POPULATION DIFFERENCES IN SENSITIVITY TO UV-B RADIATION FOR LARVAL LONG-TOED SALAMANDERS

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Abstract. Ultraviolet-B radiation (UV-B; 280–320 nm) penetrates some aquatic habitats to biologically significant depths and can alter life histories of aquatic organisms, including algae, zooplankton, fish, and amphibians. Although major species differences have been documented for UV-B sensitivity, few studies have examined differences between populations of the same species. Previous work has suggested the hypothesis that larval long-toed salamanders (Ambystoma macrodactylum) from valley populations (≈100 m elevation) were more sensitive to UV-B exposure than larvae from mountain populations (above 500 m elevation). To test this hypothesis in the absence of possible other confounding environmental effects, we brought early-stage embryos into the laboratory from three valley populations and five mountain populations from Oregon and Washington and raised them under identical conditions without UV-B for two months after hatching. Larvae from each population were then placed under UV-B lighting, and we recorded their growth and survivorship for three weeks. Larvae from all populations had higher mortality when exposed to UV-B than when shielded from UV-B. However, individuals from low-elevation populations exposed to UV-B had significantly lower survivorship than did those from high-elevation populations, suggesting an elevational difference in UV-B sensitivity. In all populations, individuals exposed to UV-B were smaller than shielded individuals after one week. If UV-B is a factor in determining the long-term persistence of some amphibian species, an understanding of within-species variation is critical.

Key words: Ambystoma macrodactylum; amphibian declines; elevation and UV-B sensitivity; long-toed salamander; ozone depletion; salamander larvae; ultraviolet-B radiation.

INTRODUCTION

Many aquatic organisms are sensitive to ultraviolet-B radiation (UV-B; 280–320 nm) exposure, including species of phytoplankton, zooplankton, aquatic invertebrates, fish, and amphibians (e.g., Shick et al. 1996, Blaustein et al. 1998, Hader et al. 1998, Beland et al. 1999). Exposure to UV-B results in cellular damage to organisms (Tevini 1993, Hader 1997), which may ultimately have consequences for species interactions (Bothwell et al. 1994, Rousseaux et al. 1998). However, as for any abiotic factor, sensitivity to UV-B is not necessarily consistent within a given taxon, and detrimental responses in one species do not imply that others within that taxon will respond similarly. For example, although some phytoplankton have decreased photosynthetic rates when exposed to UV-B, the specific biochemical effects of exposure vary greatly with species (Arts and Rai 1997, Hessen et al. 1997). As interspecific variation in sensitivity to UV-B radiation is well documented, we might also expect differences in sensitivity between populations of a single species. For example, UV-B may increase with increasing altitude (Blumthaler et al. 1997); for species existing along altitudinal gradients, we might expect differences in sensitivity between high- and low-elevation populations. Evidence from plant research indicates that there can be altitudinal variation in response to UV-B (Caldwell 1968, Rau and Hofmann 1996, Hubner and Ziegler 1998).

Amphibian responses to UV-B exposure have been studied because of the global nature of recent amphibian population declines and range reductions. As is true for other taxa, amphibian species vary in sensitivity to UV-B. Embryos of some amphibian species die when exposed to ambient UV-B, whereas other species at the same sites appear to be unaffected (e.g., Blaustein et al. 1994, Anzalone et al. 1998, Lizana and Pedraza 1998, Broomhall et al. 2000). However, few species have been examined for population-level variation in sensitivity to UV-B and, to our knowledge, no studies have examined the same species from more than two sites in the same experiment. If UV-B is a contributing factor to some amphibian population declines, then potential differences in population-level response to UV-B could be important in conservation efforts.

Although its population status is not currently known, the long-toed salamander, Ambystoma macrodactylum, is an excellent species to use in examining potential differences in UV-B sensitivity because it has a large range and occurs from low to high elevation in the Pacific Northwest, USA (Nussbaum et al. 1983).
Previous work has shown that long-toed salamander embryos are sensitive to UV-B radiation (Blaustein et al. 1997), and work with long-toed salamander larvae from a single valley and a single mountain population suggested the possibility that mountain larvae were more resistant to UV-B exposure (Belden et al. 2000). In this study, we addressed whether there are differences in UV-B sensitivity for larval long-toed salamanders from valley and mountain populations.

**METHODS**

To determine if there were differences in UV-B sensitivity between valley and mountain populations, we raised individuals from multiple populations in the laboratory from the early-embryo stage and tested UV-B sensitivity in the laboratory eight weeks after hatching. We collected fresh eggs (Harrison stages 5–12; Harrison 1969) from eight different populations, three in the Willamette Valley (Linn and Benton Counties, Oregon) and five in the Cascade Mountains (Klickitat County, Washington; Deschutes County, Oregon) soon after oviposition (January–February for valley populations; April–June for mountain populations). The valley populations were 13–17 km apart. Elevations for the three valley sites were 78, 86, and 105 m. The mountain populations were 15–205 km apart. They had elevations of 564, 1957, 1988, 2015, and 2038 m. Once in the laboratory, eggs were reared by population in plastic boxes (32 × 18 × 8 cm) filled with dechlorinated tap water (~25 eggs per box and 40–50 eggs per population).

For each population, when hatching commenced, new larvae were separated from the eggs each day and maintained in the same type of boxes at a density of six individuals per box. We recorded the day of hatching for all larvae. For each population, we used individuals in the experiment that had all hatched within three days of one another to avoid potential differences in sensitivity due to developmental differences. Larvae were maintained at 12.7–14.5°C with 12L:12D fluorescent lighting for eight weeks prior to testing. They were fed brine shrimp ad libitum three times per week, with a complete water change done the day after feeding. We tested larvae at eight weeks of age, which is approximately half of the mean time required to metamorphose that we have previously observed in this species in the laboratory. When larvae from a single population reached eight weeks post-hatching, larvae from that population were pooled into a single tank and then randomly assigned singly into plastic petri dishes (15 cm diameter × 1.5 cm depth) filled with 1 cm of dechlorinated tap water. Larvae can be found at 1 cm depth in the field, and, using this depth in the laboratory, ensured that there could not be behavioral avoidance of UV-B by individuals choosing different locations in the water column. The total number of individuals used for each population (half went to each UV-B treatment) varied between 16 and 30 larvae (valley A, 30; valley B, 20; valley C, 30; mountain 1, 16; mountain 2, 16; mountain 3, 24; mountain 4, 30; mountain 5, 24). Sample sizes were limited by the space available for UV-B exposure and the number of larvae that were available that hatched within three days of one another.

After transfer to the dishes, we recorded the total length of each larva to the nearest mm. Each dish was then randomly assigned to either a UV-B exposed (acetate filter) or UV-B shielded (mylar filter) treatment. Mylar blocks almost 100% of UV-B radiation, whereas acetate allows 80% of UV-B to pass through (Blaustein et al. 1994). All petri dishes were then randomly placed under UV-B enhanced full-spectrum lighting. We used a parallel array of lights, consisting of four UV-B lights (Q-Panel UVB313; Q-Panel, Cleveland, Ohio, USA), alternated with four fluorescent full-spectrum lights (VitaLite; Durotest Corporation, Fairfield, New Jersey, USA). These were suspended above the table to result in levels of UV-B at a range of 4–6 μW/cm² in the portion of the table that we used. The range in UV-B radiation under mylar filters was 0.2–0.4 μW/cm²; under acetate, the range was 1.3–3.1 μW/cm². These levels are within the range experienced by *A. macrodactylum* larvae during development in the Cascade Mountains of Oregon (Belden et al. 2000), and are probably higher than levels experienced by valley larvae.

Larvae were exposed to UV-B for three weeks. Survival was recorded daily during that time, and the body length of all surviving individuals was recorded at the end of each week. During the exposure, individuals were fed approximately eight *Tubifex* worms every other day. The day after feeding, leftover food and waste was removed from all dishes and water was added so that the depth remained at 1 cm. Once a week, all of the dishes had a complete water change.

In addition, at approximately 1430 h on five clear days during larval development at a single site in the Cascade Mountains and a single site in the Willamette Valley, UV-B penetrance of the water column was recorded at 0, 5, 10, 15, and 20 cm depth using a hand-held Solar Light meter with a UV-B detector (meter model PMA2100 and detector model PMA2102; Solar Light Company, Philadelphia, Pennsylvania, USA).

We analyzed mean larval growth at the end of one week, prior to any mortality, in a two-way ANOVA, with valley vs. mountain and UV-B vs. no UV-B as the factors. All ANOVA assumptions were met for the growth data. Prior to completing a similar ANOVA on the survivorship data, we tested the data for normality and homogeneity of variance. To meet normality assumptions, survivorship data were arcsine square-root transformed prior to analysis. However, variance was not homogenous for survivorship (ANOVA on residuals of transformed data by four groups: UV-mountain, UV-valley, no UV-mountain, no UV-valley; $F_{1,12} = 4.814, P = 0.02$). We therefore proceeded with three individual hypothesis tests on the four groups, using separate variance F tests with a Bonferroni correction.
FIG. 1. Percentage of larval long-toed salamanders surviving after three weeks (top panel) and larval growth after one week (bottom panel) for UV-B exposed (black bars) and non-exposed (light gray bars) individuals from the three valley (A–C) and five mountain (1–5) populations. Statistics were performed on the means for mountain and valley populations, which are also graphed here. Sample sizes are given in the text; error bars represent 1 SE.

FIG. 2. Attenuation of UV-B radiation in the water column (0–20 cm depth) on five days during larval development at a mountain (●) and valley (○) site.

Results

Survival was significantly lower for UV-B exposed larvae from valley populations than it was for individuals from mountain populations ($F_{1,5.49} = 28.5$, $P = 0.002$). Mean survival for UV-B exposed larvae from the valley populations was 7%, vs. 74% for the UV-B exposed mountain larvae (Fig. 1). Mean survival for non-exposed larvae did not differ significantly between the mountains and the valley ($F_{1,2} = 1.0$, $P = 0.423$). Indeed, survival for non-exposed individuals was high in all populations (mean = 100% for mountain populations and 97% for valley populations; Fig. 1). However, even though survival for UV-B exposed individuals was lower in the valley than in the mountains, there was still a significant decrease in survival for UV-B exposed mountain individuals when compared to non-exposed mountain individuals ($F_{1,4} = 19.9$, $P = 0.011$).

Growth of salamander larvae at one week was significantly affected by UV-B exposure ($F_{1,12} = 11.49$, $P = 0.005$), but the location was not significant (mountain or valley; $F_{1,12} = 0.046$, $P = 0.834$), nor was the interaction between the two variables ($F_{1,12} = 0.433$, $P = 0.523$). Regardless of the population, larvae that were shielded from UV-B grew approximately twice as much as exposed individuals, on average, during the first week (Fig. 1).

At the Cascade Mountain site, UV-B radiation was $19.3 \pm 1.3 \mu W/cm^2$ (mean $\pm 1$ SD) at the surface of the water, and at 20 cm it was $4.2 \pm 0.9 \mu W/cm^2$ (Fig. 2). In the valley, UV-B at the surface of the water was $5.1 \pm 0.5 \mu W/cm^2$, and at 20 cm it was $0.7 \pm 0.7 \mu W/cm^2$ (Fig. 2).

Discussion

To our knowledge, this study is the first to demonstrate population differences in UV-B sensitivity for a single amphibian species. Long-toed salamander larvae from valley populations experience greater mortality when exposed to UV-B than do larvae from mountain populations, although individuals from all populations experience sublethal effects on growth after one week. We do not have genetic or dispersal data to demonstrate that the sites we sampled within the valley or within the mountains represent genetically distinct populations. Like many amphibians, *Ambystoma macrodactylum* is thought to have relatively low levels of dispersal, although within single mountain basins in Idaho and Montana, populations appear to be very similar genetically and colonization can occur in lakes when introduced fish are removed (Funk and Dunlap 1999, Tallmon et al. 2000).
In our study, the sampling of multiple populations in the valley and mountains provides strong evidence that there are regional, and probably elevational, differences in UV-B sensitivity. In fact, populations from the valley and mountains have previously been identified as different subspecies, with *A. m. macrodactylum* in the Willamette Valley and *A. m. columbianum* in the Cascade Mountains. This designation is based on morphometric measures and pigmentation in adults, with no consistent differences reported for the larvae (Ferguson 1961). Although there is accumulating evidence illustrating interspecific differences in tolerance to UV-B radiation in amphibians (Blaustein et al. 1998), there are few studies on intraspecific differences. Different populations may exist under different ecological conditions, different elevations, and under different light regimes. Knowing if populations, and even subspecies as in our case, differ in their response to UV-B radiation allows us to better understand the overall effects of UV-B on amphibians. Moreover, data at the population level will provide us with a basis for examining the adaptations that animals may have as a defense against UV-B radiation exposure.

Blaustein et al. (1994) looked at embryonic sensitivity in two populations each of Pacific treefrogs (*Hyla regilla*), Cascades frogs (*Rana cascadae*), and western toads (*Bufo boreas*) and obtained the same results within each species, with Pacific treefrogs being consistently more resistant to UV-B exposure than the other two species, regardless of population. Embryos from two populations of western spotted frogs (*Rana pretiosa*) were also found to be resistant to UV-B (Blaustein et al. 1999).

Several independent studies have examined UV-B sensitivity in the same species in different geographic regions. Three separate studies on embryonic Pacific treefrogs (*H. regilla*) completed in British Columbia, Canada (Ovaska et al. 1997), Oregon (Blaustein et al. 1994), and California (Anzalone et al. 1998), have all reported embryonic resistance of this species to ambient UV-B radiation. Similar results have been obtained for red-legged frog (*Rana aurora*) embryos in Oregon (Blaustein et al. 1996) and British Columbia (Ovaska et al. 1997). Embryos from multiple populations of common frogs (*Rana temporaria*) also appear to be resistant to UV-B (Langhelle et al. 1999, Merilä et al. 2000, Pahkala et al. 2000), although there may be sublethal effects on growth (Pahkala et al. 2000).

In our study, several differences in the UV environments between the valley and the mountain sites suggest that selection for UV-B tolerance could be occurring in mountain populations of salamanders. In the Willamette Valley, *A. macrodactylum* breeding and development of larvae normally occur during the winter, when UV-B levels tend to be low. Additionally, heavy cloud cover decreases exposures even further and animals are exposed to shorter day lengths during development. Many of the temporary aquatic habitats used by long-toed salamanders in the Willamette Valley are dense with aquatic vegetation, which can further shade developing larvae. In contrast, many of the Cascade Mountain sites are devoid of vegetation and development occurs in these sites during summer months, when there is little cloud cover and periods of daylight are long. Overall, UV-B exposure levels at these mountain sites are likely to be much higher than those in the Willamette Valley during development of larval *A. macrodactylum*. This is supported by our field measurements of UV-B (Fig. 2).

Because none of the individuals were able to escape from exposure in our experiments and some individual larvae survived, it appears that behavioral avoidance alone cannot explain survival in high-UV environments in nature. There appear to be physiological and/or biochemical differences regulating the variation in sensitivity. The difference in UV-B exposure during development could have resulted in selection for a number of traits providing tolerance to UV-B in the mountain populations. For example, differences in production of photolyase, the enzyme responsible for repairing a majority of UV-B induced DNA damage, may be important. There could also be differences in the production of screening compounds, such as melanin, that may effectively absorb and dissipate the energy from UV-B exposure (Jablonski 1998, Cockell and Knowland 1999).

Behavior could potentially mitigate the sublethal effects on growth that we observed. However, we have no evidence at this point that *A. macrodactylum* larvae directly avoid UV-B exposure in the field (Belden et al. 2000). In fact, field observations suggest that larvae in the mountains spend time in shallow water environments where they are exposed to UV-B radiation (Belden et al. 2000; L. K. Belden, unpublished data).

As part of the global amphibian population decline phenomenon, some amphibian species have disappeared completely, and many others have suffered population and range reductions (Blaustein and Wake 1990, Alford and Richards 1999, Houlihan et al. 2000). Investigations into the causes of population declines typically focus on the variation in environmental factors at the site that could be responsible for the decline. Our study emphasizes the need to also examine the potential of within-species variation in response to environmental factors, which could result in a similar pattern of population and range reductions. Within-species variation to environmental factors, such as UV-B, may have a significant effect on the future of specific amphibian populations.

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LITERATURE CITED


Hader, D. P. 1997. The effects of ozone depletion on aquatic ecosystems. RC Landes Company, Austin, Texas, USA.


Tevini, M. 1993. UV-B radiation and ozone depletion: effects on humans, animals, plants, microorganisms and materials. Lewis Publishers, Boca Raton, Florida, USA.