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Larval amphibians seek warm temperatures and do not avoid harmful UVB radiation

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Habitat use by animals often reflects the balance between conflicting demands such as foraging and avoiding predation. Environmental stressors such as temperature can also affect habitat use in many organisms, particularly in ectothermic animals. For example, warm, shallow thermal regimes in ponds can optimize growth and developmental rate of amphibian larvae but may also expose larvae to potentially harmful levels of ultraviolet B (UVB) radiation. Thus, optimally, amphibians seeking sunlight for thermoregulation must balance this behavior while limiting their exposure to harmful UVB radiation. We conducted a series of laboratory and field experiments to test the hypothesis that larval amphibians avoid UVB by selecting microhabitats with lower exposure to UVB. We then quantified habitat use of the larvae of 4 amphibian species using field transects in 3 ponds with different UVB transmission. Tadpoles did not avoid UVB radiation in laboratory or field experiments and preferred warmer temperatures in laboratory thermal gradients regardless of UVB exposure. The majority of anuran larvae were observed in water less than 10–15 cm deep in field surveys, whereas salamander larvae were most often observed in deeper, cooler water. The similarity in habitat use across different sites and the lack of evidence of UVB avoidance in choice tests suggest that larval anuran amphibians may be exposed to harmful levels of UVB radiation due to habitat choice behaviors that have been established over evolutionary time. Levels of UVB radiation at the earth's surface have recently increased due to stratospheric ozone depletion. Thus, long-term selection pressures such as thermoregulation may override the relatively recent selection pressure of increased UVB radiation. *Key words: Ambystoma macrodactylum, Bufo boreas*, ephemeral pond, habitat use, *Pseudacris regilla, Rana cascadae*, selection pressure, thermoregulation, ultraviolet B radiation, UVB avoidance. *[Behav Ecol 19:879–886 (2008)]*

Organisms are faced with multiple environmental and biological factors in natural systems. Some may act as stressors (e.g., predation and competition), such that an organism may exhibit avoidance behavior of any one stressor at a particular time. Others may attract organisms to certain locations within habitats (e.g., prey availability). Reaction to the simultaneous combination of several stressors and other factors molds an animal's pattern of habitat use. Generally, the selection and use of habitat is assumed to have fitness consequences, as selecting and using advantageous or optimal habitats will increase the fitness of organisms within a population (Fretwell and Lucas 1970; Jaenike and Holt 1991). Environmental parameters such as light intensity, temperature, soil moisture, and nutrient availability can influence habitat use and selection in many species (e.g., Huk and Kühne 1999). Biotic factors such as food resources (prey), competition, facilitation, and predation can also influence the distribution of animals (Fretwell and Lucas 1970; Kats et al. 1988; Rosenzweig 1991). These abiotic and biotic agents can act as selection pressures, resulting in trade-offs between different components of fitness such as growth or reproduction (Sih 1980; Lima 1998). In addition, the behavior resulting from conflicting selection pressures may be context dependent. For example, physiological condition or food availability can influence antipredator behavior in lizards (Martín et al. 2003).

Abiotic and biotic pressures may result in trade-offs in how an animal uses a particular microhabitat. For ectothermic vertebrates, abiotic regimes may be especially important. For example, temperature greatly influences the behavior and ecology of vertebrate ectotherms (Magnuson et al. 1979), and many ectothermic vertebrates can actively thermoregulate using microhabitat variation to control body temperature (Huey and Slatkin 1976; Magnuson et al. 1979; Hutchison and Dupré 1992). However, thermoregulatory demands must be balanced with biological demands such as foraging, avoiding predation, and reproduction (Holomuzki 1986; Downes and Shine 1998; Martín 2001; Martín and López 2003). These trade-offs can result in complex spatial and temporal patterns of habitat use in ectothermic organisms.

Trade-offs due to conflicting selection pressures may be particularly important in ectotherms with complex life cycles, such as amphibians. The early life-history stages of many amphibian species develop in aquatic habitats of varying temporal stability (e.g., ephemeral ponds). Larvae in ephemeral ponds must develop quickly and undergo metamorphosis before the pond dries or freezes (Wilbur 1980; Blaustein et al. 2001). Thermoregulation is particularly important for these amphibians, as growth rate is closely tied to temperature (Atlas 1935; Lillywhite et al. 1973; Sype 1974). Moreover, size at metamorphosis is related to adult size (Werner 1986), and adult size is positively correlated with reproductive success (Semlitsch et al. 1988; Altwegg and Reyer 2003). Shallow margins of ponds are generally warmer than deeper regions, but shallow regions can contain more predators than deeper water, particularly predaceous diving beetle larvae (family Dyticidae; Holomuzki 1986; Fairchild et al. 2003). Larval amphibians in ephemeral ponds are therefore exposed to conflicting abiotic and biotic selection pressures.

Amphibians, like numerous other aquatic organisms, are harmed by ambient but increasing levels of ultraviolet B (UVB; 280–320 nm) radiation (Bancroft et al. 2007). Sensitivity to UVB radiation may vary interspecifically between life

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stages within a species, between populations, and with environmental conditions (Pahkala et al. 2002; Blaustein and Belden 2003). Exposure to ambient levels of UVB can cause mortality (e.g., Blaustein et al. 1998; Häkkinen et al. 2001), reduce growth (Belden et al. 2000), alter behavior (Nagl and Hofer 1997; Kats et al. 2000), and increase susceptibility to disease (Kiesecker and Blaustein 1995; Kiesecker et al. 2001).

The varied negative effects of UVB on amphibians suggest that under many circumstances, it is a significant stressor for amphibians. Thus, optimally, amphibians should avoid regions of high UVB exposure. One way by which amphibians may avoid high UVB levels is to seek regions with low UVB transmittance such as shaded areas or deeper water (Belden et al. 2000; Blaustein and Belden 2003; Licht 2003). In choice experiments, larvae and adults of some species prefer areas with lower UV irradiance (Nagl and Hofer 1997; van de Mortel and Buttemer 1998; Belden et al. 2000; Garcia et al. 2004; Han et al. 2007). Palen et al. (2005) measured oviposition depth relative to percentage of surface irradiance at 10 cm, a common indicator of UVB penetration in aquatic habitats. The authors found a positive relationship between oviposition depth and UVB penetration for a salamander, *Ambystoma gracile*, such that eggs were found in deeper water in higher UVB environments. Two other species, however, did not alter oviposition behavior with varying UVB penetration (Palen et al. 2005). Clearly, some species avoid high levels of UVB radiation. However, avoiding UVB by seeking cooler, deeper water may reduce growth and create a conflict between thermal requirements and avoiding exposure to harmful levels of UVB. Thus, we expect habitat use to reflect the conflict between avoiding harmful UVB radiation and maintaining thermal requirements for development.

We conducted a series of experiments and used field transect information to test the hypothesis that larval amphibians avoid UVB radiation by seeking microhabitats with low UVB exposure. Thus, we 1) measured the preferred temperature of larvae in thermal gradients with a simultaneous UVB choice test, 2) conducted UVB choice tests in isothermic field enclosures to explore avoidance behaviors in the absence of a trade-off between UVB and temperature, and 3) quantified habitat use of amphibian larvae by conducting transect surveys in different amphibian habitats. We examined the larvae of 4 amphibian species known to be harmed by ambient levels of UVB radiation. Thus, we reasoned that selection pressure for these larvae to avoid UVB radiation is apparent.

MATERIALS AND METHODS

Study sites

Our study sites were selected to include a range of pond-breeding amphibian habitats. We collected and sampled larvae in a large lake (Todd Lake, elevation 1875 m), a large ephemeral pond (Susan's pond, elevation 1954 m), and an alpine meadow containing ~38 small ephemeral pools (potholes, elevation 2300 m), all located in Deschutes County, OR.

UVB avoidance in thermal gradients

These experiments tested if larval amphibians alter thermoregulatory behavior to avoid UVB radiation. *Pseudacris regilla* (Pacific tree frog) larvae were collected from the potholes on 17 July 2005 and *Bufo boreas* (western toad) larvae were collected from Todd Lake on 22 July 2005. Larvae were returned to the laboratory and kept in 38 L glass aquaria filled with treated dechlorinated tap water. Animals were fed a 3:1 mix of alfalfa pellets and fish flakes (TetraMin, Melle, Germany) ad libitum. To prevent acclimation to a constant temperature in the laboratory, all animals were kept at 15 °C from 2000 to 0800 h and warmed to 22 °C during daylight hours. All trials were completed within 10 days of field collection.

Experimental lanes were created in 1.2-m aluminum rain gutters painted with Pratt & Lambert PalGard™ epoxy paint (Sherwin–Williams Company, Cleveland, OH) and filled to a depth of 3.5 cm with dechlorinated tap water. Thermal gradients were created by filling a small metal pocket at one end of each lane with dry ice while the opposite end was placed on a hot plate. This method allowed the establishment of a ~10 °C difference between the 2 ends. Temperature was recorded throughout each trial with iButton temperature loggers (Maxim Integrated Products, Sunnyvale, CA) placed every 27 cm along each experimental lane for a total of 6 iButtons per lane. In addition, thermometers were placed at each end to directly monitor temperatures throughout the trials. Each lane was covered by 2 filters, one which blocks the passage of UVB radiation (Mylar-D) and one which permits the passage of UVB radiation (acetate). Each filter covered half the length of the lanes, and 2 filters were placed such that the entire lane was covered by filters. During each trial, 4 lanes had the blocking filter placed over the warm end with the transmitting filter over the cool end, whereas the remaining 4 lanes had the transmitting filter over the warm end and the blocking filter over the cool end. Experimental lanes were placed under an array of UVB (Q-Panel UVB 313; Q-Panel, Cleveland, OH) and full-spectrum bulbs (Vita-Light; Durotest Corporation, Fairfield, NJ), such that each lane was illuminated by both types of lights. UVB radiation was measured using a hand-held Solar Light meter with a UVB probe (meter model PMA2100, probe model 2102; Solar Light Company, Philadelphia, PA). In each experiment, UVB was approximately 10 $\mu W/cm^2$ under acetate filters, whereas UVB under Mylar filters was virtually undetectable ($\leq 0.05\ \mu W/cm^2$). These levels are within the range of ambient UVB levels in the Oregon Cascades (Belden et al. 2000; Kiesecker et al. 2001).

Two sets of trials per day were conducted over 3 days for each species. For the first set of trials, observations began at approximately 1100 h each day, whereas the second set of trials began at approximately 1400 h each day. Our intent was to test UVB avoidance behavior of tadpoles during peak UVB hours (1100–1700 h). Once a stable gradient was established in each of the 8 lanes, tadpoles were randomly assigned to one of the 2 UVB treatments. Each unit contained one tadpole, for a total of 24 tadpoles in each treatment. Tadpoles were allowed to acclimate for 15 min prior to observation. After the acclimation period, the location of the tadpole in each gradient was recorded from behind an observation blind every 10 min for 100 min (10 observations per tadpole). Observers were blind to treatment combinations during the course of the experiment. The temperature closest to each tadpole for each observation was used to determine average temperature selected by each tadpole. If a tadpole was located equidistant between 2 probes, the average of the 2 temperatures was used as the preferred temperature.

Field UVB choice experiments

These experiments were designed to determine if amphibian larvae selectively avoided areas with higher UVB exposure in the absence of a thermal gradient. Trials were conducted between 1215 and 1415 h on 22 July 2005 (*B. boreas*, Todd Lake), 20 July 2006 (*Rana cascadae*, Susan's pond), and 10 August 2006 (*P. regilla*, potholes). Experimental units were constructed based on the methods of Belden et al. (2000) and consisted of plastic boxes (34 cm length × 21 cm width × 11 cm depth) floated in the pond with Styrofoam floats on all 4 sides with mesh panels to allow for water circulation. One half of each container was randomly assigned a UVB blocking filter (Mylar-D). The other half of each container was covered with a UVB transmitting filter (acetate). Tadpoles ($N = 24$) were placed singly in each box and allowed to acclimate for 15 min prior to observation. The location of each tadpole within the unit was recorded by an observer blind to treatment every 10 min for 120 min, for a total of 12 observations per tadpole. Halfway through the trials, each box was rotated 180° to avoid bias due to cardinal direction. After rotating the boxes, tadpoles were allowed 10 min to acclimate before resuming the trials. Temperatures on each side were recorded in a subsample of units at the end of each trial. UVB radiation was measured at the beginning of each trial under the filters using a hand-held Solar Light meter with UVB probe (see model information under "laboratory trials"). The Mylar filters transmitted approximately 10% of ambient UVB, and the acetate filters transmitted 75–80% of ambient UVB. UVB levels differed on each day but were approximately 10–16 μW/cm^2 under acetate filters.

Field transects

Field transects were used to supplement our experimental tests. We applied field transects at Susan's pond, Todd Lake, and the potholes to quantify thermoregulatory behaviors and UVB exposure in natural systems. The distance between transects and length of each transect varied between locations based on water body size and shape. Transects at Susan's pond (19–20 July 2006) were 10 m long with 20 m separating the 3 transects. At Todd Lake (9–10 August 2006), transects were 7 m long, with 35 m between the 3 transects. Transects were run perpendicular to shore and were divided into 1-m^2 sections. We attached vertical arrays of iButton temperature loggers at 2- (Susan's pond) or 3-m (Todd Lake) intervals to record temperature at different depths. Each array consisted of iButtons located at 20-cm intervals from the surface to the bottom of the pond. At Susan's pond and Todd Lake, an individual iButton was placed in the shallowest portion of each transect next to shore. Individual ponds at the potholes site (10 August 2006) were too small to contain more than one transect; thus, we selected 4 representative ponds and aligned each transect along the widest axis of each pond. Transect length varied based on the size of the pond and were 4–7 m long. The 4 ponds selected at the potholes were physically different from each other and from both Susan's pond and Todd Lake. With the exception of pond C, the ponds were steep sided without a shallow margin. Thus, iButtons were placed approximately 1.5 and 3.5 m from shore in 2 of the 4 ponds.

All transects were in place by 1000 h. We allowed sediment to settle for 30 min prior to walking along transects. For each observation, 2 observers slowly walked either side of each transect, counting the number of larvae of any observed amphibian species within his or her half of the square meter. Care was taken not to disturb animals or sediment. We measured UVB radiation at the surface of the water and at every depth from 10 to 50 cm (or the bottom of the pond) before each observation period using a hand-held light meter with a UVB probe (see model listed under UVB avoidance in thermal gradients). Our goal was to capture larval movement due to diel thermal fluctuations. At Susan's pond and Todd Lake, we walked along each transect 3 times a day for 2 consecutive days. We walked along the transects at Susan's pond and Todd Lake in the morning (0900–1100 h), afternoon (1400–1500 h), and in the evening (1800–1900 h). Transects at the potholes were in place for 1 day. We walked along the transects at the potholes twice, once in the afternoon (1200 h) and once in the evening (1700 h).

The number of tadpoles observed in each transect varied widely across transects and at each sampling time. We normalized the data using the square root transformation (all species at the potholes and *Ambystoma macrodactylum* in Susan's pond), the logit transformation (*P. regilla* and *R. cascadae* at Susan's pond), and the natural log transformation (*B. boreas* at Todd Lake). After transformation, we used mixed effects models to explore the effects of observation date, observation time, water depth, and the second-order interactions among these 3 factors on the distribution of amphibian larvae. Transect number was considered a random effect in these mixed models. Each site and species were analyzed separately.

RESULTS

UVB avoidance in thermal gradients

Thermal gradients ranged from 20 to 31 °C in the Pacific tree frog trials and from 21 to 31 °C in the western toad trials. No difference in average temperature selected was detected between treatments in either *P. regilla* ($P = 0.885$) or *B. boreas* larvae ($P = 0.293$; Mann–Whitney *U* test; Table 1). Both species selected relatively warm temperatures (Table 1). Because we did not detect a difference in mean temperature selection, we used equivalency tests and calculated the least significant value using power analysis. The equivalency test and power analysis support our findings of no difference between mean temperatures selected in the 2 UVB treatments (Table 1).

Field UVB choice experiments

We found no evidence for UVB avoidance in larvae of the 3 species in these experiments (binomial test; *P. regilla*, $P = 0.16$; *B. boreas*, $P = 0.15$; *R. cascadae*, $P = 0.14$). Three *B. boreas* larvae escaped during the trials and were excluded from the analysis. Several tadpoles from each species exhibited no choice between sides (i.e., equal number of observations on each side of the container). However, as the majority of tadpoles exhibited a choice (16/21 *B. boreas*, 19/24 *R. cascadae*,

Table 1

Results from Mann–Whitney *U* test, equivalency test, and power analysis in thermal gradient laboratory trials

Species	Treatment	Average temperature selected (1 standard error)	*n*	df	*Z* value	*P* value	Equivalence *P* value[*]	Least significant value
Western toad	UV shielded	28.2 (0.5)	24	1	1.05	0.29	0.03	1.34
	UV exposed	27.6 (0.4)	24					
Pacific tree frog	UV shielded	25.2 (0.4)	24	1	−0.13	0.89	0.002	1.26
	UV exposed	25.1 (0.5)	24					

Treatment indicates the UVB exposure of the warm end of the gradient.

[*] Practical difference = 2 °C.

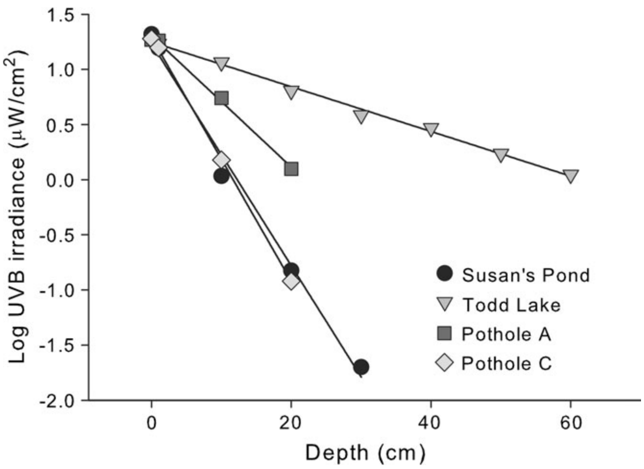

Figure 1
UVB profiles from all sites during midday. Todd Lake had the highest UVB transmittance. Only ponds A and C are shown from the potholes for clarity. The transmittance of pond B was nearly identical to pond A, whereas pond D was similar to pond C. Note the log scale used on the *y* axis.

and 21/24 *P. regilla*), tadpoles with no preference were excluded from the analysis. No difference in temperature was observed between sides in any trial (Wilcoxon signed-rank test; *B. boreas*: $P = 0.99$, degrees of freedom [df] = 5; *R. cascadae*: $P = 0.13$, df = 4; *P. regilla*: $P = 0.99$, df = 5).

Field transects

Abiotic parameters
As expected, UVB irradiance was highly correlated with water depth at all sites during all observation times ($r^2 > 0.97$; Figure 1). In addition, water temperature was generally negatively correlated with water depth, particularly at midday (Table 2). The similarity in the direction of the correlation between depth and water temperature and UVB irradiance results in warm regions with high UVB irradiance and cool regions with low UVB irradiance. Thus, using water depth as a parameter allowed us to test for a trade-off between thermoregulation and UVB avoidance. In addition, using water depth as a proxy for both UVB radiation and temperature avoided multicollinearity in our statistical models. Maximum temperature gradients varied widely among sites, with the potholes having the least difference between maximum and minimum temperatures (4.5 °C, Table 2).

Species distributions
Similar trends in distribution were observed for all anuran species at Todd Lake and Susan's pond. An interaction between amphibian distribution (number of tadpoles observed at each depth) and time of observation was detected for all anuran species (Table 3). This interaction is the result of diurnal movements toward shore, such that more tadpoles were observed in shallow water at midday (Figures 2 and 3). Both *B. boreas* and *R. cascadae* were more common in deep water during morning and evening observations, whereas *P. regilla* were observed in deep water only during the evening. In contrast, salamander larvae were more common in deeper water at all observation times (Table 3, Figure 2). Fewer salamander larvae were observed on 20 July, the warmer of the 2 observation days at Susan's pond (Table 3). No relationship between depth and amphibian distribution was observed for any species at the potholes (Figure 4).

DISCUSSION

Amphibian larvae did not avoid UVB radiation in any of our experimental trials, even in the absence of other environmental cues such as temperature. Our field transects showed that the majority of anuran larvae were observed in very shallow regions during midday, resulting in exposure to high levels of UVB radiation. Our experimental data and surveys are consistent with others who have shown that larval and postmetamorphic amphibians seek sunlight, bask, and are often found in shallow water, most probably for thermoregulation (e.g., Lillywhite 1970; Bradford 1984; Wollmuth et al. 1987; Wollmuth and Crawshaw 1988; Hutchison and Dupré 1992). UVB radiation negatively affects all 4 of the species we used in this study (e.g., Blaustein et al. 1994, 1997, 2005; Kiesecker and Blaustein 1995; Belden et al. 2000; Kiesecker et al. 2001; Bancroft 2007). These negative effects include mortality and sublethal effects that may reduce growth and development in larvae and postmetamorphic individuals (e.g., Belden et al. 2000; Blaustein et al. 2005). Our UVB avoidance hypothesis was generally not supported by our experimental data. In laboratory thermal gradients, larval *P. regilla* (Pacific tree frog) and *B. boreas* (western toad) did not avoid UVB by selecting cooler temperatures with lower UVB levels. Rather, larvae preferred relatively high temperatures, regardless of UVB exposure, thus illustrating the strong selection pressures for thermoregulation. In the field, larvae of these 2 species, in addition to *R. cascadae* (Cascades frog) larvae, showed no preference for low UVB areas in choice tests. Using similar methods, Belden et al. (2000) found that larval *A. macrodactylum* (long-toed salamander) preferred shade to full sun but

Table 2
Correlation between water depth and water temperature at 3 amphibian breeding habitats in Deschutes County, OR

Site	Time (h)	Spearman's rho	*P* value	Maximum temperature gradient (°C)
Todd Lake	0900	0.21	0.15	16.5–18.5
	1400	−0.46	<0.0001	17.0–25.5
	1800	−0.057	0.66	18.5–22.5
Susan's pond	1000	−0.5585	<0.0001	19.0–24.5
	1400	−0.5605	<0.0001	18.0–30.0
	1800	−0.5198	<0.0001	23.5–28.5
Potholes[a]	1200	−0.89	—	17.5–20.0
	1600	−0.83	—	20.0–24.5

[a] Too few observations to generate *P* values at the potholes (only 5 temperature loggers per pond).

Table 3

Quantifying habitat use in larvae of 4 amphibian species in Deschutes County, OR

Site	Species	Source of variation	*F* ratio (*P*)	df
Todd Lake	Western toad	Date	0.07 (NS)	1, 114
		Time	15.84 (***)	2, 114
		Depth	0.96 (NS)	1, 115
		Date × time	2.11 (NS)	2, 114
		Date × depth	3.77 (NS)	1, 114
		Time × depth	6.50 (**)	2, 114
Susan's pond	Long-toed salamander	Date	6.19 (*)	1, 168
		Time	0.19 (NS)	2, 168
		Depth	77.95 (***)	1, 166
		Date × time	2.00 (NS)	2, 168
		Date × depth	1.72 (NS)	1, 168
		Time × depth	1.68 (NS)	2, 168
	Cascades frog	Date	0.056 (NS)	1, 148
		Time	1.99 (NS)	2, 149
		Depth	6.90 (**)	1, 131
		Date × time	0.04 (NS)	2, 146
		Date × depth	1.74 (NS)	1, 148
		Time × depth	6.68 (**)	2, 148
	Pacific tree frog	Date	1.2573 (NS)	1, 120
		Time	11.7943 (***)	2, 118
		Depth	15.4972 (***)	1, 120
		Date × time	1.0895 (NS)	2, 120
		Date × depth	0.1919 (NS)	1, 118
		Time × depth	3.5018 (*)	2, 118

Fixed effect results from mixed models testing the hypothesis that larval amphibians avoid UVB radiation by seeking cooler, deeper water during peak UV hours. Transect number (3 per observation time per site) was included as a random effect in these models. NS, Not significant.

*$P < 0.05$.

**$P < 0.01$.

***$P < 0.0001$.

did not discriminate between differences in UVB levels manipulated by plastic filters. The results of the 2 experiments presented here, in conjunction with previous research, suggest that the larvae of the 4 species in this study do not avoid sunlight with potentially harmful levels of UVB radiation. Our field transects corroborate our experimental data. If amphibian larvae avoid UVB in the field, we would expect to see fewer larvae in shallow water at Todd Lake due to the relatively high UVB transmittance at this lake. Yet, we observed large numbers of tadpoles in shallow water (<10 cm), despite high levels of UVB at these depths in Todd Lake. Although the transect data are a 1- or 2-day snapshot of the field conditions, the similarity between our experimental results, transect data, and data collected by Belden et al. (2000) are striking and suggest that we accurately captured the habitat use of these species in the larval stage.

The habitat use exhibited by the anuran larvae in this study exposes them to potentially damaging levels of UVB radiation. Some studies have suggested that habitat characteristics such as dissolved organic carbon (DOC) may fully protect amphibians from damage caused by UVB radiation (Adams et al. 2001; Palen et al. 2002). DOC provides protection from UVB by reducing the penetration of harmful wavelengths through the water column. However, these studies did not consider temporal and spatial habitat use within a pond and the potential for other selection pressures to affect exposure to UVB. Our results suggest that anuran larvae preferentially exploit warm, shallow water during midday when UVB radiation is high. Ignoring larval behavior and temporal fluctuation in environmental parameters such as temperature can result in erroneous conclusions about the functional role of DOC as a photoprotectant. Even high amounts of DOC cannot provide protection in very shallow water, as larvae will be very close to the surface with little intervening water to provide protection. Although the effects of UVB radiation on survival of amphibians vary among species (e.g., Häkkinen et al. 2001), the overall effect of UVB on amphibians tends to be large and frequently acts synergistically with other environmental stressors such as contaminants (Bancroft et al. forthcoming). Thermoregulatory behaviors combined with increasing levels of UVB radiation result in exposure to higher, potentially damaging, levels of UVB that may interact with other anthropogenic stressors in aquatic habitats.

The lack of UVB avoidance in our field trials and the strong influence of temperature on habitat use in our laboratory trials suggest that the patterns we observed in our field surveys are the result of thermoregulatory behavior. The absence of a relationship between the number of larvae observed and water depth at the potholes provides further support for this assertion. The pothole ponds had the smallest thermal gradient, 4.5 °C, compared with 8.5 °C at Todd Lake and 12 °C at Susan's pond. Temperature is frequently the dominant physical factor affecting physiology and behavior of larval amphibians (Ultsch et al. 1999). The diel movements of *B. boreas* tadpoles at Todd Lake closely followed diel temperature fluctuations, suggesting a strong relationship between temperature and habitat use. Wollmuth et al. (1987) found the highest densities of Cascades frog tadpoles in the warmest (and shallowest) regions of a pond, and tadpole aggregations moved throughout the afternoon to track the warmest temperatures. The importance of temperature as a cue guiding habitat use is not surprising given the influence of temperature on growth rate in amphibians (Atlas 1935; Ryan 1941; Álvarez and Nicieza 2002).

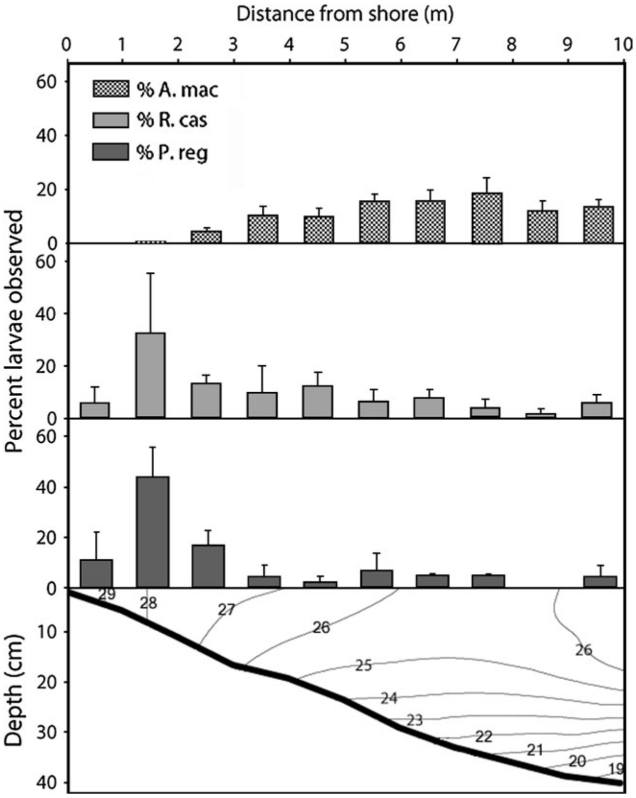

Figure 2
Distribution of the larvae of 3 species at midday on 20 July 2006 in Susan's pond, Deschutes County, OR. Thick black line in the bottom panel represents the bottom of the pond, and the numbers are temperature in degrees Celsius. Bars represent the mean percentage of larvae of each species observed at each depth ± standard error (mean of 3 transects). The majority of anuran larvae (*Rana cascadae* and *Pseudacris regilla*) were observed closer to shore in water <15 cm deep. Most salamander larvae (*Ambystoma macrodactylum*) were observed in water >20 cm deep.

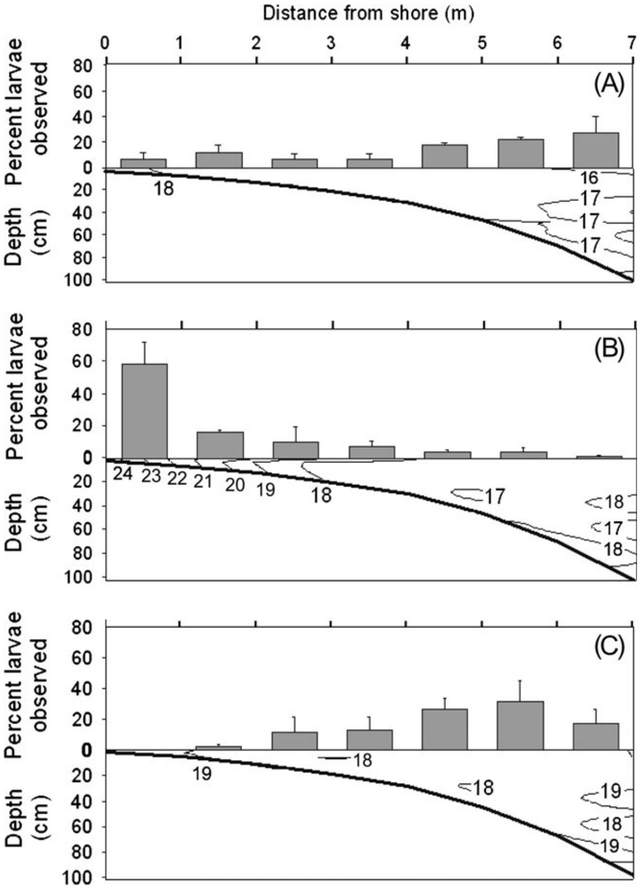

Figure 3
Distribution of *Bufo boreas* larvae in Todd Lake, Deschutes County, OR, on 10 August 2006. The thick black line indicates the bottom of the lake. Contour lines represent thermal stratification in degrees Celsius. Bars indicate the mean percentage (±SE) of *B. boreas* tadpoles observed in 3 transects in the morning (A), midday (B), and evening (C).

Amphibians developing in any of the 3 sites in our study are under pressure to develop quickly and emerge from the water before the habitat either dries (pothole and Susan's ponds) or freezes (Todd Lake). Selection for thermoregulatory behaviors that speed development may have been intense and taken precedence over selection for UVB avoidance in these populations due to the extreme nature of these sites. Because all 3 of the sites in the current study undergo rapid environmental changes near the end of larval development, we may be unable to detect any context-dependent behavior in response to conflicting selection pressures in these populations. UVB avoidance behavior might be more common in systems where thermoregulation is less important. In addition, several other factors not measured in the current study likely contribute to habitat use in larval amphibians, including distribution of food resources, predators, and other abiotic parameters such as dissolved oxygen. Temperature likely interacts with all these factors, which may result in strong selection for seeking warm shallow water during peak UV hours, despite the negative consequences of UVB exposure.

Our results suggest that, over evolutionary history, UVB radiation has been a lower order selective pressure, where the benefits of rapid development resulting from seeking warm temperatures outweighed the costs associated with UVB exposure in these sites. Amphibians have been exposed to UVB radiation throughout evolutionary history (Cockell 2001) and are able to mediate damage caused by UVB via several mechanisms (reviewed in Blaustein and Belden 2003). The presence of several types of mediators suggests that UVB has exerted selective pressure on amphibians over evolutionary time. However, UVB levels reaching the earth's surface have increased to unprecedented levels in modern time (<100 years ago) due to stratospheric ozone depletion (Solomon 1999). We suggest that UVB avoidance behaviors may not have had time to evolve in this system (Blaustein and Bancroft 2007). Furthermore, our results suggest that larval amphibians are exposed to potentially damaging doses of UVB as a consequence of thermoregulatory behaviors.

Habitat selection behavior is a reflection of many potentially interacting pressures. Conflicting selection pressures may result in inefficient avoidance behaviors (Sih 1992) and patterns of habitat use that are unpredictable and nonoptimal. Including the interplay among various stressors in natural systems is vital to our understanding of how conflicting selection pressures may affect organisms.

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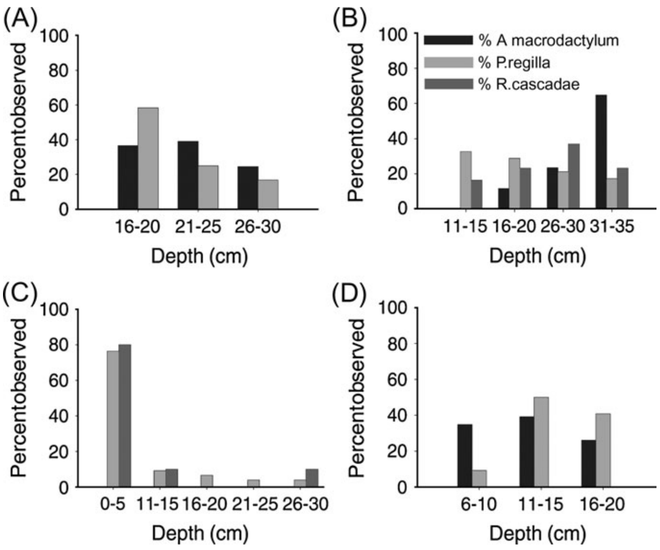

Figure 4
Distribution of larvae at midday on 10 August 2006 at the potholes, Deschutes County, OR. Individual potholes (A–D) are shown separately. Bars indicate percentage of each species observed within each depth class.

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