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EFFECTS OF ULTRAVIOLET RADIATION ON BOREAL TOADS IN COLORADO

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Abstract. Field exposures of *Bufo boreas* embryos to fractions of ambient UV-B radiation at two sites in Rocky Mountain National Park, Colorado, USA, were conducted to evaluate UV-B as a possible cause of recent severe declines of this species. There were no differences in hatching success of *B. boreas* embryos exposed to 0–100% of ambient UV-B radiation at either study site, results that are different from those of recent studies in Oregon that found increased mortality of *B. boreas* embryos exposed to ambient UV-B. The reasons for these differing results are not apparent, and several possible explanations exist, including differences in experimental design, presence or absence of a pathogenic fungus, and geographic genetic variation. *Bufo boreas* embryos were probably not receiving higher doses of UV-B radiation during the experiments in Oregon compared to the experiments in this study. Results of this study do not support UV-B radiation alone as the cause of the decline of *B. boreas* during the past 20 yr in the southern Rocky Mountains, but UV-B cannot be dismissed because of the contradictory results from other studies.

Key words: amphibian population declines; amphibians; Bufo boreas; *ozone depletion; Rocky Mountain National Park, Colorado, USA; ultraviolet radiation and amphibian declines.*

INTRODUCTION

Ozone depletion and resulting increased ultraviolet-B radiation (Kerr and McElroy 1993, Zerefos et al. 1995) is under increasing scrutiny as a potential cause of amphibian declines. Worrest and Kimeldorf (1976) observed that enhanced UV-B radiation (290–315 nm) caused developmental abnormalities and mortality of boreal toad (*Bufo boreas*) tadpoles as they approached metamorphosis. The ecological relevance of this experiment is uncertain, however, because tadpoles were confined in small dishes and continuously exposed to relatively high doses of UV-B radiation (7.5–41 $kJ \cdot m^{-2} \cdot d^{-1}$, compared to 4.3 $kJ \cdot m^{-2} \cdot d^{-1}$ used by Long et al. [1995] as a "normal" high elevation UV-B dose).

Recently, Blaustein et al. (1994) generated considerable interest with their report of mortality of amphibian embryos exposed to ambient UV-B in sunlight. Embryos of two species, *B. boreas* and the Cascades frog (*Rana cascadae*), had lower hatching success in field enclosures exposed to full sunlight compared to enclosures with UV-B filtered out. Blaustein et al. (1994) found differences among amphibian species in activity of photolyase, an enzyme involved in repair of photo-damaged DNA, extracted from eggs. The species with lower hatching success in the field, *B. boreas* and *R. cascadae,* had low photolyase activity, and Pacific treefrogs (*Hyla regilla*), which did not differ in mortality among UV-B treatments, had relatively high photolyase activity. Blaustein et al. (1995*a*) demostrated similar reduced hatching success in field enclosures in full sunlight and reduced photolyase activity in northwestern salamanders (*Ambystoma gracile*).

However, not all studies have demonstrated convincing links between increased UV-B radiation and amphibian mortality. Blaustein et al. (1996) found no effect of ambient UV-B radiation on hatching success of red-legged frogs (*Rana aurora*), but that *R. aurora* oocytes had relatively high levels of photolyase activity. Long et al. (1995) observed increased mortality of leopard frog (*Rana pipiens*) embryos under enhanced UV-B radiation (about twice normal, reflecting a 30% reduction in ozone), but only in combination with low pH. However, such acidic waters (pH = 4.5) are observed infrequently in amphibian breeding habitats in the western United States (Bradford et al. 1992, Corn and Vertucci 1992, Vertucci and Corn 1996).

Two recent studies in Canada did not find a relationship between current levels of UV-B radiation and mortality of amphibian embryos. Grant and Licht (1995) did not observe mortality of wood frog (*Rana sylvatica*) embryos, exposed in the laboratory to UV-B doses comparable to current mid-summer exposure in southern Ontario (~44° N latitude, approximately the same as study sites used by Blaustein et al. [1994, 1995*a*]). Grant and Licht (1995) also did not observe UV-B-related effects on survival and growth of *R. sylvatica,* American toad (*Bufo americanus*), or green frog (*Rana clamitans*) tadpoles. Ovaska et al. (1997) reported that ambient UV-B did not affect hatching suc-

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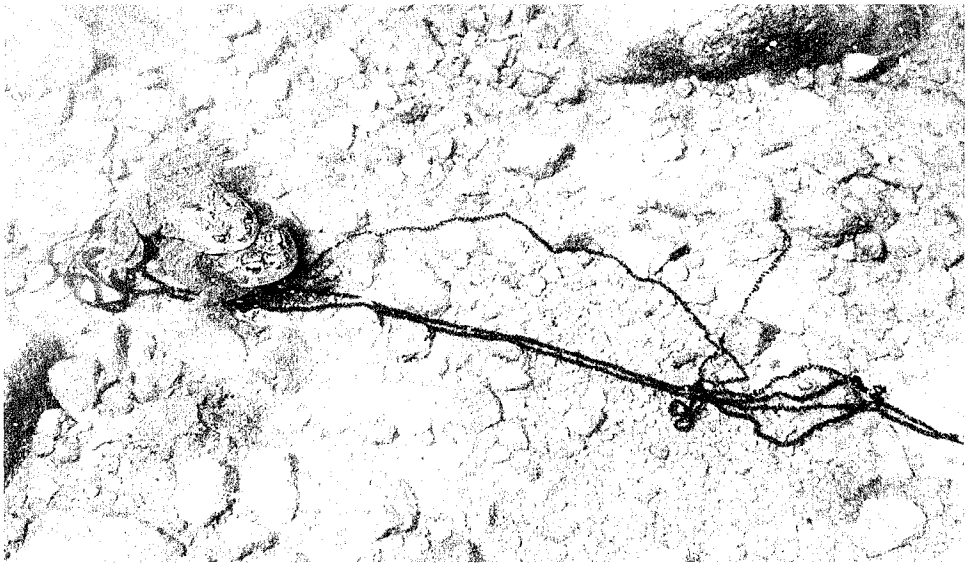

FIG. 1. *Bufo boreas* in amplexus at Kettle Tarn in Rocky Mountain National Park. Oviposition is occurring in water 4–5 cm deep.

cess of *R. aurora* and *H. regilla* in experiments conducted on Vancouver Island, British Columbia, in 1995, but that hatching success of *R. aurora* was lower when UV-B was enhanced to 15–30% above ambient levels. In field experiments in Australia similar to Blaustein et al (1994), van de Mortel and Buttemer (1996) found higher hatching success of green and golden bell frog (*Litoria aurea*) embryos when UV-B was filtered out. However, there was no UV-B effect in a second experiment with *L. aurea* or with two other species of *Litoria* (van de Mortel and Buttemer 1996). The differing results of studies of UV-B radiation and amphibians are difficult to evaluate because of the variety of methods used (laboratory vs. field, continuous exposure to sunlight vs. brief exposure to high-intensity UV lamps, etc.).

Despite these conflicting results and problems of interpretation, mortality from increased UV-B radiation is an attractive hypothesis to explain declines of amphibians in regions without obvious habitat destruction or pollution (Blaustein 1994, Corn 1994). Populations of *B. boreas* in the southern Rocky Mountains in Colorado, southeast Wyoming, and northern New Mexico have undergone a severe decline in relatively pristine areas (Carey 1993, Corn 1994, Stuart and Painter 1994). This species is listed as "Endangered" by the State of Colorado and is a candidate for Federal listing (U.S. Fish and Wildlife Service 1995). The cause(s) of the decline of *B. boreas* have not been discovered. Acid precipitation, the only anthropogenic cause so far thoroughly investigated, is not considered severe enough to have had a regional effect (Corn and Vertucci 1992, Vertucci and Corn 1996; but see Harte and Hoffman 1989). Carey (1993) observed toads with red-leg disease and extinction of toad populations in central Colorado in the early 1980s. Carey hypothesized that immune-system failure caused by stress could have caused the decline of *B. boreas,* but a stressor has not been identified.

By depositing strings of eggs (compared to clumps) in shallow water, *B. boreas* may be predisposed to have difficulty dealing with increased UV-B radiation. However, the degree to which these factors are important has been disputed. Grant and Licht (1995) criticized the study design of Blaustein et al. (1994), contending that the species used typically do not deposit their eggs in extremely shallow (5–10 cm) water without silt or vegetation that might provide shielding from UV-B radiation. Blaustein et al. (1995*b*) responded that their experiments were conducted at the sites where oviposition was taking place, but oviposition sites are variable. Olson (1989) reported that between 1982 and 1986 *B. boreas* deposited eggs at depths of 5 cm–2 m at one location used by Blaustein et al. (1994) and 5–25 cm at the other location. However, since 1986 oviposition has not been observed in deep water (>1 m) at these locations (D. Olson [U.S. Forest Service, Pacific Northwest Research Station, Corvallis, Oregon, USA], *personal communication*). *Bufo boreas* in Colorado often deposits eggs in shallow (<10 cm), exposed situations (Fig. 1; Hammerson 1984, Corn et al. 1997). Grant and Licht (1995) measured reduced transmission of UV-B radiation through the jelly matrix surrounding amphibian egg masses, and they suggested that Blaustein et al. (1994) artificially increased exposure to UV-B radiation by breaking apart egg masses to distribute among their experimental enclosures. This may be a valid criticism for *R. cascadae,* which deposits eggs in spherical masses, but this criticism does not hold for *B. boreas,* which deposits eggs in long

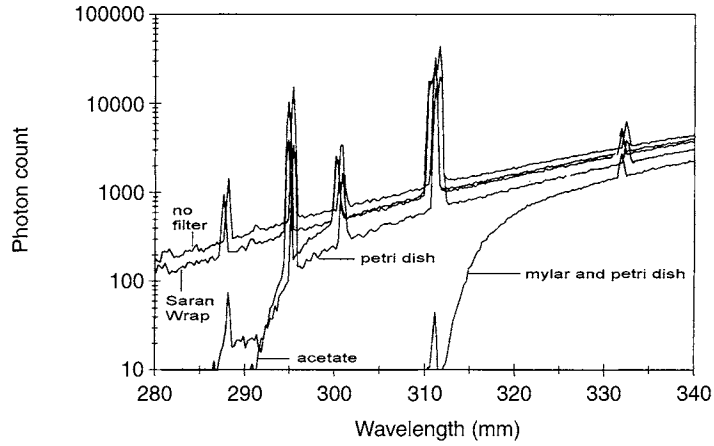

FIG. 2. Transmission of UV-B radiation through materials used to filter ambient sunlight. See Table 1. I did not use acetate (5-mil) in this study, but I presume this to be the same as that used by Blaustein et al. (1994).

strings, so that every embryo receives the same maximal exposure to solar radiation (Fig. 1). *Bufo boreas* in Colorado typically breeds from mid-May through June (Hammerson 1984; P. S. Corn, *personal observation*), the time of year when solar intensity is maximal.

Here, I report the results of a field experiment similar to Blaustein et al. (1994). *Bufo boreas* embryos were exposed to ambient UV-B radiation and fractions thereof in situ at two breeding locations in the mountains of Colorado. The objectives of this experiment were to conduct a field experiment similar to that of Blaustein et al. (1994) using *B. boreas* in Colorado, and to evaluate UV-B radiation as a cause of the decline of *B. boreas*.

METHODS

I conducted experiments at Kettle Tarn (2810 m elevation) and Lost Lake (3266 m) in the North Fork drainage of the Big Thompson River in Rocky Mountain National Park, Colorado, USA (Corn et al. 1997; see Vertucci and Corn [1996: Fig. 3] for a photograph of *Bufo boreas* breeding habitat at Lost Lake). Kettle Tarn is a shallow, fishless, glacial kettle pond and Lost Lake is a drainage lake with a restored resident population of endangered greenback cutthroat trout (*Oncorhynchus clarki stomias*).

The experiments were performed in 1994 from 21 May to 3 June at Kettle Tarn and 5 to 16 June at Lost Lake. At each site, recently deposited *B. boreas* eggs (2- to 32-cell stage embryos; most were at 8- or 16-cell stage) from five clutches were distributed among three UV-B treatments. I placed samples of 18 to 37 embryos ($\bar{X} = 25$) in 8.9-cm diameter polystyrene petri dishes, the sides of which were perforated to allow water circulation. I used petri dishes instead of large enclosures as in Blaustein et al. (1994; 38 × 38 × 7 cm) for two reasons: I wished to test for differences in mortality among clutches, and large enclosures were impractical to pack into my remote sites and would have been intrusive in a national park. Total numbers of embryos used per clutch were very similar to the 150 embryos used by Blaustein et al. (1994).

TABLE 1. Percentage of unfiltered lamp light transmitted through various filter types at 10-nm intervals.

Filter type	Wavelength (nm)						
	280	290	300	310	320	330	340
Saran Wrap	68	66	77	83	80	81	87
Petri dish	0	5	39	52	61	64	71
Mylar and petri dish	0	0	0	0	29	46	51
Acetate	0	0	76	87	86	88	93

Each dish had one of three UV-B filters (Fig. 2 and Table 1): Saran Wrap held in place with a rubber band, which allowed transmission of ~80% of ambient UV-B radiation from 280 to 315 nm; the cover to the dish, which transmitted ~50% ambient UV-B; and the cover plus a mylar disk and mylar collar around the side of the dish, which transmitted 0% ambient UV-B. Exact relative transmission of UV-B through each filter type is unknown, because the correct action spectrum for affects on toad embryos is unknown. (The action spectrum is the weighted biological response as a function of wavelength [Caldwell et al. 1986, Horneck 1995]. If *B. boreas* embryos are most sensitive at 290 nm, the effective transmission of the filters would be different than if the embryos are most sensitive at 310 nm.) UV-B transmission (Fig. 2) was measured as photon counts at 1-nm intervals by interposing each filter type between mercury and tungsten lamps and a UV meter composed of a 5-cm integrating sphere optically coupled to a prism-grating double monochromator, with a bi-alkalai photo multiplier tube set up for photon counting (Patrick Disterhoft [Air Resources Laboratory, National Oceanic and Atmospheric Administration, Boulder, Colorado], *personal communication*).

Each treatment was repeated three times for each clutch. Samples were distributed randomly among five blocks, each consisting of nine dishes arranged in three columns (80%, 50%, and 0%). Dishes were placed in shallow water on the north shoreline at each site and

were held in place with short twigs pushed into the substrate. Because of topographic differences between sites, the average depth of treatment dishes (surface of water to top of dish) was 3.4 cm at Lost Lake and 9.8 cm at Kettle Tarn. Additionally, at Kettle Tarn I also examined embryos in three short segments (13 to 37 embryos) of each original egg string just before hatching, which constituted a 100% UV-B treatment. These data were not obtained at Lost Lake. There, the original egg strings were laid in very shallow water and the level of the lake dropped during development, resulting in complete mortality of all five clutches.

When embryos hatched I recorded the numbers of developing (normal and abnormal) and undeveloped embryos in each dish. Undeveloped embryos either did not develop beyond gastrulation or may have been unfertilized eggs. They turned a grayish-whitish color and decayed rapidly. Abnormal embryos developed beyond tail-bud stage but were grossly deformed and failed to hatch. Hatching success was measured for all embryos (the proportion of normal embryos relative to all embryos) and for developing embryos only (the proportion of normal embryos relative to embryos that developed beyond gastrulation). Variation among UV-B treatments and clutches was tested using arcsine-transformed hatching success.

I recorded temperature every 9.6 min at Kettle Tarn and every 12 min at Lost Lake from two dishes in each treatment (one dish at Lost Lake) and in the water using thermistor probes and single-channel data loggers (Hobotemps, Onset Computer Corporation, Pocasset, Massachusetts, USA).

UV-B radiation was not measured directly in this experiment or by Blaustein et al. (1994). I did not have access to a meter that could be transported to my remote study sites. However, some comparisons between study locations in Colorado and Oregon are possible using remote-sensing data. The Russian satellite Meteor 3 carried a total-ozone mapping spectrometer (TOMS) that operated 22 August 1991 to 27 December 1994, that measured UV scattered by the Earth's atmosphere (McPeters and Beach 1996). Surface UV-B data are not currently available from this data set, but ozone thickness and reflectivity, two primary determinants of surface UV-B (Herman and Celarier 1996), are available (McPeters and Beach 1996). The satellite data have a resolution of 1° latitude × 1.25° longitude. I compared mean stratospheric ozone thickness in Dobson units (DU) and percentage reflectivity for the area in Colorado that included my study sites (block centered at 40.5° N, 105.625° W) with the area in Oregon (44.5° N, 121.875° W) that included the study sites of Blaustein et al. (1994), for the periods 1 May to 30 June in 1993 (Oregon) and 1994 (Colorado). (One DU is 10^{-3} cm of ozone at standard temperature and pressure, or $\sim 2.69 \times 10^{16}$ molecule/cm^2 [Wayne 1991].) Unfortunately, the satellite's orbit was such that data are missing periodically, including 7–30 June 1993 and 1–31 May 1994 (McPeters and Beach 1996). To compensate for these missing data and to examine long-term differences in UV-B exposure between the Colorado and Oregon study sites, I used data from an earlier TOMS instrument carried on NASA's Nimbus 7 satellite from November 1978 to March 1993 (Herman and Celarier 1996). These data are available as erythemal exposure (dimensionless), UV-B weighted by the action spectrum for human skin irritation. For each location, I calculated mean erythemal exposure in May and June (n = 61 d) for 1979 through 1992 and the mean daily exposure (n = 14 yr) for 1 May through 30 June. Daily measurements from TOMS potentially have relatively large errors but data averaged over several days (≥1 wk) agree well with ground-based measurements (Jay Herman [Goddard Space Flight Center, Beltsville, Maryland], *personal communication*).

Results

There were no significant block or block × UV-B interaction effects at either Kettle Tarn or Lost Lake for proportions of all embryos and developing embryos only that hatched ($P > 0.25$ in all cases). Therefore, the numbers of normal, abnormal, and undeveloped embryos were pooled across blocks for each clutch at each UV-B level for all further analyses. Hatching success was higher at Kettle Tarn than at Lost Lake. Hatching success of all embryos averaged 0.76 at Kettle Tarn (Table 2) and 0.58 at Lost Lake (Table 3; $F = 14.3$, df = 1, 100; $P < 0.001$). Hatching success of developing embryos only was also higher: 0.98 at Kettle Tarn (Table 4) and 0.92 at Lost Lake (Table 5; $F = 13.7$, df = 1, 100; $P < 0.001$).

There were significant UV, clutch, and UV × clutch interaction effects for hatching success of all embryos at Kettle Tarn (Table 2) and for clutch at Lost Lake (Table 3). Embryos from clutch 3 at Kettle Tarn and clutch 4 at Lost Lake had very low hatching success. Differences in hatching success among UV-B treatments did not relate to the amount of UV-B radiation. Hatching success was lowest at the 80% UV-B (Saran Wrap filter) treatment at Kettle Tarn, but this treatment had the highest hatching success at Lost Lake. The significant interaction at Kettle Tarn was mostly due to clutch 3, which had low hatching success of manipulated embryos (0, 50, and 80% UV-B) and high hatching success of unmanipulated embryos (100% UV-B). If sensitivity to handling stress is related to embryo age, then this difference was possibly an artifact. Embryos from clutch 3 were placed in petri dishes at the 2-cell stage, the earliest of any clutch at either study site.

When only those embryos that developed successfully past gastrula stage were considered, there were no significant UV, clutch, or UV × clutch interaction effects for hatching success at Kettle Tarn (Table 4) or at Lost Lake (Table 5).

Temperatures were slightly higher inside the petri

TABLE 2. Hatching success of all *Bufo boreas* embryos at Kettle Tarn, Rocky Mountain National Park, Colorado, and ANOVA results. The experiment was conducted from 21 May to 3 June 1994. Sample size (*N*) for each UV × clutch cell is 3 dishes unless noted otherwise.

A) Hatching success

	Mylar filter (0% UV-B)		Petri dish cover (50% UV-B)		Saran Wrap (80% UV-B)		Egg mass (100% UV-B)		All	
Clutch	Mean	1 SD	Mean	1 SD	Mean	1 SD	Mean	1 SD	Mean	1 SD
1	0.82	0.07	0.81	0.03	0.75	0.20	0.76	0.32	0.78	0.17
2	0.86	0.04	0.97	0.02	0.90	0.11	0.92	0.08	0.91	0.07
3	0.23	0.04	0.30	0.19	0.21	0.11	0.85	0.12	0.40	0.30
4	0.97	0.02	0.89	0.02	0.86	0.12	0.83	0.13	0.89	0.10
5	0.88†	0.03	0.79†	0.14	0.62	0.14	0.97	0.02	0.81	0.19
All	0.74	0.29	0.75	0.28	0.67	0.28	0.87	0.16	0.72	0.28

B) Analysis of variance

Source of variation	df	SS	MS	*F*	*P*
UV	3	0.536	0.179	4.49	0.009
Clutch	4	3.096	0.774	19.44	<0.001
UV × clutch	12	1.190	0.099	2.49	0.016
Error	38	1.512	0.040		

† $N = 2$.

dishes compared to the surrounding water, and the dishes with the additional mylar filter (0% UV-B) were the warmest (Table 6). Average differences between treatments and open water were 0.2–0.4°C at Kettle Tarn and 0.7–1.2°C at Lost Lake. Mean temperatures were higher at Kettle Tarn, but the temperature range was greater at Lost Lake. The higher treatment temperatures and greater range of temperatures at Lost Lake were probably because treatment dishes were in shallower water at Lost Lake than at Kettle Tarn.

Relative UV-B exposures at Kettle Tarn and Lost Lake are difficult to determine. UV-B radiation was probably greater at Lost Lake than at Kettle Tarn, because Lost Lake is 450 m higher than Kettle Tarn and UV-B radiation increases with increasing elevation (Caldwell et al. 1980). Also, the experiment at Lost Lake was conducted closest to summer solstice. Ozone thickness and atmospheric reflectivity data for 1994 were missing for most of the period of the experiment at Kettle Tarn (McPeters and Beach 1996), but erythemal exposure from 1979 to 1992 averaged 16% greater during 5 to 15 June compared to 21 May to 3 June (Fig. 3). Embryos were placed 6 cm deeper below the surface of the water at Kettle Tarn, which may have further reduced the amount of UV-B reaching them.

TABLE 3. Hatching success of all *Bufo boreas* embryos at Lost Lake, Rocky Mountain National Park, Colorado, and ANOVA results. The experiment was conducted from 5 to 15 June 1994. The source egg masses were stranded by receding water and destroyed before data on hatching could be collected. Sample size (*N*) for each UV × clutch cell is 3 dishes unless noted otherwise.

A) Hatching success

	Mylar filter (0% UV-B)		Petri dish cover (50% UV-B)		Saran Wrap (80% UV-B)		All	
Clutch	Mean	1 SD	Mean	1 SD	Mean	1 SD	Mean	1 SD
4	0.18	0.06	0.11	0.14	0.32	0.09	0.20	0.13
11	0.69	0.18	0.78	0.21	0.88	0.04	0.79	0.16
17	0.81	0.25	0.63	0.23	0.72	0.29	0.72	0.24
18	0.51†	0.25	0.53	0.05	0.56	0.08	0.53	0.11
19	0.60	0.32	0.66	0.06	0.63	0.15	0.63	0.18
All	0.56	0.30	0.54	0.28	0.62	0.23	0.58	0.26

B) Analysis of variance

Source	df	SS	MS	*F*	*P*
UV	2	0.103	0.051	1.13	0.34
Clutch	4	2.607	0.652	14.33	<0.001
UV × clutch	8	0.246	0.031	0.68	0.71
Error	29	1.319	0.045		

† $N = 2$.

Evaluating relative UV-B exposures between this study and Blaustein et al. (1994) is also complicated by the missing Meteor 3 TOMS data. Daily ozone thickness averaged 317 DU (Dobson units) from 1 May to 6 June 1993 in Oregon and 298 DU from 1 to 30 June 1994 in Colorado. Reflectivity during the same periods averaged 30.5% in Oregon and 17.7% in Colorado. These data indicate greater UV-B in Colorado than in Oregon; but the Oregon data are mostly from May and the Colorado data are from June, and UV-B radiation is greater in June than in May (Fig. 3). Long-term erythemal data indicate, however, that my study sites in Colorado receive a greater dose of UV-B than the Oregon sites of Blaustein et al. (1994). Average daily exposure for 1 May to 30 June was greater in Colorado compared to Oregon in every year, and the 14-yr average for any one day was greater in Colorado than in Oregon for every day except 17 May (Fig. 3).

DISCUSSION

Results of this experiment differed considerably from similar experiments on *Bufo boreas* embryos (Blaustein et al. 1994, Kiesecker and Blaustein 1995). I obtained no differences in hatching success of *B.*

TABLE 4. Hatching success of developing embryos only (embryos that developed past gastrula stage) at Kettle Tarn, Rocky Mountain National Park, Colorado, and ANOVA results. The experiment was conducted from 21 May to 3 June 1994. Sample size (*N*) for each UV × clutch cell is 3 dishes unless noted otherwise.

A) Hatching success

	Mylar filter (0% UV-B)		Petri dish cover (50% UV-B)		Saran Wrap (80% UV-B)		Egg mass (100% UV-B)		All	
Clutch	Mean	1 SD	Mean	1 SD	Mean	1 SD	Mean	1 SD	Mean	1 SD
1	0.92	0.06	0.98	0.03	0.85	0.26	1.00	0	0.94	0.13
2	1.00	0	1.00	0	1.00	0	0.96	0.04	0.99	0.02
3	1.00	0	1.00	0	1.00	0	0.88	0.14	0.97	0.08
4	1.00	0	1.00	0	1.00	0	1.00	0	1.00	0
5	0.98†	0.02	1.00†	0	1.00	0	0.99	0.02	0.99	0.01
All	0.98	0.04	0.99	0.01	0.97	0.12	0.96	0.07	0.98	0.07

B) Analysis of variance

Source	df	SS	MS	*F*	*P*
UV	3	0.060	0.020	1.17	0.34
Clutch	4	0.154	0.038	2.24	0.08
UV × clutch	12	0.373	0.031	1.82	0.08
Error	38	0.650	0.017		

† $N = 2$.

boreas embryos that were related to UV-B exposure in Colorado in 1994. Blaustein et al. (1994) reported a 50% increase in hatching success when *B. boreas* embryos were shielded from ambient UV-B radiation in Oregon in 1993, and Kiesecker and Blaustein (1995) reported similar 40–50% increases in hatching success of toad embryos shielded from ambient UV-B at the same sites in 1994. However, when Kiesecker and Blaustein (1995) removed the water mold *Saprolegnia ferax,* they obtained slightly greater hatching success of *B. boreas* embryos under ambient sunlight compared to sunlight with UV-B removed.

There are numerous possible causes for the differences between results in Colorado and Oregon. The apparent synergism between UV-B and *S. ferax* suggests two: that this pathogen was either not present in Colorado or that virulence varies geographically. This hypothesis cannot be evaluated with current data and needs further research.

Another potential explanation, that toad embryos were exposed to greater doses of UV-B radiation in Oregon than in Colorado, seems unlikely, given the data from the TOMS instruments. Erythemal exposures typically are 20–25% greater in Colorado than Oregon in May and June, when *B. boreas* breeding occurs and embryos are present in shallow water. However, incident UV-B is not necessarily the dose that reaches the

TABLE 5. Hatching success of developing embryos only (embryos that developed past gastrula stage) at Lost Lake, Rocky Mountain National Park, Colorado, and ANOVA results. The experiment was conducted from 5 to 15 June 1994. The source egg masses were stranded by receding water and destroyed before data on hatching could be collected. Sample size (*N*) for each UV × clutch cell is 3 dishes unless noted otherwise.

A) Hatching success

	Mylar filter (0% UV-B)		Petri dish cover (50% UV-B)		Saran Wrap (80% UV-B)		All	
Clutch	Mean	1 SD	Mean	1 SD	Mean	1 SD	Mean	1 SD
								0.32
								0.05
								0.05
								0.06
4	0.88	0.12	0.67	0.58	0.97	0.06	0.84	0.07
11	0.93	0.09	0.97	0.03	0.93	0.05	0.94	
17	0.96	0.03	0.94	0.10	0.99	0.02	0.96	0.13
18	0.91†	0.04	0.95	0.09	0.95	0.05	0.94	0.02
19	0.91	0.07	0.98	0.03	0.87	0.15	0.92	0.08
All	0.92	0.07	0.99	0.26	0.94	0.06	0.92	0.16

B) Analysis of variance

Source	df	SS	MS	*F*	*P*
UV	2	0.024	0.040	0.14	0.87
Clutch	4	0.159	0.012	0.47	0.76
UV × clutch	8	0.439	0.055	0.65	0.73
Error	29	2.444	0.084		

† $N = 2$.

TABLE 6. Air, water, and UV-B treatment temperatures at Kettle Tarn and Lost Lake (Rocky Mountain National Park, Colorado, USA). Temperatures were recorded every 10–12 min with single-channel data loggers.

	Temperature (°C)		
Site	Mean	Maximum	Minimum
Kettle Tarn			
Air	5.6	26.8	−0.8
Water	11.9	19.0	6.0
80% UV-B	12.1	20.1	6.1
50% UV-B	12.2	20.3	6.3
0% UV-B	12.3	20.1	6.2
Lost Lake			
Air	10.1	23.6	−3.5
Water	9.5	23.6	0.5
80% UV-B	10.2	24.3	1.7
50% UV-B	10.5	25.0	1.7
0% UV-B	10.7	26.2	1.7

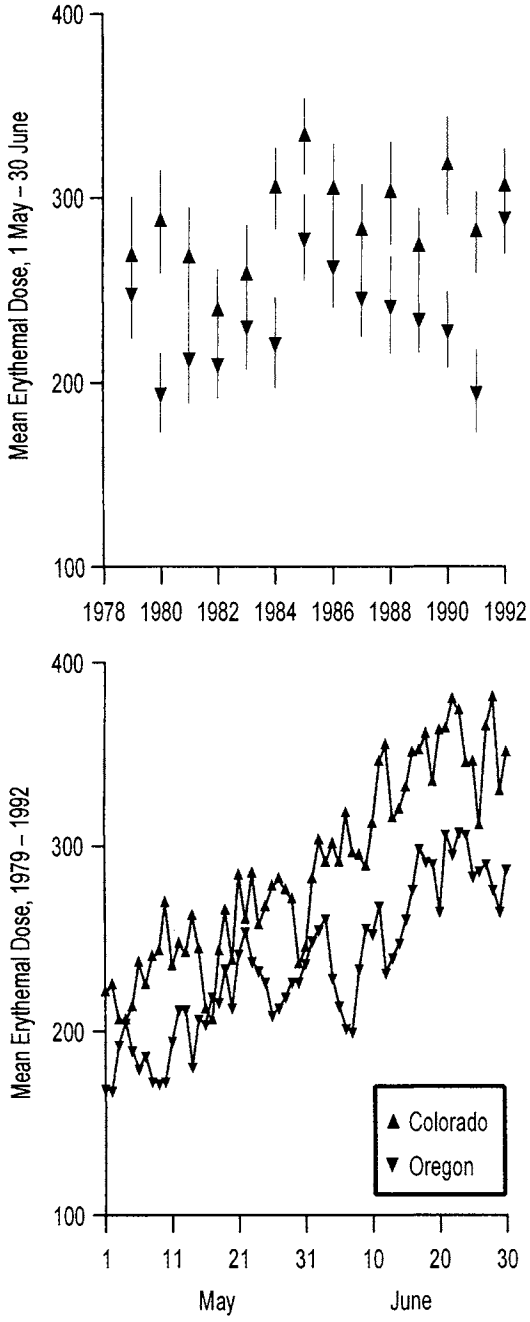

FIG. 3. Erythemal exposure in Colorado and Oregon calculated from data measured by a total-ozone mapping spectrometer onboard the Nimbus 7 satellite. Erythemal exposure is dimensionless; UV-B was weighted by the action spectrum for human skin irritation. Top, mean daily erythemal exposure for 1 May to 30 June, 1979–1992; bottom, mean erythemal exposure for each day.

embryos. UV-B penetration in water decreases with depth, increased turbidity, and increasing concentration of dissolved organic carbon (DOC; Grant and Licht 1995, Morris et al. 1995, Schindler et al. 1996, Schindler and Curtis 1997). I do not know what the conditions were at the Oregon locations, but both sites are permanent lakes with emergent vegetation and woody debris in the water (Olson 1992), and so should have significant DOC. In Colorado, Kettle Tarn and Lost Lake had low apparent turbidity, and although DOC was not measured in this study, measurements in 1990 (P. S. Corn, *unpublished data*) indicate low DOC at Lost Lake (2.89 mg/L) and moderate DOC (7.69 mg/L) at Kettle Tarn. These values are somewhat higher than in another, higher elevation watershed in Rocky Mountain National Park (McKnight et al. 1997) but within the range of other mountain lakes in Colorado (Morris et al. 1995).

This study and Blaustein et al. (1994) differed in experimental design. I used small enclosed dishes to expose embryos compared to the larger open chambers used in Oregon. I was concerned before the experiment in Colorado that temperatures inside the dishes would be higher than ambient water temperatures. This occurred to a small degree, but temperatures differed little among UV-B treatments in Colorado or Oregon. The materials used for filters in both studies were the same or had similar properties. I used Saran Wrap instead of acetate as the UV-B transmitting filter, but Saran Wrap appears to have a more consistent effect than acetate throughout the entire UV-B spectrum (Fig. 2; Table 1). A mechanism for how mechanical differences in experimental design could account for the different results is not apparent.

Grant and Licht (1995) observed that developmental rates of amphibian embryos vary among species and are related to temperature. They suggested that the interspecific variation in tolerance to UV-B observed by Blaustein et al. (1994) between *B. boreas* and Pacific treefrogs (*Hyla regilla*), which did not suffer UV-B-related mortality, may have been due to prolonged development of *B. boreas* embryos at low temperatures and consequent increased UV-B exposure. This hypothesis does not apply to the different results between Colorado and Oregon. Mean temperatures and developmental rates of *B. boreas* embryos were similar between this study and Blaustein et al. (1994). Additionally, in the present study, mortality was greater at Lost Lake, where embryos required ~11 d to hatch, than at Kettle Tarn, where embryos hatched in 13 d. Although mean temperatures were greater at Kettle Tarn, embryos at Lost Lake generally experienced higher daytime temperatures. This may have sped up development, but high temperatures could also have caused higher mortality.

Another potential explanation for different results in Colorado and Oregon is that toads in the southern Rocky Mountains may be better adapted to high-UV-B, high-elevation habitats than toads in the Pacific Northwest. This could be because of geographic genetic variation, or because the recent severe decline in the southern Rocky Mountains has selected for increased UV-B tolerance. Available evidence provides support for the former hypothesis. Blaustein et al.

(1994) and Hays et al. (1996) report considerable variation in photolyase activity among amphibian species, and Pedraza and Lizana (1997) report differences in mortality of *Bufo bufo* (high mortality) and *Bufo calamita* (low mortality) embryos exposed to ambient sunlight in a design similar to Blaustein et al. (1994). Goebel (1996) described genetic differentiation among *B. boreas* and concluded that populations in Colorado and the Pacific Northwest represent distinct evolutionary units. A selection hypothesis is difficult to evaluate because, if true, the magnitude of declines in the southern Rocky Mountains suggests that toads with low tolerance to UV-B radiation should be scarce. I did obtain a significant interaction between clutch and UV-B at Kettle Tarn (Table 2), but this seemed more due to experimental error than genetic variation.

It is not possible to identify the reason(s) the results of this study differed from those of Blaustein et al. (1994). Both were field studies using experimental designs with unique features that were not measured (water chemistry, UV-B dose), so true replication is not possible. A field study of geographic variation in UV-B tolerance in *B. boreas* that uses a common experimental design is underway. Site differences are still uncontrolled, so the experiment is being repeated by bringing toads from the different field locations to a common site.

Results of this study do not support a hypothesis that increased UV-B radiation has caused the decline of *B. boreas* in the southern Rocky Mountains. The most serious decline of *B. boreas* in Colorado, Wyoming, and New Mexico apparently took place in the late 1970s and early 1980s (Carey 1993, Corn 1994, Stuart and Painter 1994). There is little evidence for increased UV-B radiation in the United States during that time (Scotto et al. 1988), although UV-B measurements during that time were largely made in urban areas and are likely biased by increasing levels of air pollution (Grant 1988). Transient episodes of increased UV-B radiation, either temporally or geographically constrained, could have gone undetected by the sparse UV-B monitoring network present 15–20 yr ago, but *B. boreas* is long-lived and populations persist despite periodic reproductive failures (Olson 1992, Corn et al. 1997). Also, observed mortality during the period of greatest decline was of adult and juvenile toads (Carey 1993), not embryos or tadpoles.

Conversely, the results of this study are not sufficient to eliminate UV-B radiation as either the primary or a contributing cause of the decline in *B. boreas*. The results of studies in Oregon and elsewhere clearly indicate the potential for UV-B radiation, either alone or acting synergistically, to decrease amphibian reproductive success. The differing results between this study and Blaustein et al. (1994) outline an interesting paradox. Blaustein et al. (1994) and Kiesecker and Blaustein (1995) document a link between ambient UV-B radiation and mortality of *B. boreas* embryos and have observed significant embryo mortality at a number of locations. However, there is no published evidence that *B. boreas* in the Pacific Northwest has undergone a decline in distribution and abundance similar to that observed in the southern Rocky Mountains, where this study did *not* obtain a relationship between embryo mortality and UV-B radiation. Additional research is necessary to determine the role of UV-B in amphibian declines, including determining the various action spectra necessary for measuring biologically effective UV doses.

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