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Combined Effects of UV-B, Nitrate, and Low pH Reduce the Survival and Activity Level of Larval Cascades Frogs (*Rana cascadae*)

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Abstract. We investigated interactions between low pH, high nitrate level, and ultraviolet-B (UV-B) light on the survival and activity level of larval Cascades frogs (*Rana cascadae*). We used a fully factorial experimental design, with pH levels of 5 and 7; initial "pulse" nitrate exposure levels of 0, 5, and 20 mg/L; and UV-B present or absent. After a 3-week laboratory exposure, we measured survival and activity level of the larvae. The experiment was repeated two times, in two separate years. Similar effects on survival and activity level were observed in both experiments. *R. cascadae* survival was not significantly reduced in treatments with individual factors alone (*i.e*., UV-B control, pH 5 control, or high nitrate level without pH or UV-B). However, in experiments from both years, survival and activity levels of larval *R. cascadae* were significantly reduced in the treatment with low pH, high nitrate, and UV-B together. In both years, analysis of variance (ANOVA) indicated that pH and nitrate had the greatest effect on survival and that UV-B and nitrate had the greatest effect on activity level. Additional effects were observed in the 1998 experiment on both survival and activity level. In 1998, UV radiation and the interaction term between pH and nitrate (pH × nitrate) had a significant effect on survival. Also in the 1998 experiment, activity level was significantly reduced in treatments at neutral pH with UV, at initial nitrate doses of 5 and 20 mg/L and at neutral pH without UV at an initial nitrate dose of 20 mg/L. We suggest that the adverse effects were due to the multiple stressors acting together.

Amphibian populations at several locations worldwide appear to be declining (*e.g*., Pounds *et al*. 1999; Blaustein *et al*. 1994a, 1994b; Richards *et al*. 1993). Several anthropogenic factors likely contribute to these population declines, including habitat destruction, climate change, increased intensity of ultraviolet-B radiation (UV-B; 280–315 nm), pathogens, introduced species, and chemical pollution (*e.g*., Pounds *et al*. 1999; Blaustein *et al*. 1997, 1994a, 1994b, 1994c). Amphibians may be particularly sensitive to these environmental impacts because of their thin, permeable skin and biphasic life history, exposing them to environmental stressors in both aquatic and terrestrial environments (Blaustein *et al*. 1994b).

Although several environmental factors may contribute to amphibian population declines, there is probably not a single pervasive cause for the declines. In fact, developing amphibians are simultaneously exposed to a variety of stressors in nature. Moreover, detrimental effects due to synergistic interactions have been demonstrated between several environmental stressors. For example, synergistic effects were observed between pathogens and UV (Kiesecker and Blaustein 1995), pH and UV (Long *et al*. 1995), and between chemical contaminants and UV (Hatch and Burton 1998; Zaga *et al*. 1998). In these studies, survival was reduced greatly by simultaneous exposure to stressors, in comparison to exposure to a single stressor alone.

Often, the response of developing amphibians to environmental stressors depends on other abiotic conditions or stressors that are present. For example, the effects of the fungicide triphenyltin on the growth and development of larval *Rana lessonae* and *Rana esculenta* depended on pH (Fioramonti *et al*. 1997). In a series of multifactorial experiments, Horne and Dunson (1994) demonstrated that some metals ameliorate the toxicity of low pH, but at higher pH survival was lower in the presence of some nontoxic metals (Horne and Dunson 1995a). These investigators have further shown that different amphibian species respond to different combinations of environmental stressors. For example, in a study of the combined effects of metals, low pH, and dissolved organic carbon (DOC) concentration, wood frogs (*Rana sylvatica*) were most negatively affected by high DOC in combination with low pH, while the salamanders *Ambystoma jeffersonianum* and *Ambystoma maculatum* were most affected by low pH in combination with high metal concentration (Horne and Dunson 1995b).

Environmental stressors may alter biological interactions with unexpected ecological consequences. For example, low dissolved oxygen alters the behavior of *Rana clamitans* tadpoles, making them more susceptible to predation by fishing spiders (*Dolomedes triton*) (Moore and Townsend 1998). Interactions between biotic and abiotic factors may differ between amphibian species. Density interacted with low pH to delay metamorphosis and decrease weight at metamorphosis for *Hyla gratiosa* but not for *Hyla femoralis* (Warner *et al*. 1991).

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In many cases, greater toxicity or altered biological interactions are observed when animals are simultaneously exposed to more than one stressor than when animals are exposed to only a single stressor. Synergism may be due to a mechanism in which the toxic action of one factor is altered or enhanced by the presence of another, for example, in the photo-induced toxicity of some chemical contaminants (*e.g*., Hatch and Burton 1998, 1999; Zaga *et al*. 1998). Alternatively, interactions may be due to reduced tolerance for stressors in the presence of other stressors.

In this study, we investigated the combined effects of UV, nitrate (fertilizer runoff), and low pH on the survival and activity level of larval *Rana cascadae* (the Cascades frog) using a full factorial experimental design in the laboratory. All three factors are currently or potentially relevant to breeding sites of these two species in montane regions of Oregon.

The intensity of UV-B radiation that reaches the Earth's surface may be increasing due to depletion of the ozone layer (McKenzie *et al*. 1999; Kerr and McElroy 1993). Declines in the ozone layer have been linked to increases in biologically active UV-B in several different locations worldwide, including Hawaii, Germany, Toronto Canada, Greece, New Zealand, and the South Pole (Madronich *et al*. 1998). In the Cascade Mountain range of Oregon, ambient levels of UV-B increase egg mortality of *R. cascadae* and *Bufo boreas* (Western toad) (Blaustein *et al*. 1994a) and cause mortality and deformities in *Ambystoma macrodactylum* (long-toed salamander) embryos (Blaustein *et al*. 1997). In the Santa Monica Mountains in southern California, UV-B reduces the survival and the hatching success of embryonic *Hyla cadaverina* and *Taricha torosa* (Anzalone *et al*. 1998). Alpine newts (*Triturus alpestris*) in central Europe are sensitive to UV-B, exhibiting skin damage, behavioral abnormalities, and mortality when exposed to UV (Nagl and Hofer 1997).

Nitrate may contaminate amphibian breeding sites as a result of runoff from agricultural use or, in the Cascades Mountain range, as a result of the application of forest fertilizers. Nitrate can be toxic to some species of developing amphibians at environmentally realistic levels (Marco *et al*. 1999; Hecnar 1995). Several species commonly occurring in the Cascades range are sensitive to low levels of nitrate and nitrite (Marco *et al*. 1999). In a study of amphibians in southern Ontario, exposure to levels of nitrate typical of agricultural areas reduced the survival of *Pseudacris triseriata* (chorus frog) and *Rana pipiens* (leopard frog) (Hecnar 1995). Acute nitrate exposure reduced the size of newt (*Triturus vulgaris*) larvae (Watt and Oldham 1995). Ammonia (NH_3), which may be associated with high nitrate input into freshwater systems from organic decomposition or animal excretion, is also toxic to amphibians at environmentally realistic levels (Jofre and Karasov 1999). Nitrite ($N\text{-}NO_2$), which also may be associated with nitrogen-based fertilizer application, delayed metamorphosis and altered the behavior of larval *R. cascadae*; larvae occupied shallow water more frequently than control larvae, possibly in an effort to increase their oxygen intake (Marco and Blaustein 1999).

Finally, low pH alone may impair the development of amphibians (*e.g*., Rowe *et al*. 1992; Freda 1986; Kiesecker 1996). Although acidification is not an immediate threat to freshwater systems in the Pacific Northwest, even slight acidification may be a potential problem when considering the possibility for synergistic interactions with other environmental stressors (*e.g*., increasing UV-B radiation; Long *et al*. 1995). Water in the Pacific Northwest region may be at risk for acidification because of its low buffering capacity. Moreover, small temporary ponds or ditches where some amphibians breed may have a particularly low buffering capacity (Freda 1986).

Materials and Methods

Larvae Collection and Rearing

R. cascadae larvae (Gosner stage 13–15; embryonic, neural fold developing; Gosner 1960) were collected from Parrish Pond (62 km east of Lebanon in Linn County, OR) upon breeding in 1998 (May 6) and 1999 (June 1). Experiments began when developing larvae were at Gosner stage 23–24 (free-swimming larvae, operculum developing, external gills disappearing; Gosner 1960) and lasted for 3 weeks. Larvae were at Gosner stages 30–33 (toes developing on hind limb bud; Gosner 1960) at the end of the experiment. Prior to experimental exposure, larvae were housed in 38-L aquaria with approximately 100 larvae/tank, under a 14 h light:10 h dark photoperiod. Larvae were fed ground rabbit chow *ad libitum* during rearing and during experiments. Dechlorinated tap water was used for rearing larvae and in experimental trials.

Experimental Design and Water Quality

UV, pH, and nitrate were manipulated in a $2 \times 2 \times 3$ factorial design, for a total of 12 treatments. Each treatment was replicated four times in 500-ml plastic cups filled with 400 ml water, containing five larvae per cup. Twenty larvae were exposed to each of the 12 treatments.

To maintain the controlled pH levels, water was changed (approximately 30%) every 48 h. Temperature, UV-B level, and pH were measured at each water change. pH was measured in the renewal water, and UV-B and temperature were measured in the constant-temperature experimental room. pH was measured using an Orion 290A pH/ISE meter with a pH electrode. UV-B was quantified at the water's surface using a UV-B meter (model 2100 PMA meter with model 2102 detector, Solar Light Co., Philadelphia, PA). UV-A was quantified after the experiment ended using the same meter and a PMA2111 detector.

We measured nitrate, dissolved oxygen, conductivity, alkalinity, and hardness levels in the water weekly. Nitrate was quantified with an Orion 290A pH/ISE meter with a nitrate electrode. Conductivity was measured using a Hanna Instruments hand-held conductivity meter. Dissolved oxygen was calculated using the Winkler titration method (American Public Health Association 1995). Water hardness and alkalinity were measured by titration with EDTA and 0.02 N HCl, respectively (APHA 1995).

Experimental Variables

UV lighting was achieved in the laboratory using UV-B 313 light bulbs (Q Panel Inc., Cleveland, OH) alternated with full spectrum Vita-Lite light bulbs (Duro-Test Corporation, Fairfield, NJ). We used equal numbers of UV-B bulbs and Vita-Lite bulbs. Vita-Lite bulbs were included to ensure the presence of UV-A radiation (approximately 315–400 nm), a natural component of sunlight and essential for photorepair processes in the developing amphibians (Blaustein *et al*. 1994a and references therein). Two levels of UV were included in the experimental design: with UV (using acetate filters) and without UV (using Mylar® filters). The use of these filters in UV experiments has

Table 1. Measured nitrate concentrations (in units mg/L) for nominal concentrations used in the experiment

	pH 5			pH 7		
	0 mg/L	5 mg/L	20 mg/L	0 mg/L	5 mg/L	20 mg/L
Initial	0.49	4.95	20.8	0.31	5.01	19.7
One week						
Without UV	0.24	2.91	11.8	ND	3.02	9.2
With UV	0.47	2.98	10.0	0.32	2.76	10.2
Two weeks						
Without UV	0.11	1.02	4.76	0.22	1.52	3.28
With UV	ND	1.76	3.63	0.41	1.32	4.21
Three weeks						
Without UV	0.20	0.95	2.54	0.23	0.62	2.65
With UV	ND	0.98	3.20	0.15	0.35	1.51

ND = Not detectable.

been described elsewhere (*e.g.*, Blaustein *et al*. 1994a, 1997). Acetate filters transmit approximately 80% of UV-B and UV-A intensity.

We used levels of pH 5 and pH 7 in our tests. pH 7 was unmanipulated dechlorinated tap water. pH 5 was achieved by acidifying dechlorinated tap water using a ratio of 2:1 sulfuric acid (H_2SO_4) to nitric acid (HNO_3) (Kiesecker 1996). At every water change, pH was measured and adjusted in the source water before adding water to exposure containers.

We used sodium nitrate ($NaNO_3$) to obtain three initial concentrations of nitrate: 0, 5, or 20 mg/L. Nitrate levels in the treatments were measured weekly. Although renewal water contained nitrate at the appropriate concentration, degradation of nitrate occurred over the experiment, and the concentration was not maintained over the entire experiment's duration. This exposure regime may represent a "pulse" type of exposure similar to the type of exposure at amphibian breeding sites, where runoff of nitrate fertilizers occurs following fertilizer application and then decreases over time.

Response Variables

We assessed survival and activity level in each treatment. Dead animals were removed when observed at each water change, and overall survival was determined at the end of the experiment by counting the number of animals alive. To measure activity level of *R. cascadae*, individual larvae were placed in plastic boxes and stacked in a manner so that they could not see one another. After a 15-min acclimation period, the observer watched animals at 30-s intervals for 10 min. Each animal was scored as moving or not moving, and the proportion of time spent active was calculated.

Statistical Analysis

Differences between treatment groups in the percent survival and proportion of time spent active were analyzed using analysis of variance (ANOVA) with SAS version 6.12 software. Survival was analyzed as the mean proportion of surviving animals per cup. Activity level was analyzed as the proportion of time spent active for each individual. Prior to analysis, data were arc-sine-squareroot transformed to achieve normality. Following ANOVA, we used Tukey's test to compare mean responses between treatments. We also examined the effect of each variable and its interactions with other variables using ANOVA. To examine interactive effects, two-way and three-way interactions were included in the model.

Results

Water Quality

Water quality variables were within the same range in both years, with the exception of temperature and pH. Temperature in the exposure containers ranged from 14 to 17°C during the 1999 experiment and from 12 to 14°C during the 1998 experiment. pH in the pH 5 treatment varied from 4.7 to 5.3 in 1998 and from 4.8 to 5.2 in 1999. In both experiments, pH in the pH 7 treatments varied from 6.8 to 7.4. UV-B at the water's surface varied from 9–11 $\mu W/cm^2$ during both years. The UV-A intensity, measured after the completion of the experiment, was 57 $\mu W/cm^2$. Alkalinity in the water ranged from 15–20 mg $CaCO_3$/L, hardness (measured as EDTA) ranged from 32–48 mg $CaCO_3$/L, and conductivity ranged from 220–248 μmhos/cm. Nitrate levels were within expected ranges based on the treatment levels. As expected, nitrate levels decreased somewhat during the 3-week exposure (Table 1).

1998 Experiment

Both survival ($F_{11,36} = 5.92$; $p = 0.0001$) and activity level ($F_{11,193} = 2.44$; $p = 0.0073$) of Cascades frog larvae were significantly affected by the experimental regime. Survival was significantly reduced in the treatment group in which all three factors were augmented (pH 5, high nitrate level, with UV) in comparison to treatment groups in which only one factor was augmented (Table 2). Nitrate, UV, and pH level individually contributed significantly to reduced survival, as well as the interaction between nitrate and pH (Table 3).

Activity level was significantly reduced in the treatment group in which all three factors were augmented, as well as some additional treatments at pH 7; at initial nitrate doses of 20 mg nitrate/L without UV; and at 5 and at 20 mg nitrate/L with UV. Nitrate level and UV contributed most significantly to the reduced activity level (Table 3). The three-way interaction term was suggestive but not significant in the ANOVA model for *R. cascadae* activity level ($p = 0.08$) (Table 3). The three-way interaction term in the ANOVA model for survival was not significant ($p = 0.5953$). Activity level of individual animals

Table 2. *Rana cascadae* survival and activity level, 1998 experiment

pH 5			pH 7		
0 mg/L	5 mg/L	20 mg/L	0 mg/L	5 mg/L	20 mg/L
Percent survival, 1998 experiment:					
Without UV					
94% (5)[ab]	65 (10)[ab]	70 (10)[ab]	99 (0)[a]	94 (5)[ab]	90 (6)[ab]
With UV					
90 (6)[ab]	45 (15)[c]	40 (8)[c]	90 (6)[ab]	90 (6)[ab]	85 (5)[ab]
Proportion of time active, 1998 experiment					
Without UV					
.26 (.04)[ab]	.22 (.05)[ab]	.21 (.04)[ab]	.34 (.07)[ab]	.39 (.08)[a]	.15 (.05)[b]
With UV					
.18 (.04)[ab]	.25 (.08)[ab]	.14 (.07)[b]	.23 (.06)[ab]	.16 (.05)[b]	.14 (.03)[b]

Mean percent survival or mean proportion of time active with standard error in parentheses. Treatments that share a letter are not statistically significantly different from one another (ANOVA followed by Tukey's test; alpha = 0.05).

was measured, but because we actually exposed animals in groups (five per replicate) we also investigated the effect of "replicate" on the activity level. There was no significant effect ($p = 0.25$) due to "replicate"; therefore the term was removed from the statistical analysis.

1999 Experiment

Both survival ($F_{11,36} = 2.54$; $p = 0.0172$) and activity level ($F_{11,164} = 2.80$; $p = 0.0025$) of Cascades frog larvae were significantly affected by the experimental regime. Survival was significantly reduced in the treatment group in which all three factors were augmented (pH 5, high nitrate level, with UV) in comparison to treatment groups in which only one factor was augmented (Table 4). Activity level was also lower in this treatment. Nitrate and pH level contributed significantly to reduced survival (Table 5), while nitrate level and UV contributed significantly to reduce activity level (Table 5). ANOVA did not indicate significant two- or three-way interaction terms. However, as in the 1998 experiment, the three-way interaction term was marginally significant in the ANOVA model for *R. cascadae* activity level ($p = 0.06$) (Table 5). The three-way interaction term in the ANOVA model for survival was not significant ($p = 0.9289$). As in the previous experiment, there was not a significant effect due to container ("replicate") within the treatment ($p = 0.33$).

Table 3. ANOVA models for *Rana cascadae* survival and activity level, 1998 experiment

Source	DF	F-Value	p-Value
Model for *Rana cascadae* survival, 1998 experiment			
Model	11	5.92	0.0001
NO_3	2	10.25	0.0003
UV	1	7.51	0.0095
pH	1	25.69	0.0001
NO_3 * UV	2	0.05	0.9523
NO_3 * pH	2	4.93	0.0128
UV * pH	1	0.43	0.5168
NO_3 * UV * pH	2	0.53	0.5953
Model for *Rana cascadae* activity level, 1998 experiment			
Model	11	2.44	0.0073
NO_3	2	3.94	0.0211
UV	1	9.14	0.0029
pH	1	0.04	0.8484
NO_3 * UV	2	1.07	0.3455
NO_3 * pH	2	0.70	0.5001
UV * pH	1	1.23	0.2679
NO_3 * UV * pH	2	2.51	0.0837

Discussion

In both of our experiments, the combined effects of high nitrate, low pH, and UV resulted in lower survival of larval *R. cascadae*. Similar effects were observed in the experiment in both years: UV and pH had the most significant effects on survival, while UV and nitrate had the most significant effects on activity level. Additionally, in the 1998 experiment, the interaction term between nitrate and pH had a significant effect on survival. In both experiments, the three-way interaction term (pH × nitrate × UV) had a suggestive but not significant effect on activity level but not survival.

In previous studies, *R. cascadae* appears sensitive to the effects of nitrate as larvae (*e.g.*, Marco and Blaustein 1999) and to the effects of UV as embryos (Blaustein *et al*. 1994a). Larvae were sensitive to treatments with these agents in this study. In our experiments, combined effects of high nitrate, low pH, and UV light resulted in lower survival and activity in *R. cascadae*. *R. cascadae* embryos are sensitive to the effects of UV-B alone in field experiments, demonstrating reduced survival and hatching success when exposed to UV-B, and typically demonstrating relatively low levels of the repair enzyme photolyase (Blaustein *et al*. 1994a). *R. cascadae* larvae are sensitive to the sublethal effects of nitrite, exhibiting reduced growth and increased time to metamorphosis (Marco and Blaustein 1999). Furthermore, *R. cascadae* larvae exhibited behavioral effects when exposed to nitrite; larvae spent more time near the water's surface than did controls, possibly in an effort to increase their oxygen intake (Marco and Blaustein 1999). In another study, other species common to the Cascades Mountain range were sensitive to the effects of both nitrate and nitrite (Marco *et al*. 1999). Nitrite was generally more toxic

Table 4. *Rana cascadae* survival and activity level, 1999 experiment

pH 5			pH 7		
0 mg/L	5 mg/L	20 mg/L	0 mg/L	5 mg/L	20 mg/L
Percent survival, 1999 experiment					
Without UV					
100 (0)[a]	94 (5)[ab]	90 (5)[ab]	94 (9)[ab]	100 (0)[a]	100 (0)[a]
With UV					
100 (0)[a]	90 (5)[ab]	75 (9)[b]	100 (0)[a]	100 (0)[a]	94 (5)[ab]
Proportion of time active, 1999 experiment					
Without UV					
.65 (.02)[a]	.52 (.02)[ab]	.53 (.10)[ab]	.51 (.02)[ab]	.53 (.04)[a]	.50 (.07)[ab]
With UV					
.45 (.06)[ab]	.58 (.04)[a]	.29 (.13)[b]	.46 (.05)[ab]	.45 (.04)[ab]	.34 (.05)[ab]

Mean percent survival or proportion of time spent active with standard error in parentheses. Treatments that share a letter are not statistically significantly different from one another (ANOVA followed by Tukey's test; alpha = 0.05).

Table 5. ANOVA model for *Rana cascadae* survival and activity level, 1999 experiment

Source	DF	F-Value	p-Value
Model for *Rana cascadae* survival, 1999 experiment			
Model	11	2.54	0.0172
NO_3	2	5.01	0.0120
UV	1	2.14	0.1521
pH	1	4.65	0.0377
NO_3 * UV	2	2.50	0.0961
NO_3 * pH	2	2.69	0.0815
UV * pH	1	0.59	0.4468
NO_3 * UV * pH	2	0.07	0.9289
Model for *Rana cascadae* activity level, 1999 experiment			
Model	11	2.80	0.0025
NO_3	2	7.27	0.0010
UV	1	5.79	0.0174
pH	1	0.09	0.7674
NO_3 * UV	2	0.71	0.4938
NO_3 * pH	2	0.55	0.5767
UV * pH	1	2.13	0.1462
NO_3 * UV * pH	2	2.84	0.0619

than nitrate, although survival of spotted frog (*Rana pretiosa*) and salamander (*Ambystoma gracile*) larvae was reduced significantly by exposure to nitrate greater than 10 mg/L (Marco *et al*. 1999).

Sublethal behavioral endpoints are useful and should be included in studies of lethal effects of stressors on developing amphibians. Behavior may be altered as a result of exposure to environmental stressors (*e.g*., Hatch and Burton 1999; Marco and Blaustein 1999), with the potential for altered biological interactions with species. UV-B exposure alters the orientation behavior of roughskin newts (*Taricha granulosa*) and the response to chemical cues from predators in juvenile Western toads (*B. boreas*) and larval *T. granulosa* and *R. cascadae* (Blaustein *et al*. 2000; Kats *et al*. 2000). Exposure to nonlethal levels of nitrate increased the feeding behavior of smooth newt (*T. vulgaris*) larvae, and caused newt prey (*Daphnia*) to spend more time near the top of the water column (Watt and Oldham 1995). The insecticide carbaryl reduced tadpole activity level and sprinting performance (speed and distance) (Bridges 1997). Low pH reduced the swimming activity of *Ambystoma laterale* larvae and increased the risk of predation by diving beetles to *A. maculatum* larvae (Kutka 1994).

Altered behavior may also result in altered biological interactions (Kiesecker 1996; Moore and Townsend 1998). For example, if a predator is more susceptible to low pH than its prey, the prey could potentially have enhanced survival (*e.g*., Kiesecker 1996). Alternatively, environmental conditions might alter the behavior of prey making them more conspicuous to predators (*e.g*., Moore and Townsend 1998). In our study, we noted lethal effects at all treatments where sublethal effects were also observed. However, measuring activity level provided an additional measure of the effects of the three variables and an additional measure of the effects of interactions between the variables. For example, ANOVA analysis of survival indicated that nitrate and pH contributed most to reduced survival, whereas nitrate and UV contributed most to reduced activity level (Tables 4–5).

The three factors that we manipulated in this study are potentially relevant to anuran breeding sites in the Cascades Range, where these animals live. While nitrate levels higher than about 5 mg/L are probably rare in breeding ponds in the Cascades, there is a potential for fertilizer runoff to enter amphibian breeding ponds. Forest stands are fertilized every few years with urea (U.S. Forest Service, Sweet Home Ranger Station, personal communication). In the agricultural Willamette Valley, where fertilizer use is more prevalent, nitrate levels in runoff areas may reach as high as 20 mg/L (ACH unpublished data). pH and UV levels used in this study are also relevant to field conditions in the Cascades. Amphibian breeding sites have been measured with water pH in the range of 4.8–5.5 (ACH unpublished data). Also at known anuran breeding sites, UV levels of 9–25 $\mu W/cm^2$ have been recorded at the water's surface (Blaustein *et al*. 1997; ACH unpublished data). The combined effect of these stressors is particularly relevant considering that low pH can increase water clarity, resulting in greater UV penetrance (Yan *et al*. 1996). When such potential interactions are considered, we believe that all of the environmental factors that we studied have the potential to affect developing amphibians in the aquatic environment.

In conclusion, we observed significantly reduced survival and activity level of *R. cascadae* larvae at high nitrate level and low pH in the presence of UV. We conclude that the increased toxicity

in the treatment in which all three factors were augmented is likely due to the combined effects of the three stressors, rather than to a specific mechanism of toxicity. We do not know of a mechanism for UV or low pH to enhance the toxic mechanism for nitrate. We therefore suggest that results are due to a reduced ability to cope with stressors in the presence of other stressors, rather than to a mechanistic action between the three stressors.

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