

## Effects of Ultraviolet Radiation on Amphibians: Field Experiments<sup>1</sup>

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**SYNOPSIS.** Numerous reports suggest that populations of amphibians from a wide variety of locations are experiencing population declines and/or range reductions. In some cases, unusually high egg mortality has been reported. Field experiments have been used with increasing frequency to investigate ultraviolet radiation as one of the potential factors contributing to these declines. Results from field experiments illustrate that hatching success of eggs is hampered by ultraviolet radiation in a number of species, while other species appear to be unaffected. Continued mortality in early life-history stages may ultimately contribute to a population decline. Although UV-B radiation may not contribute to the population declines of all species, it may play a role in the population decline of some species, especially those that lay eggs in open shallow water subjected to solar radiation and in those that have a poor ability to repair UV-induced DNA damage.

### INTRODUCTION

The fundamental problem in ecology is to determine the causes that limit the distribution and abundance of organisms (Krebs, 1994). Examining this problem is especially relevant today because populations of many organisms are undergoing declines and range reductions as part of an overall loss in biodiversity (Wilson, 1988; Ehrlich, 1997). As part of this "biodiversity crisis," many amphibian populations have been declining and undergoing range reductions (e.g., Semb-Johansson, 1989; Wake, 1991; Crump *et al.*, 1992; Richards *et al.*, 1993; Pounds and Crump, 1994; Blaustein and Wake, 1995; Stebbins and Cohen, 1995; Drost and Fellers, 1996; Fisher and Shaffer, 1996; Laurance *et al.*, 1996; Reaser, 1996; Pounds *et al.*, 1998; Lips 1998). Unfortunately, the causes for amphibian population declines have been difficult to assess. Much of the information on amphibian declines comes from observational or anecdotal accounts (Blaustein *et al.*, 1994a; Stebbins and Cohen, 1995). Hy-

pothesized causes for the declines include habitat destruction (the most obvious cause), pathogens, introduced exotic species, pollution, and increased ultraviolet radiation (Blaustein and Wake, 1995; Stebbins and Cohen, 1995). These agents may act alone or in combination to contribute to the decline of amphibian populations. However, few experimental tests of key hypotheses have been conducted, especially in the field under natural conditions. This is surprising because field experiments have been extremely useful tools in examining basic ecological questions such as how amphibians interact with one another, with other organisms, and with their abiotic environment (Hairston, 1989, 1994).

The diversity of locations where amphibian populations have declined prompted consideration of atmospheric factors such as increased ultraviolet irradiance associated with depletion of stratospheric ozone. Although still relatively rare, several investigators have used field experiments to examine the potential role of ultraviolet-B radiation (UV-B; 280–315 nm) in amphibian population declines by measuring mortality of embryos (e.g., Blaustein *et al.*, 1997a; Broomhall *et al.*, 1999; Anzalone *et al.*,

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1998; Corn, 1998; Lizana and Pedraza, 1998). Continuous high mortality in early life stages may ultimately contribute to a decline at the population level.

In this review, we briefly describe the methods, evidence and implications of studies that have incorporated field experiments to test the effects of UV-B radiation on amphibian eggs and embryos. We also discuss some concerns and misinterpretations regarding the use of field experiments in studies of UV and amphibians. We have attempted to include all studies that have incorporated field experiments or a field component to investigate the effects of UV-B on amphibian embryos. Details of the methods are included so that a more thorough interpretation can be made of the results. Laboratory studies are not reviewed.

#### UV SENSITIVITY HYPOTHESIS

Since 1979 we have been studying the ecology of amphibians at several locations in the Pacific Northwest (USA). As part of this research, we have monitored egg development and hatching success of Cascades frog (*Rana cascadae*), Pacific treefrog (*Hyla regilla*) and western toad (*Bufo boreas*) embryos in the field (Blaustein and Olson, 1991; Blaustein *et al.*, 1994b; Kiesecker and Blaustein, 1997). From the 1950s through the mid 1980s, egg mortality in Oregon was no more than 10% for these species (Kiesecker and Blaustein, 1997). Through the 1990s this remains the case for *H. regilla* but not for the other two species. At several sites, *R. cascadae* and *B. boreas* suffered high embryo mortality (greater than 50%). In some years, mortality was over 90% at some sites (Kiesecker and Blaustein, 1997). Moreover, when eggs were taken from these high mortality sites and reared in the laboratory, more than 99% survived through metamorphosis (unpublished data, A.R.B.). Heavy metals and pesticides were not detected in lake water and pH values were near neutral. Consequently, we formulated the hypothesis that ambient levels of UV-B radiation contribute to amphibian egg mortality in nature. We proposed this for several reasons: 1) egg mortality was occurring at relatively high elevation sites (>1,500 m), which under cer-

tain circumstances may receive more ambient UV-B than sites at lower elevations, 2) eggs were usually laid in open, shallow water exposed to solar radiation 3) previous laboratory studies showed that amphibian embryos were susceptible to UV-B radiation and 4) there were no other obvious causes of egg mortality at the sites of oviposition.

As we planned our experiments, our working hypothesis was modified and stated as the "UV Sensitivity Hypothesis." One basic prediction of this hypothesis is that the effects of ambient UV-B radiation on eggs and embryos will vary according to their natural exposure to sunlight and their ability to repair UV-induced DNA damage (Blaustein *et al.*, 1994b). For example, the eggs of species that are not laid in sunlight (*e.g.*, under forest canopies, in deep water, under debris) would not be damaged by UV-B radiation. Furthermore, we predicted that the eggs of those species with a relatively high capacity to repair UV-induced DNA damage would be more resistant to UV-B than those with a lower capacity.

#### *Field experiments in Oregon and Washington: General methods*

Most of our field experiments were conducted at natural oviposition sites. Methods and materials were similar in all studies. We placed newly laid eggs, with their surrounding jelly matrix in enclosures (38 × 38 × 7 cm). Eggs were exposed to ambient levels of UV-B radiation or shielded from UV-B radiation with filters (50 × 50 × 7 cm). All enclosures had clear Plexiglas frames with floors of 1 mm<sup>2</sup> fiberglass mesh screen. In all experiment but one (Blaustein *et al.*, 1997a; see below), we used the following treatments: 1) a UV-B blocking filter made of mylar was placed over one third of the enclosures; 2) an acetate filter that transmitted UV-B was placed over another third of the enclosures (a control for placing a mylar filter on some enclosures); 3) the remaining enclosures had no filters. Transmitting properties of the mylar and acetate used on enclosures were assessed before and after experiments.

Enclosures were always placed in a lin-

ear array in a randomized block design, with the three treatments randomly assigned to enclosures within each block (e.g., Blaustein *et al.*, 1994b, 1995). Each treatment was replicated four times. Experiments were terminated when the original embryos either hatched or died, as assessed by daily count of embryos. All embryos were accounted for at the end of each experiment; there was no predation. Survival was measured as the proportion of hatchlings produced per enclosure. Temperatures were taken within enclosures in each treatment.

*Pacific treefrog, Cascades frog, and western toads in Oregon*

Our initial field experiments were designed to assess the effects of ambient levels of UV-B radiation on embryos of the Pacific treefrog (*H. regilla*), western toad (*B. boreas*), and Cascades frog (*R. cascadae*) as they developed at natural oviposition sites in the Cascade Range of Oregon (Blaustein *et al.*, 1994b). We chose these species because their eggs are laid in open, shallow water and are highly exposed to sunlight (Blaustein *et al.*, 1994b; Nussbaum *et al.*, 1983). Moreover, populations of *B. boreas* and *R. cascadae* are in decline, whereas those of *H. regilla* appear to be robust (Blaustein *et al.*, 1994b). Furthermore, unusual embryo mortality has been documented in both *B. boreas* and *R. cascadae* but not in *H. regilla* (Blaustein and Olson, 1991; Kiesecker and Blaustein, 1997). Therefore, differences in sensitivity to UV-B may help us explain differences in the population status of these species.

Experiments were conducted at several sites from 1,220–2,000 m elevation in spring 1993 (Blaustein *et al.*, 1994b). To ensure that effects were not due to site differences, we tested each species at two different sites, including one site where all three species were found together. We placed 150 newly deposited eggs (<24 hr old) in each enclosure. Enclosures were placed parallel to the water's edge at depths of 5–10 cm, where eggs are laid naturally (Blaustein *et al.*, 1994b).

There were no differences in *H. regilla* hatching success among the three treatment regimes. Thus, *H. regilla* embryos were

highly resistant to ambient levels of UV-B radiation (Table 1). The hatching success of *B. boreas* and *R. cascadae*, however, was significantly greater under sunlight lacking UV-B than under either unfiltered sunlight or filtered controls (Table 1). Therefore, we concluded that embryos of *B. boreas* and *R. cascadae* in Oregon were susceptible to deleterious effects of UV-B radiation.

*Red-legged frogs, and Northwestern and long-toed salamanders in Oregon*

We conducted field experiments similar to those described above to investigate the effects of UV-B radiation on Northwestern salamander (*Ambystoma gracile*) and red-legged frog (*R. aurora*) embryos (Blaustein *et al.*, 1995, 1996). Experiments on red-legged frog embryos were conducted in the Willamette Valley, Oregon (76 m elev.) where they were formerly abundant but are now rare (Nussbaum *et al.*, 1983). Studies of northwestern salamanders were conducted at 183 m elev. in the Coast Range of Oregon. Long-toed salamanders (*A. macrodactylum*) were studied at 2,000 m elev. at a site in the Oregon Cascade Range.

The red-legged frog has disappeared from large portions of its historical range (Nussbaum *et al.*, 1983; Blaustein *et al.*, 1996; Kiesecker and Blaustein, 1998). The population status of the Northwestern salamander and the long-toed salamander is unknown. All three species commonly lay eggs in shallow water, exposed to solar radiation (Blaustein *et al.*, 1995, 1996, 1997a). However, there are no reports of unusual egg mortality in any of these species.

We used the same methods and materials described above for *R. cascadae* and *B. boreas* except that enclosures were placed in small plastic pools (110 cm diameter, 18 cm deep). Within the pools, eggs were immersed in 5–10 cm of natural pond water, a depth at which eggs are naturally laid. Because there were fewer eggs available, we used only the mylar (UV-B blocking) and acetate (mylar control) regimes when testing *A. macrodactylum*. We have never seen significant differences in hatching success among the acetate and open regimes.

Hatching success of *A. gracile* and *A.*

TABLE 1. *Effects of ultraviolet radiation on amphibians in field experiments.*

Species	UV Effect	Location	Population status	Investigators
<i>Rana cascadae</i> Cascades frog	Yes	Oregon	Declining	Blaustein <i>et al.</i> , 1994b
<i>Rana aurora</i> Red-legged frog	No	Oregon	Declining	Blaustein <i>et al.</i> , 1996
<i>Rana aurora</i>	No	Western Canada	Declining	Ovaska <i>et al.</i> , 1997
<i>Rana pretiosa</i> Oregon Spotted Frog	No	Washington	Declining	Blaustein <i>et al.</i> , unpublished
<i>Rana luteiventris</i> Columbia Spotted Frog	No	Washington	Declining	Blaustein <i>et al.</i> , unpublished
<i>Bufo boreas</i> Western toad	Yes	Oregon	Declining	Blaustein <i>et al.</i> , 1994b
<i>Bufo boreas</i> <sup>1</sup>	No	Colorado	Declining	Corn, 1998
<i>Bufo bufo</i> Common toad	Yes	Spain	Declining in some areas of Europe	Lizana and Pedraza, 1998
<i>Bufo calamita</i> Natterjack toad	No	Spain	Declining in some areas of Europe	Lizana and Pedraza, 1998; see also Gasc, 1997
<i>Hyla regilla</i> Pacific treefrog	No	Oregon	Persistent	Blaustein <i>et al.</i> , 1994b
<i>Hyla regilla</i>	No	California	Persistent	Anzalone <i>et al.</i> , 1998
<i>Hyla regilla</i>	No	Western Canada	Persistent	Ovaska <i>et al.</i> , 1997
<i>Hyla cadaverina</i> California treefrog	Yes	California	Unknown	Anzalone <i>et al.</i> , 1998
<i>Litoria verreauxii</i> Alpine treefrog	Yes	Australia	Declining	Broomhall <i>et al.</i> , 1999
<i>Litoria aurea</i> <sup>2</sup> Bell frog	?	Australia	Declining	van de Mortel and Buttemer, 1996
<i>Litoria dentata</i> Keferstein's treefrog	No	Australia	Persistent	van de Mortel and Buttemer, 1996
<i>Litoria peronii</i> Peron's treefrog	No	Australia	Persistent	van de Mortel and Buttemer, 1996
<i>Crinia signifera</i> Eastern froglet	Yes	Australia	Persistent	Broomhall <i>et al.</i> , 1999
<i>Ambystoma macrodactylum</i> Long-toed salamander	Yes	Oregon	Unknown	Blaustein <i>et al.</i> , 1997a
<i>Ambystoma gracile</i> Northwestern salamander	Yes	Oregon	Unknown	Blaustein <i>et al.</i> , 1995
<i>Taricha torosa</i> California newt	Yes	California	Unknown	Anzalone <i>et al.</i> , 1998

<sup>1</sup> Goebel (1996) suggests that *B. boreas* in Oregon and Colorado may belong to different species.

<sup>2</sup> Possible effects; see text for discussion.

*macrodactylum* was significantly greater under treatments blocking UV-B radiation than under those in which eggs were exposed to UV-B. Furthermore, more than 90% of *A. macrodactylum* embryos were deformed under the UV-transmitting regime (Blaustein *et al.*, 1997a). There were no significant differences in hatching success among the three sunlight regimes in tests of *R. aurora*.

#### Spotted frogs in Washington

Effects of UV-B radiation on embryos of spotted frogs, *Rana pretiosa* and *R. luteiv-*

*entris*, were studied at three elevations in Washington (unpublished data, A.R.B.). Populations of both species are in decline (McAllister *et al.*, 1993; Leonard *et al.*, 1993). Unusual egg mortality has not been reported in these species, but detailed information on their reproductive dynamics is lacking.

*Rana pretiosa* was examined near sea level and at a site about 600 m elev. *Rana luteiventris* was examined at about 1,700 m elev. Hatching success was not affected by UV-B radiation in any of the spotted frog experiments.

### *Treefrogs and newts in California*

Effects of UV-B radiation on California and Pacific treefrog (*H. cadaverina* and *H. regilla*) embryos and on embryos of the California newt (*Taricha torosa*) were studied at a low elevation site (290 m) in the Santa Monica Mountains, near Los Angeles, California (Anzalone *et al.*, 1998). Embryos were placed in small plastic chambers (25 cm long, 15 cm wide). Both open ends were covered with fiberglass screen to allow water flow, and chambers were placed in shallow areas of a stream so that the open ends were parallel with the current. Embryos were immersed in 4–8 cm of water under the surface of the stream. Chambers were exposed to three treatments: 1) uncovered chambers allowing unfiltered sunlight 2) chambers with UV-B blocking mylar filters and 3) chambers with Acrylite UV-B transmitting filters.

Solar UV-B radiation significantly reduced the hatching success of *H. cadaverina* and *T. torosa* embryos. *Hyla regilla* embryos were not affected by ambient UV-B radiation.

### *Toads in Colorado*

Populations of toads (*Bufo boreas boreas*) have experienced widespread declines in Colorado (Carey, 1993; Corn, 1998). Corn (1998) studied the effects of UV-B on toads at two Colorado lakes (2,810 and 3,266 m elev.). Between 18 and 37 recently laid eggs at the 2–32 cell stage were placed in 8.9 cm diameter polystyrene petri dishes and assigned to one of three treatments: 1) petri dishes with Saran Wrap<sup>®</sup> over them that transmitted about 80% of ambient UV-B, 2) petri dishes with covers that transmitted about 50% ambient UV-B, and 3) petri dishes with a cover, a mylar disk and a mylar collar around them that blocked all UV-B transmission. The depths at which eggs were placed varied from 3.4 to 9.8 cm. These depths also varied between sites and within sites. There were no differences in hatching success among regimes at either site.

### *Treefrogs and red-legged frogs in Western Canada*

Experiments were conducted at 90 m elev. in a forest clearing in British Colum-

bia, Canada, to examine the effects of UV-B radiation on *H. regilla* and *R. aurora* (Ovaska *et al.*, 1997). Thirty-six 12-liter plastic buckets were buried up to their rims in a 6 × 6 grid within a 4 × 4 meter area. Plastic tubs (24 × 24 cm, 10 cm height) were fitted into the upper portion of each bucket and filled with pond water. Approximately equal numbers of newly laid eggs were placed in each bucket. In one treatment, an acrylic filter blocked UV wavelengths <450 nm. In another treatment, an acrylic filter allowed transmission of all UV wavelengths. A third treatment had no filters and was open to solar radiation. By using fluorescent lamps, two UV enhancement regimes were also employed: lamp with a filter, which provided a 15% increase over noon ambient UV; and lamp without a filter which provided a 30% increase.

Hatching success of *H. regilla* did not differ among regimes. Hatching success of *R. aurora* was similar in the treatments where UV was blocked and those where eggs were exposed to ambient levels. However, it was lower in the UV-enhanced treatments when data from both enhanced treatments were combined.

These results are similar to those of Blaustein *et al.* (1994b) and Anzalone *et al.* (1998) who showed that hatching success of *H. regilla* was unaffected by UV-B in Oregon and California, respectively. Moreover, as in Blaustein *et al.* (1996), hatching success of *R. aurora* was unaffected by ambient UV-B radiation.

Ovaska *et al.* (1997) also observed larvae of *H. regilla* and *R. aurora* for two months post-hatching under the UV regimes described above and found that survival was similar in no UV and ambient UV regimes. However, larval survival was lower in enhanced regimes.

### *Frogs in Australia*

Populations of several species of frogs have disappeared in Australia (Richards *et al.*, 1993). At least two studies have used field experiments to investigate the possible role of UV-B radiation in these declines.

van de Mortel and Buttemer (1996) studied the effects of UV-B on *Litoria aurea*, *L. dentata* and *L. peronii* at sites in the Il-

lawarra and Southern Highlands of New South Wales. Populations of *L. aurea* seem to be in decline while populations of the other two species appear to be stable (van de Mortel and Buttemer, 1996).

An aluminum raft was constructed at each site. Each raft had nine Perspex boxes (40 cm wide  $\times$  40 cm long  $\times$  7 cm deep) with 1 mm<sup>2</sup> fiberglass mesh bases. The boxes were placed on the rafts in 2 cm of water. A muslin insert was placed over the mesh to prevent egg and tadpole losses. Freshly laid eggs (150 per box) were subjected to three UV treatments: 1) unfiltered sunlight, 2) a mylar sunlight filter to remove UV-B, and 3) a polyethylene control filter that allowed 80% UV-B transmission.

In one experiment, the hatching success of *L. aurea* was significantly higher in regimes where UV-B was blocked. However, in other experiments with this species and the other species there were no differences in hatching success among treatments.

Using similar methods, Broomhall *et al.* (1999) studied the effects of UV-B on the hatching success of *Crinia signifera* and *Litoria verreauxii alpina* at three different sites (1,365 m, 1,600 m and 1,930 m elev.) in the Snowy Mountains of southeast Australia. Populations of *L. v. alpina* are in decline while those of *C. signifera* do not appear to be declining (Broomhall *et al.*, 1999). However, high numbers of dead *C. signifera* eggs have been observed in shallow alpine ponds (Broomhall *et al.*, 1999).

Tanks containing six Perspex trays (41 cm long  $\times$  41 cm wide  $\times$  9 cm deep) with a 1 mm<sup>2</sup> mesh base were supported on an aluminum floating raft. A layer of curtain mesh lining was attached to all four rims of each tray to retain larvae. Trays were immersed in 6–9 cm of water. Three treatments were used: 1) unfiltered sunlight, 2) sunlight with mylar filters that removed UV-B, and 3) polyethylene filters that transmitted UV-B. The numbers of embryos hatching and larvae reaching metamorphosis were recorded for four weeks.

The exclusion of UV-B radiation significantly enhanced the survival of both species at all sites. The probability of dying was considerably higher in *L. v. alpina*, a

species whose populations are in decline, than in *C. signifera*, a nondeclining species.

### Toads in Spain

Field experiments investigating the effects of UV-B radiation on the hatching success of two toad species, *Bufo bufo* and *Bufo calamita*, were conducted at 1,920 m elev. in the Sierra de Gredos, Central System of Spain (Lizana and Pedraza, 1998). Populations of both species have declined in various parts of Europe (Blaustein *et al.*, 1994a; Gasc, 1997).

One hundred fifty newly laid eggs (<24 hr old) were placed in each of 12 plastic enclosures (27  $\times$  27  $\times$  14 cm) that were perforated to allow water and air circulation. Enclosures were placed under 1) Llu-mar Window Film stuck to a PVC plastic sheet that blocked UV-B radiation, 2) PVC plastic that blocked 80–85% UV-B, or 3) 2-cm plastic mesh netting that permitted transmission of UV-B radiation. Eggs were placed in 15 cm of water.

There were significant differences in hatching success of *Bufo bufo* eggs among the three regimes. Survival was about 16% in open enclosures, 49% in enclosures that partially blocked UV-B, and 78% in enclosures that totally blocked UV-B radiation. There were no differences among treatments in *Bufo calamita*.

### SYNERGISTIC EFFECTS

In nature, more than one environmental agent may affect amphibians as they develop. Field experiments have been used to examine at least three factors that may interact synergistically with UV-B: a pathogenic alga (*Saprolegnia ferax*), low pH, and fluoranthene, a polycyclic aromatic hydrocarbon that may pollute aquatic environments impacted by petroleum contamination.

### UV/Alga

The alga *Saprolegnia ferax* has contributed to mortality in several amphibian species inhabiting the Pacific Northwest (Blaustein *et al.*, 1994c; Kiesecker and Blaustein, 1997). In a series of field experiments similar to those described above, Kiesecker and Blaustein (1995) tested the hypothesis that there is a synergism between UV-B radia-

tion and *Saprolegnia*. This hypothesis would be supported if the effects of both UV-B radiation and *Saprolegnia* together were greater than those of either factor alone. Tests were conducted on eggs of *R. cascadae*, *B. boreas*, and *H. regilla*.

Experiments were conducted at three natural oviposition sites in the central Oregon Cascade Range. Each species was tested at two sites including one site where all three species were tested (from 1,190–2,000 m elev.). Enclosures identical to those described in Blaustein *et al.* (1994b) were placed in small plastic pools so that the presence or absence of *Saprolegnia* could be controlled. Within the pools eggs were immersed in 5–10 cm of natural lake water. Pools with enclosures were placed in a linear array parallel to the water's edge in a 2 × 3 randomized block design, with three sunlight treatments crossed with two algal treatments (four replicates per treatment; 150 eggs per enclosure) at each site.

*Saprolegnia* was cultured in the laboratory and randomly added to half of the enclosures in each sunlight treatment. An antifungal agent was added to the remainder of the enclosures to remove any naturally occurring *Saprolegnia*. A second experiment was conducted simultaneously with the first one using identical techniques except that enclosures were placed directly into the lake. Thus, in the second experiment, embryos were exposed to all three sunlight regimes and natural levels of *Saprolegnia*.

In the first experiment, all three species had reduced hatching success in the presence of *Saprolegnia*. In addition, when exposed to both *Saprolegnia* and UV-B, embryos of *Bufo* and *Rana* experienced significantly higher mortality than when they were exposed to either factor alone. Hatching success of *Hyla regilla* was not affected by UV-B radiation but it was affected in the presence of *Saprolegnia*.

In the second experiment, hatching success of *B. boreas* and *R. cascadae* was also greater in treatments shielded from UV-B radiation. Hatching success of *H. regilla* did not differ among the regimes.

#### UV/pH

Several studies have shown that habitat acidification can have adverse effects on

amphibians (*e.g.*, Pierce, 1985; Dunson *et al.*, 1992; Kiesecker, 1996). In regions where acid pollution is a concern, there may be synergistic effects between low pH and other factors such as UV-B radiation. Long *et al.* (1995) investigated the potential effects of such a synergism on amphibian embryos. They studied eggs of the leopard frog (*Rana pipiens*) exposed to combinations of three levels of UV-B and three levels of pH.

Experiments were conducted outdoors (250 m elev.) but natural UV-B was supplemented with artificial sources. One regime had no UV-B levels. Another regime had UV-B levels simulated for high elevations (intermediate UV-B level) and a third regime had UV-B levels simulated to approach levels predicted with a 30% loss in ozone (high UV-B level). These UV levels were crossed with pH levels of 4.5, 5, and 6.

With low pH and simulated intermediate and high UV-B levels, hatching success was significantly lower compared to the other regimes. There was no increase in mortality with either UV-B or pH alone. This study demonstrates the potential effects of a synergism between UV-B radiation and low pH.

#### UV/Fluoranthene

Hatch and Burton 1998 investigated the potential synergistic effects of UV and fluoranthene on two species of frogs, *Xenopus laevis* and *Rana pipiens*, and the spotted salamander, *Ambystoma maculatum*. Experiments were conducted outdoors in experimental chambers on the roof of a building in Ohio. In one experiment, they monitored the hatching success of *R. pipiens* embryos under full spectrum sunlight and under approximately 6% sunlight. In a second experiment using the same materials they monitored the survival of *X. laevis* and *A. maculatum* larvae under three light regimes crossed with three concentrations of fluoranthene. The light regimes were full sunlight, a regime that transmitted about 40% solar radiation (by covering the beakers with cheesecloth), and a regime that transmitted about 4–12% solar radiation. The lowest light regime in all experiments was obtained by coating the beakers with

Coppertone<sup>™</sup> Sport SPF 30 sun block and covering them with Saran Wrap<sup>™</sup> and black plastic. In all experiments, eggs or larvae were placed in 250-ml beakers (10 individuals per beaker; two replicates per treatment). Water depth was 5 cm. A randomized block design was used in all experiments and beakers were placed in a tub full of water to control for evaporation and overheating.

There were no effects on hatching success in any treatment regime. However, under the highest fluoranthene doses and full solar radiation, mortality rates of larvae were significantly increased in *X. laevis* and *A. maculatum* in a manner that was correlated with increasing UV intensity and increasing fluoranthene concentration.

#### SUMMARY OF FIELD EXPERIMENT RESULTS

Results of field experiments by several different investigations strongly indicate that the hatching success of at least nine species of amphibians, from widely separated locales, is reduced under *ambient* UV-B radiation (Table 1). This includes two frog species, one toad species, two salamander species and a new from North America, two frog species from Australia, and a species of toad from Europe. These species comprise a diverse group taxonomically that includes two orders, six families and seven genera of amphibians. Some of these species are found in montane areas, others at sea level. A key characteristic shared by these species is that they often lay their eggs in shallow water, where they are exposed to solar radiation.

Hatching success of several other species of frogs in North America and Australia, and toads in Europe and Colorado, were not affected by UV-B radiation (Table 1). This is not surprising because many studies have demonstrated differential sensitivity of amphibians to various abiotic factors (e.g., Blaustein, 1994).

Results of several experiments examining synergistic interactions suggest that there is a potential for UV radiation to act synergistically with a wide variety of agents.

#### DISCUSSION

The power of field experiments in ecology has been discussed extensively (e.g., see discussions in Hairston, 1989; Jaeger and Walls, 1989; Wilbur, 1989; Real and Brown, 1991; Walls 1991; Ramsey and Shafer, 1997). It is apparent, however, that there is some misunderstanding regarding the design, power and interpretation of the results of field experiments.

##### *Where should field experiments be conducted?*

We believe that field experiments investigating particular causes for amphibian population declines, egg mortality and related phenomena should be done in the precise area, and if possible, microhabitat where these phenomena are occurring. In some cases, field experiments may be conducted in areas where a species was formerly abundant but where it is now rare or perhaps extinct. Most of the studies described above were performed in areas where the animals live or where they were historically found. For example, Blaustein *et al.* (1994b) erected enclosures among egg masses that were laid communally to examine egg mortality in frogs and toads in the Pacific Northwest. Anzalone *et al.* (1998) conducted their experiments in areas of streams where the amphibians they examined naturally lay their eggs. Interpreting the results of an experiment where a species is brought into an area far from its native site is difficult and should be done with caution.

##### *What varies in a field experiment?*

Field experiments are designed so that all factors vary naturally and simultaneously between experimental and control treatments, except for the variable(s) of interest (see Hairston, 1989 and papers cited therein). Thus, in a randomized block design, (e.g., Blaustein *et al.*, 1994b; 1997a, b) treatment combinations are assigned randomly to subjects within a block and separate randomizations are conducted for each block (Ramsey and Shafer, 1997). This concept seems to be insufficiently understood by some investigators. For example, Licht (1996) erroneously claimed that in earlier

UV/amphibian experiments investigators "control[ed] all variables but UV-B transmission." Actually, UV-B transmission is the only variable that is strictly controlled. All other factors vary naturally.

Variability of abiotic and biotic factors is what makes a field experiment ecologically relevant. For example, it may be cloudy one day and sunny the next. But if the experiment is designed appropriately, all enclosures are subjected to the same amount of sunny and cloudy days. The inherent variability of field experiments may seem unintuitive or unacceptable to laboratory scientists who strictly control all parameters in an artificial setting. A good experimental design contains adequate replication (to capture a range of the variability present), controls treatments (to account for the effects of any manipulations), and often uses blocking (to find environmental gradients within the study area).

### *Controls*

Obviously, adequate controls are necessary in a field experiment. Controls should be treated the same as experimental units. If enclosures are used, the control and experimental enclosures should be the same dimensions and made of the same material. If shields are placed over experimental enclosures such as those used in many of the UV-B experiments described above, shields should be placed over control enclosures because there may be a "shield effect."

### *Replicates*

"Replication means applying exactly the same treatment to more than one experimental unit" (Ramsey and Shafer, 1997). True replicates have to be conducted with the same methods on the same species and in the same experiment. Employing an adequate number of replicates for each treatment will help to ensure that results are not unique to a particular series of treatments or blocks. In addition, we suggest, that there be replicate sites whenever possible. It is possible that the results obtained from one site are unique to that site. Some of the UV-B studies described above do not use more than one site. Site replicates enhance

the significance and generality of the results.

### *Comparisons between studies*

Even if the exact same methods and materials are used it is difficult to compare the results of studies that are conducted in different systems. It is even more difficult to compare studies that use different methods, conduct tests at different times, and examine different species. This is especially true for studies examining the effects of UV-B radiation on amphibians. The amount of UV-B radiation exposure varies with many factors, including cloud cover, precipitation, altitude, latitude, and local pollution events. Aquatic habitats differ in turbidity, amount of dissolved organic carbons, temperature, animals, plants, microorganisms and many other factors. These differences may result in differences in how UV radiation affects amphibians between habitats. Thus, it is not surprising that Corn's (1998) results on the effects of UV-B on *B. boreas* differed from ours. Our group (e.g., Blaustein *et al.*, 1994b) used relatively large open enclosures with mylar and acetate filters and Corn (1998) used small petri dishes, some covered with Saran Wrap<sup>®</sup>. The Colorado system differs dramatically from Oregon in several abiotic and biotic parameters, as well as atmospheric conditions. There also may be geographic variation in the effects of UV-B in such a wide-ranging species. Indeed, based on molecular-systematic data, toads examined in Colorado may constitute a different species from *B. boreas* in Oregon (Goebel, 1996). Comparisons between studies are difficult to make and they certainly should not be considered replicates of one another. We suggest greater caution when comparing results from different studies.

### *Will measuring levels of UV-B radiation enhance the significance of experimental results?*

Measurements of UV-B radiation are not necessary to document a detrimental effect of radiation on amphibian development. In a well-designed experiment, if hatching success is lower in enclosures where eggs are exposed to UV-B than in enclosures

where they are shielded from UV-B it is obvious that UV-B radiation has affected hatching success.

Measuring UV-B levels under shields obviously should show that levels under blocking regimes are less than levels under transmitting regimes. If done precisely enough, UV-B measurements may reveal the minimum dose that will cause egg mortality. Nevertheless, UV-B levels change constantly. They change with weather patterns, cloud cover, water flow, and turbidity. Due to temporal changes, UV-B levels must be monitored constantly for a meaningful interpretation of their effects on biological systems. Because there are no long-term data on levels of UV-B radiation from sites where amphibian tests are being conducted, levels measured at present cannot be compared with any historical data. However, accumulating long-term data on terrestrial UV radiation will allow us to have a baseline for comparing current and future levels.

Carey *et al.* (1996) measured UV-B irradiance at midday in air and water at breeding sites of three species of amphibians in Colorado. They also conducted laboratory experiments in which amphibian eggs and larvae were exposed to various doses of UV-B radiation. Salamanders experienced significant mortality at relatively low levels of simulated UV-B radiation. Eggs and larvae of two toad species were more tolerant to simulated UV-B radiation than were salamanders. Based on these laboratory tolerance limits and field measurements, they concluded that field levels of exposure were not sufficient to be a causative factor in the decline of the species of amphibians examined.

We regard this conclusion as premature. No field experiments were conducted. Under field conditions there are many factors that vary and which could potentially influence how UV varies and how it impacts amphibians. Biotic parameters also vary. Performing an experiment in a dynamic natural system such as a lake, pond or stream will be the most rigorous test of the effects of ambient UV-B on amphibians. Without such a test, UV-B measurements will not provide much information on UV-B tolerance levels of amphibians in nature.

Corn (1998) used remote satellite data in an attempt to estimate UV levels in Oregon and Colorado. However, in the absence of corroborative ground measurements, satellite estimates may not be very useful. Moreover, even if satellite data showed the same levels of UV-B at the precise microhabitats where experiments in Colorado and Oregon were conducted (which was not possible), this would not influence the conclusions of the studies conducted in those regions.

#### *Natural selection*

Why are the eggs of some species of amphibians affected by UV-B whereas the eggs of others are not? One possible reason is that, over evolutionary time, there were strong selection pressures for certain species to evolve mechanisms that counteracted the harmful effects of UV-B. When UV radiation penetrates a cell, photoproducts may form that can lead to mutations or cell death. Embryos of some amphibian species may be more resistant to UV-B because they can repair UV damage more efficiently than others. One important repair process is enzymatic photoreactivation. A single enzyme, photolyase, uses visible light energy to remove the most frequent UV-induced lesion in DNA, cyclobutane pyrimidine dimers (CPDs) (Friedberg *et al.*, 1995). Moreover, multi-protein broad specificity excision repair processes can remove CPDs and other UV photoproducts. Both mechanisms may be used simultaneously. However, photoreactivation appears to be the first level of defense for many organisms exposed to sunlight (Pang and Hays, 1991; Friedberg *et al.*, 1995).

We suggest that greater photolyase activity makes some amphibian species more resistant to UV-B radiation than others, by removing more of the primary cytotoxic/mutagenic photoproducts from their DNA. If eggs are damaged by UV-B in field experiments, obviously none of the repair mechanisms is working efficiently enough to repair the damage. However, if eggs are resistant, it is difficult to determine whether excision repair, photolyase or both are repairing damage. Because photolyase repair is probably the most important repair mechanism in amphibians, a parsimonious expla-

TABLE 2. Photolyase levels in North American amphibian eggs from the Pacific Northwest for which field experiments examining the effects of UV on hatching success have been conducted.

Species	Activity of photolyase $10^{11}$ CPDs* per hr per $\mu\text{g}$ ( $\pm$ SE)	Reference
<i>Hyla regilla</i>	7.5 (0.35)	Blaustein <i>et al.</i> , 1994b
Pacific treefrog		
<i>Rana luteiventris</i>	6.84 (0.3)	Blaustein and Hays, unpublished
Columbia spotted frog		
<i>Rana pretiosa</i>	6.62 (0.01)	Blaustein and Hays, unpublished
Oregon spotted frog		
<i>Rana aurora</i>	6.09 (0.22)	Blaustein <i>et al.</i> , 1996
Red-legged frog		
<i>Rana cascadae</i>	2.4 (0.23)	Blaustein <i>et al.</i> , 1994b
Cascades frog		
<i>Bufo boreas</i>	1.3 (0.08)	Blaustein <i>et al.</i> , 1994b
Western toad		
<i>Ambystoma gracile</i>	1.0 (0.10)	Blaustein <i>et al.</i> , 1994b
Northwestern salamander		
<i>Ambystoma macrodactylum</i>	0.8 (0.72)	Blaustein <i>et al.</i> , 1994b
Long-toed salamander		

\* Cyclobutane pyrimidine dimers.

nation is that those species with the highest photolyase activity are the most resistant to UV damage. Indeed, the amount of photolyase in eggs is positively correlated with survival of embryos in field experiments (Table 2). Thus, eggs of the most resistant species in our field experiments (*H. regilla*, *R. aurora*, *R. pretiosa*, and *R. luteiventris*) had much higher levels of photolyase activity than eggs of the more susceptible species (*R. cascadae*, *B. boreas*, *A. macrodactylum*, *A. gracile*).

Behavioral and ecological attributes also may limit UV-B damage to amphibians. For example, many salamander species (and some frogs and toads) lay their eggs under leaf litter, in crevices or under logs where they are not subjected to high levels of sunlight. Many amphibians lay their eggs in muddy water where light penetration may not be significant to damage eggs. Thus, in the clear, high mountain lakes in Oregon, where we have conducted our research, the potential for UV penetration is greater than in relatively turbid sea-level ponds where many eastern and some western species lay their eggs (Stebbins, 1954; Nussbaum *et al.*, 1983).

Even if eggs are laid in the open at high altitudes (where under certain conditions UV levels may be high), take very long to develop and are subjected to intense levels of UV-B radiation, they may not be affected

by UV-B radiation if they have efficient DNA repair mechanisms. Conversely, species at sea level (where UV levels may be low) with very low photolyase levels may be quite sensitive to UV-B radiation as we have shown (Blaustein *et al.*, 1995, 1996).

Within a species it is possible that individuals from one population may differ from members of another population in their sensitivity to UV-B radiation. This may be due to differences in their ability to repair DNA damage or differences in individual egg-laying behavior. Thus, the eggs of one species may differ between populations in their exposure to solar radiation. For example, long toed-salamanders (*A. macrodactylum*) deposit their eggs in small discrete clutches in shallow temporary ponds in the Willamette Valley of Oregon. However, in the Cascade Range of Oregon they scatter eggs singly with some being laid in open shallow water and others in deeper water under rocks in temporary or permanent ponds (personal observations).

#### *Why is there mortality under UV-B shields?*

Hatching success in nature is usually less than 100% (Duellman and Trueb, 1986). Thus, in field experiments, there was some mortality even under UV-B shields (e.g., Blaustein *et al.*, 1994b; Anzalone *et al.*, 1998; Lizana and Pedraza, 1998). Mortality

may occur because of species differences in hatching success, individual genetic variability, pathogens, other biotic factors and numerous abiotic factors including UV-A (315–400 nm) radiation. Importantly, however, in several species described in this review, hatching success was greater under UV-B shielded regimes compared with regimes where embryos were exposed to UV-B radiation. Thus, UV-B was a significant source of mortality in several species (Table 1).

#### *How do we interpret negative results?*

It is difficult to interpret negative data. Thus, if field experiments fail to find a “UV effect,” one must be cautious about interpreting the results because it is not possible to prove that something did not occur. As discussed above, several studies have failed to find an effect of ambient UV-B radiation on the eggs of some amphibian species as assessed by hatching success. It is possible, however, that UV-B radiation did affect the embryos but the effects may not become manifest until later life-history stages (*e.g.*, larvae, metamorphs, adults). It is possible that the methods, materials and measurements used in certain studies were not sensitive enough to detect detrimental effects of UV-B on eggs. It is also possible that the eggs found at some sites are UV-resistant remnants of a population whose other eggs were less resistant and that perished.

Studies of the same species at different sites add some assurance that negative results are real. For example, the effects of ambient UV-B radiation on *H. regilla* were examined at three sites in Oregon, and one site each in Canada and California. It is difficult to compare the results of different investigations. However, based on the number of locales used and the consistency of the results, we believe that it is most appropriate to conclude that hatching success of *H. regilla* is unaffected by UV-B radiation across the species’ range.

#### *The role of UV-B radiation in population declines*

UV-B radiation is affecting the eggs and embryos of several species of amphibians in widely scattered locales around the world

(Table 1). Continued mortality at the egg stage may eventually lead to a population decline. However, the effects of mortality at the egg or embryo stage may not be observed at the population level for many years, especially in relatively long-lived species (Blaustein *et al.*, 1994a). For example, well documented and continuing mortality at the egg stage in western toads (*B. boreas*) in Oregon (*e.g.*, Kiesecker and Blaustein, 1997) may eventually lead to a population decline in adults. However, adult *B. boreas* may live for at least 16 years (unpublished data, A.R.B.) and the effects of mortality in eggs and embryos may not be observed in adult populations until adults eventually disappear and recruitment is insufficient to maintain the population. Moreover, if a population has been recruiting poorly for several years it may rebound quickly with one good bout of recruitment. Populations of those species whose eggs and embryos are susceptible to UV-B radiation are at risk, but the extent of this risk is unknown.

UV-B radiation obviously is not the only agent that may contribute to a population decline. It would be an unlikely factor in the declines of species that lay their eggs under logs, in crevices, in deep water or under dense forest canopy. As we have shown in this review, several species of amphibians are unaffected by UV-B in early life stages but still are in decline. Nevertheless, the hatching success of many amphibian species is affected by UV-B radiation. Several factors, such as pathogens, low pH, and pesticides may act synergistically with UV-B radiation to enhance mortality in early life stages. These synergistic interactions may eventually contribute to a population decline.

The available evidence suggests that more than one agent is contributing to amphibian population declines. A number of investigators have employed field experiments to examine the effects of UV-B radiation on amphibians. UV-B radiation is one agent that is significantly hampering the hatching success of some amphibian species at the egg and embryo stage. We do not have enough information on how UV-induced mortality in early life stages is af-

fecting adult amphibian populations. However, we suggest that field experiments are an excellent method for examining agents, such as UV, that may be involved in amphibian population declines.

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