OPTICAL CHARACTERISTICS OF NATURAL WATERS PROTECT AMPHIBIANS FROM UV-B IN THE U.S. PACIFIC NORTHWEST

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Abstract. Increased exposure to ultraviolet-B (UV-B) radiation has been proposed as a major environmental stressor leading to global amphibian declines. Prior experimental evidence from the U.S. Pacific Northwest (PNW) indicating the acute embryonic sensitivity of at least four amphibian species to UV-B has been central to the literature about amphibian decline. However, these results have not been expanded to address population-scale effects and natural landscape variation in UV-B transparency of water at amphibian breeding sites: both necessary links to assess the importance of UV-B for amphibian declines. We quantified the UV-B transparency of 136 potential amphibian breeding sites to establish the pattern of UV-B exposure across two montane regions in the PNW. Our data suggest that 85% of sites are naturally protected by dissolved organic matter in pond water, and that only a fraction of breeding sites are expected to experience UV-B intensities exceeding levels associated with elevated egg mortality. Thus, the spectral characteristics of natural waters likely mediate the physiological effects of UV-B on amphibian eggs in all but the clearest waters. These data imply that UV-B is unlikely to cause broad amphibian declines across the landscape of the American Northwest.

Key words: Ambystoma gracile; Ambystoma macrodactylum; amphibian declines; Bufo boreas; Cascades frog; dissolved organic matter; DOM; long-toed salamander; northwestern salamander; Pacific Northwest; Rana cascadae; UV-B radiation; western toad.

INTRODUCTION

Substantial evidence exists that many amphibian populations have declined in the past 40 yr across the Earth (Blaustein and Wake 1990, Fellers and Drost 1993, Lips 1999, Pounds et al. 1999, Houllahan et al. 2000, Knapp and Matthews 2000). Several causes have been proposed, including ultraviolet-B radiation (UV-B) (Blaustein et al. 1994), non-native predators (Knapp and Matthews 2000), climate change (Lips 1999, Pounds et al. 1999), habitat degradation (Davidson et al. 2001), and pathogens (Berger et al. 1998). Examination of amphibian sensitivity to various environmental stressors has focused on individual- rather than population-level consequences, and the importance of stressors for natural population dynamics remains largely unexplored. Here, we highlight the possible consequences of this disconnect for the UV-B hypothesis by placing individual-level results in a landscape context for the Olympic and Cascade Ranges of the U.S. Pacific Northwest.

The decline of amphibian populations in relatively pristine habitats across wide geographic areas suggests that global atmospheric factors could be involved (Blaustein and Wake 1990, Lips 1999, Pounds et al. 1999). Recent decreases in stratospheric ozone have resulted in increased UV-B flux (wavelengths: 290–320 nm), the most energetic and potentially damaging range of ultraviolet radiation for biological systems, especially at polar and mid-latitudes (Blumthaler and Ambach 1990, Stolarski et al. 1992, Kerr and McElroy 1993, Madronich 1994). Ultraviolet-B radiation may contribute to the decline of amphibian populations by reducing hatching success and larval growth rates (Blaustein et al. 1994, 1995, 1997, van de Mortel and Buttemer 1996, Kiesecker et al. 2001), elevating morphological abnormalities (Blaustein et al. 1997, Ankley et al. 2000), and increasing susceptibility to fungal pathogens (Kiesecker and Blaustein 1995). Physiolog-
Ical tests have demonstrated different levels of embryonic susceptibility to ambient UV-B for a range of amphibian species (Blaustein et al. 1994). One common source of UV-B induced mortality is associated with reduced resistance to the fungal pathogen Saprolegnia ferax (Kiesecker and Blaustein 1995). Recent evidence demonstrates that the depth at which eggs are deposited and develop may also affect amphibian egg sensitivity to UV-B (Kiesecker et al. 2001). At Lost Lake, in the Oregon Cascade Mountains, variation in winter precipitation results in changes in the average depth where western toad (Bufo boreas) eggs develop. Bufo boreas eggs deposited at shallower depths experienced increased egg mortality, suggesting that the overlying water column directly influences UV-B attenuation (Kiesecker et al. 2001).

To evaluate the significance of UV-B sensitivity of amphibian populations at large spatial scales, we compared the level of UV-B that has been demonstrated to reduce hatching success (Kiesecker et al. 2001) to estimated levels at other sites in the region. Exposure of embryonic and larval amphibians to UV-B is a function of the ambient flux of UV-B, optical properties of the aquatic environment, and behavioral and physiological adaptations of individuals. Biotic adaptations may include the selection of oviposition sites by adults, melanin pigmentation of eggs, and the size and shape of jelly covering embryos (Licht and Grant 1997). Physiological sensitivity to UV-B may be of limited importance to embryonic and larval amphibians if UV-B is substantially reduced in the aquatic environment. Colored dissolved organic matter (DOM) directly reduces the intensity of UV-B in the water column of breeding sites through the specific absorption of high-energy wavelengths (Kirk 1994, Morris et al. 1995). Even modest concentrations of DOM can virtually eliminate UV-B within a few centimeters of the water surface. Most (85–92%) of the natural variation in the UV-B attenuation properties of natural waters is explained by DOM concentration (Scully and Lean 1994, Morris et al. 1995). Most DOM originates in forest soils and wetlands, and its concentration in surface waters can vary across landscapes by three to four orders of magnitude depending on a variety of watershed features (Schindler et al. 1992, Morris et al. 1995, Williamson et al. 1996, Gergel et al. 1999). For amphibian breeding sites in the PNW, spatial variation in DOM produces a gradient of UV-B exposure for aquatic organisms (Adams et al. 2001). We contend that estimates of organism sensitivity to UV-B without consideration for the optical properties of water produce results of limited relevance to the global UV-B hypothesis.

**METHODS**

We quantified the variation in UV-B transparency for 136 potential breeding ponds in the Olympic and Cascade Ranges in the U.S. Pacific Northwest, and determined the proportion of sites that may experience UV-B irradiance levels shown to produce significant embryonic mortality in B. boreas at another site in the region (Kiesecker et al. 2001). Sites used in this analysis included ponds and small lakes from the states of Washington (counties: Clallam, Jefferson, Mason) and Oregon (counties: Lane, Deschutes), and occur within ±1200 m of the current tree-line elevation (breeding site elevation range: 859–1844 m). All sites were in protected areas where direct sources of anthropogenic disturbance are limited, including Olympic National Park, Washington, and Three Sisters Wilderness Area, along the crest of the Cascade Range, Oregon. Sites were not selected according to any a priori hypothesis regarding the distribution of UV-B irradiance, and in many watersheds they represent all potential amphibian breeding sites encountered. We also compared the overall pattern of UV-B exposure in 136 ponds to the subset of sites used for breeding by four widespread species of pool breeding amphibian with known embryonic sensitivity to UV-B (Blaustein et al. 1994, 1995): Cascades frog (Rana cascadae), western toad (B. boreas), long-toed salamander (Ambystoma macrodactylum), and northwestern salamander (Ambystoma gracile).

We determined the landscape variation in UV-B transparency among amphibian breeding sites using established rapid assessment methods to estimate the attenuation of UV-B with water depth in each pond (Baker and Smith 1982, Morris et al. 1995, Williamson et al. 1996). In a subset of sites (seven ponds in Olympic National Park, Washington), we evaluated the strength of the relationships between in situ measures of UV-B attenuation (Macam UV-203 underwater spectroradiometer, Macam, Livingston, Scotland, UK) and analytical measures of DOM concentration (g/m³), and absorption of 440 nm light (per meter) from water samples. Absorption at 440 nm explains most of the variation in the UV-B attenuation coefficients (K₄) of ponds and is strongly correlated with DOM concentration. Based on K₄ estimates for each of the 136 sites, we estimated the UV-B exposure at a common water depth (10 cm) by applying the ambient UV-B intensity measured by Kiesecker et al. (2001) to all sites. We used an estimated average depth of eggs laid by the four focal species based on a combination of individual accounts of species breeding habits (Slater 1936, Kezer and Farner 1955, Kiesecker et al. 2001) and field observations (C. A. Pearl and W. J. Palen, unpublished data; K. O. Richter, personal communication). The UV-B values reported by Kiesecker et al. (2001) represent irradiance measures integrated over UV-B wavelengths and weighted by the action spectra of human skin (Difffey erythermal weighting, Madronich and Flocke 1997). While UV-B irradiance does not represent the total UV-B dose over time for each site, irradiance allows us to evaluate the maximum potential for UV-B regulation of amphibians. Incorporating additional factors that explain variation in the UV-B exposure between sites (local topography, vegetation, and as-
RESULTS AND DISCUSSION

We found strong relationships, similar to those published from extensive surveys of the optical properties of lakes (Scully and Lean 1994, Morris et al. 1995), between the attenuation coefficient \( K_d \) for UV-B and both assessment techniques: DOM concentration \( K_d = -0.0371 \times [\text{DOM}], r^2 = 0.96, P < 0.0001 \) (Fig. 1A), and absorbance at 440 nm \( K_d = -0.0795 \times \text{absorption}_{440}, r^2 = 0.98, P < 0.00001 \) (Fig. 1B). The concentration of DOM is a strong predictor of the absorption of 440 nm light (absorption_{440} = 0.421 \times [\text{DOM}]+ 0.198, r^2 = 0.91, P < 0.01) (Fig. 1C).

Based on these relationships, we estimated the UV-B irradiance at 10 cm depth for 136 ponds and compared them to levels of UV-B irradiance that reduced egg survivorship for \( B. \) boreas (Kiesecker et al. 2001). The 136 ponds in our dataset span the entire range of possible UV-B exposures at 10 cm depth, with some ponds experiencing undetectable levels of UV-B at 10 cm and others experiencing a level of UV-B at 10 cm that is indistinguishable from surface irradiance (Fig. 2A). We calculated the proportion of available breeding ponds exposed to levels of UV-B at 10 cm water depth that correspond to a significant UV-B effect (33% reduction in egg survivorship, 22.4 \( \mu \text{W/cm}^2 \)) and no UV-B effect (12.7 \( \mu \text{W/cm}^2 \), Kiesecker et al. 2001). We found that 85% of the available breeding sites sampled would not experience levels of UV-B irradiance that reduced egg survivorship for \( B. \) boreas (Kiesecker et al. 2001), and that 50% of sites experience UV-B irradiance below levels shown to have no effect on embryonic survival (Fig. 2A). If we assume that amphibian eggs occur at 5 cm or 20 cm water depth, we estimate that 70% and 90%, respectively, of sites receive UV-B levels <22.4 \( \mu \text{W/cm}^2 \).

URL: (http://oz.physast.uga.edu/)
Diffuse attenuation coefficient ($K_d$; per centimeter) values for UV-B as a function of (A) dissolved organic matter (g/m$^2$; $K_d = -0.0371 \times [\text{DOM}], r^2 = 0.96, P < 0.0001$), and (B) absorption of 440 nm light (per meter; $K_d = -0.0795 \times \text{absorption}_{440\text{nm}}, r^2 = 0.98, P < 0.00001$). (C) Absorption of 440-nm light is predicted by dissolved organic matter concentration (absorption$_{440\text{nm}} = 0.421 \times [\text{DOM}] + 0.198, r^2 = 0.91, P < 0.01$). Values of $K_d$ were determined from the extinction of UV-B radiation measured in situ at seven sites in Olympic National Park, Washington.

We also compared the distribution of UV-B transparency of actual breeding sites to the UV-B levels that produced increased mortality in $B$. boreas (Kiesecker et al. 2001), and found that 100% of $B$. boreas breeding sites included in our survey are not likely to receive critical doses of UV-B at 10 cm. Similarly most breeding sites of $R$. cascadae (89%), $A$. macrodactylum (79%), and $A$. gracile (76%) receive <22.4 $\mu$W/cm$^2$ of UV-B at 10 cm depth (Fig. 2B). Because the physiological sensitivity of $R$. cascadae, $B$. boreas, $A$. gracile, and $A$. macrodactylum are comparable (Blaustein et al. 1994, 1995), our results suggest that most current breeding sites for these species are well protected from UV-B levels that can reduce embryonic survival (Table 1).

There has been recent discussion of complex interactions between fluctuations in climate and UV-B exposure for amphibians, where water depth, temperature, and seasonal exposure to UV-B flux (Kiesecker et al. 2001, Pounds 2001; Corn and Muths 2002) have all been shown to have substantial effects on amphibian embryos. We propose that one of the more important consequences of global climate change for aquatic organisms is the effect that changes in DOM inputs and residence time in aquatic environments will have on UV-B transparency (see Leavitt et al. 1997, Pienitz and Vincent 2000). Because the movement of DOM from terrestrial systems into breeding ponds is driven by precipitation, wetter climates may increase DOM inputs to many aquatic systems, resulting in higher DOM concentrations and reduced UV-B transmission (Schindler et al. 1992, Gergel et al. 1999). In contrast, drier climates may result in reduced DOM concentrations due to increased residence time and potential for degradation (Webster et al. 1996). Furthermore, tree-line migration in response to warmer climates will increase DOM loading to aquatic ecosystems in alpine areas, and therefore reduce UV-B exposure (Leavitt et al. 1997, Williamson et al. 2001).

Our results suggest that the current focus on individual physiological responses of amphibians to UV-B may lead to conclusions that overstate the importance of increasing ambient UV-B for natural populations. Physiological tests and experiments conducted at a small number of sites must be interpreted in the context of landscape variation in UV-B exposure to understand their effect across large spatial scales. Furthermore, the relevance of UV-B-induced mortality of embryos to large-scale population dynamics of amphibians remains a critically untested assumption of many UV studies (but see Diamond et al. 2002). The potential exists that even significant reductions in embryonic survival may never impact population growth rates if there is strong larval density-dependent competition, predation, or disease transmission (Vonesh and De la Cruz, in press). Further, despite anecdotal observations and much speculation, there are few published studies that quantitatively document amphibian population losses in the PNW, making an evaluation of potential mechanisms leading to declines in the PNW difficult at present.

Though our results suggest that current levels of UV-B coupled with the spatial pattern of DOM likely pro-
Northwest (sites in the Olympic and Cascade Ranges in the U.S. Pacific and associated species distributions for amphibian breeding (DOM) are based on direct measures of absorbance at 440 nm (UV-B flux from Kiesecker et al. (2001) (26.4 W/cm²) of water samples from each site, using ambient weighted UV-B irradiance (mW/cm²). For comparison, thin solid lines represent the equivalent diffuse attenuation coefficients (Kd), based on absorption of light at 440 nm of water samples from each site, using ambient UV-B flux from Kiesecker et al. (2001) (26.4 W/cm²). Max depth represents the percentage occurrence of R. cascadae, B. boreas, A. macrodactylum, and A. gracile at 10 cm water depth in Lost Lake. (B) Box and whisker plots representing the relative UV-B exposure of breeding locations for each species. Data were collected at Olympic and Cascade breeding sites between 1997 and 2000.

Table 1. Summary of the optical characteristics of amphibian breeding ponds.

<table>
<thead>
<tr>
<th>Species</th>
<th>N</th>
<th>A_{abs}</th>
<th>I_{UVB} (μW/cm²)</th>
<th>[DOM] (mg/L)</th>
<th>Max depth (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rana cascadae</td>
<td>50</td>
<td>1.610 ± 0.191</td>
<td>10.885 ± 1.072</td>
<td>3.369 ± 0.452</td>
<td>58.0%, &lt;1 m</td>
</tr>
<tr>
<td>Bufo boreas</td>
<td>9</td>
<td>1.639 ± 0.391</td>
<td>9.227 ± 1.705</td>
<td>3.424 ± 0.929</td>
<td>55.6%, &gt;2 m</td>
</tr>
<tr>
<td>Ambystoma macrodactylum</td>
<td>29</td>
<td>1.606 ± 0.268</td>
<td>11.770 ± 1.699</td>
<td>3.413 ± 0.624</td>
<td>72.4%, &lt;1 m</td>
</tr>
<tr>
<td>Ambystoma gracile</td>
<td>25</td>
<td>1.243 ± 0.230</td>
<td>13.073 ± 1.658</td>
<td>2.550 ± 0.532</td>
<td>36%, &lt;1 m</td>
</tr>
<tr>
<td>No breeding amphibians</td>
<td>53</td>
<td>0.998 ± 0.117</td>
<td>14.287 ± 1.007</td>
<td>1.940 ± 0.272</td>
<td>39.6%, 1–2 m</td>
</tr>
</tbody>
</table>

Notes: Data are means ± 1 se. Estimates of UV-B irradiance at 10 cm depth (I_{UVB}) and dissolved organic matter concentration ([DOM]) are based on direct measures of absorbance at 440 nm (A_{abs}). Max depth represents the percentage occurrence of the dominant category (<1 m, 1–2 m, >2 m) for breeding sites of each species.
size that the current distribution of amphibian breeding sites is due not to a recent elimination of amphibians from high UV-B areas, but rather a long-term adaptation to avoid high UV-B or factors that covary with UV-B in aquatic environments.

Due to high landscape level variation in photo-protective DOM, damaging UV-B exposure of the most sensitive amphibian stages appears to be limited to a small proportion of sites in the PNW. Our results combined with the limited observations of widespread embryonic mortality at even the most UV-B exposed sites suggests that the actual impact of UV-B exposure on amphibian populations may be quite small and highly variable across the landscape. Until tested at the landscape scale and related to actual population dynamics, the UV-B explanation for amphibian declines should be reconsidered.

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LITERATURE CITED


