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A Meta-Analysis of the Effects of Ultraviolet B Radiation and Its Synergistic Interactions with pH, Contaminants, and Disease on Amphibian Survival

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Abstract: *Human alterations to natural systems have resulted in a loss of biological diversity around the world. Amphibian population losses have been more severe than those of birds and mammals. Amphibian population declines are likely due to many factors including habitat loss, disease, contaminants, introduced species and ultraviolet-B (UVB) radiation. The effect of UVB, however, varies widely among species and can vary within populations of the same species or at different life-history stages. This variation has often led to opposing conclusions about how UVB affects amphibians. We used meta-analysis techniques to explore the overall effects of UVB radiation on survival in amphibians. We also used recently developed factorial meta-analytic techniques to quantify potential interactions between UVB radiation and other stressors on amphibians. Ultraviolet-B radiation reduced survival of amphibians by 1.9-fold compared with shielded controls. Larvae were more susceptible to damage from UVB radiation compared with embryos, and salamanders were more susceptible compared with frogs and toads. Furthermore, UVB radiation interacted synergistically with other environmental stressors and resulted in greater than additive effects on survival when 2 stressors were present. Our results suggest that UVB radiation is an important stressor in amphibians, particularly in light of potential synergisms between UVB and other stressors in amphibian habitats.*

Keywords: anura, caudata, life-history stage, meta-analysis, synergistic, UVB radiation

Un Meta-Análisis de los Efectos de la Radiación Ultravioleta B y sus Interacciones Sinergéticas con el pH, Contaminantes y Enfermedades sobre la Supervivencia de Anfibios

Resumen: *Las alteraciones humanas a los sistemas naturales han resultado en la pérdida de diversidad biológica en todo el mundo. Las pérdidas de poblaciones de anfibios han sido más severas que las de aves y mamíferos. Las declinaciones de anfibios probablemente se deben a muchos factores incluyendo la pérdida del hábitat, enfermedades, contaminantes, especies introducidas y radiación ultravioleta-B (UVB). Sin embargo, el efecto del UVB varía ampliamente entre especies y puede variar en poblaciones de la misma especie o en diferentes etapas de la historia de vida. A menudo, esta variación ha llevado a conclusiones opuestas sobre el efecto de UVB sobre anfibios. Utilizamos técnicas de meta-análisis para explorar los efectos totales de la radiación UVB sobre la supervivencia de anfibios. También utilizamos técnicas meta-analíticas factoriales, recientemente desarrolladas, para cuantificar las interacciones potenciales entre la radiación UVB y otros estresantes sobre anfibios. La radiación ultravioleta-B redujo la supervivencia de anfibios 1.9 veces en comparación con condiciones protegidas. Las larvas fueron más susceptibles a los daños de la radiación UVB en comparación con embriones, y las salamandras fueron más susceptibles en comparación con las ranas y sapos. Más aun, la radiación UVB interactuó sinérgicamente con otros estresantes ambientales y resultó en mayores efectos aditivos sobre la supervivencia cuando estaban presentes 2 estresantes. Nuestros resultados*

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sugieren que la radiación UVB es un estresante importante en anfibios, particularmente a la luz de sinergias potenciales entre UVB y otros estresantes en los hábitats de anfibios.

Palabras Clave: anura, caudata, etapa de historia de vida, meta-análisis, radiación UVB, sinergético

Introduction

Anthropogenic changes to the environment have altered the abiotic and biotic habitat of many organisms. These changes include the addition of contaminants (Fleeger et al. 2003), introduction of exotic organisms (Sakai et al. 2001), alteration of flood (Gergel et al. 2005) and fire regimes (Allen et al. 2002), and increases in mean temperatures (IPCC 2007). In addition, reduction in the stratospheric ozone layer has resulted in increases in ultraviolet-B (UVB) radiation reaching the Earth's surface (e.g., Kerr & McElroy 1993; Madronich et al. 1998; Solomon 1999). Ultraviolet-B radiation negatively affects numerous freshwater and marine organisms (Bancroft et al. 2007). The effects of UVB range from increased mortality to sublethal effects on growth, development, photosynthesis, and immunity (Tevini 1993; Caldwell et al. 1998). These effects may scale up to the population or community level, causing changes in community structure and function (Bothwell et al. 1994; Mostajir et al. 1999; Marinone et al. 2006; but see Wahl et al. 2004).

Alteration of the abiotic and biotic habitat has negatively affected many organisms, including amphibians. Amphibians are of particular conservation concern, because their populations are declining more rapidly than either birds or mammals (Stuart et al. 2004); perhaps as many as 122 species have become extinct since 1980 (Mendelson et al. 2006). Many factors appear to contribute to amphibian population declines, including habitat loss, pathogens, contaminants, climate change, and increases in UVB radiation (Blaustein & Kiesecker 2002; Collins & Storfer 2003).

The effects of UVB radiation on amphibians include increased mortality, reduced growth, developmental abnormalities, increased susceptibility to disease, and behavioral changes (reviewed in Blaustein et al. 1998; Blaustein & Kiesecker 2002). In addition, the magnitude of the effect of UVB may vary among species or among different populations or life-history stages of the same species (e.g., Blaustein et al. 1998). For example, survival of moor frog (*Rana arvalis*) embryos was higher when UVB radiation was experimentally filtered out compared with embryos exposed to UVB radiation (Häkkinen et al. 2001). Nevertheless, survival of larval moor frogs was not affected by UVB exposure (Häkkinen et al. 2001).

Numerous factors may influence the results and interpretation of experimental studies reporting the effects of UVB radiation on living organisms (Bancroft et al. 2007). These factors may include aspects of the environment (e.g., elevation, climate, latitude, water transparency), animal behavior (UV avoidance), and methods used by the investigators. For example, venue (laboratory or field) and duration of an experiment often vary among studies, which may make it difficult to compare results. Venue may be particularly important because the quality and quantity of UVA (315–400 nm) and photosynthetically active radiation (PAR) in laboratory experiments rarely approximate conditions in the field (e.g., Ankley et al. 2000). These wavelengths are necessary for effective DNA repair after exposure to UVB radiation (i.e., photorepair; Sancar & Sancar 1988). Lack of efficient repair or exposure to high doses of UVB radiation that overwhelm repair mechanisms may be responsible for the observed negative effects of UVB in amphibians in laboratory studies.

The results of many studies suggest that UVB may interact synergistically with other stressors such as contaminants, climatic factors, or pathogens (Blaustein et al. 2001, 2003). For example, western toad (*Bufo boreas*) embryos are susceptible to a complex interaction between UVB radiation, a pathogenic water mold (*Saprolegnia* sp.), and changes in precipitation (Kiesecker et al. 2001). Thus, mortality in western toad embryos increases when they are infected with *Saprolegnia* in the presence of increasing UVB radiation that occurs during years of lower precipitation when water levels are low and the UVB shielding property of the water is diminished.

Several researchers report no effects of UVB alone or in conjunction with other stressors on amphibians (e.g., Blaustein et al. 1996; Merilä et al. 2000; Starnes et al. 2000; Pahkala et al. 2001). The lack of a UVB effect reported by many researchers has led to controversy regarding the importance of UVB as an environmental stressor of amphibians. This controversy stems from using formal or informal "vote counts" of results in the literature, in which the numbers of studies reporting significant effects are summed and a conclusion about the overall importance of a factor is estimated (e.g., Licht 2003). Nevertheless, vote counting suffers from low statistical power and is biased toward finding no effect (Rosenberg et al. 2000).

Meta-analytic techniques are designed to avoid issues associated with vote counting and have been used to explore the effects of UVB radiation and other global environmental factors (e.g., Parmesan & Yohe 2003; Root et al. 2003; Bancroft et al. 2007). Furthermore, recent advances in meta-analytic techniques now allow one to assess the potential interaction among factors in fully factorial experiments (Gurevitch et al. 2000; Hawkes &

Sullivan 2001). Meta-analytic techniques are the most appropriate and statistically rigorous methods for summarizing independent experiments. Nevertheless, these techniques can only synthesize available data and may therefore suffer from publication or research bias (Gurevitch & Hedges 1999). Tools such as Rosenthal's number can estimate the potential importance of unpublished studies by quantifying the number of additional, unpublished studies with an average effect size of zero necessary to change the results of a meta-analysis (Rosenthal 1979; Rosenberg et al. 2000).

We used meta-analytic techniques to quantify the overall effect of UVB radiation on survival of amphibians. Although UVB radiation has many effects on amphibians (reviewed in Blaustein & Belden 2003), we selected survival as the response variable because the majority of studies on the effects of UVB in amphibians focused on survival. Moreover, mortality is the most severe end point after UVB exposure, making our analyses conservative. We then used multiple linear regression to explore the sources of variation among effect size estimates and to identify important predictors of effect size in published studies. Finally, we used factorial meta-analysis to quantify the interaction between UVB and additional stressors. Our results suggest that UVB is indeed an important stressor for amphibians, as it is for many other aquatic organisms (Bancroft et al. 2007).

Methods

Effects of UVB on Survival

We searched 6 electronic databases (BIOSIS, Web of Science, Aquatic Sciences and Fisheries Abstracts, Fish and Fisheries Worldwide, Wildlife and Ecology Studies Worldwide, and Biological and Agricultural Index) to identify papers used in these analyses. We searched for all possible combinations of the terms *ultraviolet*, *UV*, *UVB* with *survival* and *amphibian**, *frog**, *toad**, *salamander** or *newt** and identified peer-reviewed articles that provided a measure of amphibian survival after exposure to ambient levels of UVB. We limited our search to include only experimental manipulations of UVB that used the standard technique of applying plastic filters that differentially transmitted or filtered UVB radiation. All articles were included that provided a sample mean for each treatment (UVB exposed and UVB shielded). We included all study locations and species within each article. When multiple sampling dates were reported, we selected the final sampling day. If multiple doses of UVB radiation were used, we randomly selected only one dose from each study. Because several individual studies contained more than one species or location, we define a *study* as the original article and a *comparison* as an estimate for a single species or location within the study. The 41 studies generated 89 comparisons and included 2 of the 3 amphibian orders (anura and caudata), 8 genera, and 32 individual species (full list available from B.A.B.). We extracted data from figures with TechDig 2.0 (Jones 1998). No evidence of publication bias was detected in the data: Rosenthal's number calculated for this analysis (135) was larger than the number of comparisons in the analysis (Rosenthal 1979).

We calculated the log-response ratio (lnR) as a measure of effect size for each comparison. The lnR is the natural log of the ratio of survival with and without UVB radiation within each comparison (Hedges et al. 1999). We selected the lnR because of the clear biological interpretation (proportional survival of experimental organisms compared with controls) and good statistical properties (Osenberg et al. 1997; Shurin et al. 2002). We calculated the grand mean effect size for the full model and generated bias-corrected bootstrapped 95% confidence intervals (CIs) (Adams et al. 1997). We were unable to use sample variance as a measure of precision because approximately half the comparisons did not include a measure of variance (e.g., standard deviation). We ran our analyses with unweighted effect sizes and effect sizes weighted by sample size (n). The results were qualitatively similar, so we report only the unweighted effect sizes. Effect size calculations and summary analyses were conducted in MetaWin 2.0 (Rosenberg et al. 2000).

We explored the variables contributing to residual variance in our model with multiple linear regression (Borer et al. 2005). The variables examined included biological, geographical, and methodological factors reported in each study (Table 1). Nevertheless, some studies did not include enough information to estimate study duration, days of UVB exposure, or elevation of study location.

Table 1. Biological and methodological explanatory variables used in regression analysis of the effects of ultraviolet B (UVB) on survival in amphibians.

Variable	*Possible values*	*Number of comparisons including the variable*
Latitude	continuous variable	88
Duration of study	long (>7 days) short (≤7 days)	75
Days of UVB exposure	continuous variable	70
Elevation (altitude)	continuous variable	79
Venue*	laboratory field	89 (all)
Life-history stage*	embryo larva metamorphic	89 (all)
Taxonomic order*	anura caudata	89 (all)

* *Variables available for all studies.*

Table 2. Additional stressors used in factorial meta-analysis of the effect of UVB radiation and an additional stressor on survival of amphibians.

Additional stressor	*Life-history stage*	*Species*	*Reference*
Saprolegnia (pathogenic water mold)	embryo	*Bufo boreas*	Kiesecker & Blaustein 1995
		Pseudacris regilla	
		Rana cascadae	
pH	embryo	*R. arvalis*	Pahkala et al. 2001
	larva	*R. pipiens*	Long et al. 1995
		R. temporaria	Pahkala et al. 2002
		R. cascadae	Hatch & Blaustein 2002
Nitrate	embryo	*P. regilla*	Hatch & Blaustein 2003
	larva	*R. cascadae*	Hatch & Blaustein 2002
Landfill leachate	embryo	*R. blairi*	Bruner et al. 2002
Copper	larva	*P. crucifer*	Baud & Beck 2005
Methoprene	larva	*R. pipiens*	Ankley et al. 1998
Carbaryl	embryo	*Xenopus laevis*	Zaga et al. 1998
	larva	*Hyla versicolor*	
		X. laevis	
		H. versicolor	
Bisphenol A	larva	*R. temporaria*	Koponen & Kukkonen 2002

Therefore, we conducted 2 separate analyses: the first explored all variables, effectively excluding the comparisons without all variables reported ($n = 58$ comparisons) and the second was conducted on the subset of variables reported by every study ($n = 89$ comparisons). We used backward selection to identify the best-fit model. Multiple linear regressions were conducted in JMP V.6 (SAS Institute Incorporated, Cary, North Carolina). We generated mean effect sizes and bias-corrected bootstrapped 95% CIs for groups with a significant term in our regression model. When fewer than 10 comparisons were available within a group, we used parametric 95% CIs as a more conservative estimate of the CI. Mean effect sizes were considered significantly different from zero if the 95% CI did not overlap with zero.

Effects of UVB and Additional Stressors on Survival

To explore the possible interaction between UVB and additional environmental stressors, we used factorial meta-analysis (Gurevitch et al. 2000). This type of meta-analysis allowed the examination of main effects (i.e., UVB and the other stressor alone) and the interaction between the 2 stressors. Nevertheless, only studies that were originally factorial in design were used (Gurevitch et al. 2000; Hawkes & Sullivan 2001). We conducted a separate search in the same 6 databases (see above) for studies testing the effects of UVB and an additional stressor on survival alone and in combination (Table 2). All studies that reported means for each treatment combination were included.

We used the log-response ratio as our effect-size metric. We calculated the main effects of each stressor and the interaction between the stressors. We based our calculations on previously published factorial meta-analyses (Hawkes & Sullivan 2001; Borer et al. 2006) as follows:

$$\begin{aligned}
\ln R_{\mathrm{UVB}} &= [\ln(Y_{\mathrm{US}}) + \ln(Y_{\mathrm{UA}})] \\
&\quad - [\ln(Y_{\mathrm{NS}}) + \ln(Y_{\mathrm{NA}})], \\
\ln R_{\mathrm{STRESS}} &= [\ln(Y_{\mathrm{NS}}) + \ln(Y_{\mathrm{US}})] \\
&\quad - [\ln(Y_{\mathrm{NA}}) + \ln(Y_{\mathrm{UA}})], \quad \text{and} \\
\ln R_{\mathrm{INTERACTION}} &= [\ln(Y_{\mathrm{US}}) + \ln(Y_{\mathrm{UA}})] \\
&\quad - [\ln(Y_{\mathrm{NS}}) + \ln(Y_{\mathrm{NA}})],
\end{aligned}$$

where for each comparison, Y is the mean survival in the UVB treatments (present, U; absent, N) and the stressor treatment (present, S; absent, A). A negative value for the main effects indicates the stressor had a negative effect on survival. The interaction term as presented here can be read as the mean effect of the stressor when UV is present $[\ln(Y_{\mathrm{US}}) - \ln(Y_{\mathrm{UA}})]$ minus the mean effect of the stressor when UV is absent $[\ln(Y_{\mathrm{NS}}) - \ln(Y_{\mathrm{NA}})]$. Therefore, an effect size estimate of zero for the interaction term suggests that the effect of the stressor is the same with and without UVB. A negative value for the interaction term indicates a synergistic (more than additive) effect of the 2 stressors together. Effect-size estimates were considered significantly different from each other if the 95% CIs did not overlap. Furthermore, effect-size estimates were considered significantly different from zero if the 95% CIs did not overlap with zero. Two comparisons were removed from the analysis due to mathematical incompatibility (zero survival in all treatments except controls).

Results

Effects of UVB on Survival

Exposure to UVB resulted in a 1.85-fold decrease in survival compared with controls (Fig. 1a). When all explanatory variables were included in the regression analysis,

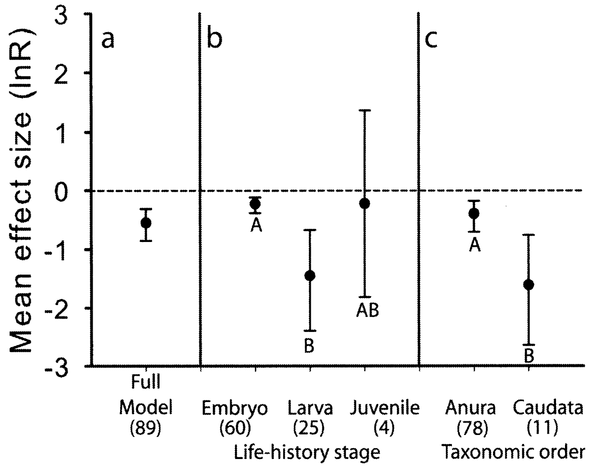

Figure 1. Mean effect sizes (log-response ratio) for all comparisons included in the analysis of the effect of ultraviolet-B radiation on amphibian survival alone: (a) full model, (b) life-history stage, and (c) taxonomic order. All effect sizes are significantly different from zero except the metamorphic life-history stage. In each panel, means that share a common uppercase letter are not significantly different. Numbers in parentheses indicate sample size for each estimate. Data are means and bias-corrected bootstrapped CIs, except the CI for the metamorphic life-history stage is a parametric CI.

days of UVB exposure, life-history stage, and taxonomic order accounted for 41.4% of the variation in survival (Table 3). Surprisingly, the correlation coefficient for days of UVB exposure was positive (0.012), suggesting that amphibian survival was higher with longer exposure times. When all comparisons were included in the analysis, and therefore fewer explanatory variables, life-

Table 3. Final regression models of the effect of UVB on survival of amphibians.[a]

Factor	*df*	*Type II sum of squares*	F	p
All explanatory variables included[b]				
days of UVB	1	3.07	4.10	0.048
life-history stage	1	12.89	17.22	0.0001
taxonomic order	1	11.94	15.95	0.0002
All studies included[c]				
life-history stage	1	12.82	9.26	0.0031
taxonomic order	1	12.75	9.21	0.0032

[a]Variables were iteratively removed until only significant terms remained in the model. All explanatory variables were included (studies that did not report all information were excluded, information on which is available from B.A.B.) or all studies were included (using a subset of variables indicated by an asterisk in Table 1).
[b]$n = 58$, residual $df = 54$, model $r^2 = 0.414$.
[c]$n = 89$, residual $df = 86$, model $r^2 = 0.182$.

Table 4. Final regression models of the effect of UVB on survival of amphibians after excluding Blaustein studies.[a]

Factor	*df*	*Type II sum of squares*	F	p
All explanatory variables included[b]				
days of UVB	1	5.75	8.96	0.0047
life-history stage	1	21.52	33.55	<0.0001
taxonomic order	1	7.28	11.40	0.0016
All studies included[c]				
life-history stage	1	14.84	8.23	.0056
taxonomic order	1	10.69	5.93	0.018

[a]Variables were iteratively removed until only significant terms remained in the model.
[b]$n = 45$, residual $df = 41$, model $r^2 = 0.5760$.
[c]$n = 64$, residual $df = 61$, model $r^2 = 0.1862$.

history stage and taxonomic order explained 18.2% of the variation in survival after exposure to UVB. Days of UVB exposure was not included in the second model because this information was not available for all comparisons (Table 1). Larvae were more sensitive to UVB than embryos (Fig. 1b), and caudates (salamanders) were more susceptible to UVB than anurans (frogs and toads) (Fig. 1c) in both models. We did not include study as a random effect in our analyses because comparisons within the same study were frequently variable. Nevertheless, we also ran our analyses with a mixed model (i.e., including study as a random effect with the same explanatory variables as fixed effects) and the predicted effect of study was not significant ($t = -0.63$, $p = 0.6440$). Furthermore, the results were qualitatively similar regardless of the model used.

Nearly one-third (28%) of the comparisons in these analyses were from the work of A. R. Blaustein and colleagues. To explore the possibility of bias, we removed all comparisons generated by these researchers and reran the analyses. The final regression models were qualitatively the same without studies conducted by this group (Table 4). Without the Blaustein comparisons in the analysis, UVB reduced survival by 1.90-fold.

Effect of UVB and Additional Stressors on Survival

In the factorial analysis the main effects (UVB and additional stressors) were significantly different from zero, but not different from each other (Fig. 2). There was evidence of a synergistic (more than additive) effect of UVB and an additional stressor: the interaction effect size was negative and significantly different from zero (Fig. 2). We were unable to conduct further regression analyses exploring variance on the factorial data because sample sizes in each group (e.g., taxonomic order, life-history stage, stressor type) were too low.

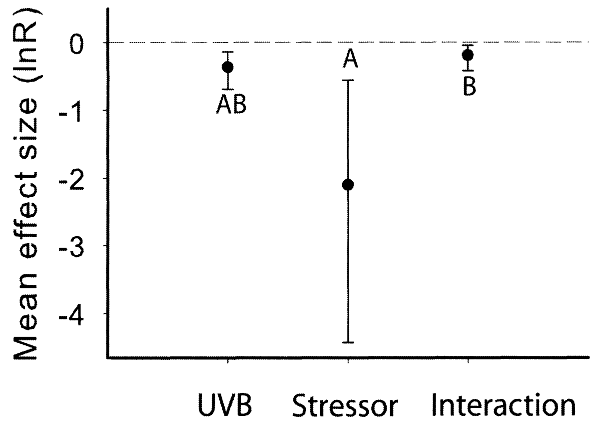

Figure 2. Mean effect size estimates for the factorial meta-analysis of the interaction between ultraviolet-B (UVB) radiation and additional stressors on survival of amphibians. All effect estimates are significantly different from zero. Estimates that share a common uppercase letter are not significantly different. Data are means and bias-corrected 95% CIs.

Discussion

Ultraviolet-B radiation alone and in combination with other factors is an important stressor in natural systems. The synergistic interaction between stressors can lead to "ecological surprises" even when one stressor is not considered a major stressor in the system (Christensen et al. 2006).

Effect of UVB Alone

Exposure to UVB radiation nearly halved survival of amphibians in these comparisons. This result was surprising because only 32 of the original 89 comparisons (35%) reported a significant reduction in survivorship under UVB radiation. The majority of the variation in effect size was explained by life-history stage, taxonomic order, and number of days of exposure.

The average effect size was much smaller for embryos than larvae, suggesting that UVB radiation has a larger effect on survival of larvae. Only 4 studies on the effects of UVB on metamorphic amphibians were found and included in our analysis. Clearly, more research is necessary on the effects of UVB at this important life-history stage. Embryos may have been less susceptible to UVB radiation because they were exposed to UVB for fewer days on average (embryos, 12.5 days; larvae, 34.5 days), most likely due to the shorter period spent in this life-history stage. In addition, embryos may be better protected from UVB by the jelly envelope, pigmentation, or cellular repair mechanisms such as photolyase (Epel et al. 1999; Blaustein & Belden 2003). Larvae may be less defended from UVB radiation because they have the potential to behaviorally avoid areas with high UVB levels in natural systems. The experimental design of the comparisons in this analysis did not allow larvae to seek refuge from UVB. Larvae of some species appear to avoid regions with high UVB exposure (Nagl & Hofer 1997; van de Mortel & Buttemer 1998; Garcia et al. 2004). In contrast, larvae of other species do not avoid UVB and are frequently observed in very shallow water (O'Hara 1981; Wollmuth et al. 1987; van de Mortel & Buttemer 1998; Belden et al. 2000; Belden et al. 2003).

Larger effects of UVB on survival of larvae may have important implications for population persistence. A recent demographic model suggests that reduction in postembryonic survival results in decreases in equilibrium density in the adult portion of the population (Vonesh & De la Cruz 2002). Therefore, reduction in larval survival due to UVB may result in reductions in the adult population over time, particularly in habitats with high transmission of UVB and in species that do not avoid UVB radiation.

The average estimate of effect size was much larger for caudates compared with anurans, suggesting that UVB has a much larger effect on survival in salamanders and newts compared with frogs and toads. This result is in accordance with previous work on relative photolyase activity in embryos. Photolyases are a group of enzymes responsible for the majority of DNA repair after exposure to UVB radiation (Sancar & Sancar 1988). In general, salamander and newt species have much lower levels of photolyase than frogs and toads (Blaustein et al. 1994; Smith et al. 2002). Nevertheless, these differences and the observed difference in effect size between caudates and anurans may reflect the differences in expected exposure to UVB in natural systems, particularly at the egg stage (Blaustein et al. 1994). For example, oviposition occurred at deeper depths on average for 2 salamander species compared with 5 frog species (Smith et al. 2002). Nevertheless, this relationship is not always observed, and some caudates lay their eggs in shallow water. Oviposition depth varies between species and even between individuals of the same population (Palen et al. 2005). Mobile larvae, however, may be exposed to varying levels of UVB due to habitat use (Bancroft et al. 2008). If larvae do not avoid UVB radiation, other factors influencing habitat use may lead to increased UVB exposure.

Temperature is one important predictor of habitat use in amphibians, including caudates (Lucas & Reynolds 1967; Keen & Schroeder 1975; Dupré & Petranka 1985; Hutchison & Dupré 1992). Temperature preferences vary by species and life-history stage, but many amphibians select warmer temperatures, where growth and development are maximized (Hutchison & Dupré 1992). Seeking warm, shallow water for thermoregulation also may expose larvae to high levels of UVB during development. Thus, caudate larvae may be exposed to similar UVB levels as anuran larvae, and these UVB levels may result in larger effects on survival in caudates.

We expected the relationship between days of exposure and effect size to be negative because the effects of UVB on an organism are closely related to total dose of UVB (e.g., Ankley et al. 2002). Nevertheless, we observed the opposite trend in this analysis. It is possible that organisms surviving the first day of UVB exposure could develop a "UV-hardening" response, similar to the temperature-hardening response seen in many organisms (Hutchison & Maness 1979; Nobel 1982; Lee et al. 1987). Ultraviolet hardening in amphibians may involve melanin, used as a sunscreen pigment (Jablonski 1998; Blaustein & Belden 2003), or upregulation of cellular mechanisms, such as photolyases, heat-shock proteins, or antioxidant enzymes (Feder 1999; Lesser et al. 2001; Blaustein & Belden 2003). Alternatively, a priori estimates of UV tolerance could result in longer exposure times for species or stages with higher tolerance for UVB. Interestingly, a significant negative correlation between days of UVB exposure and effect size was observed for embryos ($r = -0.38$, $p = 0.013$), whereas a significant positive correlation was observed for larvae ($r = 0.57$, $p = 0.006$). Thus, the relationship between effect size and days of UVB exposure is in the expected direction for embryos but not larvae. This difference in the relationship between exposure days and effect size suggests differences may exist in physiological tolerance for UVB exposure between life-history stages. Embryos may be more likely to accrue damage from longer exposure, whereas larvae may be more likely to exhibit UV hardening or acclimation. More research on the physiological mechanisms of UV tolerance at the larval life-history stage is necessary.

Effect of UVB and Additional Stressors on Survival

Our results suggest that the combination of UVB radiation and an additional stressor leads to a synergistic interaction and greater than additive mortality in amphibians. These additional stressors were diverse and ranged from pathogens to agricultural contaminants and low pH. The effect of these stressors was large and significantly different from zero when amphibians were exposed to the stressor alone. Nevertheless, amphibians in natural systems are frequently exposed to more than one stressor and these stressors commonly interact synergistically (Sih et al. 2004). The finding that UVB radiation interacts with other stressors is particularly important because many amphibian habitats in temperate regions are exposed to at least some amount of UVB radiation (Diamond et al. 2005). Levels of UVB radiation vary widely between years and habitats and at smaller spatial and temporal scales. Thus, the effects of these stressors in natural populations could vary on similar spatial and temporal scales.

The potential for interaction with other stressors suggests that UVB is an important factor even in species that are relatively resistant to UVB radiation. For example, Pacific treefrogs (*Pseudacris regilla*) are generally considered resistant to damage from UVB radiation. Nevertheless, when Pacific treefrog embryos are exposed to UVB and nitrate, survival is lower than that observed after exposure to either stressor alone (Hatch & Blaustein 2003). Experiments on the same species found no interaction between UVB and the pathogen *Saprolegnia ferax* (Kiesecker & Blaustein 1995). Thus, strength of the interaction may vary within a species depending on the stressors involved. Results of this analysis support the observation that amphibian population declines are likely due to multiple causes that may interact (Blaustein & Kiesecker 2002).

The main effect of UVB was smaller in the factorial analysis than in the analysis of effects of UVB alone. Fewer comparisons were included in the factorial analysis because only fully factorial studies were included. Nevertheless, none of the studies in the factorial analysis were on caudates. In the analysis of UVB alone, UVB had a much larger negative effect on caudates than on anurans. Furthermore, only 4 of the 19 comparisons in the factorial analysis were on larvae. The rest of the comparisons were on embryos, the least susceptible life-history stage. Thus, the factorial analysis may underestimate strength of the interaction between UVB and additional stressors on amphibian survival and development. More research on caudates and later life-history stages is necessary to understand the potential for nonadditive effects in amphibians exposed to UVB radiation.

Conclusions

Results of our analyses suggest that UVB radiation is an important stressor for amphibians. A negative effect of UVB radiation on survival was detected, despite the apparent lack of significant UVB effects in much of the literature. Moreover, UVB radiation interacts synergistically with many common stressors in natural systems. Future work should focus on underrepresented life-history stages and should explore the potential for interactions among stressors in natural systems. These types of data are necessary for including the effects of stressors in demographic models of amphibian populations. In addition, previous research has identified several sublethal effects of UVB exposure, including reduced growth and behavioral changes. These sublethal effects did not fall within the scope of our analyses, as we focused on the most extreme effect (lethality). Nevertheless, sublethal effects should be examined in future analyses to enhance understanding of how UVB affects amphibians. Understanding the independent and interactive effects of various stressors on multiple species of amphibians at multiple life-history stages is vital to clarifying causes of amphibian population declines. Similarly, techniques such as meta-analysis can be used to explore factors involved in the decline of other taxonomic groups and are important tools in conservation biology.

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