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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, Houlahan 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 hypoth-

esis 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-

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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/m^3), 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 (K_d) of ponds and is strongly correlated with DOM concentration. Based on K_d 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 (Difey 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-

pect) will only act to reduce the potential for UV-B effects by reducing the ambient UV-B flux.

Water samples (125 ml) were taken at each location during and following early summer breeding seasons between 1997 and 2000. For sites with multiple samples, absorption values and DOM concentrations are arithmetic means used to characterize UV-B transparency. Water samples were filtered through a 0.2-micron nylon syringe filter (Gelman Acrodisc, 25 mm diameter, Pall Corporation, Ann Arbor, Michigan, USA) and refrigerated in the dark at 4–10°C until spectrophotometric analysis. Absorbance (A) of 440 nm light passed through a 10-cm quartz cuvette (path length, z) containing each filtered water sample was determined using a Shimadzu UV-2100 model double beam spectrophotometer (Shimadzu America, Columbia, Maryland, USA), and related to absorption (a) at 440 nm according to Lambert's and Beer's Laws, where $a_{440} = ((2.303 \times A_{440})/z)$. Dissolved organic matter samples were filtered through 0.7-micron ashed glass filters (Gelman GF-F, 47 mm diameter in the field, and transported in ashed amber glass bottles maintained in the dark at 4–10°C. Dissolved organic matter concentrations were determined by catalytic oxidation infrared detection using a Tekman-Dohrman model DC-190 carbon analyzer (Tekman-Dohrman, Mason, Ohio, USA) within 21 d of sample collection. Estimates of in situ UV-B attenuation coefficients (K_d) made at seven sites in Olympic National Park were determined as part of the U.S. EPA's (Environmental Protection Agency) PRIMENet program, and were collected on clear sky days within 2 h of solar noon (Kirk et al. 1994; Peterson et al. 2002). Attenuation coefficients reported for a given site represent the average of three consecutive light profiles, where five to six measurements were made for each profile from just above the water surface to a depth where 5% of the surface UV-B irradiance remained or the bottom of the pond was reached. Amphibian surveys at each site were conducted according to the established Visual Encounter Search method (Bury and Major 1997, Thoms et al. 1997), where two trained surveyors wade the shallow water edge of each pond extensively searching the shoreline, substrate, and macrophytes for amphibian species presence and evidence of breeding (indicated by the presence of eggs or larvae). Sites were visited and surveyed multiple times during the open water season.

Based on the published data showing Diffey-weighted UV-B irradiance vs. depth (d) from Lost Lake, Linn County, Oregon (Kiesecker et al. 2001), we determined the diffuse attenuation coefficient (K_d) by fitting these data to the function $UVB_z = UVB_0 e^{(-K_d \times z)}$. Based on this equation and a surface UV-B irradiance (UVB_0) of 26.4 $\mu\text{W}/\text{cm}^2$ (Kiesecker et al. 2001), we estimated the UV-B flux at 10 and 50 cm depths that correspond to levels that produced a 33% reduction (10 cm), and no reduction (50 cm) in *B. boreas* embryonic survivorship in Lost Lake. To compare these UV-B irradi-

ance levels to the expected levels in other breeding sites throughout the Oregon Cascades and Olympic National Park, we calculated the UV-B irradiance at 10 cm depth for each site given the same ambient UV-B level (26.4 $\mu\text{W}/\text{cm}^2$ from Kiesecker et al. 2001) and site-specific K_d values determined from our surveys. We compared this level of ambient UV-B to values recorded at Olympic National Park (data available online from the Environmental Protection Agency/National Park Service Ultraviolet Monitoring Network)⁵ by a high resolution UV spectrophotometer (Brewer #147, Mark IV Spectrophotometer, Kipp and Zonen Incorporated, Bohemia, New York, USA) within 1.5 h of solar noon each day of May, June, and July 1998–2000. Once corrected for the average elevation of our sites (1432 m, Blumthaler et al. 1997), we found that a Diffey-weighted UV-B value of 26.4 $\mu\text{W}/\text{cm}^2$ was a liberal estimate of the UV-B intensity at these sites, and falls in the top 13% of recordings (range: 1.20–33.58 $\mu\text{W}/\text{cm}^2$, mean: 18.34 $\mu\text{W}/\text{cm}^2$).

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 ($K_d = -0.0795 \times \text{absorption}_{440\text{nm}}$, $r^2 = 0.98$, $P < 0.00001$) (Fig. 1B). The concentration of DOM is a strong predictor of the absorption of 440 nm light ($\text{absorption}_{440} = 0.421 \times [\text{DOM}] + 0.198$, $r^2 = 0.91$, $P < 0.01$) (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 $\mu\text{W}/\text{cm}^2$) and no UV-B effect (12.7 $\mu\text{W}/\text{cm}^2$, 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 $< 22.4 \mu\text{W}/\text{cm}^2$.

⁵ URL: (<http://oz.physast.uga.edu/>)

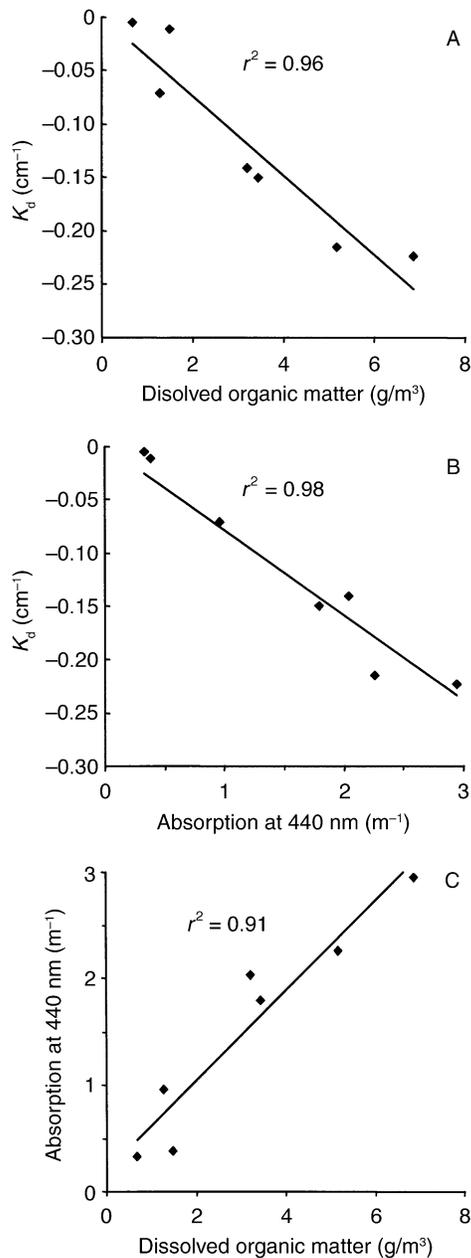


FIG. 1. Diffuse attenuation coefficient (K_d ; per centimeter) values for UV-B as a function of (A) dissolved organic matter (g/m^3 ; $K_d = -0.0371 \times [\text{DOM}]$, $r^2 = 0.96$, $P < 0.0001$), and (B) absorption of 440 nm light (per meter; $K_d = -0.0795 \times \text{absorption}_{440\text{nm}}$, $r^2 = 0.98$, $P < 0.00001$). (C) Absorption of 440-nm light is predicted by dissolved organic matter concentration ($\text{absorption}_{440} = 0.421 \times [\text{DOM}] + 0.198$, $r^2 = 0.91$, $P < 0.01$). 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 $<22.4 \mu\text{W}/\text{cm}^2$ 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 survivorship (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-

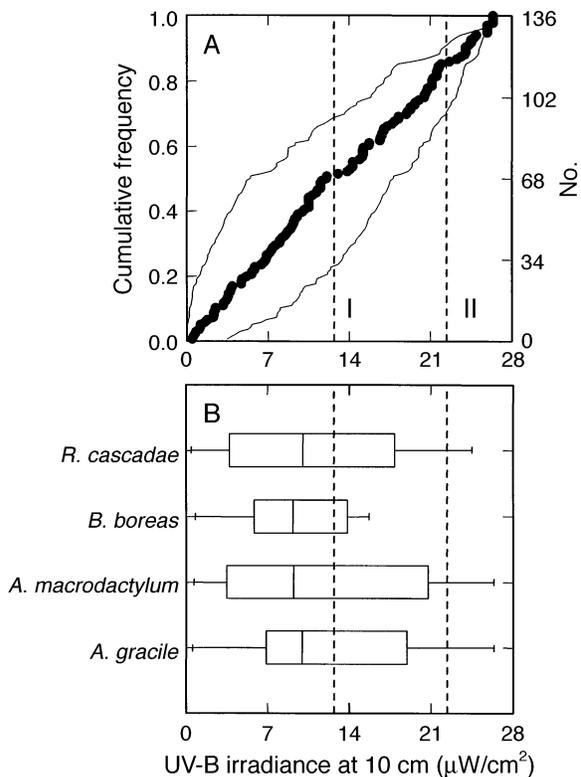


FIG. 2. Cumulative frequency of estimated Diffey-weighted UV-B irradiance ($\mu\text{W}/\text{cm}^2$) at 10 cm water depth and associated species distributions for amphibian breeding sites in the Olympic and Cascade Ranges in the U.S. Pacific Northwest ($N = 136$). (A) UV-B values were calculated from diffuse attenuation coefficients (K_d), based on absorption of light at 440 nm of water samples from each site, using ambient UV-B flux from Kiesecker et al. (2001) ($26.4 \mu\text{W}/\text{cm}^2$ at depth = 0). For comparison, thin solid lines represent the equivalent distributions for 5 cm (bottom line) and 20 cm (top line) incubation depths. Dashed lines represent UV-B flux from Kiesecker et al. (2001) that (I) produced no reduction of survivorship of *B. boreas* embryos ($12.7 \mu\text{W}/\text{cm}^2$ corresponding to 50 cm water depth in Lost Lake, Oregon, USA, elevation 1220 m), and that (II) produced a 33% reduction in survivorship of *B. boreas* embryos ($22.4 \mu\text{W}/\text{cm}^2$ corresponding to 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.

ffects most amphibian embryos from harmful levels of UV-B, there is the potential that nonlethal doses of UV-B may have lasting effects on individual growth, reproduction, and survival later in life. For example, we evaluated the importance of UV-B for amphibian embryos based on levels known to be associated with either significant mortality ($22.4 \mu\text{W}/\text{cm}^2$) or with no change in survival ($12.7 \mu\text{W}/\text{cm}^2$, from Kiesecker et al. 2001). The potential exists that nonlethal effects may occur between these two levels, or that the response of amphibians to levels of UV-B may vary between local populations (Belden et al. 2001). Furthermore, while most physiological research has focused on evaluating the embryonic sensitivity of amphibians, larval stages of some species may show equal or greater sensitivity to UV-B radiation. Recent studies of several amphibian species have demonstrated low embryonic mortality associated with UV-B exposure, but significant mortality and malformation in response to UV-B exposure in the larval stage (Ankley et al. 2000, Tietge et al. 2001; Ankley et al. 2002). However, little research has thus far focused on evaluating the potential long-term consequences of different levels of UV-B exposure in embryonic and larval stages (but see Pakkala et al. 2001). Additionally, our analysis shows that a small fraction of the breeding sites of all four species is likely exposed to harmful levels of UV-B, although under limited circumstances. While we have attempted to evaluate the exposure of amphibian embryos to UV-B under conditions that would represent a worst case scenario, the impact of species specific exposures to UV-B will ultimately depend on a variety of physiological, ecological, and physical habitat factors (Williamson 1995), many of which we are not able to explicitly evaluate. Therefore, UV-B may still be an important factor controlling amphibian population dynamics of some local systems.

While we propose that increasing ambient UV-B flux may not be an immediate threat for amphibian populations in the PNW, over longer time scales UV-B is likely to have been an important factor contributing to the current distribution of breeding sites for these species of pool-breeding amphibians. Given the level of mortality reported in physiological studies of amphibian embryonic sensitivity to UV-B (15–50%; Blaustein et al. 1994, 1995, Kiesecker et al. 2001), we hypoth-

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

| Species | N | A_{440} | $I_{\text{UV-B}}$ ($\mu\text{W}/\text{cm}^2$) | [DOM] (mg/L) | Max depth (m) |
|--------------------------------|-----|-------------------|---|-------------------|---------------|
| <i>Rana cascadae</i> | 50 | 1.610 ± 0.191 | 10.885 ± 1.072 | 3.369 ± 0.452 | 58.0%, <1 m |
| <i>Bufo boreas</i> | 9 | 1.639 ± 0.391 | 9.227 ± 1.705 | 3.424 ± 0.929 | 55.6%, >2 m |
| <i>Ambystoma macrodactylum</i> | 29 | 1.606 ± 0.268 | 11.770 ± 1.699 | 3.413 ± 0.624 | 72.4%, <1 m |
| <i>Ambystoma gracile</i> | 25 | 1.243 ± 0.230 | 13.073 ± 1.658 | 2.550 ± 0.532 | 36%, <1 m |
| No breeding amphibians | 53 | 0.998 ± 0.117 | 14.287 ± 1.007 | 1.940 ± 0.272 | 39.6%, 1–2 m |

Notes: Data are means \pm 1 SE. Estimates of UV-B irradiance at 10 cm depth ($I_{\text{UV-B}}$) and dissolved organic matter concentration ([DOM]) are based on direct measures of absorbance at 440 nm (A_{440}). Max depth represents the percentage occurrence of the dominant category (<1 m, 1–2 m, >2 m) for breeding sites of each species.

esize 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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