Ambient Levels of Ultraviolet-B Radiation Cause Mortality in Juvenile Western Toads, Bufo boreas

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ABSTRACT.—Numerous anthropogenic and natural factors affect living organisms in nature. Anthropogenic factors include a wide array of contaminants and processes that alter the habitat on both local and global scales. For example, chlorofluorocarbons (CFCs) and other industrial gases contribute to the depletion of the earth's protective ozone layer, resulting in increased amounts of cell damaging ultraviolet-B (UV-B) radiation reaching the surface of the earth. Recent experiments provide evidence that increasing ambient levels of UV-B radiation harm many amphibian species. UV-B radiation can kill amphibians and can cause sublethal damage to them. However, most studies that have examined the effects of UV-B radiation on amphibians have focused on developing embryos. There is little information on how UV-B radiation affects amphibians at later stages of development. In experimental laboratory tests, we exposed one group of juvenile western toads (Bufo boreas) to full spectrum lighting with ambient levels of UV-B radiation and control toads to full spectrum lighting excluding most UV-B. Juvenile toads exposed to ambient levels of UV-B radiation showed significantly greater mortality rates compared with controls. These results add to a growing body of literature demonstrating that UV-B is harmful to amphibians. Furthermore, our results suggest that investigators should look at the effects of UV-B radiation on different life stages before making conclusions about the overall impact of UV-B on amphibians.

INTRODUCTION

Numerous abiotic and biotic agents affect living organisms in nature. These include both natural and anthropogenic factors. Anthropogenic factors include a wide array of contaminants and processes that alter the habitat on both local and global scales. For example, chlorofluorocarbons (CFCs) and other commonly used industrial gases have contributed to the depletion of the earth's protective ozone layer, increasing the amount of cell damaging ultraviolet-B (UV-B; 280–315 nm) radiation that reaches the terrestrial surface (van der Leun and Bornman, 1998). Significant increases in UV-B radiation have been measured in both temperate and tropical regions (Kerr and McElroy, 1993; Herman *et al.*, 1996; Middleton *et al.*, 2001). All other things being equal, current ozone loss and related UV-B increases relative to those in the 1970s in the northern hemisphere at mid latitudes are about 4% in summer/fall and 7% in winter/spring (Madronich *et al.*, 1998).

Many plant species, microorganisms, invertebrates and vertebrates are negatively affected by current levels of UV-B radiation (van der Leun and Bornman, 1998; Cockell and Blaustein, 2001). This includes members of the class Amphibia. Indeed, recent experimental tests have shown that more than 40 amphibian species representing at least six families (of both frogs and salamanders) from different habitats around the world are adversely affected by exposure to UV-B radiation (Blaustein *et al.*, 2001, 2003).

For amphibians, like other organisms, the most extreme effect of exposure to UV-B radiation is death. However, mortality after exposure to UV-B radiation has been observed primarily in embryonic stages (Blaustein *et al.*, 1998, 2001). Furthermore, experimental studies have shown that there are interspecific differences in how UV-B radiation affects

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embryos. Thus, for some species, the hatching success is significantly lower when embryos are exposed to ambient levels of UV-B radiation in the field, whereas the hatching success of other species is unaffected (reviewed in Blaustein *et al.*, 1998). For example, the hatching success of western toads (*Bufo boreas*) and Cascades frogs (*Rana cascadae*) in Oregon, when exposed to ambient levels of UV-B radiation, is lower than eggs shielded from UV-B (Blaustein *et al.*, 1994). However, hatching success of Pacific treefrogs (*Hyla* regilla) throughout their range, including Oregon appears to be unaffected by exposure to ambient levels of UV-B radiation (Blaustein *et al.*, 1994; Ovaska *et al.*, 1997; Anzalone *et al.*, 1998). Similarly, in Europe and Australia when eggs of some amphibian species were exposed to ambient levels of UV-B radiation the hatching success was lower than those shielded from UV-B, whereas hatching success in other species was unaffected after eggs were exposed to UV-B (*e.g.*, van de Mortel and Buttemer, 1996; Lizana and Pedraza, 1998; Broomhall *et al.*, 2000; Häkkinen *et al.*, 2001).

At later life history stages (larval and postmetamorphic) sublethal effects after UV-B exposure, rather than mortality, have been more commonly documented. Sublethal effects include hampered growth and development, physiological anomalies, anatomical malformations and changes in behavior (reviewed in Blaustein *et al.*, 2003). These effects occur in later life history stages even in species whose embryos appear to be unaffected after being exposed to UV-B radiation (*e.g.*, Smith *et al.*, 2000; Pahkala *et al.*, 2001; Belden and Blaustein, 2002a). Both mortality rates and sublethal effects after exposure to UV-B radiation vary among species, between populations of the same species, with life stage and with ecological conditions (Blaustein and Kiesecker, 2001).

Although there have been more than a dozen studies investigating the effects of UV-B on embryos, there have been few studies on the effects UV-B exposure after metamorphosis even though several investigators have suggested the importance of such studies (*e.g.*, Pahkala *et al.*, 2001). Fite *et al.* (1998) showed that prolonged exposure to ambient levels of UV-B radiation in adult frogs resulted in severe retinal damage. This effect was observed in wild captured frogs (*Rana cascadae*) and was experimentally induced in *R. pipiens*. Hays *et al.* (1996) reported that juvenile frogs of several species displayed physiological abnormalities after being exposed to "modest" levels of UV-B radiation. In another study, juvenile toads (*Bufo boreas*) exposed to ambient levels of UV-B radiation altered their response to predator cues (Kats *et al.*, 2000). These studies suggest the importance of examining the effects of UV-B in later stages of development.

It is important to understand how UV-B radiation affects amphibians after metamorphosis for several reasons. Changes in behavior after metamorphosis may alter an amphibian's ability to find food or escape predation (Fite *et al.*, 1998; Kats *et al.*, 2000). After metamorphosis, amphibians may seek sunlight for thermoregulation. Yet, prolonged basking may actually damage amphibian eyes (Fite *et al.*, 1998), cause immune dysfunction and cause skin lesions (Blaustein *et al.*, 2003). Post-metamorphic mortality may be a particularly important factor contributing to amphibian population declines (Biek *et al.*, 2002; Vonesh and de la Cruz, 2002). Because of the limited numbers of studies regarding the effects of UV-B on amphibians after metamorphosis, we examined further the effects of ambient levels of UV-B on juvenile (after metamorphosis but before adult stage) western toads, a species that is particularly sensitive to UV-B exposure in early life stages (Worrest and Kimeldorf, 1976; Blaustein *et al.*, 1994; Kats *et al.*, 2000; Kiesecker *et al.*, 2001).

METHODS

Juvenile western toads (*Bufo boreas*) were collected on 16 and 23 September 2001 at Lost Lake in the Oregon Cascade Range (approx. 145 km NE of Corvallis, OR; Linn County;

elevation 1220 m) and brought back to our laboratory at Oregon State University. They were maintained in large plastic tubs (81 cm diameter; height = 62 cm) with about 40–120 per tub at 14 ± 1 C on a natural photoperiod via natural sunlight. Moist towels were placed in each tub. Toads were fed crickets ad lib. Crickets were occasionally sprinkled with a powdered

tub. Toads were fed crickets ad lib. Crickets were occasionally sprinkled with a powdered vitamin supplement (either Reptocal[©] or VitaLife[©]). Tubs were cleaned approximately every ten days. After quarantine in the laboratory and making sure animals were well fed and apparently healthy, experiments began in January 2002.

We chose 100 toads similar in snout-vent length (SVL) and mass to use in experiments; $21.0 \pm 0.10 \text{ mm}$ (mean SVL \pm sE) and a mass of $0.92 \pm 0.09 \text{ g}$ (mean \pm sE). Five toads were randomly assigned to each of 20 round plastic tubs (diameter = 14 cm, depth = 7 cm). Tubs had moist paper towels on the bottom to keep toads hydrated. Tubs were then transferred to a room maintained at 17 C and placed under experimental light regimes. Within the room, tubs were placed on a laboratory bench below an array of six horizontally-placed lamps, each containing a UV-B light bulb (UV-313, Q-Panel, Cleveland, Ohio, USA) and a full spectrum light bulb (Vita-Lite). Presence and absence of UV-B was manipulated by placing plastic shields over containers. In the control treatment (UV-B-absent), plastic mylar shields blocked approximately 99% of UV-B (280–315 nm), but allowed transmission of longer wavelengths. In the UV-B treatment (UV-B-radiation and longer wavelengths.

We used a randomized block design to control for variation in lighting across the bench. The 20 containers were arranged in five blocks, each consisting of a row of four tubs running parallel to the light bulbs. Within each block each tub was randomly assigned to a treatment; half exposed to UV-B and half not exposed to UV-B serving as controls. UV-B levels inside tubs (on bottom) were measured immediately before and after the experiment using a hand-held meter (model PMA2100, Solar Light Co., Philadelphia, PA, USA). UV-B levels ranged from 19.0 to 23.4 μ W/cm² in the UV-B treatment and from 0.16 to 0.26 μ W/cm² in the control treatment. The UV-B levels in the laboratory are within the ranges of ambient UV-B under field conditions where toads are found (Blaustein *et al.*, 1997; Kiesecker *et al.*, 2001; Palen *et al.*, 2002). For example, ambient UV-B radiation taken on land amongst an aggregation of newly metamorphic *Bufo boreas* on 2 August 2002 at 1200 hours at Scott Lake, Oregon in the Willamette National Forest (elevation = 1470 m) was 19.6 μ W/cm². Ambient levels are highest in spring and summer where toads breed and bask and can reach levels of more than 25 μ W/cm² (Hatch, 2002; unpublished data).

We want to emphasize, however, that it is the dose received (exposure over time) that is critical, not specific levels at a particular time. Thus, even if an amphibian is subjected to a relatively high level of UV-B radiation, it may not be harmed if exposure is short-term and accumulated doses of UV-B are relatively low.

A 10:14 light:dark photoperiod was used during the experiment. The UV-B and full spectrum light bulbs (simulated ambient light containing visible light, UV-A and UV-B) were synchronized. Therefore, each day, toads in the UV-B treatment received a continuous 10-h exposure of full spectrum light with ambient levels of UV-B each day, while toads in the UV-B absent treatment received a continuous 10-h exposure of full spectrum light only. Lights turned on 0900 and off at 1900 hours. The experiment was run for 1 wk. Thus, toads in the UV-B exposed regime received relatively small "doses" of UV-B (1 wk at ambient levels) because in nature, as discussed below, toads are active in sunlight for months and accumulate much higher doses of UV-B radiation.

During the experiment, toads were checked twice per day (once in the morning and once in the afternoon). Mortality and posture of toads were noted. Percent survival after 7 d was

Effect	MS	DF	F	Р
Block	412.46	4	2.285	0.132
Treatment	11561.80	1	64.059	< 0.001
$Block \times treatment$	53.77	4	0.298	0.873
Error	180.49	10		

TABLE 1.—Analysis of variance (ANOVA) results for survival of western toads (*Bufo boreas*) in UV-B and control treatments

arcsine square root transformed as a safeguard against violation of the parametric assumption of normality and analyzed using analysis of variance (ANOVA).

RESULTS

After 1 d, toads exposed to UV-B radiation displayed different behaviors than toads shielded from UV-B. One d after UV-B exposure, eight out of 50 (16%) displayed a slumped body posture as opposed to an upright posture. All or part of their ventrum was in contact with the paper towel substrate. At the same time, all 50 toads in the UV-B absent treatment sat upright.

We first observed mortality in the UV-B exposed treatments 4 d after the experiment began. At the end of the experiment (after 1 wk), survival was significantly lower in the UV-B-exposed treatment compared with the control treatment (P < 0.001; Table 1, Fig. 1). This difference in mortality was pronounced. Thirty-five toads died in the UV-B exposed treatment compared with only four in the control treatment. There were no block effects



FIG. 1.—Percent survival (+1 sE) of western toads (Bufo boreas) in UV-B and control treatments

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(P = 0.1312), nor was there a significant effect of the block by treatment interaction (P = 0.8723; Table 1).

DISCUSSION

Previous experimental tests suggest that western toads from Oregon are especially sensitive to UV-B exposure compared with other amphibian species. Exposure to UV-B radiation causes increased embryonic mortality, physiological and anatomical abnormalities in larvae and hampers antipredator behavior in juveniles (Worrest and Kimeldorf, 1976; Blaustein *et al.*, 1994; Hays *et al.*, 1996; Kats *et al.*, 2000). Our results suggest that prolonged exposure to ambient levels of UV-B radiation also causes mortality in juvenile western toads.

The results of our experiments are especially relevant regarding the natural history of western toads. Juvenile western toads are exposed to prolonged levels of solar radiation from the time they emerge from hibernation in spring to early fall. Juvenile toads can be exposed to as much as 10 h/day of solar radiation from April–September as they bask in sunlight often en masse by the thousands along the shore or above water (pers. obs.). In late summer toads metamorphose synchronously as thousands of toads sit on shore usually for all daylight hours (Arnold and Wassersug, 1978; Samollow, 1980; pers. obs.). By the time they become juveniles, western toads may have accumulated the adverse effects of large doses of UV-B radiation because they are exposed to UV-B as eggs, larvae and after metamorphosis. Western toads often lay eggs in shallow water or eggs are exposed to UV-B as water levels recede from spring through summer. Western toad larvae school in shallow water and during the breeding season, adults amplex above the surface of the water (O'Hara, 1981; O'Hara and Blaustein, 1982; Blaustein, 1988; Blaustein *et al.*, 1994; Kiesecker *et al.*, 2001).

Millions of years of selection pressure have apparently influenced juvenile toad behavior towards seeking sunlight. For thermoregulation, for finding food and perhaps as an antipredator behavior, juvenile toads often seek open sunlit areas along the shores after they metamorphose and just before hibernation. Because ozone depletion and increasing levels of UV-B radiation are relatively recent phenomena (<100 y; van der Leun and Bornman, 1998), primarily due to human production of CFCs, it is unlikely that strong selection pressures influencing toad behavior for seeking sunlight will be overridden in the short-term (Blaustein and Belden, 2003). Yet, toads face obvious counter selection pressures. Toads seeking sunlight may accrue sublethal effects associated with UV-B exposure. If they prolong their stay in sunlit areas, they may die.

Our results add to the increasing experimental evidence showing that amphibians are harmed by UV-B radiation. However, the harmful effects of exposure to UV-B are context dependent and vary with species, population and life stage (Blaustein and Kiesecker, 2001). For some amphibian species, UV-B affects early life history stages more than later stages. In other species, later stages may be most sensitive. Even populations of the same species may have different sensitivities to UV-B radiation (*e.g.*, Belden and Blaustein, 2002b; Pahkala *et al.*, 2002). Examining only one life stage can lead to erroneous conclusions regarding the effects of UV-B on amphibians (discussed in Pahkala *et al.*, 2002; Blaustein *et al.*, 2004). Moreover, one model (Biek *et al.*, 2001) suggests that mortality after metamorphosis is especially important in influencing population declines of western toads in Oregon.

At one site we have studied for more than 20 y, 90–95% of the western toads do not disperse but disappear within the first year (Samollow, 1980). Undoubtedly, numerous factors, including predation, disease and stochastic events, contribute to these disappearances.

We suggest that some of these disappearances could be the result of mortality due to the accumulation of harmful doses of UV-B radiation.

Our study shows how chronic exposure to UV-B may affect amphibians. Our study used UV-B levels that were in the range of natural levels they would encounter in nature. In fact, amphibians develop in the wild under UV-B intensities that are at times stronger than those supplied here and for prolonged periods of time. For example, several studies have reported levels exceeding 25 μ W/cm² (*e.g.*, Kiesecker *et al.*, 2001; Palen *et al.*, 2002). However, our study used chronic artificial UV-B radiation whereas solar intensities rise and fall temporally. Another aspect of our laboratory study is that artificial visible light might be suboptimal for photoreactivation for induced synthesis of repair enzymes or for production of other protective compounds (as discussed in Hays *et al.*, 1996). More studies examining the effects of UV-B radiation on amphibians metamorphose are needed to assess the overall relevance of UV-B radiation on amphibians and how it may affect amphibians at the population level.

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