Does UV-B Radiation Affect Embryos of Three High Elevation Amphibian Species in California?

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Declines and extinctions of amphibians in well-protected habitats suggest that global atmospheric factors may be responsible. We tested effects of field exposures of ultraviolet radiation (UV-B) on embryo hatching success and time to hatching in three anurans that inhabit high elevation areas of the Sierra Nevada in California, USA. While few obvious environmental impacts have occurred in the high elevation area of the Sierra Nevada, two of the three most common anurans, the Yosemite Toad (*Bufo canorus*) and the Southern Mountain Yellow-legged Frog (*Rana muscosa*), have suffered severe population declines while the sympatric Pacific Treefrog (*Pseudacris regilla*) has remained relatively abundant. Previous studies have shown that hatching of *P. regilla* embryos at lower elevations are not affected negatively by UV-B radiation. We hypothesized that differences in UV sensitivity may help explain why *P. regilla* remain abundant while *B. canorus* and *R. muscosa* have declined sharply. We conducted field experiments at two remote sites above 3030 m elevation over two years. No effect of UV-B was found on hatching success or rate of development in embryos of *B. canorus*, *P. regilla*, or *R. muscosa*, except for a small, context-dependent increase in time to hatching in *R. muscosa*. We recommend that research efforts on these species in the Sierra Nevada concentrate on post-hatching effects of UV-B, or on other decline hypotheses.

GLOBAL biodiversity is being lost at an unprecedented rate, and amphibians have attracted a lot of attention because species are suffering population declines and extinctions even in well-protected habitats (Blaustein and Wake, 1990; Alford and Richards, 1999; Houlahan et al., 2000). Ultraviolet radiation (Blaustein et al., 1994), climate change (Pounds, 2001; D'Amen and Bombi, 2009), environmental contaminants (Davidson et al., 2001; Bank et al., 2006), introduced species (Knapp and Matthews, 2000; Gillespie, 2001; Pilliod and Peterson, 2001), and infectious agents (Berger et al., 1998; Bosch et al., 2001; Daszak et al., 2003) have each been investigated as causes associated with declining amphibians in protected areas.

The UV-B hypothesis has probably generated more controversy than any other about amphibian population decline (Licht, 2003; Blaustein et al., 2004; Corn and Muths, 2004). The hypothesis states that increased mortality rates have occurred in amphibians due to recent increases in ambient levels of harmful ultraviolet radiation. Anthropogenic activities have altered the global atmosphere resulting in a reduction of stratospheric ozone (Blumthaler and Ambach, 1990; Muller et al., 1997; Rex et al., 1997). A thinner ozone layer allows more biologically damaging ultraviolet radiation (in the UV-B waveband 280–315 nm) to reach the Earth's surface (Middleton et al., 2001; Helbling and Zagarese, 2003). Most of the reduction in ozone occurs in winter and spring, and thus could be significant to amphibians in areas such as North America, where animals breed in early spring (Kerr and McElroy, 1993; Muller et al., 1997; Rex et al., 1997). Most of the focus has been on embryo mortality, and much of the controversy arises because there are many factors that can affect the amount of damaging light reaching the embryos (Licht, 2003).

Previous field experiments have shown that a variety of amphibian species are sensitive to lethal and sublethal effects of UV-B radiation at various life history stages (Blaustein et al., 1998, 2003; Pahkala et al., 2000; Bancroft et al., 2008a; Croteau et al., 2008a); however, not all species that are sensitive are in decline (Blaustein and Kats, 2003). Water chemistry, cloud cover, precipitation patterns, and natural defense mechanisms all play key roles in protecting aquatic species from harmful solar radiation. Dissolved organic carbon (DOC) naturally occurring in water effectively blocks harmful UV-B radiation, and many aquatic systems seem to have enough DOC to protect aquatic species even if UV-B radiation increases (Palen et al., 2002; Brooks et al., 2005). Seasonal variations in breeding and the amount of precipitation experienced at sites can also affect amphibian exposure to high UV-B doses (Kiesecker et al., 2001; Corn and Muths, 2002, 2004). Some species of amphibians are able to overcome the damaging effects of UV-B radiation with DNA repair mechanisms (Blaustein et al., 1994) and other defenses (Hofer and Mokri, 2000; Lesser et al., 2001; Hansen et al., 2002a). All of these factors have led to complications when interpreting the potential effects of increasing UV-B on amphibians and, as stated above, some species appear to be sensitive though others are not.

UV-B radiation has been rising in recent decades (Blumthaler, 1993; Herman et al., 1999), and because the effects of increased UV-B radiation occur without any visible change in the environment, we believe this hypothesis deserves further attention. This may be especially relevant to cases in which amphibians have declined in protected areas (Lizana and Pedraza, 1998; Broomhall et al., 2000; Middleton et al., 2001). National parks in the United States contain some of the best protected habitat in North

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America, yet UV-B radiation could still be a problem for amphibians in those areas, although there has been considerable debate about this (Licht, 2003; Blaustein et al., 2004; Corn and Muths, 2004). Techniques to measure UV-B dose in the field are relatively new. A recent study found that UV-B dose in Sequoia and Kings Canyon National Parks in the Sierra Nevada (California, USA) is the highest among the six national parks that were part of the study (Diamond et al., 2005).

The Sierra Nevada mountain range is an ideal place to look for possible UV-B effects on amphibians, not only because the aquatic systems generally contain little DOC, but also because of well-documented amphibian declines in the region. Four of the five native anuran species at high elevation sites (>2500 m) in the region have disappeared from significant portions of their ranges during the past 20 years despite remarkable habitat protection (Jennings and Hayes, 1994; Drost and Fellers, 1996). The Sierra Nevada is nearly 600 km long and contains one of the largest contiguous roadless areas in the continental United States. Most of the alpine areas above 2000 m elevation where these anurans historically occurred are remote and protected as state and national parks, national forests, and wilderness areas (Sherman and Morton, 1993; Bradford et al., 1994; Stebbins and Cohen, 1995).

Here, we focus on three co-occurring high elevation anurans in the Sierra Nevada and experimentally test for sensitivity to ambient UV-B radiation on hatching success and time to hatching. One species, the Pacific Treefrog (*Pseudacris regilla*) has remained relatively abundant and widespread, but the three other anurans inhabiting the high Sierra Nevada, the Sierra Nevada Mountain Yellow-legged Frog (*Rana sierrae*), the Southern Mountain Yellow-legged Frog (*Rana muscosa*), and the Yosemite Toad (*Bufo canorus*), have experienced severe population declines and local extinctions (Bradford, 1989; Jennings and Hayes, 1994; Stebbins and Cohen, 1995; Drost and Fellers, 1996). *Rana muscosa* are highly vulnerable to predation by introduced trout, with the latter playing an important role in their overall decline (Vredenburg, 2004; Knapp et al., 2007). However, other factors are also involved in the decline of *R. muscosa* (Davidson, 2004; Briggs et al., 2005; Rachowicz et al., 2006). Introduced trout probably have less effect on *P. regilla* and *B. canorus* because these species do not have the long larval phase present in *R. muscosa* and can reproduce in ephemeral bodies of water that do not retain introduced trout (Knapp, 2005). Previous researchers have suggested that UV-B radiation may be at least partially responsible for the decline of *B. canorus* (Sherman and Morton, 1993), yet we are not aware of any published studies that addressed this. Earlier studies have shown that *P. regilla*, tested at a range of elevations (90, 290, 1190, 1655, 2000 m) and in multiple regions, appear to be relatively resistant to UV-B radiation (Blaustein et al., 1994; Kiesecker and Blaustein, 1995; Ovaska et al., 1997; Anzalone et al., 1998; Hatch and Blaustein, 2003), which may help explain why this species remains relatively abundant in the Sierra Nevada. In our experiments, we tested the effects of ambient levels of ultraviolet radiation on all three species concurrently at natural breeding sites.

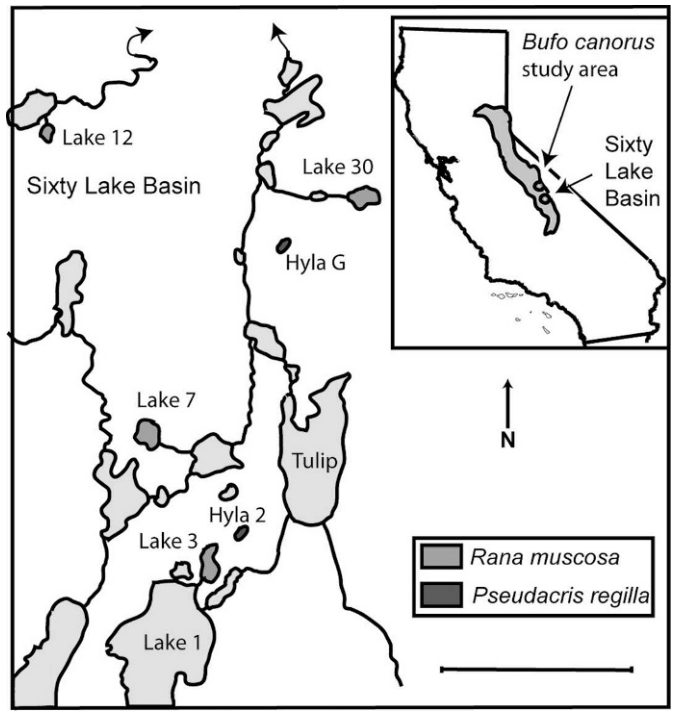

Fig. 1. The inset map shows the location of the two study areas in California, USA. The shaded region in California approximates the high elevation area (2,500–3,660 m) in the Sierra Nevada where the three species in this study co-occur. The *Bufo canorus* field experiments were conducted in and near Cloverleaf Lake (n = 2 water bodies), ~60 km north of the second study area, Sixty Lake Basin (shown in the foreground). The water bodies shaded in gray and black are the study lakes for *Rana muscosa* (n = 4) and *Pseudacris regilla* (n = 2), respectively. Water samples analyzed for DOC and spectral analysis were collected from all six amphibian study lakes in Sixty Lake Basin and also from Lake 1 and Tulip Lake. Scale bar shown on bottom right is 0.5 km.

MATERIALS AND METHODS

Field experiments.—We conducted field experiments from 9 July–8 August 1998 on *R. muscosa* at three water bodies in the high elevation Sixty Lake Basin of Kings Canyon National Park, Fresno County, California, USA (36°49′13″N, 118°25′30″W; 3,340 m elevation) and from 13 June–12 July 1999 on *R. muscosa* at two sites (one of which we used to test *R. muscosa* in 1998) and *P. regilla* at two sites in Sixty Lake Basin (Fig. 1, foreground). We tested *R. muscosa* and *P. regilla* in Sixty Lake Basin, one of the last areas containing large numbers of *R. muscosa*, to allow us to work simultaneously on a long-term study of *R. muscosa* in the basin (Vredenburg, 2004). In both years, *R. muscosa* was tested at water bodies chosen randomly from the seven breeding sites in the basin, all permanent ponds, with ≥25 *R. muscosa* embryo masses (enough to allow experimentation without disturbing >20% of embryos). *Pseudacris regilla* was tested at two water bodies chosen randomly from approximately 50 breeding sites in Sixty Lake Basin, all temporary ponds, with ≥180 *P. regilla* embryo masses (enough to avoid disturbing >20% of embryos). From 20 June–July in 1999, two of our research members conducted our experiments on *B. canorus* approximately 60 km north of the Sixty Lake Basin in Cloverleaf Lake (Cloverleaf 1) and in a nearby unnamed wet meadow (Cloverleaf 2) in the high elevation Convict Creek drainage in Mono County, California (37°32′57″N, 118°53′52″W; 3,159 m elevation; Fig. 1, inset). We chose this area for logistical reasons; out of the few areas still containing large numbers *B. canorus*, it was the closest to Sixty Lake Basin. We

tested *B. canorus* at all two of their breeding sites in the Convict Creek drainage, both of which had large numbers of embryos (≥10,000).

We constructed rectangular grids (1.5 m × 1 m) of bamboo poles and attached 15 mesh-sided containers to the array. We anchored experimental grid arrays to pond bottoms at natural breeding sites (one site per pond) before breeding commenced. The containers served as the experimental units. For each array, we randomly assigned each container to one of three filter treatments (UV-B blocking filters, UV-B transmitting filters, and no filters, or open) for a total of five experimental replicates per treatment per array. The UV-B transmitting filters controlled for the presence of filters in the UV-B blocking filter treatment. The containers were round (20 cm diameter × 10 cm deep) and allowed for water exchange between the containers and the pond, but prevented tadpole escape. Filters were affixed to the bamboo array directly above each container and allowed for air flow between the water surface and the filters. We adjusted the depth of containers such that the top of each egg mass ranged from just below the surface to approximately 7 cm depth, within the range of natural egg deposition sites of all three species.

For each array, we collected egg masses from the same pond in which the array was placed. We selected egg masses that were laid during the previous 3–8 hours within five m of the array at a depth no deeper than 6 cm. For experiments on *R. muscosa* and *B. canorus*, each egg mass was split into thirds by hand and divided equally among the three UV-B treatments. Because egg masses of *P. regilla* have many fewer eggs and are more delicate, we placed 1–3 whole egg masses in each container and did not split them as we did with the other two species.

Hatching success was recorded as the percentage of embryos hatching/container. Rates of development were quantified for each container as the average number of days from the start of the experiment to hatching of all surviving embryos/container. Each day, dead embryos were removed from containers to prevent the accumulation of mold. In addition, we took daily water temperatures at each array from randomly selected containers for each of the three treatments and at nearby natural oviposition sites located in the same pond as the array, less than five m away, using a handheld thermometer. We placed the tip of the thermometer at the shallowest edge of the embryo mass for each measurement. Each array was terminated four days after the last of its embryos hatched. Tadpoles in each container were counted, inspected for gross abnormalities, and released.

Statistical analyses.—Each species was analyzed separately, and separate analyses were conducted for each of the two years of data for *R. muscosa*. Analysis of variance (ANOVA) blocked by lake was used to test for treatment effects on embryo hatching success and rates of development (average time to hatching) and temperature differences among the four conditions: UV-B blocking filter, UV-B transmitting filter, and no filters, and natural egg deposition sites. If we found a significant effect of lake ($P < 0.05$), we then conducted separate *post-hoc* ANOVAs for each lake to test for trends within each block/lake. *Post-hoc* ANOVAs had treatment as the sole factor.

When ANOVA indicated a significant treatment effect, Tukey tests were used to test for pair-wise differences between particular treatments. In addition, within each ANOVA for hatching success or time to hatching, we used a planned contrast to make a pair-wise comparison between the UV-B blocking filter treatment and the UV-B treatments (UV-B transmitting and open). All ANOVAs were performed with and without an interaction factor (hatching success and time to hatching analyses: lake × treatment; temperature analyses: lake × condition).

Hatchling counts for *Rana muscosa* in 1999 and one container of *B. canorus* embryos in the UV-B blocking filter treatment at Cloverleaf 1 were incomplete and thus we excluded them from time to hatching analyses. All results met parametric assumptions of normality and homoscedasticity except for the presence of outliers in the hatching success and time to hatching data. Outliers were defined as data points with an externally studentized residual >1.96 (Ramsey and Schafer, 1997). Transformation was not successful in removing these outliers, so their influence was investigated by performing separate analyses with all possible combinations of inclusion and exclusion of each outlier. Where outliers were influential, the nonparametric Wilcoxon rank sum test was performed using the data with all outliers included.

For the multi-lake ANOVAs of hatching success and time to hatching, we used power tests to provide statistical confidence in our results (Cohen, 1988; Reed and Blaustein, 1995). For hatching success, we ran *post-hoc* power analyses with an effect size of 10% and determined the minimum effect size necessary for us to have enough power ($\hat{I}^2 > 0.8$) of detection for *P. regilla*, which had higher variance than the other species. For time to hatching, we simply determined the minimum effect size necessary for us to have $\hat{I}^2 > 0.8$. All statistical analyses were conducted using JMP, ver. 8.0 statistical software (SAS Institute, Inc., Cary, NC; http://www.jmp.com).

Collection and analysis of water samples.—We collected water samples at all sites used for experimentation in Sixty Lake Basin except for Lakes 3 and 7. To provide a broader context of water quality characteristics in Sixty Lake Basin, we also opportunistically collected water samples from two additional lakes not used for experiments, which were representative of the gross physical and biological characteristics of lakes in this basin (Lake 1 and Tulip Lake). The water sampling regime was uneven, and Lakes 3 and 7 were missed due to logistical constraints on the rapid collection of samples and transport by helicopter to the laboratory before degradation. Table 1 summarizes the experiments and dates of water collection for each site. We measured the amount of DOC and the spectral transmittance characteristics (the amount of UV-B that passes through the water) of water samples collected. Samples were collected 5 cm below the water surface from 1200–1400 hr within 1 m of where bamboo arrays were located, or in sites with similar characteristics if no experiment took place in the lake. Water depth at collection sites was <0.5 m, and bottom substrate was dominated by silt at all sites. Samples were filtered on-site through 0.07 μm ashed glass fiber filters (GF-F, Gelman, Inc., Ann Arbor, MI) and transported in ashed amber glass bottles at ambient temperature. Refrigeration was not possible due to the remote nature of the study sites; however, when possible, snow was used to keep samples cool. Collection, transportation, and analyses of water samples were done using previously published methods (Diamond et al., 2005). All samples were received at the USEPA, National Health and

Table 1. Summary of Data Recorded at High Elevation Lakes in the Sierra Nevada. Hatching success and time to hatching of amphibian embryos and water temperatures were measured in all lakes used in experimentation, but time to hatching was not analyzed for *Rana muscosa* in 1999 due to missing data.

Year	Area	Lake	Species studied	Dates of experimentation	Water sampling date(s)
1998	Sixty Lake Basin	3	*Rana muscosa*	21 July–8 August	None
		7	*R. muscosa*	15 July–3 August	None
		30	*R. muscosa*	15 July–1 August	None
1999	Sixty Lake Basin	1	None	NA	21 Aug., 2 Sep.
		12	*R. muscosa*	26 June–12 July	4 Aug., 21 Aug., 2 Sep.
		30	*R. muscosa*	13 June–28 June	4 Aug., 21 Aug., 2 Sep.
		Hyla 2	*Psuedacris regilla*	24 June–3 July	4 Aug.
		Hyla G	*P. regilla*	18 June–1 July	4 Aug., 21 Aug.
		Tulip Lake	None	None	21 Aug., 2 Sep.
	Convict Creek Drainage	Cloverleaf 1	*Bufo canorus*	20 June–3 July	None
		Cloverleaf 2	*B. canorus*	21 June–28 June	None

Environmental Effects Research Laboratory, Duluth, MN, USA, and were analyzed within ten days of sampling.

Field measures of UV irradiance.—Field irradiance data were derived from USEPA Ultraviolet Monitoring Program (UV-Net). The data were gathered with a Brewer Spectroradiometer located in Sequoia National Park 30 km south of Sixty Lake Basin, and are available only for the 1999 field season. To ensure that the materials used in the field experiments properly blocked UV-B, we measured the UV-B blocking qualities of the materials at a USEPA laboratory using a solar simulator (Hansen et al., 2002b).

RESULTS

Inclusion or exclusion of outliers in any combination did not influence the qualitative interpretation of any analyses, except for the planned contrasts within the *post-hoc* ANOVAs for time to hatching of *R. muscosa* in Lake 7. Therefore, we present the results with all outliers included, unless noted. In addition, block × treatment and block × condition interaction factors in full model multi-lake ANOVAs were never close to significance (all $P > 0.17$), and inclusion or exclusion of these factors never influenced the qualitative interpretation of the block, treatment, or condition factors. Hence, we present multi-lake ANOVAs and planned contrasts in reduced model form, without interactions, and Tukey tests with standard deviations derived from reduced model ANOVAs.

Visual inspections of tadpoles revealed no gross abnormalities. Our experiments included 25 *R. muscosa* egg masses, nine *B. canorus* egg masses, and 57 *P. regilla* egg masses. Hatching success was recorded for 3,177 *R. muscosa*, 1,181 *B. canorus*, and 607 *P. regilla* embryos. According to the multi-lake analyses, there were no overall differences in hatching success among treatment groups or lakes for any of the focal species, except that hatching success of *P. regilla* was higher in Hyla 2 compared to Hyla G (ANOVA: *R. muscosa* in 1998—Treatment $F_{2,40} = 0.448$, $P = 0.642$, Lake $F_{2,40} = 2.004$, $P = 0.148$, Fig. 2; *R. muscosa* in 1999—Treatment $F_{2,26} = 0.780$, $P = 0.469$, Lake $F_{1,26} = 2.136$, $P = 0.156$, Fig. 2; *B. canorus*—Treatment $F_{2,26} = 0.678$, $P = 0.516$, Lake $F_{1,26} = 0.876$, $P = 0.358$, Fig. 3; *P. regilla*—Treatment $F_{2,26} = 1.281$, $P = 0.295$, Lake $F_{1,26} = 5.806$, $P = 0.023$, Fig. 3). Hatching success of *R. muscosa* in 1998 was highest in the UV-B blocking filter treatment and lowest in the open treatment; however, this difference was not significant according to ANOVA. Within *P. regilla*, *post-hoc* ANOVAs within individual lakes also found no significant differences in hatching success among treatments (ANOVA: Hyla 2—$F_{2,12} = 0.743$, $P = 0.497$; Hyla G—$F_{2,12} = 1.051$, $P = 0.380$). Furthermore, pair-wise differences in hatching success between UV-B blocking filter and UV-B treatments were never significant for any of our species (planned contrasts: all $P > 0.12$).

Our *post-hoc* power analyses show that, based on our samples sizes for *R. muscosa* and *B. canorus*, the multi-lake ANOVAs had sufficient power ($\hat{I}^2 > 0.8$) to detect as little as a 10% difference in hatching success among treatments. Power ($\hat{I}^2$) to detect this 10% difference was 1.00, 0.99, and 1.00 for *R. muscosa* in 1998, *R. muscosa* in 1999, and *B. canorus*, respectively. In contrast, because of the higher variance in *P. regilla* hatching rates, power to detect this 10% difference in *P. regilla* was only 0.69, and we needed an 11.4% effect size to have enough power ($\hat{I}^2 > 0.8$) of

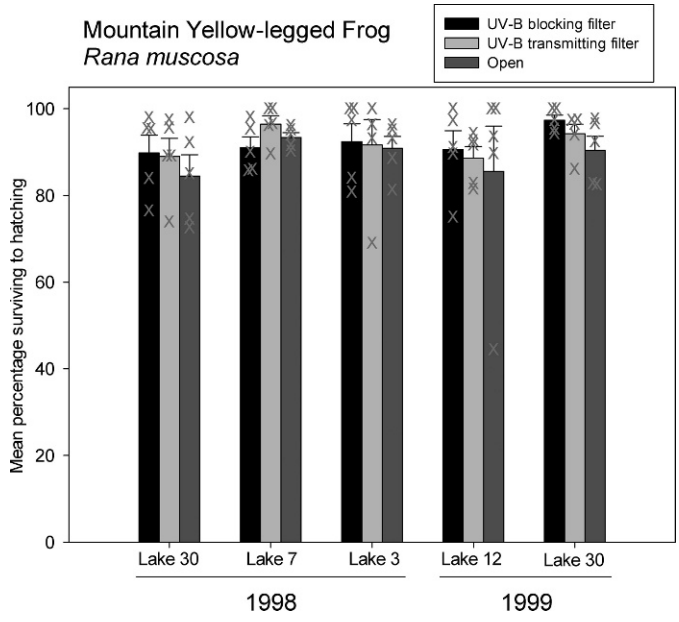

Fig. 2. Mean percentage of embryos surviving to hatching ± 1 SE in *Rana muscosa* over two years in four water bodies ($n = 25$ egg masses; 18–70 embryos/experimental unit). Hatching success of each experimental unit is represented by an "X."

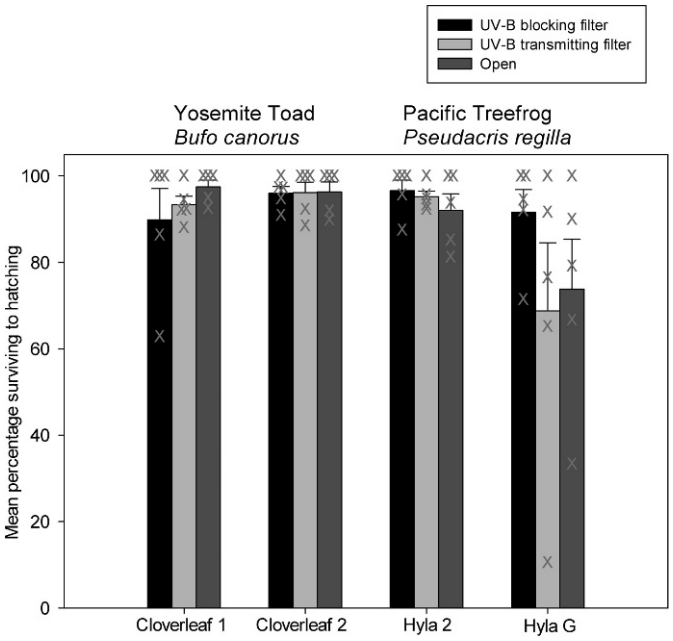

Fig. 3. Mean percentage of embryos surviving to hatching ± 1 SE in *Bufo canorus* (*n* = 9 egg masses; 30–52 embryos/experimental unit) and *Pseudacris regilla* (*n* = 59 egg masses; 13–31 embryos/experimental unit) in four breeding water bodies in 1999. Hatching success of each experimental unit is represented by an "X."

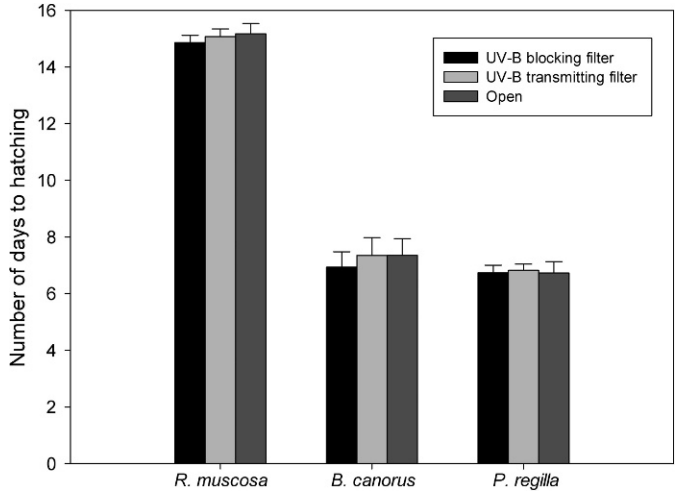

Fig. 4. Mean duration of development (fertilization to hatching) ± 1 SE in *Rana muscosa* (*n* = 1,777 hatched embryos), *Bufo canorus* (*n* = 1,119 hatched embryos), and *Pseudacris regilla* (*n* = 527 hatched embryos). Time to hatching of each experimental unit is represented by an "X".

detection. Previous studies, including those on *R. cascadae*, *B. boreas*, and *H. cadaverina*, which are ecologically similar to *R. muscosa*, *B. canorus*, and *P. regilla*, commonly report a UV-B effect on survival greater than 20% (Blaustein et al., 1994; Kiesecker and Blaustein, 1995; Anzalone et al., 1998). Therefore, even in *P. regilla* we had the power to detect effects documented in other species.

Water temperatures did not differ significantly among the four conditions (treatment groups and natural oviposition sites located less than five m from experimental arrays), although water temperatures did differ among lakes for *B. canorus* (ANOVA: *R. muscosa* in 1998—Condition $F_{3,54}$ = 0.107, $P = 0.956$, Lake $F_{4,54} = 2.137$, $P = 0.128$; *R. muscosa* in 1999—Condition $F_{3,30} = 0.040$, $P = 0.989$, Lake $F_{4,30}$ = 1.380, $P = 0.249$; *P. regilla*—Condition $F_{3,35} = 0.672$, P = 0.575, Lake $F_{1,35} = 1.200$, $P = 0.281$; *B. canorus*—Condition $F_{3,31} = 0.070$, $P = 0.976$, Lake $F_{1,31} = 16.192$, $P < 0.001$). *Post-hoc* ANOVAs for individual *B. canorus* lakes found no significant differences among conditions (both $P > 0.87$). Mean temperatures ± 1 SE for natural egg deposition sites, open containers, those with UV-B blocking filters, and those with UV-B transmitting filters were 17.7°C ± 0.8, 18.2°C ± 0.8, 17.9°C ± 0.8, and 18.3°C ± 0.9, respectively for *R. muscosa* in 1998, 20.2°C ± 0.6, 19.4°C ± 0.7, 19.4°C ± 0.6, and 19.5°C ± 0.7 for *R. muscosa* in 1999, 20.6°C ± 0.5, 20.7°C ± 0.4, 20.1°C ± 0.3, and 21.0°C ± 0.6, respectively for *P. regilla*, and 22.1°C ± 0.6, 22.3°C ± 0.6, 22.1°C ± 0.5, and 22.3°C ± 0.6, respectively for *B. canorus*.

Time to hatching was also recorded for these species under the three experimental conditions (Fig. 4). According to the multi-lake analyses, there were no overall differences in time to hatching among solar UV-B treatments for *B. boreas* or *H. regilla*, although hatching rates in embryos of these species were influenced by lake effects (ANOVA: *B. canorus*—Treatment $F_{2,25} = 0.314$, $P = 0.733$, Lake $F_{1,25} = 131.970$, $P < 0.001$; *P. regilla*—Treatment $F_{2,26} = 0.759$, $P = 0.478$, Lake $F_{1,26} = 8.322$, $P = 0.008$). In addition, for *B. boreas* and *P. regilla*, *post-hoc* ANOVAs within individual lakes found no significant differences in time to hatching among treatments (*B. canorus*: Cloverleaf 1—$F_{2,11} = 0.390$, $P = 0.686$; Cloverleaf 2—$F_{2,12} = 0.093$, $P = 0.912$; *P. regilla*: Hyla 2 $F_{2,12}$= 0.891, $P = 0.436$; Hyla G—$F_{2,12} = 2.106$, $P = 0.164$) and there were no significant pair-wise differences in time to hatching between UV-B blocking filter and UV-B treatments (planned contrasts: all $P > 0.07$).

Similarly, the multi-lake analysis of time to hatching in *R. muscosa* found no overall effect of treatment, but did find a lake effect (ANOVA: Treatment $F_{2,40} = 0.318$, $P = 0.729$, Lake $F_{2,40} = 4.02$, $P = 0.026$), with no difference in time to hatching between the UV-B blocking filter and UV-B treatments (planned contrast: $P = 0.07$). However, the *post-hoc* ANOVA results for time to hatching in this species were complex; Lake 3 showed a significant treatment effect, while Lakes 7 and 30 did not (Lake 3—$F_{2,12} = 4.856$, P = 0.029; Lake 7—$F_{2,12} = 0.235$, $P = 0.794$; Lake 30—$F_{2,12}$ = 0.541, $P = 0.596$). Within Lake 3, time to hatching was not different in the open treatment compared to the UV-B transmitting filter treatment or in the UV-B transmitting filter treatment compared to the UV-B blocking filter treatment (Tukey tests: both $P > 0.05$), but was longer in the open compared to the UV-B blocking filter treatment (Tukey test: $P < 0.05$), and longer in the UV-B treatments compared to the UV-B blocking filter treatment (planned contrast: $P = 0.038$). In Lake 3, mean time to hatching ± 1 SE was 15.8 ± 0.2, 15.3 ± 0.1, and 15.1 ± 0.2 days for the open, UV-B transmitting filter, and UV-B blocking filter treatments, respectively, and was increased by 5% in the open treatment compared to the UV-B blocking filter treatment and by 3% in the UV-B treatments compared to the UV-B blocking filter treatment. In Lakes 7 and 30, where *post-hoc* ANOVA found no differences among treatments, pairwise comparisons between UV-B treatments and the UV-B blocking filter treatment also found no differences. In Lake 7, there were three outliers, and significance of the planned contrast between the UV-B blocking filter and the UV-B treatments depended on which particular outliers were included in the contrast. When two particular outliers were excluded, the contrast was significant ($P = 0.040$), but

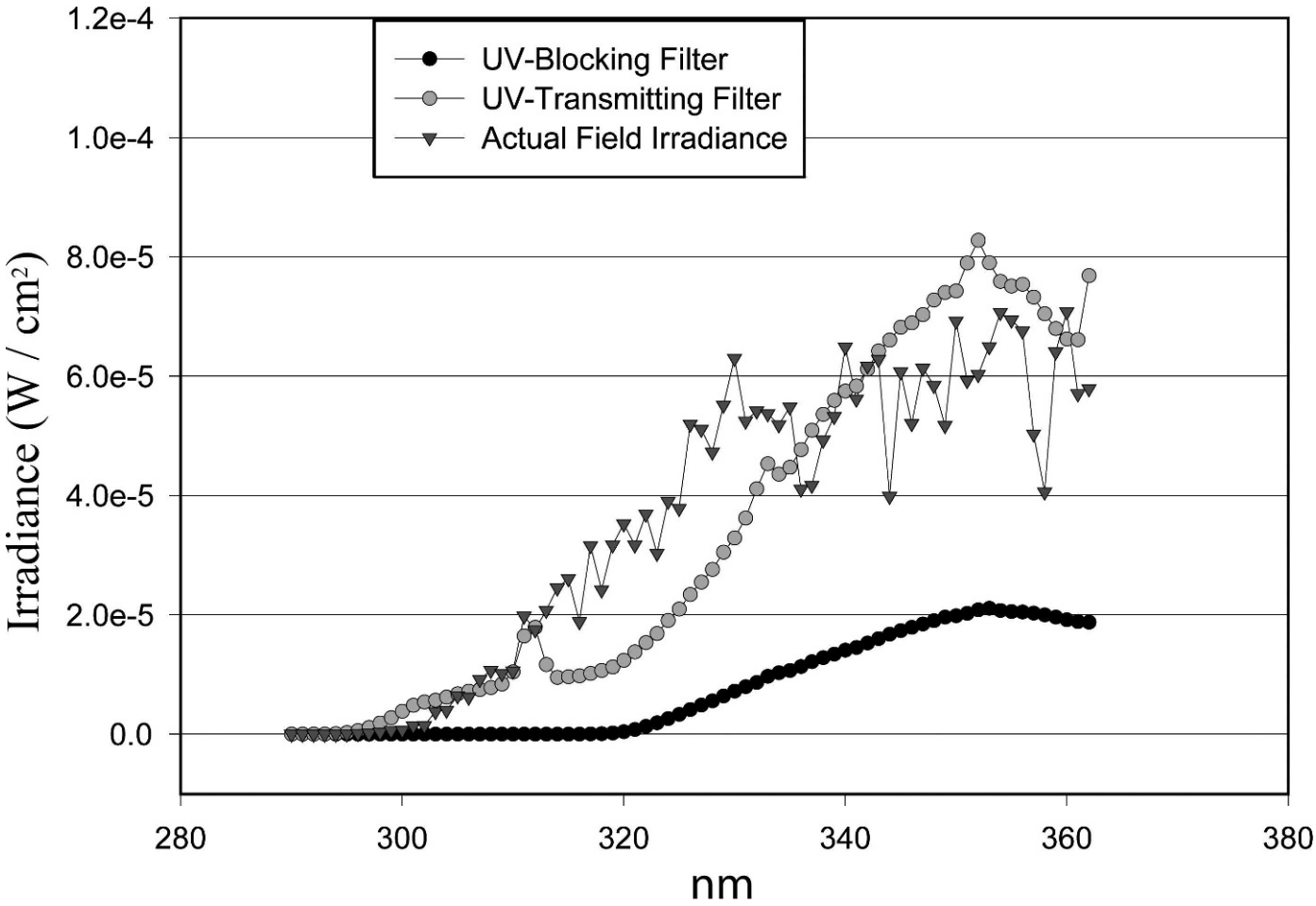

Fig. 5. Irradiance (μW/cm²) measured in the field by the USEPA Ultraviolet Monitoring Program (UV-Net) at a Brewer station in Sequoia National Park near the field experiments and UV-B transmittance of the materials used in the field experiment. Field data are peak irradiance values for 13 June 1999. Values are shown for wavelengths ranging from 290 to 363 nm.

otherwise the contrast was non-significant (all $P > 0.066$). However, nonparametric pairwise comparison of the UV-B treatments and the UV-B blocking filter treatment within this lake provided a clear result of no difference (Wilcoxon rank sum test: $Z = 0.857$, $P = 0.391$). In Lake 30, the planned contrast between the UV-B blocking filter and the UV-B treatments was non-significant ($P = 0.782$).

In the multi-lake ANOVAs for time to hatching, minimum effect size necessary to have sufficient power of detection ($\hat{I}^2 > 0.8$) was 0.53, 0.46, and 0.53 days for *R. muscosa*, *B. canorus*, and *P. regilla*, respectively. We were not able to find relevant time to hatching effect sizes in the published literature to compare with these minimum effect sizes. Previous studies (Pahkala et al., 2002a, 2002b) have reported effects of UV-B on rate of development in common frog (*R. temporaria*) embryos, but we found no studies reporting significant differences in time to hatching between amphibian embryos exposed to either ambient UV-B or ambient levels of UV-B and unexposed controls.

Mylar filters (UV-B blocking filters) successfully blocked UV-B radiation, while acetate filters (UV-B transmitting filters) were more transparent to UV-B (Fig. 5). Peak irradiance measured at the Brewer Spectroradiometer in Sequoia National Park on a representative day of our experiments in 1999 closely matched the irradiance transmitted by the acetate filters (Fig. 5), indicating that the solar simulator in the laboratory provided a realistic wavelength profile with which to test the transmittance of the filters. Daily UV-B dose (erythermally weighted) measured by the same instrument ranged from 11–23 W hr/m² over the course of the experiments in 1999 (13 June–12 July). No Brewer data are available for 1998.

The mean DOC concentration was low, as expected for high elevation oligotrophic lakes (1.81 mg carbon L^{-1}; 1 SD = 1.54), and thus allows high UV transmission through the water. The UV blocking potential of the water samples was very low; almost all samples showed transmittance near 100% at eight cm depth, with the exception of one water sample from the pond Hyla G that had reduced UV transmittance (Fig. 6). This pond had the highest UV-B blocking potential from DOC, experienced the highest embryo mortality (Fig. 3, *P. regilla*), and had a non-significant trend of highest hatching rates in the UV-B blocked treatment.

DISCUSSION

We hypothesized that differences in UV sensitivity may help explain why *P. regilla* remain abundant while *B. canorus* and *R. muscosa* have declined sharply in the Sierra Nevada. Our study sites were an ideal place to test this because the UV environment in the high elevation (>3,000 m) Sierra Nevada is extreme (Diamond et al., 2005), with high ambient UV doses and virtually no attenuation in the water

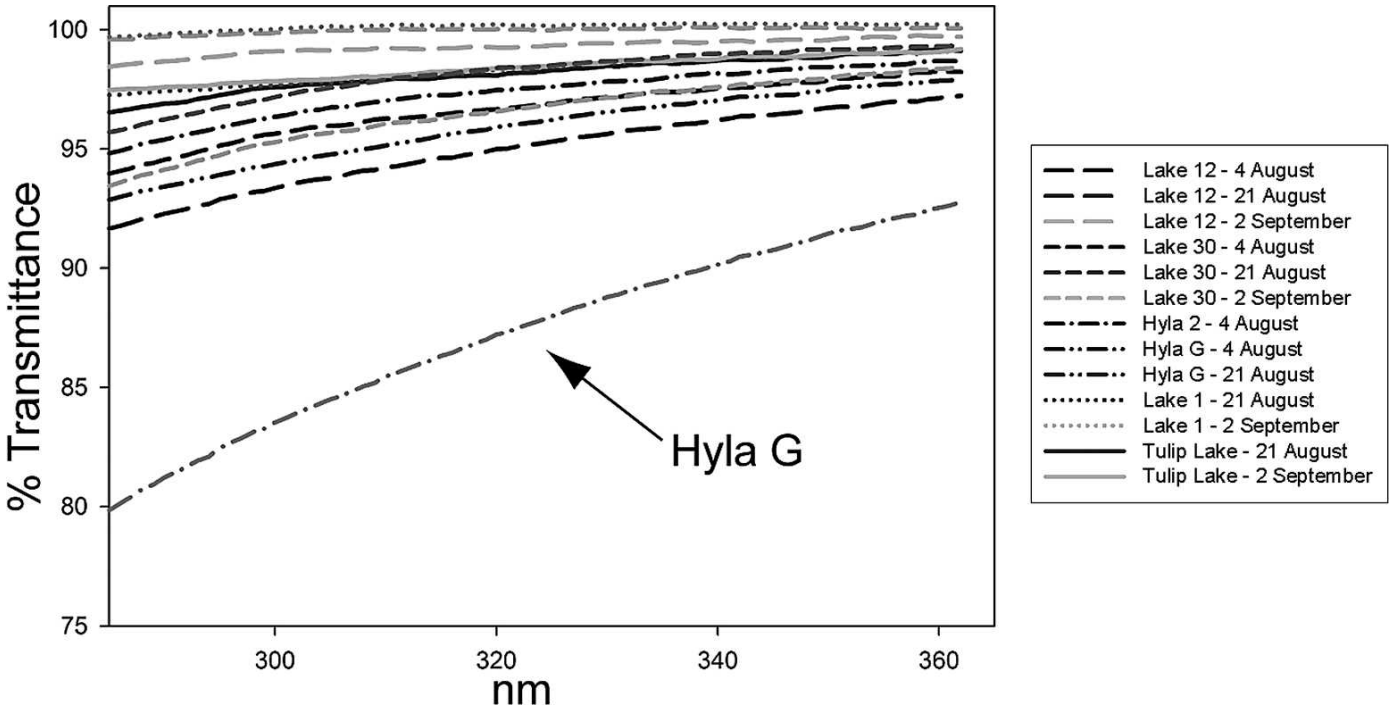

Fig. 6. Percent transmittance of UV-B though 10 cm of water from samples collected in the Sixty Lake Basin in 1999 (study sites: Lake 12, Lake 30, Hyla 2, Hyla G; other sites: Lake 1, Tulip Lake). Lake and date are given for each sample. Lakes 12 and 30 were sampled three times; Lake 1, Hyla G, and Tulip Lake were sampled twice; and Hyla 2 was sampled once.

because of low DOC concentrations (Diamond et al., 2005). In addition, our lab results suggest that the UV-blocking and transmitting filters we used functioned correctly, and the UV dose measured by the Brewer station during the course of our experiments is within the range that is known to negatively affect hatching rates of other amphibians (Blaustein et al., 2004). We found no differences in survival to hatching among UV-B-exposed and UV-B-shielded treatments for *R. muscosa*, *B. canorus*, or *P. regilla*. Hence, the available evidence does not link the differences in decline status for these three species to differential effects of ambient UV-B radiation on survival of their embryos. Furthermore, we found no differences among UV-B-exposed and UV-B-shielded embryos in hatchling size in *B. canorus* or in time to hatching in *B. canorus* or *P. regilla*. In contrast, *R. muscosa* displayed a context-dependent effect of UV-B on hatching rate, but the increase in time to hatching in UV-B-exposed compared to UV-B-shielded embryos was small and occurred in only one lake. Thus, there is little evidence that sublethal effects of UV-B on embryos have contributed to the pattern of amphibian declines in the Sierra Nevada. Like other studies that used this same field design, all differences in temperature between treatments were slight and non-significant (Blaustein et al., 1998), and it is unlikely that they significantly influenced hatching success, time hatching, or early larval development.

In the several amphibian species that have shown increased hatching success when shielded from ambient UV-B in field experiments (Blaustein et al., 1998, 2003), the effect was large and differences in hatching success among treatments were easily distinguished. For example, in *Hyla cadaverina* and *Taricha torosa*, UV-B-exposed treatments had hatching rates only half as high as controls (Anzalone et al., 1998) and in *Bufo bufo*, *B. boreas* in the Oregon Cascade Range, and *Rana cascadae* hatching success decreased by 22%, 68%, and 64%, respectively, in comparison to controls (Blaustein et al., 1994; Lizana and Pedraza, 1998), although Corn (1998) found no effect of UV-B on hatching success of *B. boreas* in the Rocky Mountain Range in Colorado. A study that compared the distribution patterns of eight species of amphibians in relation to UV-B exposure found that two species (*Taricha granulosa* and *Ambystoma macrodactylum*) had distribution patterns that ''could have resulted from negative UV-B effects'' and another species (*P. regilla*) with marginal support for the same conclusion (Adams et al., 2005). The other five species in the study (*Ambystoma gracile*, *B. boreas*, *R. cascadae*, *R. leuteiventris*, and *R. muscosa*) showed no negative association between distribution and UV-B exposure.

In contrast to some of the field experiments on other amphibians, hatching success in our study was high in all treatments for all three species. With two exceptions, we saw no trend towards decreased survival in UV-B exposed treatments. The first exception was seen in *P. regilla*. Although hatching success was higher in the UV-B blocking filter treatment compared to each of the other treatments, these differences were not significant. Interestingly, the pond with the lowest hatching success had the highest DOC and the lowest amount of UV light transmittance of all water samples collected, further supporting the conclusion that UV-B exposure probably was not involved in the decreased hatching rates at that pond. The second exception was seen in *R. muscosa* in 1998. Although hatching success was highest in the UV-blocking filter treatment and lowest in the fully exposed treatment, the pattern was not significant. It is possible that eggs that did not hatch could have been unfertilized or damaged when we placed them in the containers at the beginning of the experiment. Unfortunately, we did not save the dead eggs.

Previous studies have shown sublethal effects of UV-B exposure on amphibians at a variety of life history stages (Crump et al., 1999; Blaustein et al., 2003; Perotti and

Diegéuz, 2006; Han et al., 2007). Our study suggests that UV-B delayed hatching in *R. muscosa*, but the effect was small and occurred only in one lake. Thus, it is unclear whether UV-B-induced delays in hatching are important in the ecology of *R. muscosa* in Sixty Lake Basin. Amphibians that breed in habitats that are able to develop rapidly can escape desiccation, reduce exposure to aquatic predators (Werner, 1986; Newman, 1988; Denver et al., 1998), and escape competition and disease (Parris and Beaudoin, 2004), and thus the speed of maturation may ultimately have consequences for population size and persistence. It is difficult from our study to know the importance of the small, context-dependent delays in time to hatching induced by UV-B in *R. muscosa*. More studies should be conducted to test the role of UV-B exposure on rates of amphibian metamorphosis, especially in species that breed in ephemeral habitats where rapid development is most important.

The UV sensitivity hypothesis has been suggested previously as a potential cause for amphibian declines in the Sierra Nevada for several reasons. Firstly, three of the four anuran species that occur in the highest elevations have declined sharply even though most of the habitat is well protected (Jennings and Hayes, 1994; Stebbins and Cohen, 1995), coinciding with increases in ultraviolet radiation reaching the Earth's surface (Blumthaler and Ambach, 1990). In addition, embryos of the four most common anurans are laid in shallow water where they are naturally exposed to direct sunlight (Stebbins and Cohen, 1995; Vredenburg et al., 2005). Furthermore, UV-B exposure is high even at relatively deep depths due to naturally low concentrations of DOC (Diamond et al., 2005). Finally, the only amphibian that has not declined drastically in the area, *P. regilla*, has high levels of photolyase activity and is resistant to the negative effects of UV-B radiation at the embryonic stage (Blaustein et al., 1994).

The data presented in this study are from field experiments conducted at some of the highest elevation (>3,030 m) oligotrophic sites in North America, which experience high UV dose and transmittance (Diamond et al., 2005). Despite these conditions, which seem highly favorable to the hypothesis that UV-B contributes to the declines of *B. canorus* and *R. muscosa*, our study did not support this hypothesis. Instead, our results showing no effect of UV-B on hatching success and only a small, context-dependent increase in time to hatching in one species support conclusions from prior studies that have suggested amphibians occupying habitats with low UV blocking potential (low DOC concentrations) and high levels of exposure to UV radiation may have evolved mechanisms to deal with or avoid the negative effects of these potentially damaging rays (Blaustein et al., 1994; Belden and Blaustein, 2002; Adams et al., 2005). Another study found that long-toed salamander (*Ambystoma macrodactylum*) populations living at higher elevations were more resistant to UV-B exposure than populations from lower elevations (Belden and Blaustein, 2002). A key limitation of our study includes being able to assess UV effects only on the embryo stage. The strengths of our approach lie in the fact that we applied a widely used experimental design and replicated it spatially.

Our results showed little support for UV-B-induced effects on embryos as an important factor in the biology of *B. canorus* in the Convict Creek drainage or *R. muscosa* or *P. regilla* across the sampled breeding sites of these two species in Sixty Lake Basin. Extrapolation of our results for any of our study species outside of the basin or drainage in which we tested it must be done cautiously, because numerous relevant factors may vary across a species' range, such as resistance to UV-B (Belden and Blaustein, 2002) and synergistic cofactors including contaminants and pathogens (Kiesecker and Blaustein, 1995; Leslie et al., 1995). UV-B may have important negative effects on embryos of *B. canorus* and *R. muscosa* outside of Sixty Lake Basin and the Convict Creek drainage, respectively. However, both species are critically endangered (Drost and Fellers, 1996; Rachowicz et al., 2006) and disturbing embryos in the handful of their remaining large populations is difficult to justify. Therefore, because our results point away from UV-B-induced effects on embryos contributing to the population declines in *B. canorus* and *R. muscosa*, we suggest that research on the population declines of these species in the Sierra Nevada focus on post-hatching effects of UV-B or other hypothesized causes, such as predation from introduced fish (Vredenburg, 2004) and infectious disease (Briggs et al., 2005). Our results for *P. regilla* concur with previous experiments conducted in the Oregon Cascade Range, British Columbia, and southern California in finding no effects of ambient UV-B on hatching success of their embryos (Blaustein et al., 1994; Kiesecker and Blaustein, 1995; Ovaska et al., 1997; Anzalone et al., 1998), suggesting that this species is resistance to UV-B at the embryonic stage throughout its wide range. However, our study is the first to test *P. regilla* embryos for sublethal effects of UV-B, so the generality of their resistance to sublethal effects of UV-B across the range is unknown.

The declines and in some cases extinctions of amphibians in protected areas around the world are alarming and likely do not have a single cause (Stuart et al., 2004). Understanding the mechanisms responsible for population declines is important because these negative impacts may be unnoticed yet common even in seemingly pristine and protected areas. Because of the widespread distribution of amphibian declines, some suggested the involvement of ''global agents'' such as UV-B radiation (Blaustein et al., 1994) to help explain declines. Further testing has shown that not all species that are sensitive to UV-B radiation as embryos are in decline (Blaustein et al., 1998). With our better understanding of the underlying mechanisms, we now know that the amount of UV radiation reaching amphibian embryos can vary dramatically depending on many variables (Brooks et al., 2005). Dissolved organic carbon is critical in aquatic UV dose estimation because of the spectral characteristics of its attenuating capacity (Williamson, 1995; Diamond et al., 2002; Palen et al., 2002). Several studies have shown that the optical characteristics of the water reduce the exposure of amphibians to UV-B (Adams et al., 2005; Brooks et al., 2005; Trenham and Diamond, 2005); however, some amphibian species are nonetheless exposed to harmful levels of UV-B (Blaustein et al., 2004, Bancroft et al., 2008b), and there remains considerable interest in understanding the role of UV-B in amphibian declines (Macias et al., 2007; Bancroft et al., 2008a; Croteau et al., 2008a, 2008b; Marquis et al., 2008; Oromi et al., 2008; Castañaga et al., 2009). In our study locations, DOC concentrations were very low in comparison (1.81 mg carbon L^{-1}; SD = 1.54) to sites in the Pacific Northwest (Palen et al., 2002), and, like other studies (Brooks et al., 2005; Diamond et al., 2005), we measured very low attenuation of UV light at the shallow depths where the eggs are oviposited.

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