Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 11049–11052, November 1995
Ecology

Synergism between UV-B radiation and a pathogen magnifies amphibian embryo mortality in nature

(solar radiation/fungus/egg mortality)

JOSEPH M. KIESECKER AND ANDREW R. BLAUSTEIN

Department of Zoology, Oregon State University, Corvallis, OR 97331-2914

Communicated by Gordon H. Orians, University of Washington, Seattle, WA, August 14, 1995 (received for review March 10, 1995)

ABSTRACT **Previous research has shown that amphibians have differential sensitivity to ultraviolet-B (UV-B) radiation. In some species, ambient levels of UV-B radiation cause embryonic mortality in nature. The detrimental effects of UV-B alone or with other agents may ultimately affect amphibians at the population level. Here, we experimentally demonstrate a synergistic effect between UV-B radiation and a pathogenic fungus in the field that increases the mortality of amphibian embryos compared with either factor alone. Studies investigating single factors for causes of amphibian egg mortality or population declines may not reveal the complex factors involved in declines.**

In the Pacific Northwest, high mortality of eggs in certain amphibian species has recently been attributed to two main agents, ultraviolet-B (UV-B; 290–320 nm) radiation and a pathogenic fungus, *Saprolegnia ferax* (1–3). For example, the western toad (*Bufo boreas*) has experienced 50% to nearly 100% egg mortality at several sites in the Oregon Cascade Range (3, 4). Conversely, Pacific treefrog (*Hyla regilla*) eggs in the same ponds and lakes as *B. boreas* have not experienced unusual mortality (1). Recent research showing that amphibians have differential sensitivity to ambient UV-B radiation is consistent with these observations. When shielded from UV-B radiation *B. boreas* and Cascades frog (*Rana cascadae*) eggs showed much higher hatching success than unshielded eggs (1). Hatching success in *H. regilla* was unaffected by shielding (1).

Potential detrimental effects of UV-B radiation acting with other agents (3) may enhance egg mortality in nature and ultimately affect amphibians at the population level. Indeed, populations of amphibians in widely distributed locations, including *B. boreas* and *R. cascadae*, appear to have undergone declines and range reductions in recent times (5–9), with some, perhaps, becoming extinct (10, 11). Habitat destruction and natural population fluctuations undoubtedly play a role in the declines of some populations (12, 13). However, several investigators have suggested that UV radiation may be one factor contributing to the declines of certain populations in relatively remote regions where there are no other obvious explanations (1, 11, 14).

The detrimental effects of UV-B radiation on amphibian embryos acting alone or synergistically with other agents are poorly understood in natural systems. For example, in nature, it is possible that the effects of pathogens, such as *Saprolegnia*, may be enhanced when defense systems are weakened by stressors (3, 7). One source of stress, UV-B radiation, has well-documented effects that weaken disease defense systems (15, 16).

To test the hypothesis that UV-B radiation and *Saprolegnia* interact synergistically to enhance amphibian egg mortality, we conducted field experiments on *R. cascadae, B. boreas*, and *H. regilla* to assess the hatching success of their eggs. All three species lay their eggs in open, shallow water, exposed to UV-B radiation and *Saprolegnia*.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

MATERIALS AND METHODS

Experiments were conducted at three natural oviposition sites in the central Oregon Cascade Range from March 17 to July 13, 1994. Tests of all species were conducted at Three Creeks Lake (43 km west of Bend, Deschutes County, OR; elevation 2000 m). Additionally, tests of *B. boreas* were conducted at Lost Lake (Linn County, OR, 97 km east of Albany; elevation 1220 m) and of *R. cascadae* and *H. regilla* at Small Lake (Linn County, OR, 92 km east of Albany, OR; elevation 1190 m). Enclosures (38 × 38 × 7 cm) were placed in small plastic pools (110 cm in diameter, 18 cm deep) so that we could control *Saprolegnia* densities. Within the pools eggs were emersed in 5–10 cm of natural lake water, a depth at which eggs are naturally laid (17). Pools with enclosures were placed in a linear array parallel to the water's edge in a 2 × 3 randomized block design (18) with three sunlight treatments crossed with two fungal treatments. There were four replicates for each treatment. Thus, there were 24 enclosures at each site.

Enclosures had clear Plexiglas frames with floors of 1-mm^2 fiberglass mesh screen. For *R. cascadae* and *B. boreas*, 25 eggs from each of six different clutches (total = 150 eggs per enclosure) were placed in each enclosure. Because of their small clutch size, for *H. regilla* we used eggs from more than six clutches and randomly assigned 25 eggs from at least six clutches to each enclosure (total = 150 eggs per enclosure). A UV-B-blocking filter (50 × 50 × 7 cm) made of Mylar was placed over one-third of the enclosures. An acetate filter that transmitted UV-B (a control for using a filter over the eggs) was placed over another third of the enclosures. The remaining enclosures had no filters. Analyses with an Optronics International (Chelmsford, MA) model 752 spectroradiometer showed that the Mylar blocked 100% of UV-B. The acetate allowed about 80% UV-B transmission.

Saprolegnia was cultured in the laboratory on 20 ml of corn meal agar in standard Petri dishes. Using the standardized culture protocol, boiled hemp seeds (19) were added to cultures and cultures were allowed to incubate at 20°C for ≈168 hr. In the pools where *Saprolegnia* was added, we introduced three hemp seeds laden with *Saprolegnia* (≈3000–5000 zoospores per liter).

In experiment 1, we placed 150 newly deposited eggs (<24 hr old) in each of 24 enclosures at natural oviposition sites of each species. The enclosures at each site were randomly assigned to unfiltered sunlight, sunlight filtered to remove UV-B and shorter wavelengths, and sunlight filtered to remove wavelengths shorter than UV-B. We randomly added *S. ferax* to half of the enclosures in each sunlight treatment (see Fig. 1). To the remainder of the enclosures, we added the antifungal agent Malachite green (20, 21) to remove any *Saprolegnia* that may be present naturally. Enclosures were placed in plastic pools. Within the pools, eggs were emersed in natural lake water.

Experiment 2 was conducted at Three Creeks Lake simultaneously with the tests conducted for experiment 1 at Three Creeks Lake. In experiment 2, we used procedures identical to those in experiment 1 except that enclosures were placed directly into the lake. Thus, in this experiment, embryos were exposed to all three sunlight regimes and natural levels of *Saprolegnia*.

The experiments ended when all embryos either hatched or died. Survival was measured as the proportion of hatchlings produced per enclosure. The proportion of hatchlings produced per enclosure (survivorship through hatching) was assessed using analysis of variance (ANOVA) to test for differences among the treatments.

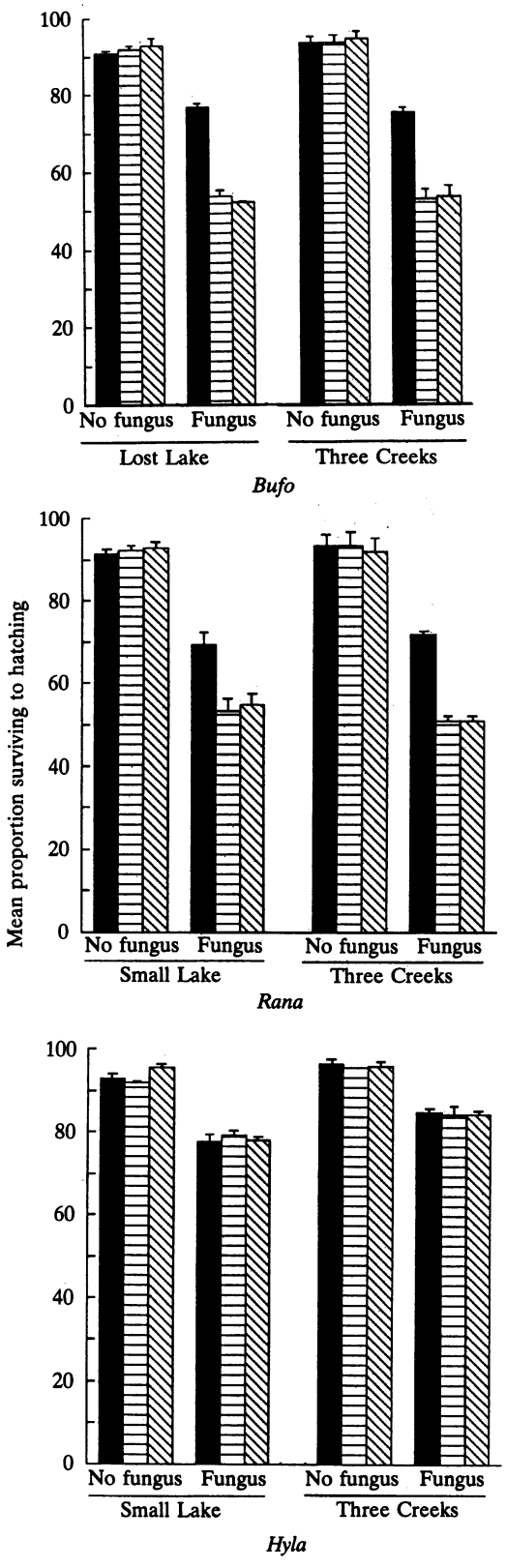

FIG. 1. Experiment 1: Effects of UV-B radiation and manipulated amounts of *S. ferax* on hatching success ($\bar{x} \pm$ SE) in *B. boreas*, *R. cascadae*, and *H. regilla*. ■, UV-B-blocking filter; ▤, UV-B-transmitting filter; ▧, no filter.

RESULTS

In experiment 1, the ANOVA indicated a significant UV-B effect by itself. However, the effect is secondary to the

Table 1. Experiment 1: ANOVA of hatching success in three amphibian species

Source of variation	MS	df	*F*	*P*
B. boreas				
Lost Lake				
UV	0.034	2	55.920	<0.001
Fungus	0.574	1	938.024	<0.001
UV × fungus	0.043	2	69.484	<0.001
Error	0.001	18		
Three Creeks				
UV	0.023	2	16.958	<0.001
Fungus	0.493	1	363.966	<0.001
UV × fungus	0.025	2	18.455	<0.001
Error	0.001	18		
R. cascadae				
Small Lake				
UV	0.014	2	7.544	0.004
Fungus	0.658	1	360.660	<0.001
UV × fungus	0.018	2	10.079	<0.001
Error	0.002	18		
Three Creeks				
UV	0.032	2	15.879	<0.001
Fungus	0.748	1	368.494	<0.001
UV × fungus	0.027	2	13.529	<0.001
Error	0.002	18		
H. regilla				
Small Lake				
UV	0.001	2	0.977	0.396
Fungus	0.137	1	266.734	<0.001
UV × fungus	0.001	2	2.185	0.141
Error	0.001	18		
Three Creeks				
UV	<0.001	2	0.300	0.746
Fungus	0.060	1	150.834	<0.001
UV × fungus	<0.001	2	0.018	0.982
Error	<0.001	18		

A preliminary analysis indicated no significant block effects (i.e., no differences between temperature or other variables among blocks). Therefore, the block and error terms were pooled for remaining tests (18). Post hoc comparisons (Tukey Test) (18) were performed to test for differences between means among the six regimes. Temperatures were taken within enclosures for each species in each treatment. Mean temperatures (and ANOVAs) are given for each species at each site for the unfiltered, UV-B-transmitting, and UV-B-blocking regimes with and without the fungus respectively: *Bufo* at Lost Lake = 8.3°C, 8.7°C, 8.8°C, 9.1°C, 8.6°C, and 8.5°C, $F_{5,18} = 0.822$, MS = 0.034, $P = 0.649$; *Bufo* at Three Creeks = 12.0°C, 12.9°C, 13.2°C, 12.1°C, 12.8°C, and 12.6°C, $F_{5,18} = 1.294$, MS = 0.039, $P = 0.160$; *Rana* at Small Lake = 10.8°C, 11.3°C, 10.7°C, 11.3°C, 10.7°C, and 10.3°C, $F_{5,18} = 1.061$, MS = 0.034, $P = 0.464$; *Rana* at Three Creeks = 10.9°C, 12.6°C, 11.5°C, 11.4°C, 11.6°C, and 12.5°C, $F_{5,18} = 2.237$, MS = 0.043, $P = 0.227$; *Hyla* at Small Lake = 11.2°C, 10.9°C, 11.6°C, 11.4°C, 10.7°C, and 11.9°C, $F_{5,18} = 1.561$, MS = 0.005, $P = 0.271$; *Hyla* at Three Creeks = 12.3°C, 13.8°C, 14.3°C, 13.6°C, 13.7°C, and 12.9°C, $F_{5,18} = 0.798$, MS = 0.006, $P = 0.667$. MS, mean square; *F*, *F* statistic [with degrees of freedom (df)]; *P*, probability.

interaction effect of UV-B radiation and fungus. All three species had reduced hatching success in the presence of *Saprolegnia*. However, with *Saprolegnia* present, UV-B enhanced this effect in *Bufo* and *Rana* (Fig. 1; Table 1). *H. regilla* hatching success was not affected by UV-B and its hatching success was reduced only in the presence of *Saprolegnia*.

In experiment 2, the hatching success of *B. boreas* and *R. cascadae* was also greater in regimes shielded from UV-B (Fig. 2; Table 2). *H. regilla* hatching success did not differ among the regimes.

DISCUSSION

We have shown a synergistic interaction between a pathogen and UV-B radiation that is killing amphibian embryos in nature. Although UV-B radiation and *Saprolegnia* alone may damage *Rana* and *Bufo* embryos, the results of experiment 1 showed that UV-B radiation and *Saprolegnia* together produced an effect that was greater than the effect of UV-B radiation or *Saprolegnia* alone. Thus, complex interactions among several factors may affect amphibians in nature that could potentially lead to a population decline. *R. cascadae* populations have virtually disappeared from the southern portion of their range in California and have shown declines in Oregon (8, 9). *B. boreas*, a previously ubiquitous species in western North America (22), has undergone drastic declines in numbers throughout its range and has exhibited unusually high egg mortality at certain montane sites (3, 7, 8).

Saprolegnia is a well-known pathogen of amphibians and fishes (3). Yet, it has been largely overlooked in the context of amphibian declines (3). *Saprolegnia* occurs in lakes and ponds with amphibians in Oregon. The origin of *Saprolegnia* at our study sites is unknown, but one likely source is infected hatchery-reared fishes that are stocked throughout the Cascade Range (3).

UV-B radiation probably does not contribute to the declines of all amphibian populations. For example, UV-B radiation is less likely to affect species that lay their eggs in relatively deep water or under dense foliage, shielded from solar radiation (10, 11). However, we suggest that the anticipated progressive expansion of UV-impacted areas to lower latitudes (23–25) could potentially lead to increased mortality of amphibian embryos as they develop in nature. Moreover, individuals not directly exposed to UV-B radiation or *Saprolegnia* may become contaminated as *Saprolegnia*-infected individuals disperse.

Multifactor studies investigating stressors that may compromise disease defense mechanisms are warranted (3, 7, 16). Furthermore, our results suggest the importance of multifactor studies when investigating mortality factors in early life stages that could eventually lead to a population decline.

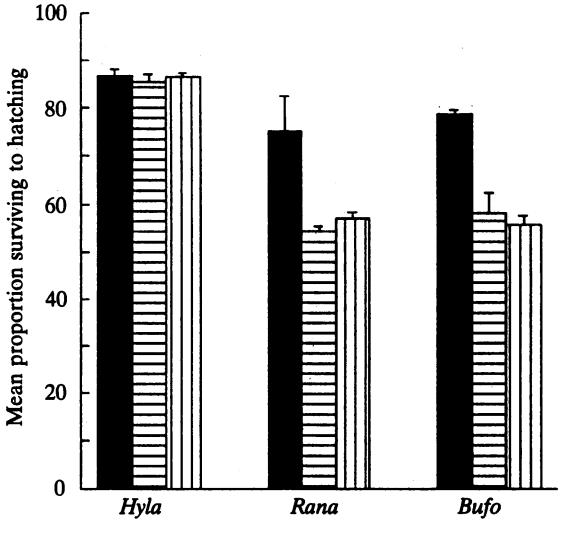

FIG. 2. Experiment 2: Effects of UV-B radiation on hatching success ($\bar{x} \pm$ SE) of eggs emersed directly into a lake. This experiment was conducted at Three Creeks Lake simultaneously with the tests conducted at this site described in the legend to Fig. 1. The methods and materials were identical to those in Fig. 1, except that enclosures were placed directly into a lake rather than into pools and *Saprolegnia* was not added to enclosures. Thus, if *Saprolegnia* was present, eggs were exposed to natural levels of the pathogen. Enclosures were placed in a linear array, in a randomized block design, with three replicates for each treatment. ■, UV-B-blocking filter; ▤, UV-B-transmitting filter; ▥, no filter.

Table 2. Experiment 2: Univariate analysis (ANOVA) of hatching success in three amphibian species

Source of variation	MS	df	*F*	*P*
B. boreas				
Three Creeks				
Treatment	0.048	2	19.97	0.002
Error	0.002	6		
R. cascadae				
Three Creeks				
Treatment	0.396	2	12.98	<0.001
Error	<0.001	6		
H. regilla				
Three Creeks				
Treatment	<0.001	2	3.43	0.723
Error	<0.001	6		

A preliminary analysis indicated no significant block effects. Therefore, the block and error terms were pooled for remaining tests (18). Post hoc comparisons (Tukey Test) (18) were performed to test for differences between means among the three regimes. Temperatures were taken within enclosures for each species in each treatment. Mean temperatures (and ANOVAs) are given for each species for the unfiltered, UV-B-transmitting, and UV-B-blocking regimes, respectively: *Bufo* = 13.9°C, 14.3°C, and 14.1°C, $F_{2,6} = 0.497$, MS = 0.011, $P = 0.786$; *Rana* = 11.6°C, 12.1°C, and 11.3°C, $F_{2,6} = 4.599$, MS = 12.671, $P = 0.345$; *Hyla* = 14.8°C, 15.3°C, and 15.6°C, $F_{2,6} = 0.175$, MS = 0.001, $P = 0.952$. MS, mean square; *F*, *F* statistic [with degrees of freedom (df)]; *P*, probability.

We thank C. Miller, D. G. Hokit, S. Walls, P. Raven, E. Graham, M. Crane, and W. Gates for helping with field work and reviewing the manuscript. H. Whisler and G. Mueller advised us on all aspects of *Saprolegnia* biology. Support was provided by the National Science Foundation and the U.S. Forest Service through the cooperation of Deanna H. Olson.

1. Blaustein, A. R., Hoffman, P. D., Hokit, D. G., Kiesecker, J. M., Walls, S. C. & Hays, J. B. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 1791–1795.
2. Blaustein, A. R., Edmond, B., Kiesecker, J. M., Beatty, J. J. & Hokit, D. G. (1995) *Ecol. App.* **5,** 740–743.
3. Blaustein, A. R., Hokit, D. G., O'Hara, R. K. & Holt, R. H. (1994) *Biol. Conserv.* **67,** 251–254.
4. Blaustein, A. R. & Olson, D. H. (1991) *Science* **253,** 1467.
5. Wake, D. B. (1991) *Science* **253,** 860.
6. Blaustein, A. R., Wake, D. B. & Sousa, W. P. (1994) *Conserv. Biol.* **8,** 60–71.
7. Carey, C. (1993) *Conserv. Biol.* **7,** 355–362.
8. U.S. Fish and Wildlife Service, Department of the Interior (1991) Fed. Regist., Part 8.
9. Fellers, G. M. & Drost, C. A. (1993) *Biol. Conserv.* **65,** 177–181.
10. Pounds, J. A. & Crump, M. L. (1994) *Conserv. Biol.* **8,** 72–85.
11. Richards, S. J., McDonald, K. R. & Alford, R. A. (1993) *Pac. Conserv. Biol.* **1,** 66–77.
12. Blaustein, A. R. (1994) *Herpetologica* **50,** 85–97.
13. Pechmann, J. H. K. & Wilbur, H. M. (1994) *Herpetologica* **50,** 65–84.
14. La Marca, E. & Reinthaler, H. P. (1991) *Herpetol. Rev.* **22,** 125–128.

15. Orth, A. B., Teramura, A. H. & Sisler, H. D. (1990) *Am. J. Bot.* **77,** 1188–1192.
16. Tevini, M. (1993) *UV-B Radiation and Ozone Depletion: Effects on Humans, Animals, Plants, Microorganisms, and Materials* (Lewis, Boca Raton, FL).
17. O'Hara, R. K. (1981) Ph.D. thesis (Oregon State Univ. Corvallis).
18. Zar, J. H. (1984) *Biostatistical Analysis* (Prentice–Hall, Englewood Cliffs, NJ).
19. Laskin, A. I. & Lechevalier, H. A. (1978) *Handbook of Microbiology* (CRC, West Palm Beach, FL), Vol. 2.
20. Coborn, J. (1992) *The Proper Care of Amphibians* (TFH, Neptune City, NJ).
21. Mattison, C. (1988) *The Care of Reptiles and Amphibians in Captivity* (Sterling, New York).
22. Stebbins, R. C. (1985) *Western Reptiles and Amphibians* (Houghton Mifflin, Boston).
23. Kerr, J. B. & McElroy, C. J. (1993) *Science* **262,** 1032–1034.
24. Worrest, R. C. & Grant, L. D. (1989) in *Ozone Depletion: Health and Environmental Consequences*, eds. Russell, J. & Wigley, T. (Wiley, New York), pp. 197–206.
25. Zurer, P. S. (1993) *Chem. Eng. News* **71,** 8–18.