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Ambient UV-B radiation causes deformities in amphibian embryos

(ultraviolet/salamanders/abnormalities)

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ABSTRACT **There has been a great deal of recent attention on the suspected increase in amphibian deformities. However, most reports of amphibian deformities have been anecdotal, and no experiments in the field under natural conditions have been performed to investigate this phenomenon. Under laboratory conditions, a variety of agents can induce deformities in amphibians. We investigated one of these agents, UV-B radiation, in field experiments, as a cause for amphibian deformities. We monitored hatching success and development in long-toed salamanders under UV-B shields and in regimes that allowed UV-B radiation. Embryos under UV-B shields had a significantly higher hatching rate and fewer deformities, and developed more quickly than those exposed to UV-B. Deformities may contribute directly to embryo mortality, and they may affect an individual's subsequent survival after hatching.**

Recent attention has focused on the apparent increase in deformities in amphibians (1, 2). However, most reports are anecdotal, and few have been based on quantitative field observations. Although pesticides (3), parasites (4), and UV-B radiation (5–7) may all play roles as potential causes for these deformities, the causes are unknown and no experiments have been performed in the field under natural conditions to examine the problem. Indeed, recent evidence suggests that ambient UV-B (280–315 nm) radiation, perhaps linked to stratospheric ozone depletion or pollution (8–12), causes significant embryonic mortality in some amphibian species (13–15). However, the sublethal effects of UV-B on developing amphibian embryos in nature are unknown.

To test whether UV-B radiation induces deformities in amphibians in nature, we conducted a field experiment using long-toed salamanders (*Ambystoma macrodactylum*). Long-toed salamanders are ideal subjects for such a study because (*i*) they are found at relatively high elevations in the Oregon Cascade Range, (*ii*) they lay their eggs in a variety of microhabitats, including open shallow water, and (*iii*) their eggs have relatively little photoreactivating enzyme, photolyase (13). Photolyase can remove harmful photoproducts induced by UV light such as cyclobutane pyrimidine dimers (CPDs), which have cytotoxic and mutagenic effects (13, 16). Thus, *A. macrodactylum* eggs are potentially exposed to high levels of UV-B and have potentially little ability to remove CPDs.

MATERIALS AND METHODS

Experiments were performed from May 20 through June 7, 1997 at a natural oviposition site in a pond in the Oregon Cascade Range (2,000 m elevation, 24.2 km south of Sisters, Deschutes Co., OR; 44° 06′ N; 121° 37′ W). Individual eggs (<24 hr old) were collected from shallow water (<20 cm deep) from natural oviposition sites in the pond. We randomly placed 50 newly laid eggs in enclosures under mylar filters that removed more than 94% of ambient UV-B and 50 eggs in enclosures under acetate control filters that allowed about 90% of ambient UV-B transmission. UV-B measurements were taken with a Solar Light (Philadelphia) PMA2100 UV meter. We first measured ambient UV-B levels and then measured UV-B levels under mylar and acetate filters twice per day on three separate days to assess percent transmission of UV-B under each regime. Each regime (UV-B blocking and UV-B transmitting) was replicated four times in a repeated measures randomized block design (13). Enclosures (38 × 38 × 7 cm) with either mylar or acetate filters on top were placed in small, unperforated plastic pools (one enclosure per pool; 110 cm in diameter, 18 cm deep) containing pond water, and the pools were placed in the pond. Thus, eggs and developing embryos were exposed to ambient physical parameters. Temperatures were recorded in each enclosure to ensure that there were no differences between treatments. We monitored developmental rates, assessed deformities and tabulated mortality in each regime. The experiment ended when all eggs either hatched or died.

RESULTS

Embryos under the UV-B shields had a significantly higher hatching rate and fewer deformities, and developed more quickly compared with those under UV-B transmitting filters (Tables 1 and 2). Only 29 individuals hatched after being reared under acetate, and 25 of these had deformities. In contrast, under mylar, 190 individuals hatched and only one individual showed abnormalities. Deformities consisted primarily of lateral flexure of the tail, blistering, and edema (swollen, fluid-filled areas). Edema appeared on all parts of the body but was concentrated on the anterior, usually on the dorsal surface of the head (Fig. 1). These results illustrate deformities in amphibian embryos exposed to ambient levels of UV-B in controlled experiments under field conditions.

DISCUSSION

Our results show that ambient levels of UV-B adversely affect the development and induce deformities in some amphibian species in their natural habitat. High mortality and a greater frequency of deformities under UV-B-transmitting conditions may be associated with relatively low photolyase activity in this species. Apparently, higher levels of photolyase would more

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Table 1. Mean survival, deformities and developmental stages (± SE) of long-toed (*A. macrodactylum*) salamander embryos under UV-shielding and UV-transmitting filters

	% survival		% developmental abnormalities		Developmental stage	
Day	UV-transmitting	UV-shielding	UV-transmitting	UV-shielding	UV-transmitting	UV-shielding
3	100 (0)	100 (0)	0 (0)	0 (0)	15.5 (0.2)	15.2 (0.2)
6	100 (0)	100 (0)	4.0 (1.4)	0 (0)	22.8 (1.1)	23.8 (0.4)
10	84.5 (4.3)	98.0 (0.8)	56.0 (8.0)	0 (0)	32.1 (0.9)	35.2 (0.4)
13	14.5 (8.1)	95.0 (1.9)	91.9 (4.7)	0.5 (0.5)	Hatched	Hatched

Ambient UV-B ranged from 4.77–25.5 $\mu W/cm^2$. The mean (± SE) percent transmission under mylar filters was 5.74 (1.35) and under acetate filters was 90.0 (1.15). Developmental stage was according to Harrison (27).

efficiently remove photoproducts and limit cytotoxic and mutagenic effects (13, 16).

The high mortality rate and number of deformities associated with ambient UV-B radiation suggest that UV-B may contribute to deformities in other amphibian species. These results may be especially significant in those species that lay their eggs in open shallow water exposed to relatively high levels of UV-B and those with little photolyase (13). Furthermore, we suggest that those species with little photolyase would have a greater chance of displaying deformities because of their relatively low capacity to remove UV-induced photoproducts. Thus, species such as *A. macrodactylum* that may lay its eggs under rocks, on aquatic vegetation, or in deep or shallow water (17), may be under intense selection pressure to lay its eggs hidden from solar radiation.

The deformities we observed in our field study in conjunction with those found in earlier laboratory studies suggest that UV-B radiation can induce deformities in some amphibian species. In the laboratory, western toad (*Bufo boreas*) tadpoles exposed to enhanced UV-B radiation developed anomalous, concave curvatures of their spine during early development (5, 6). They also developed abnormally thick pigmented corneas and areas of hyperplasia in the integument. Moreover, compared with control groups, individuals in UV-B-enhanced groups had significantly lower survival rates.

In *A. macrodactylum*, deformities may contribute directly to embryonic mortality, and they may affect individuals after they hatch. Although, most embryos failed to hatch in the UV-B-transmitting regime in our study, deformities may persist in those few individuals that do hatch. Larvae with severe deformities may not be able to acquire food or avoid predators efficiently. In *A. macrodactylum* and other amphibian species, deformities may persist throughout the larval stage and after metamorphosis, potentially hampering survival. Some individuals may reach the adult stage, and these individuals may persist with these deformities as has been observed in various locales (2–4).

UV-B radiation may act alone, or in conjunction with other agents such as pesticides to induce developmental defects (3, 18, 19). Furthermore, UV-B radiation may impair disease defense mechanisms, making an individual more susceptible to pathogens and parasites that may hamper normal development and increase mortality (20–23). For example, in this study, a pathogenic fungus (*Saprolegnia ferax*) infected some dead and dying embryos that were exposed to UV-B (acetate regimes) but not under mylar filters.

Our results suggest that there is potential for UV-B to cause deformities in other species and in other ecosystems that could ultimately be manifest at the population level. Thus, continued embryo mortality and an increasing frequency of deformities in amphibians may lead to population declines that are evident in many amphibian species (24–26).

Table 2. Repeated measures ANOVA of survival, deformities, and developmental time in long-toed salamander (*A. macrodactylum*) embryos

Source of variation	MS	DF	F	*P*
Survival				
Treatment	0.456	1	6.343	0.045
Error	0.072	6		
Time	0.360	3	6.135	0.005
Time X treatment	0.308	3	5.259	0.009
Error	0.059	18		
Deformities				
Treatment	1.145	1	7.659	0.033
Error	0.150	6		
Time	0.392	3	4.682	0.014
Time X treatment	0.385	3	4.588	0.015
Error	0.084	18		
Developmental rate				
Treatment	16.335	1	8.868	0.025
Error	1.842	6		
Time	661.022	2	933.940	<0.0001
Time X treatment	7.875	2	11.027	0.002
Error	0.708	12		

A preliminary analysis indicated no block effects (i.e., no differences between temperature or other variables among blocks) so the block and error terms were pooled for remaining tests (28). Percent survival and percent deformities data were Arcsine transformed before analysis. Temperatures were taken on the four occasions when embryos were checked (Table 1). Student's *t* tests revealed no significant temperature differences between treatments ($T_{30} = 0.251$, $P = 0.804$; mean (±SE) temperatures were 23.28°C (0.35) and 23.41°C (0.35) for the mylar and acetate regimes, respectively. MS, mean squares; DF, degrees of freedom; F, F statistic.

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FIG. 1. Developing long-toed salamander (*A. macrodactylum*) embryo in egg reared under UV-B-transmitting regime in the Oregon Cascade Range at 2,000-m elevation exhibiting anterior-dorsal edema and tail malformation.

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