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Author(s): Leslie E. Long, Lorelei S. Saylor, Michael E. Soule

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A pH/UV-B Synergism in Amphibians

LESLIE E. LONG,*‡ LORALEI S. SAYLOR,†§ AND MICHAEL E. SOULÉ†

*Marine Science Board, University of California, Santa Cruz, Santa Cruz, CA 95064, U.S.A.

†Environmental Studies Board, University of California, Santa Cruz, Santa Cruz, CA 95064, U.S.A.,
email soule@cats.ucsc.edu

Introduction

Some workers have proposed that synergistic interactions may contribute to the decline and disappearance of amphibians (Blaustein & Wake 1990; Carey 1993; Blaustein et al. 1994a). In this pilot study, we tested the effects of low pH and increased ultraviolet-B radiation (UV-B) on frog-embryo survival. Blaustein et al. (1994b) correlated differences in declines in three anuran species in the Cascades with differences in their abilities to repair UV-B-induced DNA damage and in embryo survival in UV-B-screened enclosures in the field. Low pH has been shown by numerous researchers to decrease the survival of amphibian embryos (Freda 1986). In a review, however, Dunson et al. (1992) found no evidence that low pH was a factor in declines of *Rana muscosa* and *Bufo canorus* in the Sierra Nevada, and they reported similar results for *Rana pipiens* and *Bufo boreas* in the Rocky Mountains. Several ranid frogs have apparently suffered recent population declines (Bradford 1991; Fellers & Drost 1993; Blaustein 1994b). We studied the possibility of a pH/UV-B synergistic effect on the mortality of *Rana pipiens* embryos.

Materials and Methods

R. pipiens were obtained from the northeastern U.S.A., (Connecticut Valley Biological Supply) where declines have not been noted in this species. Artificially fertilized *R. pipiens* eggs were simultaneously exposed to combinations of three levels of pH and three levels of UV-B in a 3×3 factorial experiment. The main effects of pH and UV-B and the interaction of pH and UV-B were tested us-

ing a two-way ANOVA, fixed-effects model. The UV-B levels employed were (1) zero, (2) "normal" at high-elevation (approximately $4341 \text{ effective } J \text{ m}^{-2} \text{ day}^{-1}$), and (3) predicted at high-elevation (approximately $9507 \text{ effective } J \text{ m}^{-2} \text{ day}^{-1}$), assuming 30% ozone depletion during the current episode (CISC & CARCE 1979; Caldwell 1981). The term "effective" ("eff") $J \text{ m}^{-2} \text{ day}^{-1}$ refers to biologically weighted levels calculated to correspond to absolute measured irradiance (Setlow 1974; Caldwell et al. 1986) using the DNA action spectrum normalized to 300 nm (Behrenfeld et al. 1993). These calculations were based on measurements taken by Caldwell et al. (1980) in 1979 at Snowbird, Utah (40°N , 3352 m). UV-B levels were achieved through the use of Mylar and cellulose triacetate filters following Worrest et al. (1981). Filters were changed daily.

Embryos were observed from fertilization to hatching, stages 5 or 6 to 21 or 22 (Gosner 1960). Two replicates of the experiment were performed, with 60 and 45 eggs per treatment combination respectively. Artificially fertilized eggs from one pairing of frogs were used per replicate to assure the homogeneity of experimental material. Fertile eggs were separated into groups and placed in dishes in 150 ml of treatment water that was changed daily.

Ultrapure water was aerated for 24 hours and reconstituted to "soft" according to Stephen (1975), after Bradford et al. (1992). Acidity was adjusted to either pH 6.0, pH 5.0, or pH 4.5 using 0.1 N sulfuric-nitric acid, simulating a mid- to low-range of pH values currently found in the U.S. (Charles 1991; Corn & Vertucci 1992; Bradford et al. 1994). Treatment water was prepared in three 20-L, acid-cleaned carboys; pH was not found to vary in these containers over the two-week course of each experiment. Oxygen levels were not measured.

The experiments were conducted outdoors in order to obtain natural levels of UV-A and visible light necessary for photorepair (Pang & Hays 1991). The study was conducted at the University of California, Santa Cruz (37°N , 250 m elevation). Natural UV-B was supplemented with radiation from preconditioned UVB-313

‡Current address: 28333 Ortega Highway, San Juan Capistrano, CA 92676, U.S.A.

§Present address: Nongame Branch, Arizona Fish & Game Department, 2221 W. Greenway Rd., Phoenix, AZ 85023, U.S.A.
Address correspondence to M. E. Soulé.

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bulbs (Q-panel Company) in order to attain target UV-B levels. The chambers were aligned so that each received the same amount of sunlight; no sunlight fell directly on the treatment dishes. Each 250-ml egg dish was placed on a turntable rotating at one revolution per minute, under a separate lamp, in a separate experimental chamber. Water temperature was allowed to fluctuate with ambient temperature, with a low of approximately 5°C and a high of approximately 20°C. Temperature was found to vary less than 2°C between treatment dishes at any given time. Embryos were exposed to a constant dose rate from lamps for six hours per day, centered around solar noon, throughout the embryonic period of 10 to 14 days.

Bulb output, both with and without filters, was initially measured with an Optronics OL-752 spectroradiometer, which measures intensity per wavelength. These measurements were compared with readings from a Spectronics DM-300 radiometer, which measures total watts per cm² over the UV-B band (280–320 nm). A computer-facilitated integration converted the spectroradiometer readings to radiometer values, and radiometric readings were used throughout the experiment. Based on instrument accuracy and calculation methods used, we estimate a combined error of less than 15% in total weighted UV-B irradiance. Results were measured in terms of percentage of embryos surviving to hatching. The data were arcsine (square-root)-transformed for analyses of variance (ANOVA).

Results and Discussion

Mean survival proportions and standard errors are shown in Table 1. The average survival rate in the control treatment (pH 6/zero UV-B) was 97% (SE = 1%). A two-way ANOVA (Table 2) showed strong interaction between pH and UV-B ($p < 0.001$) as well as strong main effects of pH and UV-B. A Student-Newman-Keuls multiple comparison test showed that both the treatments pH 4.5/4341 $\text{eff J m}^{-2} \text{ day}^{-1}$ and pH 4.5/9507 $\text{eff J m}^{-2} \text{ day}^{-1}$ differed significantly from the rest of the treatments ($p < 0.05$). Embryo survival was reduced to 73% and 51% respectively in these treatments.

The significant main effects of pH and UV-B appear to be the result of the strong interaction between these

Table 1. Mean survival proportions with standard errors (two replicates) for *R. pipiens* embryos.

pH	UV-B ($\text{eff J m}^{-2} \text{ day}^{-1}$)		
	0	4341	9507
6.0	0.970 ± 0.010	0.990 ± 0.010	0.975 ± 0.005
5.0	0.955 ± 0.005	0.975 ± 0.005	0.905 ± 0.025
4.5	0.955 ± 0.005	0.725 ± 0.055	0.505 ± 0.025

Table 2. Results of two-way ANOVA* testing the interaction of pH and UV-B.

	d.f.	s.s.	m.s.	F value	p value
pH effect	2	0.4731	0.2366	81.709	< 0.001
UV effect	2	0.1504	0.0752	25.979	< 0.001
pH × UV	4	0.2106	0.0527	18.188	< 0.001
residual	9	0.0261	0.0029		
total	17	0.8602	0.0506		

*ANOVA was performed on arcsine (square-root)-transformed data.

two variables. This can be seen by inspection of the marginal values in Table 1, which show no increase in embryo mortality where a high level of one variable (acid or UV-B) is associated with a control (low) level of the other variable. This is supported by the results of one-way ANOVAs testing for independent (marginal) effects of pH or UV-B; the non-significant ($p = 0.365$ for pH, $p = 0.363$ for UV-B) results indicate that the existence of high levels of either factor by itself had no detectable effect on embryonic mortality. The statistically insignificant marginal values for pH and UV-B could be due to modest treatment effects or to low power resulting from the small number of replicates, though given the low *F*-values (1.49 and 1.45 for pH and UV-B, respectively), the former is the more likely explanation.

We noted two kinds of mortality, early and late. In early mortality (days 2 to 7 of the embryonic period), embryos failed to continue developing; this occurred with low frequency (0 to 4%) across all treatments. In later mortality, embryo failure was due almost exclusively to the curling defect, which results when embryonic membranes do not expand. Curling often prevents hatching (Freda & Dunson 1985). In our experiments it occurred when pH 4.5 was combined with both 4341 $\text{eff J m}^{-2} \text{ day}^{-1}$ and 9507 $\text{eff J m}^{-2} \text{ day}^{-1}$ UV-B. Curling occurred somewhat less consistently in the pH 5/9507 $\text{eff J m}^{-2} \text{ day}^{-1}$ treatment. Curling is normally associated with low pH (Pough 1976; Freda & Dunson 1985). A greatly elevated severity of curling associated with low pH and high UV-B has not been noted previously.

Conclusions

How realistic were the experimental conditions? Pond pH values between 4.6 and 5.4 are not infrequent in the eastern U.S., Canada, and Europe (Wellburn 1988; Charles 1991). pH values as low as 4.5 have been reported at undisturbed high-elevation sites in northern California (Fellers & Drost 1993), and values as low as 5.0 have been reported in the western Rockies (Harte & Hoffman 1989). A 35% per year increase in the intensity of UV-B radiation at 300 nm has been documented over North America in winter (Kerr & McElroy 1993), a season when lowland amphibian species often begin to

breed. This increase in UV-B has been attributed to stratospheric ozone depletion at mid-latitudes; the depletion has been estimated to have been 3 to 5% between 1979 and 1992 (Gleason et al. 1993). Clearly, the experimental treatments mimicking the forecasted 30% ozone depletion represent an extreme scenario. At high elevations, amphibians generally breed immediately following snowmelt, when pH is subject to lowering (Williams et al. 1993) and ultraviolet radiation is near its peak.

These preliminary experiments indicate that neither pH nor UV-B alone had a detectable effect on the survival of *R. pipiens* embryos. Ultraviolet-B and pH acting in concert, however, led to a significant decrease in embryo survival. If embryo survival is affected by such a synergism between pH and UV-B in nature, then pH and UV-B could suppress population growth rates even where these variables appear to have no effect when experimentally studied one at a time. It should be noted, however, that amphibian declines could have many causes in any given region, that they are species specific, and that these results indicate only that a pH/UV-B synergism occurs in the laboratory for a single species.

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