine that funds are available, and then that the Grant request is worthy of funding. A majority ruling is required for both votes. When a grant is approved, the Secretary/Treasurer will so inform the recipient, send a check for the amount determined by the Committee, and inform the recipient of the requirement to publish the results in *Catesbeiana*.

FIELD NOTES

***Bufo fowleri* (Fowler's Toad).** VA: Warren Co., Shenandoah River at Co. Rt. 613 ("Indian Hollow Road"). 15 and 30 July 2001. John White & family.

An adult Fowler's toad was captured at 0935 h on 15 July 2001 while hopping across a gravel path in front of the main building of Shenandoah River Trips. On a return trip to the area on 30 July 2001 two juvenile *B. fowleri* were found. According to Mitchell and Reay (1999. Atlas of Amphibians and Reptiles in Virginia. Virginia Department of Game and Inland Fisheries, Special Publication No.1, Richmond, Virginia. 122 pp.) and Tobey (1985. Virginia's Amphibians and Reptiles: A Distributional Survey. Virginia Herpetological Survey, Purcellville, Virginia. 114 pp.), *B. fowleri* has not been previously recorded in Warren County. Slides have been sent to the Virginia Museum of Natural History.

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***Pseudemys rubriventris* (Northern Red-bellied Cooter).** VA: Warren Co., Shenandoah River at Co. Rt. 613 ("Indian Hollow Road"). 15 July 2001. John White & family.

A juvenile northern red-bellied cooter was captured at 1330 h on 15 July 2001 while basking in the Shenandoah River. According to Mitchell and Reay (1999. Atlas of Amphibians and Reptiles in Virginia. Virginia Department of Game and Inland Fisheries, Special Publication No. 1,Richmond, Virginia. 122 pp.), Mitchell (1994. The Reptiles of Virginia. Smithsonian Institution Press, Washington D.C. 352 pp.) and Tobey (1985. Virginia's Amphibians and Reptiles: A Distributional Survey. Virginia Herpetological Survey, Purcellville, Virginia.114 pp.), *P. rubriventris* has not been previously recorded in Warren County. Slides have been sent to the Virginia Museum of Natural History.

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