ECOSYSTEMS

Distribution Patterns of Lentic-Breeding Amphibians in Relation to Ultraviolet Radiation Exposure in Western North America

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ABSTRACT

An increase in ultraviolet-B (UV-B) radiation has been posited to be a potential factor in the decline of some amphibian population. This hypothesis has received support from laboratory and field experiments showing that current levels of UV-B can cause embryo mortality in some species, but little research has addressed whether UV-B is influencing the distribution of amphibian populations. We compared patterns of amphibian presence to site-specific estimates of UV-B dose at 683 ponds and lakes in Glacier, Olympic, and Sequoia–Kings Canyon National Parks. All three parks are located in western North America, a region with a concentration of documented amphibian declines. Site-specific daily UV-B dose was estimated using modeled and field-collected data to incorporate the effects of elevation, landscape, and water-column dissolved organic carbon. Of the eight species we examined (*Ambystoma gracile, Ambystoma macrodactylum, Bufo boreas, Pseudacris regilla, Rana cascadae, Rana leuteiventris, Rana muscosa, Taricha granulosa*),

two species (*T. granulosa* and *A. macrodactylum*) had quadratic relationships with UV-B that could have resulted from negative UV-B effects. Both species were most likely to occur at moderate UV-B levels. *Ambystoma macrodactylum* showed this pattern only in Glacier National Park. Occurrence of *A. macrodactylum* increased as UV-B increased in Olympic National Park despite UV-B levels similar to those recorded in Glacier. We also found marginal support for a negative association with UV-B for *P. regilla* in one of the two parks where it occurred. We did not find evidence of a negative UV-B effect for any other species. Much more work is still needed to determine whether UV-B, either alone or in concert with other factors, is causing widespread population losses in amphibians.

Key words: amphibian decline; ultraviolet-B radiation; global change; mountain ponds; national parks..

INTRODUCTION

Ecologists have hypothesized that a recent increase in surface ultraviolet-B radiation (UV-B) resulting from thinning of the ozone layer (Kerr and McEl-

roy 1993) is one factor contributing to the global decline in amphibian populations (Blaustein and others 1994). This hypothesis is supported by experiments that show higher rates of embryonic mortality and larval deformities under ambient levels of UV-B compared to reduced UV-B levels (reviewed by Corn 2000; Blaustein and others 2003; Boone and others 2003; Licht 2003). However, experiments on UV-B effects have been confined to subpopulation scales and have not been able to determine the effect of UV-B on population persistence (Hofer 2000). Without other complementary approaches, it is difficult to determine whether the deleterious effects of UV-B on individuals translate into population losses.

One of the first studies to consider the relationship between amphibians and UV-B at a larger scale used Total Ozone Mapping Spectrometer satellite data to infer that annually averaged erythemal UV-B at the atmosphere–vegetation interface had increased from 1979 to 1998 at 11 of 11 sites in Central America and at five of nine sites in South America where amphibian declines have been documented (Middleton and others 2001). However, UV-B exposure can be strongly influenced by factors such as shading and, for aquatic species, attenuation of UV-B by dissolved organic carbon (DOC) (Schindler and Curtis 1997). These factors operate at the scale of individual amphibian habitats and were therefore not addressed by Middleton and others (2001).

Two other approaches have been used to relate amphibian occurrence to UV-B at the landscape scale. One used elevation and latitude (Davidson and others 2001, 2002) and the other used the UV-B transmission properties of pond water (Adams and others 2001) as surrogates for local variation in UV-B exposure. Davidson and others (2001, 2002) found partial support for a UV-B effect for one of the eight species they examined. However, elevation and latitude are only rough surrogates for UV-B exposure because DOC is far more important in regulating UV-B exposure in aquatic habitats. Dissolved organic carbon is responsible for 85%–92% of the variation in UV-B transmission in water (Morris and others 1995; Schindler and Curtis 1997). Moreover, elevation and latitude are also correlated with climate and habitat, which may have important consequences for amphibians unrelated to UV-B. Adams and others (2001) examined the distribution of three amphibians and found that *Rana cascadae* (Cascades frog) was most likely to breed in fishless shallow ponds with relatively low transmission of UV-B radiation. This pattern is consistent with the hypothesis that UV-B influences the distribution of this species (Nagl and

Hofer 1997). However, their study also did not incorporate some major factors that can contribute to the variation in UV-B exposure among ponds.

The objective of our study was to quantify the relationship between the distribution of breeding sites and potential UV-B exposure for a suite of amphibians in western North America. We examined eight lentic-breeding amphibian species in three national parks (11 species × park combinations). We produced an estimate of the 1-day UV-B dose for each study pond using a novel approach (Diamond and others 2002, this issue) involving the use of modeled and field-collected data to incorporate the effects of location (including elevation), topography, and the optical properties of each pond. We then used these estimates as a surrogate for UV-B exposure to quantify the relationship between UV-B and amphibian occurrence.

METHODS

Study Systems

Our study ponds were located in the following three national parks in the western United States: Glacier National Park (Glacier) in northwestern Montana, Olympic National Park (Olympic) in northwestern Washington, and Sequoia–Kings Canyon National Park (Sequoia) in central California.

Glacier contains three lentic-breeding amphibian species that were sufficiently common to include in our study (Table 1). *Ambystoma macrodactylum* (long-toed salamander) and *Rana luteiventris* (Columbia spotted frog) are abundant in most areas of western Montana including Glacier, but *Bufo boreas* (western toad) may have declined in the region and extensive surveys are in progress to document its status (Maxell and others 2002). *Pseudacris regilla* (Pacific treefrog) and *Pseudacris maculata* (boreal chorus frog) are rare in this park and were not included in this study.

Olympic contains six amphibian species that breed in high-elevation ponds and lakes (Table 1). None of these lentic-breeding species are known to be declining in Olympic. However, *R. cascadae* has declined at the southern tip of its range in California (Fellers and Drost 1993), and surveys conducted in Washington for *B. boreas* found this species to be less common than expected (Richter and Azous 1995; Adams and others 1998, 1999, 2001). Few data are available for Oregon, but the numbers of *B. boreas* in several long-studied locations have not declined (Olson 2001).

Sequoia contains two lentic-breeding amphibians that were sufficiently common for inclusion in our

Table 1. Characteristics of Pond Breeding Amphibians Included in this Study

Name	Park	Elevation Range[a] (m)	Ova and Oviposition[b]
Cascades frog (*Rana cascadae*)	Olympic	800 – 2,740	Aggregations of large spherical masses in shallow, open water
Columbia spotted frog (*Rana luteiventris*)	Glacier	~2,000 – 3,050	Aggregations of large spherical masses in shallow water
Long-toed salamander (*Ambystoma macrodactylum*)	Olympic, Glacier	0 – 3,000	Variable: eggs deposited singly or in clusters in shallow to deep water
Mountain yellow-legged frog (*Rana muscosa*)	Sequoia	1,370 – 3,650+	Medium spherical masses generally in water 0–4 m deep
Northwestern salamander (*Ambystoma gracile*)	Olympic	0 – 3,100	Large spherical masses generally in water 0.5 – 1 m deep
Pacific treefrog (*Pseudacris regilla*)	Sequoia, Olympic	0 – 3,540	Small spherical masses in water 0 – 1+ m deep
Roughskin newt (*Taricha granulosa*)	Olympic	0 – 2,800	Single eggs scattered around pond on vegetation in water.
Western toad (*Bufo boreas*)	Olympic, Glacier	0 – 3,600+	Strings in shallow water

Characteristics represent those for the entire geographic range of each species.
[a]Stebbins (1985)
[b]Nussbaum and others (1983)

study (Table 1). *Rana muscosa* (mountain yellow-legged frog) has declined severely in Sequoia over the past century (Bradford and others 1994; Drost and Fellers 1996; Jennings 1996; Knapp and Matthews 2000) and is currently being considered for listing under the federal Endangered Species Act. *Pseudacris regilla* may also have experienced declines in the Sierra Nevada region (Drost and Fellers 1996; Matthews and others 2001), but this species is still common in Sequoia (R.A.K. unpublished). *Bufo boreas* and *B. canorus* (Yosemite toad) are both uncommon and were therefore not included.

Amphibian Surveys

Surveys were conducted in seven, four, and five drainages in Glacier, Olympic, and Sequoia, respectively. We selected drainages nonrandomly to ensure that the study sites encompassed much of the topographic and climatological variation present in each park and were relatively easy to access on foot. In Glacier and Olympic, we surveyed all accessible lentic habitats (hereafter "ponds") within each drainage, ranging from small ephemeral pools (around 9 m²) to larger permanent lakes (larger than 10 ha). In Sequoia, four of five drainages contained more ponds than we anticipated being able to survey (often more than 100 ponds per drainage). In each of these four drainages, 20 ponds were chosen at random. All ponds in the fifth drainage were surveyed (*n* = 12). The total number of ponds surveyed was 391 in Glacier, 215 in Olympic, and 92 in Sequoia. Most ponds in

Glacier and Olympic were surveyed at least twice during the summer months. Ponds in Sequoia had simpler habitat conditions than those in Glacier or Olympic (for example, little or no aquatic vegetation). Therefore, most ponds were surveyed only once (Knapp and Matthews 2000). Based on multiple surveys of the same sites, we estimated that the probability of missing an amphibian species in Glacier when two or more visits were conducted was 0.01 for *A. macrodactylum*, 0.02 for *B. boreas*, and 0.06 for *R. luteiventris*. The probability of missing species in Olympic ranged from zero (*B. boreas*, *A. gracile*, and *R. cascadae*) to 0.2 (*P. regilla*), with *T. granulosa* and *A. macrodactylum* having probabilities of 0.12 and 0.01, respectively. In Sequoia, the probability of missing *R. muscosa* or *P. regilla* when only a single site visit was conducted was zero. For all amphibians in this study, false negatives were most common for very small populations or for surveys conducted very early or late in the season.

Amphibian surveys in Glacier and Olympic were conducted by two people slowly walking the perimeter of a pond following a zigzag pattern through all waters less than 0.5 m deep. One worker waded while the other searched the shoreline and recorded any amphibian observations. Because the habitat conditions in Sequoia were simpler, a single worker conducted the amphibian surveys (Knapp and Matthews 2000). We recorded the presence of each stage of amphibian species encountered, including eggs, larvae, juveniles, and adults. An amphibian species was considered to be present at a pond if evidence

of breeding (that is, eggs or larvae) was detected during at least one survey at that site. Fish were considered to be present in a pond if they were seen during the amphibian survey or if they were found in any inlet or outlet streams. One of us (R.A.K. unpublished) previously tested this protocol by conducting both visual and gill net surveys in 1,103 water bodies. With inlets and outlets included in the search, visual surveys missed fish detected by gill netting in only 25 cases (2%).

Habitat Characterization

The primary substrate of each pond was characterized as silt (particle size less than 0.5 mm in diameter), sand (0.5–2 mm), gravel (more than 2–75 mm), cobble (more than 75–300 mm), boulder (more than 300 mm), bedrock, wood, or leaf litter using one of the following two techniques: (a) If the pond was small enough for a worker to observe the entire substrate from one location (typically less than 500 m^2 in surface area and less than 3 m deep), the worker noted the percentage of each substrate type occurring in a band around the pond perimeter and extending 3 m out from shore; (b) in larger ponds, the dominant substrate type was visually estimated in two to 22 plots ($\bar{x}= 14$) equally spaced around the pond perimeter and placed perpendicular to shore. Each plot was 2 m wide and extended 3 m out from shore into the water. We also recorded the proportion of each plot occupied by emergent vegetation (or for small sites, the proportion of the 3-m–wide shoreline band occupied by emergent vegetation). We visually estimated the maximum depth in each pond as less than 1, 1–2, or more than 2 m.

Ultraviolet-B Dose Estimates

Methods used to estimate UV-B doses are described in detail in Diamond and others (this issue) and are summarized here in the following steps:

1. Parkwide estimates of UV-B dose were based on ground-level, hourly UV-B (280–320-nm wavelengths) data collected by Brewer spectrometers over the past 5 years at a single location in each national park (see http://www.epa.gov/uvnet/). These data were analyzed to determine the average of the highest 95th percentile of UV-B doses. This value is an estimate of the maximal clear-sky dose typical of each Brewer location.

2. A GIS-based solar radiation model, Solar Analyst (HEMI, Los Alamos, NM, USA), was used to estimate total solar radiation dose (300–3,000 nm) for each Brewer location and for each studied pond. Solar Analyst was used to incorporate the effect of topographic and vegetative features on solar radiation dose in the vicinity of the Brewer and pond locations. The Solar Analyst–estimated dose for each pond was divided by the dose estimated for the park Brewer location to derive a proportionality factor that represented the relative effect of landscape for each pond location.

3. A second solar radiation model, SBDART (Santa Barbara DISORT [Discrete Ordinate Radiative Transfer] Atmospheric Radiative Transfer) (see http://www.crseo.ucsb.edu/esrg/pauls_dir/), was used to generate a solar spectrum (comprised of intensity values for each nm of wavelength from 280 to 3,000 nm) for each Brewer location. These spectra were adjusted so that the energy present was representative of the maximal daily dose derived from the Brewer location data. The spectrum was then multiplied by the proportionality factor calculated from the Solar Analyst values. The result was a spectrum comprised of intensity values for each nm of wavelength, from 280 to 700 nm, representing the average spectral intensity for the summer solstice at the surface of each pond.

4. The spectra were reduced by 6.5% to account for surface reflection. Water-column attenuation was incorporated by multiplying the spectrum for each pond by the proportionate transmittance derived from laboratory scans (1-cm path length) of filtered water samples taken during amphibian surveys. Water samples (60 ml) were collected on the north side of each pond, away from any inlets, 10 cm below the surface, during amphibian surveys. They were filtered in the field using 0.7-μm ashed glass fiber filters and were kept cool until analysis (approximately 1 week). The final solar spectrum was an estimate of the average solar radiation intensity on the summer solstice, at a depth of 1 cm in each pond.

5. Finally, pond UV-B doses were calculated by integrating each spectrum from 280 to 320 nm and multiplying by the length of the summer solstice at each location.

The resulting values are estimates of maximal (cloud-free conditions), summer solstice UV-B doses for a 1-cm depth in each wetland. The models incorporate average atmospheric conditions that include appropriate values for ozone, particulates, aerosols, water vapor, and other factors. Vegetative

Table 2. Description of Pond Types Developed using Cluster Analysis

Pond Type	Fish	Depth (m)	Substrate/Cover Type[a]	Sample Size		
				Glacier	Olympic	Sequoia
MixedPond	No	<1	1	76	(2)	(0)
MudPond	No	<1	2	146	116	(2)
RockPond	No	<1	3, 4	14	35	8
MixedLake	No	1–2, >2	1, 2	48	16	(3)
CobbleLake	No	1–2, >2	3	23	22	12
BoulderLake	No	1–2, >2	4	10	(2)	34
FishPond	Yes	<1	Any	34	(1)	(1)
FishLake	Yes	1–2, >2	Any	40	21	23

Pond types too rare for analysis (n < 6) are indicated by parentheses around the sample size.
[a]Type 1, mixed substrates dominated by leaves and mud and with high ($\bar{x}$= 58%) vegetative cover (n = 10); Type 2, mud substrates with moderate ($\bar{x}$= 29%) vegetative cover (n = 382); Type 3, rock substrates dominated by cobble with low ($\bar{x}$< 1%) vegetative cover (n = 132); Type 4, rock substrates dominated by boulder with low vegetative cover (n = 74)

features were assumed to have shading effects similar to topographic features that would occlude portions of the sky. No effort was made to adjust these values for typical weather conditions, because little or no climatological data are available for specific wetland locations. However, it is reasonable to assume that average weather conditions would not greatly alter the relative UV-B dose among wetlands within parks and that our UV-B estimates provide a reasonable basis for comparing relative UV-B exposure and amphibian distributions. The uncertainties in these estimates are discussed in detail by Diamond and others (this issue).

In summary, our UV-B dose estimates incorporate ground-based UV-B data and the effects of region, elevation, local topographic and vegetative features, and attenuation by water-column DOC. The strength of this approach is that it provides a consistent value for comparing potential UV-B exposure among ponds.

Statistical Analysis

To facilitate regression analyses of the influence of UV-B dose on amphibian occurrence (detected/not detected for each pond surveyed), we first created pond categories using a cluster analysis of individual habitat variables. We developed pond categories because individual habitat variables were often highly colinear (for example, % silt negatively correlated with % boulder) and could therefore not be treated as independent variables in regression analyses. Moreover, we were not interested in amphibian associations with habitat per se, but rather wanted to account for potential confounding effects of habitat in our analysis of UV-B. To create pond categories that were consistent across all three national parks, we used a single cluster

analysis of pond substrate types and the extent of emergent vegetation for all ponds from Glacier, Olympic, and Sequoia. Substrate characteristics for each pond were first summarized by calculating the proportion of plots dominated by each substrate type. The extent of emergent vegetative cover was calculated as the mean proportion of each plot occupied by emergent vegetation averaged over all plots. Estimates of substrate composition and emergent vegetative cover were then used as input into a K-means cluster analysis using NCSS software (Hintze 1998). After examining the output for two to six clusters, we elected to use the analysis that produced four clusters because further divisions became difficult to interpret (Table 2). To further categorize ponds, we then crossed the four clusters with the three pond depth categories and fish occurrence to produce 24 categories. These were reduced to eight ''pond types'' by combining similar categories that were poorly represented in one or more parks (Table 2).

To evaluate the association between amphibian occurrence and UV-B, we used an information theoretics approach that ranked models based on the small-sample version of Akaike's Information Criterion (AIC_c) (Burnham and Anderson 2002). The strength of this approach is that, unlike null hypothesis testing, it is well suited for revealing ambiguities in the results. AIC_c is a measure of the information content of a model relative to the number of parameters in the model. It provides a way to rank models, with lower AIC_c indicating a comparatively better model given the data.

We used a two-step analysis to evaluate the association between amphibian occurrence and UV-B. The first step in our analysis was to use stepwise selection to find the best habitat model.
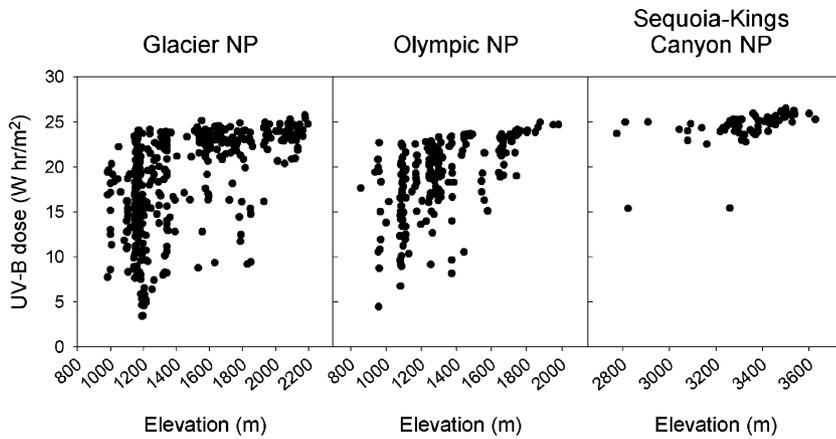
Figure 1. Relationship between estimated daily UV-B dose at 1-cm depth and elevation for ponds in Glacier, Olympic, and Sequoia–Kings Canyon national parks.

Stepwise selection began with a logistic regression model containing PondType and Elevation as predictors. For each case (each pond), the response variable was coded 1 if the species was detected and 0 if the species was not detected. The stepwise procedure was allowed to seek the lowest AIC_c by either adding the interaction term or dropping main effects. The second step in our analysis was to evaluate the effect of adding various UV-B terms to the best habitat model. This produced three or four additional models (depending on the number of terms in the habitat model): habitat + UV-B, habitat + UV-B + UV-B^2, habitat + UV-B + [UV-B × PondType] (if PondType was in the habitat model), and habitat + UV-B + [UV-B × Elevation] (if Elevation was in the habitat model). The quadratic term was evaluated because we hypothesized that negative effects might be seen only at the highest UV-B levels. The new models were then ranked using ΔAIC_c which is the difference in AIC_c between the AIC_c of the given model and the model with the lowest AIC_c. Models with ΔAIC_c of 2 or less are considered to be competing for best model status and lack a clear indication that one is better than the others (Burnham and Anderson 2002). We describe the predictive ability of the best model relative to the habitat model using Maximum Rescaled R^2 (MR-R^2) (Allison 1999). MR-R^2 is roughly analogous to the R^2 in Guassian regression but represents the predictive ability of the model rather than the percent variance explained.

This analysis was conducted using S-Plus 2000 and the S-Plus functions ''stepAIC'' and ''extractAIC'' from the MASS library (Anonymous 1999; Venables and Ripley 1999). The predictor variables were independent of each other, with Elevation (Figure 1) and PondType explaining 3%–28% of the variance in UV-B. We developed regression models for all amphibian species found at more

than 10% of the ponds we surveyed in Glacier, Olympic, or Sequoia. Only pond types represented by at least six ponds were included for any given park. This caused us to drop some ponds from the logistic regression analysis, reducing the sample sizes to 367 for Glacier, 210 for Olympic, and 77 for Sequoia. For each species–park combination, all of the pond types included in the analysis had at least one site where the species was detected and one site where the species was not detected. For amphibian species that were found at less than 10% of sites in any particular park, we evaluated the association between species occurrence (detected/not detected) and both UV-B dose and Elevation using univariate Wilcoxon rank sum tests.

RESULTS

Estimated mean daily UV-B dose at 1-cm depth on the summer solstice ranged from 3.4 to 25.8 W h/m^2 in Glacier ($\bar{x}$ = 18.5, n = 391 ponds), 4.4 to 25.0 W h/m^2 in Olympic ($\bar{x}$ = 19.2, n = 215 ponds), and 15.4 to 26.5 W h/m^2 in Sequoia ($\bar{x}$ = 24.6, n = 83 ponds) (Figure 1). Seven amphibian species were sufficiently common to be included in the logistic regression analyses. However, because *A. macrodactylum* was found in both Glacier and Olympic, we conducted eight separate analyses.

In Glacier, all eight pond types were sufficiently common to include in the analysis, and *A. macrodactylum* and *R. luteiventris* were both found in six of eight pond types. Logistic regression analyses produced a single best model (ΔAIC_c less than or equal to 2) for *A. macrodactylum*, which indicated that the odds of detecting a breeding population was a quadratic function of UV-B (Table 3). The percentage of ponds occupied by *A. macrodactylum* peaked at moderate UV-B levels (Figure 2A).

Table 3. Logistic Regressions Relating the Occurrence of Amphibians to UV-B based on Field Surveys in 1999 and 2000

Model	ΔAIC_c	df	Residual Deviance
Glacier National Park			
Ambystoma macrodactylum			
+UVB+UVB^2	0	13,353	337.47
+UVB×PondType	4.4	17,349	333.07
+UVB	6.7	12,354	346.34
+UVB×Elevation	7.9	13,353	345.39
PondType×Elevation	24.4	11,355	366.15
Rana luteiventris			
Elevation	0	1,365	405.63
+UVB	0.4	2,364	404.01
+UVB×Elevation	1.9	3,363	403.46
+UVB+UVB^2	2.4	3,363	403.99
Olympic National Park			
Ambystoma gracile			
+UVB	0	6,203	191.42
+UVB+UVB^2	0.7	7,202	190.00
+UVB×Elevation	0.9	7,202	190.17
+UVB×PondType	1.0	10,199	183.68
PondType+Elevation	6.3	5,204	199.90
Ambystoma macrodactylum			
+UVB×Elevation	0	3,206	251.28
+UVB	6.8	2,207	260.18
+UVB+UVB^2	8.9	3,206	260.16
Elevation	17.4	1,208	272.81
Rana cascadae			
+UVB+UVB^2	0	6,203	243.24
+UVB	1.0	5,204	246.35
+UVB×PondType	4.2	9,200	240.84
PondType	5.6	4,205	253.06
Taricha granulosa			
+UVB+UVB^2	0	11,198	133.98
PondType×Elevation	3.2	9,200	141.61
+UVB	5.4	10,199	141.58
+UVB×Elevation	6.7	11,198	140.64
+UVB×PondType	12.8	14,195	139.84
Sequoia–Kings Canyon National Park			
Pseudacris regilla			
+UVB	0	2,74	89.23
Elevation	2.0	1,75	93.42
+UVB+UVB^2	2.1	3,73	89.13
+UVB×Elevation	2.2	3,73	89.19
Rana muscosa			
PondType+Elevation	0	4,72	89.85
+UVB	2.4	5,71	89.84
+UVB+UVB^2	3.8	6,70	88.88
+UVB×Elevation	4.6	6,70	89.64
+UVB×PondType	6.5	8,68	86.55

Models are ranked based on ΔAIC_c. The habitat models are the only models not proceeded by a ''+''. All other models add the indicated UV-B terms to the habitat model.

Adding UV-B and UV-B^2 to the habitat model improved the MR-R^2 from 0.43 to 0.49. For *R. luteiventris*, logistic regression analysis yielded three competing models (Table 3). The best model contained only Elevation as a predictor, but two of the UV-B models could not be ruled out (Figure 2B). Models adding UV-B and the interaction between UV-B and Elevation both had ΔAIC_c less
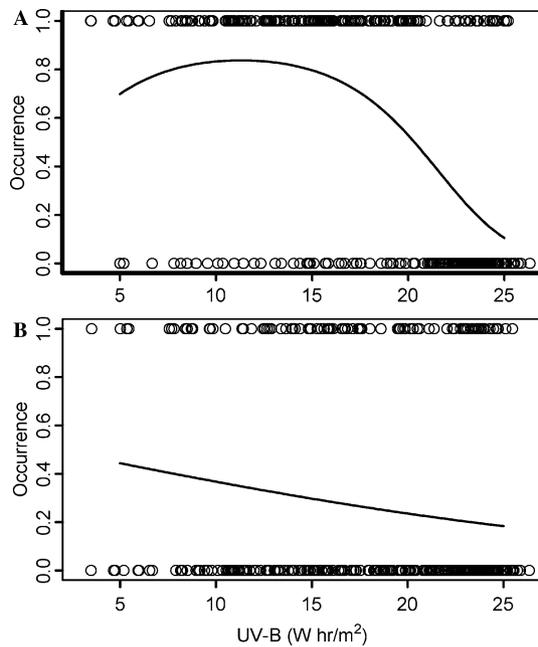
Figure 2. Relationship between amphibian occurrence and estimated daily UV-B dose for **A** *Ambystoma macrodactylum* and **B** *Rana luteiventris* in Glacier National Park based on field surveys in 1999 and 2000.

than or equal to 2 and suggested negative associations with UV-B, but were not good enough (based on ΔAIC_c values) to replace the habitat model. The best of these (UV-B main effect only) improved the MR-R^2 from 0.056 for the habitat model to 0.062, indicating that all models had low predictive ability.

In Olympic, five of eight pond types were sufficiently common to include in the analysis, and all four amphibians analyzed occurred in all five pond types. Logistic regression analysis yielded four competing models for *A. gracile* (Table 3). The simplest of these indicated that the odds of detecting *A. gracile* increased as UV-B increased, but the main effect of UV-B in the absence of the habitat variables (shown in Figure 3A) was slightly negative. All of the UV-B models had ΔAIC_c less than 2, suggesting some evidence of quadratic response functions or interactions, but none of the models were strong enough to be clearly supported over the others. Adding UV-B to the habitat model improved the MR-R^2 from 0.37 to 0.41. Logistic regression analysis for *A. macrodactylum* produced a single best model that included an interaction between UV-B and Elevation (Table 3). The odds of detecting *A. macrodactylum* increased as UV-B increased (Figure 3B), but the magnitude of the slope

varied with elevation. Adding UV-B and its interaction with Elevation to the habitat model improved the MR-R^2 from 0.08 to 0.20. Logistic regression analysis yielded two competing best models for *R. cascadae* (Table 3). The simplest of these suggested that the odds of detecting *R. cascadae* increased as UV-B increased (Figure 3C). The other model included the UV-B quadratic term. AIC_c for these models was not sufficiently different to separate them. Adding UV-B to the habitat model improved the MR-R^2 from 0.16 to 0.22. Logistic regression analysis indicated that the odds of detecting *T. granulosa* was a quadratic function of UV-B (Table 3). The proportion of occupied ponds peaked at moderate UV-B levels (Figure 3D). Adding UV-B and UV-B^2 to the model improved the MR-R^2 from 0.42 to 0.46.

In Sequoia, four of eight pond types were sufficiently common to include in the analysis, and *P. regilla* and *R. muscosa* occurred in all four pond types. Logistic regression analysis yielded two competing models for *P. regilla* (Table 3). The simplest of these indicated that the odds of detecting *P. regilla* decreased as Elevation increased. The other model indicated that the odds of detecting *P. regilla* decreased as UV-B increased (Figure 4A). The Elevation model had ΔAIC_c equal to 2, which only marginally qualifies it as competing with the UV-B main effect model for best-model status. Because the ΔAIC less than or equal to 2 rule is only a rough guideline, we conclude that this analysis provides marginal support for a negative relationship between *P. regilla* occurrence and UV-B in Sequoia. Adding UV-B to the habitat model improved the MR-R^2 from 0.04 to 0.11, indicating that predictive ability was poor for both models. Logistic regression analysis yielded a single best model for *R. muscosa* (Figure 4B and Table 3) that included only habitat variables. Adding UV-B to this habitat model had no effect on MR-R^2 to three decimal places (MR-R^2 equal to 0.235).

Bufo boreas in Glacier and Olympic and *P. regilla* in Olympic were too rare to be included in the logistic regression analysis. In all three cases, Wilcoxon rank-sum tests did not reveal differences in UV-B dose between sites where a species was detected and sites where it was not detected (*B. boreas* in Glacier: $Z = -0.37$, $P = 0.71$; *B. boreas* in Olympic: $Z = 1.04$, $P = 0.30$; *P. regilla* in Olympic: $Z = 0.82$, $P = 0.41$) (Figure 5). In contrast, elevation was significantly lower for occupied sites than for unoccupied sites for *B. boreas* in Olympic ($Z = 3.32$, $P = 0.001$) and *P. regilla* in Olympic ($Z = 2.93$, $P = 0.003$). *B. boreas* sites in Glacier were
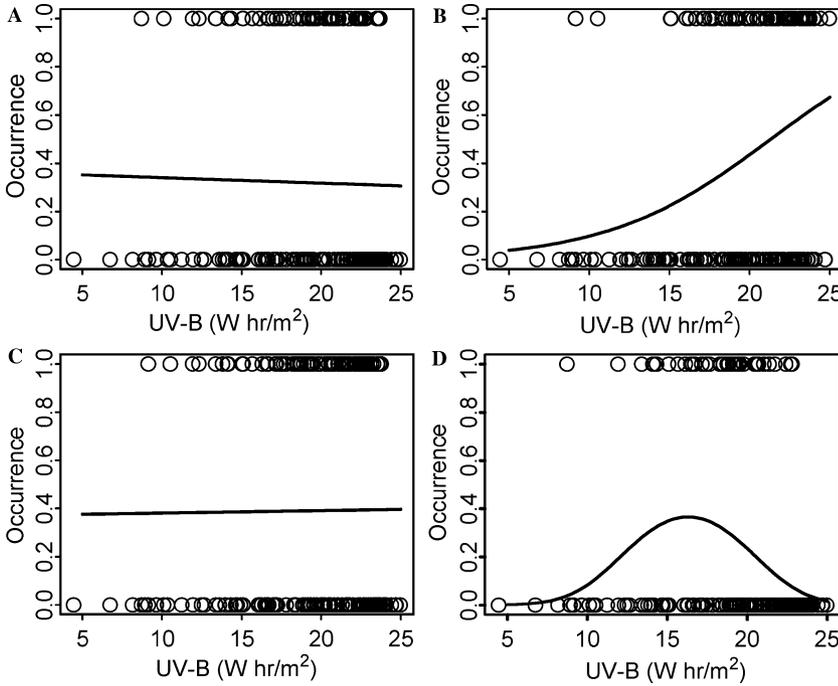
Figure 3. Relationship between amphibian occurrence and estimated daily UV-B dose for **A** *Ambystoma gracile*, **B** *A. macrodactylum*, **C** *Rana cascadae*, and **D** *Taricha granulosa* in Olympic National Park based on field surveys in 1999 and 2000.
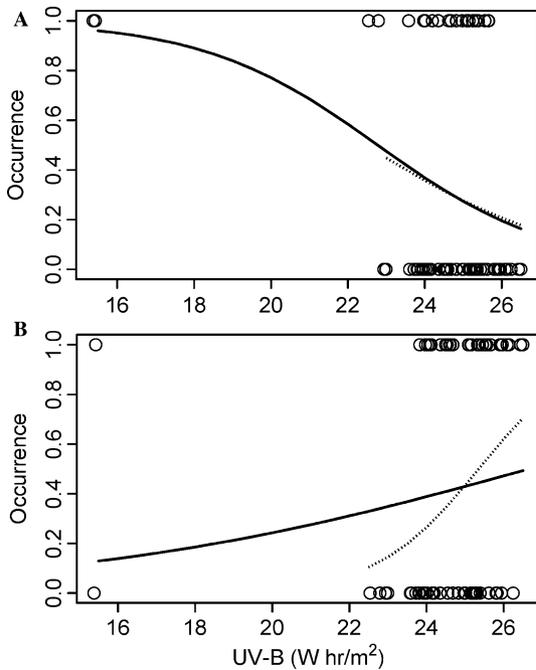
Figure 4. Relationship between amphibian occurrence and estimated daily UV-B dose for **A** *Pseudacris regilla* and **B** *Rana muscosa* in Sequoia–Kings Canyon National Park based on field surveys in 1999 and 2000. The dotted lines represent models that were fit with the low UV-B outliers omitted.

Figure 5. Relationship between breeding occurrence (present = 1, absent = 0) and estimated daily UV-B dose for three species–park combinations that were too rare to include in regression analyses. Sample sizes are given above the x-axis. Shaded boxes indicate the 25th and 75th percentiles; bars show the 10th and 90th percentiles; dots are the remaining values that fall outside the 10th and 90th percentiles. The medians are indicated by horizontal bars in the shaded boxes. NS, difference not statistically significant; **$P < 0.01$.

not significantly higher than unoccupied sites ($Z = 1.70$, $P = 0.09$) (Figure 5).

DISCUSSION

Our study focused on eight amphibian species. Prior to our work, the negative effects of UV-B on amphibians were best documented for two of these species, *B. boreas* and *R. cascadae*. In field experiments conducted in Oregon, the embryos of both species had reduced survival under ambient UV-B compared to reduced UV-B (Blaustein and others 1994; Kiesecker and Blaustein 1995), and both species are known to be declining in portions of their ranges (Corn and others 1989; Drost and Fellers 1996). However, results from experiments on *B. boreas* embryos have been inconsistent. Experiments conducted on *B. boreas* embryos from Colorado (where population declines have been documented) (Carey 1993; Muths and others 2003) and Washington using methods similar to those used by Blaustein and others (1994) did not demonstrate significantly lower mortality in ambient UV-B compared to reduced UV-B environments (Corn 1998, P. S. C. unpublished). In the current study, we failed to find convincing evidence of a negative association with UV-B for either species. Our sample size for *B. boreas* was not large enough to conduct a logistic regression analysis, but UV-B was not lower at ponds where *B. boreas* was detected compared to where it was not detected in both parks where this species occurred. *Rana cascadae* occurred in only one of the three parks we examined (Olympic), but it was the most frequently encountered species. Our results suggested that the odds of detecting *R. cascadae* increase as UV-B increases, but a subtle quadratic effect could not be ruled out.

Experiments conducted with *A. macrodactylum* and *A. gracile* have also shown negative effects of ambient UV-B levels (Kiesecker and Blaustein 1995; Blaustein and others 1997), but neither species is known to be experiencing broad declines. In our study, *A. macrodactylum* had a reduced probability of occurrence at high-UV-B sites in Glacier but the opposite was true in Olympic. These contradictory results provide mixed support for the UV-B hypothesis and suggest that there may be regional differences in effects (Pahkala and others 2002). However, UV-B levels differed little between Olympic and Glacier, which provides little basis for selection to bring about differences among populations (Cummins 2003). We did not find clear evidence of a negative association between *A. gracile* and UV-B.

Our results for *R. luteiventris* and *R. muscosa* suggested that UV-B had no negative effect on either species, but two models describing a negative association between *R. luteiventris* and UV-B could not be eliminated from consideration (both had ΔAIC equal to or less than 2). The lack of a negative effect of UV-B in the single best model for each species is consistent with the lack of response by *R. luteiventris* and *R. muscosa* embryos to experimental manipulation of UV-B (Blaustein and others 1999; Vredenburg 2002). The ambiguity of our results for *R. luteiventris* suggests that the relationship of this species with UV-B may require further examination.

Pseudacris regilla is thought to have low vulnerability to UV-B due to high photolyase activity that repairs UV-B damage to DNA (Blaustein and others 1994) and a demonstrated lack of response to experimental manipulation of UV-B (Ovaska and others 1997; Anzalone and others 1998; Vredenburg 2002). In our study, this low vulnerability was supported in Olympic, where UV-B dose did not differ between ponds with and without breeding populations of *P. regilla*. However, because only four *P. regilla* populations were encountered in Olympic, this is a weak test of the UV-B hypothesis. In Sequoia, we found marginal support for a negative association with UV-B, but the association was weak (MR-R^2 = 0.11 with habitat and UV-B in the model). Taken together, the high photolyase activity of this species, lack of response to UV-B manipulation, lack of association with UV-B in Olympic, and the low explanatory power of UV-B in Sequoia suggest that UV-B is unlikely to be exerting a large influence on the distribution of this species. However, our results provide marginal support for a negative UV-B effect, and future studies should also consider whether an interaction between UV-B and some other stressor might be causing localized population losses (see, for example, Carey 1993; Kiesecker and Blaustein 1995; Blaustein and Kiesecker 2002).

We found a quadratic relationship between *T. granulosa* and UV-B. This is consistent with the hypothesis that populations cannot persist at the highest UV-B levels. *Taricha granulosa* is thought to have low vulnerability to UV-B due to high photolyase activity (Blaustein and others 1994); however, there is some evidence of sublethal effects (Belden and Blaustein 2002). We know of no evidence that *T. granulosa* is declining.

Three potential shortcomings of our study deserve additional discussion. First, our UV-B dose estimates do not represent the dose actually experienced by an amphibian. Because of this, and because the effects of UV-B are cumulative, the 1-day UV-B doses that we estimated cannot be compared directly to any known thresholds of UV-B toler-

ance. Rather, they are appropriate for comparing relative UV-B exposure among ponds. The actual dose that an organism receives will depend on their behavior and will be a function of the time they spend at different levels of exposure.

Second, our surveys provide only a snapshot of amphibian occurrence patterns. We do not know population trends or whether the breeding populations we identified resulted in any recruitment. Because of this, it is impossible to distinguish between a decline in occurrence caused by recent increases in UV-B and limitations imposed by a species' innate tolerance of UV-B radiation. It is also possible that some populations were sinks and that we over- or underestimated the effect of UV-B. However, it is generally more appropriate to examine pond-breeding amphibians at the meta-population scale (as we did) than at the population scale because individual populations can be highly variable in abundance (Pechmann and others 1991; Green 1997).

Third, it is possible that we underestimated the effect of UV-B because we were unable to rigorously analyze data on rare species with small sample sizes. These species may be rare precisely because of the negative effects of UV-B. Species such as *B. boreas*, which some researchers have suggested may have suffered population losses due in part to UV-B (Blaustein and others 1994), require a more focused sampling effort to adequately characterize the UV-B levels at sites where they occur.

Before our study, the evidence for and against the hypothesis that the recent increase in UV-B has contributed to some amphibian declines was derived almost entirely from field and laboratory experiments conducted at subpopulation scales (reviewed by Blaustein and others 1998; Licht 2003). Although some of these experiments suggested that ambient levels of UV-B can reduce the survival of amphibian embryos, the results are difficult to link to amphibian declines for two reasons. First, most published studies did not provide estimates of the UV-B dose received by the experimental organisms (but see Crump and others 1999; Pahkala and others 2001). This does not in any way negate their results, but it makes it difficult to compare studies and to extrapolate results. For example, the conflicting results obtained for some species might simply be explained by differences in ambient UV-B or DOC. Recent work suggests that one site where many UV-B experiments have been conducted has unusually low DOC (Palen and others 2002). A second difficulty is that increased mortality of embryonic and larval amphibians does not necessarily translate into population declines (Biek and others 2002; Vonesh and de la Cruz 2002). Indeed, premetamorphic survival has long been thought to be only weakly related to the population dynamics of many amphibian species (Wilbur 1980). For example, *B. boreas* populations at two sites in Oregon where high embryonic mortality has been linked to UV-B (Kiesecker and Blaustein 1995, 1997) have not suffered declines in the past 10 years (Olson 2001). Therefore, the experiments published to date, although valuable, can only suggest the potential for any UV-B increases to cause amphibian declines.

Although UV-B may increase embryonic or larval mortality (based on previous field and laboratory experiments), our findings indicate that UV-B dose is not a strong predictor of current distribution for most species in the national park sites that we examined. Comparative studies such as ours cannot show cause and effect, but they provide an essential context for field and laboratory experiments. In this case, we found patterns consistent with the UV-B hypothesis for *T. granulosa* and, to a lesser extent, for *A. macrodactylum* and *P. regilla*. The species for which there was the most prior support for the UV-B hypothesis (*B. boreas, R. cascadae*) did not conform to the predictions of this hypothesis in our study.

We suggest four areas of research that will complement the vast body of experiments on the effects of UV-B on individuals and help to clarify whether current or future levels of UV-B may be a threat to amphibian populations: (a) additional comparative studies, including long-term monitoring, to quantify the relationship between amphibian distributions and a variety of stressors including UV-B dose; (b) studies on a range of amphibian species to identify UV-B dose thresholds above which negative population-level consequences are expected and to assess how amphibian behaviors affect the dose they receive (for example, Nagl and Hofer 1997); (c) additional collection of detailed species-specific demographic information that could be used to evaluate potential mechanisms of decline (including UV-B) in the context of population level processes (Biek and others 2002; Vonesh and de la Cruz 2002); and (d) population-scale manipulations of exposure to UV-B by amphibians.

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