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PRIMARY RESEARCH PAPER

Influence of ultraviolet-B radiation on growth, prevalence of deformities, and susceptibility to predation in Cascades frog (*Rana cascadae*) larvae

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Abstract Ambient levels of ultraviolet-B radiation (UVB) have a variety of detrimental effects on aquatic organisms. These include death and effects on growth, development, physiology, and behavior. Amphibians show all of these effects. However, the effects vary with species, life history stage, and ecological context. Little is known about the implications of the detrimental effects of UVB on ecological dynamics. Our study was designed to test how UVB may affect predator–prey interactions, an important ecological dynamic. Specifically, we tested the effect of UVB on the susceptibility of Cascades frog (*Rana cascadae*) larvae to predation by rough-skinned newts (*Taricha granulosa*). We also further examined the sublethal effects of UVB on growth and development in Cascades frog larvae. We found no direct effect of UVB exposure on survival. However, UVB-exposed frog larvae displayed decreased growth and increased prevalence of deformities. UVB also caused increased susceptibility to predation, but there was a significant treatment–block interaction. UVB increased susceptibility to predation in two out of five blocks of Cascades frogs. The other three blocks did not show an effect of UVB on susceptibility to predation. Our study suggests that UVB can alter susceptibility to predation in at least one amphibian species. UVB-induced alteration of predator–prey interactions could potentially lead to changes at the population, community, and ecosystem levels.

Keywords Ultraviolet-B radiation · Predator–prey interactions · Amphibians · Sublethal effects · Growth · Deformities

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Introduction

Anthropogenic reduction of stratospheric ozone has caused an increase in the amount of ultraviolet-B radiation (UVB; 280–315 nm) reaching the earth's surface (Blumthaler & Ambach, 1990; Kerr & McElroy, 1993; Herman et al., 1996; Muller et al., 1997; Rex et al., 1997; Zerefos et al., 1997; Middleton et al., 2001). This has raised concern about possible harmful effects of increased UVB on individuals, ecological communities, and ecosystems (Cockell &

Blaustein, 2001; Haeder & Worrest, 1991). Detrimental effects from UVB have been described in a variety of organisms, including phytoplankton (Worrest et al., 1980; Hobson & Hartley, 1983), fungi (Fourtouni et al., 1998; Moody et al., 1999), plants (Torabinejad et al., 1998; Rousseaux et al., 2001), crustaceans (Siebeck, 1978; Zagarese et al., 1994), and vertebrates (Little & Fabacher, 1994; Browman et al., 2000). In a meta-analysis of a total of 68 published experimental studies of aquatic organisms, Bancroft et al. (2007) showed that UVB exposure induces large, negative effects on survival and growth. These effects manifested in organisms with different life histories and taxonomic affinities, and in organisms from different habitats.

Although numerous factors appear to be contributing to amphibian population declines (Stuart et al., 2004), increasing ambient UVB is hypothesized to contribute, by itself, or with other stressors, to certain population declines in amphibians (Blaustein et al., 1994, 1998; Blaustein & Kiesecker, 2002). Because amphibian population declines are global (Houlahan et al., 2000; Stuart et al., 2004), it is reasonable to hypothesize that wide-scale changes in the environment, such as climate change or increased UVB over much of the earth's surface, contribute to these declines (Blaustein et al., 1998). A second meta-analysis (Bancroft et al., 2008a), which used a total of 41 published experimental studies on amphibians, found an overall negative effect of UVB on survival. The recent meta-analyses of Bancroft et al. (2007, 2008a) and the recent synthesis of Croteau et al. (2008) who reviewed the lethal and sublethal effects of UVB exposure on amphibian development and metamorphosis in relation to increasing levels of UVB at the earth's surface, strongly suggest that UVB should be investigated as one factor potentially contributing to amphibian population declines.

There is a growing literature on the effects of UVB exposure on amphibian embryos and larvae. In amphibian embryos, ambient levels of UVB can reduce hatching success, increase prevalence of deformities, decrease size at hatching, increase time to hatching, cause melanization, and induce carry-over effects of decreased growth and slower development at the larval stage (e.g., Blaustein et al., 1994, 1997; Anzalone et al., 1998; Lizana and Pedraza, 1998; Pahkala et al., 2000, 2002a, b; Smith et al., 2000; Belden & Blaustein, 2002a; Perotti & del Carmen Diéguez, 2006). In amphibian larvae, exposure to ambient levels of UVB can cause mortality, deformities, reduced growth, decreased oxygen consumption, skin darkening, and DNA damage (e.g., Worrest & Kimeldorf, 1976; Ankley et al., 2000; Bruggeman et al., 1998; Zaga et al., 1998; Kats et al., 2000; Belden et al., 2000, 2003; Häkkinen et al., 2001; Belden & Blaustein, 2002a, b, c; Formicki et al., 2003; Pahkala et al., 2003; Pandelova et al., 2006; Weyrauch & Grubb, 2006). UVB also affects behavior in larval and adult amphibians (e.g., Nagl & Hofer, 1997; Blaustein et al., 2000; Hatch & Blaustein, 2000; Kats et al., 2000; Han et al., 2007).

The effects of UVB on amphibians depend on species, life history stage, and ecological context [reviewed in Blaustein et al. (1998, 2001, 2003, 2004) and Blaustein & Kiesecker (2002)]. More knowledge about how UVB influences ecological interactions among amphibians is needed before generalizations can be made about the overall effects of UVB on these organisms. We investigated how UVB may affect one important ecological dynamic—predator–prey interactions.

We used Cascades frog (*Rana cascadae* Slater) larvae to: (1) examine effects of UVB on growth and development; (2) test whether UVB exposure increased the susceptibility of frog larvae to predation by rough-skinned newts (*Taricha granulosa* Skilton; hereafter: newts). Rough-skinned newts prey on Cascades frog larvae in nature (Peterson & Blaustein, 1991), and Cascades frog larvae respond to newt cues by reducing their activity, an anti-predatory behavior (Hokit & Blaustein, 1995). UVB exposure not only decreases activity level in Cascades frog larvae in the absence of predators (Hatch & Blaustein, 2000) but also impairs the anti-predator reduction in activity when newt cues are present (Kats et al., 2000), suggesting that UVB may alter interactions between Cascades frogs and newts.

Materials and methods

Collection and maintenance of amphibians

Cascades frog embryos [Gosner (1960) developmental stages: 8–10] were collected on 28 April 2003 at Parish Pond (elevation 1,120 m), 11 km southwest of

Marion Forks, Linn County, Oregon, USA. Cascade frogs often lay their embryos in communal masses in which embryo masses from multiple females are clumped together (Blaustein, 1988; Olson, 2005). Five non-adjacent pieces of a single communal embryo mass were collected. Pieces were collected from different parts of the communal mass such that it is unlikely that embryos from any two pieces came from the same female. Individuals from a single embryo mass piece constituted a block ($n = 5$ blocks, approximately 250 embryos/block). Each block of Cascades frogs was maintained separately in a 38-l glass aquaria (length × width × height = 50 × 25 × 31 cm; 1 aquarium/block) filled with approximately 35 l of water and aerated. All the water used in the laboratory was dechlorinated tap water conditioned with NovAqua® and Amquel® water conditioners (~0.14 ml of each conditioner/l of water).

Larvae were transferred to new aquaria with new water on 6 and 13 May. After removal of the larvae tested in the experiment, remaining larvae were maintained in the aquaria as laboratory stock for use as food for newts and fed ad libitum a mixture (approximately 3:1 by volume) of rabbit chow and Tetramin fish flakes (hereafter: tadpole food) on 16 May.

Forty-one adult male newts were collected on 18–20 May 2003 from two ponds (elevations about 1,070 and 1,090 m) separated by 100 m. These ponds are about 3.4 km northwest of Parish Pond. Each newt was brought to the laboratory and maintained in a 38-l glass aquarium (dimensions as above, 1 newt per aquarium) filled with 3.8 l of water (water depth = 3 cm). Before the predation trial, newts were given food once, during a preliminary feeding conducted 22–23 May in which each newt received five frog larvae (1 larva from each of the five blocks). Newt aquaria were checked after 25 h and all the uneaten larvae were removed. Of the newts used in the predation trial, 34 of them ate all five larvae; two ate four larvae, one ate one larva, and three did not eat any larvae during the preliminary feeding. Each newt was moved from its 38-l aquarium to a 9-l aquarium (length × width × height = 30 × 14 × 20 cm) filled with about 1.3 l of water approximately 80 min before the start of the predation trial.

Amphibians were kept at approximately 14–15°C under a natural photoperiod using fluorescent light bulbs and sunlight through unshaded windows, except that exposure of Cascades frog larvae to experimental UVB and control treatments occurred in a separate UVB exposure chamber (maintained at approximately 16°) with a different light regime (see below).

Exposure of Cascades frog larvae to UVB and control treatments

Cascades frog larvae were used in a randomized block experiment with two treatments (UVB and control) and five blocks, with each block corresponding to a single piece of the communal Cascades frog embryo mass. There were two replicates of each treatment in each block, resulting in 20 experimental units and a total of 10 replicates of each treatment. Twenty round plastic exposure containers (diameter = 20 cm, height = 6 cm) filled with 2 l of water to a depth of 5 cm were each randomly assigned to a block and a treatment. Each exposure container received 15 larvae [2–7 days post-hatching, Gosner (1960) developmental stages 23–24] haphazardly selected from the aquarium housing the assigned block of larvae. In nature and in the laboratory, Cascades frog hatchlings attach themselves to their egg jelly during early larval development (J.M.R., personal observation). Therefore, each exposure tub received approximately 10 jelly capsules (lacking embryos) from the aquarium of its assigned block. Exposure containers in the UVB treatment were covered with an acetate filter, which transmits ~43% of UVB irradiance from UV 313 bulbs (J.M.R., unpublished data). Exposure containers in the control treatment were covered with mylar, which blocks ~99% of UVB irradiance from UV 313 bulbs (J.M.R., unpublished data). Covers were placed 1 cm above the top of containers to allow airflow.

Larvae were added to exposure containers and moved to the UVB exposure chamber 1 day before the start of exposure to UVB and control treatments. Each exposure container was randomly assigned to 1 of 21 positions under an array of seven lamps. Each lamp contained one UV-generating light bulb (UVB 313, Q-Panel, Inc., Cleveland, Ohio, USA) and one full-spectrum light bulb (Vita-Lite, Duro-Test Corporation, Fairfield, New Jersey, USA).

Two days prior to the start of the UVB and control treatments, UVB irradiance was measured at the surface of the water in an acetate-covered exposure container (lacking larvae) placed in each used position under the lamps. This procedure was repeated using the same container covered with mylar instead of acetate. UVB irradiance was measured using a model 2100 PMA (personal measurement assistant) meter with model 2102 UVB detector (Solar Light, Philadelphia, Pennsylvania, USA). Mean UVB irradiance ± 1 SE was 11.1 ± 0.4 μW/cm^2 (range: 8.02–13.8 μW/cm^2) under acetate and 0.15 ± 0.01 μW/cm^2 (range: 0.10–0.22 μW/cm^2) under mylar. The levels of UVB irradiance measured under acetate are within measurements of UVB at the water's surface taken at Parish Pond and other natural breeding sites of Cascades frogs in the Oregon Cascade Range (Table 1). Cascades frog larvae were present in the pond during most measurements (see Table 1). UVB attenuates as water depth increases (Table 1). However, large numbers of Cascades frogs larvae can frequently be observed in water <10 cm deep, where UVB levels are high (Bancroft et al., 2008b; B.A.B., unpublished data), suggesting that the level of UVB irradiance used in this study is realistic for larvae of this species in the Oregon Cascade Range. Furthermore, the water depth in our experiment (5 cm) was also realistic. In ponds with a large amount of very shallow microhabitat, large numbers of Cascades frog larvae can often be found in water <5 cm deep (Bancroft et al., 2008b; J.M.R., personal observation). There was no shade in our experiment; this was also realistic, since shade from aquatic vegetation is sparse or absent in many Cascade frog breeding ponds in the Oregon Cascade Range (J.M.R., personal observation).

On the day larvae were transferred to the UVB exposure chamber, the chamber was illuminated with a single 52-W light bulb (Super Saver XL, Sylvania, Danvers, Massachusetts, USA) until 1915 h, after which the chamber was kept in darkness until 0630 on the following day, when the lamps containing UVB and full-spectrum bulbs were turned on. These lamps were operated on a 10 L:14D photoperiod from this point until larvae were removed from the UVB chamber 11 days later. At the end of the light period on the 11th day of exposure to UVB and control treatments, the UVB bulbs were replaced with full-spectrum bulbs. Thus larvae were exposed to 11 days of UVB and control treatments.

Each tub received 5 mg of tadpole food on 16 May. On 22 May, larvae and the egg jelly in their exposure containers were transferred to new tubs with fresh water and each new tub received 10 mg of food. All larvae were visually inspected for deformities at least once per day. We checked for the blistering, edema, and axial, eye, head, face, and profound deformities described in Bantle et al. (1998). We compared UVB-exposed larvae with laboratory stock Cascades frog larvae which were not in UVB or control treatments to check for other deformities including curled or frayed tails. Dead larvae or parts of larvae were removed. Final mass was quantified by weighing all the larvae remaining after removal of the larvae used in the predation trial. Survival and prevalence of deformities (prevalence of individuals displaying one or more deformities) were quantified on the last day of the experiment but before the predation trial. Prevalence of deformities was calculated using the total number of individuals present, including live and dead individuals.

Predation trial

Newts were weighed 1 day before the predation trial. Newts ranged from 9.43 to 18.98 g (mean ± 1 SE = 13.47 ± 0.36 g). Two newts rather than one were assigned to each experimental unit to minimize variation associated with differences between newts. Newts were ranked according to mass and assigned to experimental units randomly, except that the mass rankings were used to size-match newts across all combinations of treatment and block.

The predation trial was conducted on the day after the end of exposure to UVB and control treatments. Within each experimental unit, two sets out of five larvae were haphazardly selected for use in the predation trial, resulting in the inclusion of both deformed larvae and larvae not displaying deformities in the predation trial. Table 2 displays the mean prevalence of deformities among the larvae from the UVB treatment which were used in the predation trial. All deformed larvae in the predation trial had a deformed tail.

Selected larvae were inspected for deformities again as described above and each set of five larvae

was randomly paired up with one out of the two newts assigned to that experimental unit. Each pairing of larvae and newts was randomly assigned to a predation trial arena. Thus each set of five larvae was tested with one newt in one arena, and two sets of five larvae were tested per experimental unit. Each set of larvae was tested simultaneously.

Predation trial arenas were 38-l glass aquaria (dimensions as above) filled with 5 l of water to a depth of 4 cm. Each arena contained a wooden newt

Table 1 Measurements of UVB at Cascades frog breeding sites in Oregon, USA

Site	Location	Elevation (m)	Year	Dates	Time of day (h)	Depth (cm)	UVB irradiance (range, $\mu W/cm^2$)	Reference
Todd Lake	30 km W of Bend, Deschutes Co.	1,875	2005	9–10 August	0945–1045	0	8.24–12.0	Bancroft et al. (2008b) and B.A.B. (unpublished data)
						1	7.2–11.5	
						10	4.68–6.7	
						20	2.63–4.9	
					1400–1445	0	6.51–18.6	
						1	13.8–17.1	
						10	10.1–11.6	
						20	1.95–6.4	
					1750–1830	0	1.05–2.07	
						1	0.84–1.79	
						10	0.31–1.19	
						20	0.13–0.74	
Potholes (Pond A)	~0.6 km NW of Todd Lake, Deschutes County	1,980	2006	10 August	1206	0	18.6	Bancroft et al. (2008b) and B.A.B. (unpublished data)
						1	18.2	
						5	9.86	
						10	5.5	
						15	3.28	
						20	1.25	
Potholes (Pond B)	~0.6 km NW of Todd Lake, Deschutes County	1,980	2006	10 August	1206	0	19.4	Bancroft et al. (2008b) and B.A.B. (unpublished data)
						1	18	
						5	9.6	
						10	5.05	
						15	2.15	
						20	1.25	
Potholes (Pond C)	~0.6 km NW of Todd Lake, Deschutes County	1,980	2006	10 August	1206	0	19	Bancroft et al. (2008b) and B.A.B. (unpublished data)
						1	15.7	
						5	4.86	
						10	1.51	
						15	0.38	
						20	0.12	
Potholes (Pond D[a])	~0.6 km NW of Todd Lake, Deschutes County	1,980	2006	10 August	1206	0	17.6	Bancroft et al. (2008b) and B.A.B. (unpublished data)
						1	14	
						5	4.29	
						10	0.56	
						15	0.13	

Table 1 continued

Site	Location	Elevation (m)	Year	Dates	Time of day (h)	Depth (cm)	UVB irradiance (range, $\mu W/cm^2$)	Reference
Susan's Pond	25 km W of Bend, Deschutes Co.	2,020	2005	19–20 July	1015	0	13.4	Bancroft et al. (2008b) and B.A.B. (unpublished data)
						1	9.6	
						10	0.3	
						20	0.04	
					1400–1500	0	20.9	
						1	15.6	
						2	9.06	
						4	4.88	
						6	2.33	
						8	1.38	
						10	1.02–1.08	
						12	0.47	
						14	0.22	
						16	0.15	
						18	0.08	
						20	0.05–0.15	
					1801	0	2.8	
						1	2.2	
						10	0.12	
						20	0.03	
			1997	20 May–7 June	Not reported	0	4.77–25.5	Blaustein et al. (1997)
Hitchhiker Pond	10 km W of McKenzie Pass, Lane Co.	1,600	2002	13–17 August	1240–1415	0	17.5–21.2	J.M.R., unpublished data
Site 1	12 km S of Marion Forks, Linn. Co.	1,140	1998	11–18 June	1130–1230	0	14.5–16.6	Belden et al. (2003)
Parish Lake	12 km SW of Marion Forks, Linn. Co.	1,020	1999	25 June–6 August	1000–1100	0	5.2–13.2	Belden et al. (2003)
Parish Pond	11 km SW of Marion Forks, Linn. Co.	1,120	2000	14 June–5 July	Not reported	0	18.7–21.2	Hatch & Blaustein (2003)
			2001	1–21 June	Not reported	0	7.77–15.3	Hatch & Blaustein (2003)

All measurements were taken with a 2100 PMA meter with a model 2102 UVB detector (see Materials and methods) between the times of day listed. Measurements at depth >20 cm are not included. Cascades frog larvae were present in the water body during all measurements except for some measurements in Susan's Pond in 1997

[a] Maximum depth <20 cm

cage (length × width × height = 24 × 12 × 6 cm) with sides of 1-mm fiberglass mesh to allow water flow and lacking a bottom. Each cage was placed at one randomly selected end of its arena. Approximately 40 min before the start of the trial, each newt was added to the wooden cage of its arena. Each arena also contained a tadpole cage made from a round plastic cup (diameter at bottom = 8 cm, diameter at top = 11 cm, height = 11 cm) placed in the center of its assigned arena. These tadpole cages lacked bottoms and had sides with two 1-mm fiberglass mesh windows (length × height = 8 × 3.5 cm) on opposite sides to allow water flow. All the cages sat flat against the bottom of the arena and prevented exit or entrance of

Table 2 Mean proportion of deformed individuals (proportion of individuals with at least 1 deformity) ± 1 SE among the Cascades frog larvae in the predation trial

Block	Mean proportion deformed ± 1 SE
1	0.80 ± 0.10
2	0.60 ± 0.30
3	0.25 ± 0.05
4	0.70 ± 0.30
5	0.70 ± 0.10
Total	0.61 ± 0.09

Only the data for the UVB treatment are shown

animals. Approximately 20 min after the addition of newts, each set of five larvae was added to its arena by placing them inside the tadpole cage. Larvae were allowed to acclimate for 10 min before the tadpole cages were lifted out of the arenas, releasing the larvae. About 8 min later, at 1530 h, the trial was begun by lifting the newt cages out of the arenas, which released newts. Thereafter, the number of larvae remaining was counted in each predation trial arena every 10 min for 250 min.

Statistical analyses

Each dependent variable was analyzed separately. Survival was not analyzed using parametric statistics due to heteroscedasticity that could not be removed by transformation. Instead, we tested for a difference in survival between treatments by using a Wilcoxon rank sum test to compare the 10 experimental units in the UVB treatment with the 10 experimental units in the control treatment. In addition, for each block, we tested for a difference in survival between treatments with randomization tests (Ramsey & Schafer, 1997) using the original scale of the response variable. In addition, to provide tests on ranks corresponding to the rank-based Wilcoxon rank sum test, we performed alternative randomization tests using the rank-transformed results. We used a Bonferroni adjustment to maintain $\alpha = 0.05$ within the analysis of survival while making multiple comparisons regarding survival (Quinn & Keough, 2002). The Bonferroni-adjusted *P*-value for rejection of null hypothesis regarding survival was $\alpha/x = 0.05/6 = 0.0083$, where $x =$ the number of comparisons made. Results for proportion of individuals deformed also had heteroscedasticity that could not be removed by transformation, and thus were analyzed the same way as the results for survival.

Average final mass was analyzed using ANOVA followed by Tukey tests for pair-wise comparisons between different combinations of treatment and block. Mass data met all parametric assumptions after log-transformation to remove non-normality. Two individuals that were found dead on the last day of the study (1 individual in the UVB treatment in block 1 and 1 individual in the UVB treatment in block 5) were eaten by conspecifics before they could be massed. Final developmental stages were not analyzed because all larvae at the end of the experiment were at the same stage.

We examined survival during the predation trial by analyzing time to predation for each individual larva in every block using a Cox Proportional Hazards (Cox PH) model (Parmar & Machin, 1995). Block, treatment, and the block × treatment interaction were included as factors, experimental unit (exposure container) was included as a factor nested within block and treatment, and predation trial arena was included as a factor nested within experimental unit. Additionally, we tested each block for an effect of treatment, using separate Cox PH models for each block. Within-block Cox PH models had treatment as a factor, experimental unit as a factor nested within treatment, and predation trial arena as a factor nested within experimental unit. Because we compared treatments in five separate within-block Cox PH models, we employed a Bonferroni adjustment to maintain $\alpha = 0.05$ while making these five separate comparisons. The Bonferroni-adjusted *P*-value for rejection of null hypothesis in within-block Cox PH models was $\alpha/x = 0.05/5 = 0.01$. The nesting design of each Cox PH model took into account the fact that each experimental unit consisted of two statistically dependent predation trial arenas, each of which, in turn, consisted of five statistically dependent larvae.

In addition, we evaluated the possible influence of deformities on susceptibility to predation, using just the experimental units in the UVB treatment, since no individuals in the control treatment displayed any deformities. Using the experimental unit as the unit of replication, we tested for a correlation between the prevalence of deformities in larvae used in the predation trial and time to predation during the trial using Spearman rank correlation corrected for ties (Zar, 1999). Each experimental unit was ranked

according to the median time to predation for its 10 larvae in the predation trial. Because Spearman rank correlation analysis only tests for a positive correlation between ranks, we first tested for a positive correlation between deformities and time to predation by ranking experimental units in prevalence of deformities from the lowest to the highest and then tested for a negative correlation between deformities and time to predation by ranking experimental units in prevalence of deformities from highest to lowest.

Results

Survival

Median survival after exposure to UVB and control treatments was 96.7% (95% CI: 93.3–100%) in the UVB treatment and 100% (95% CI: 93.3–100%) in the control treatment. This difference was not significant ($Z = 1.0086$, $P = 0.3132$). In addition, there were no within-block differences between treatments (results on original scale: all $P \geq 0.0500$; rank-transformed results: all $P \geq 0.0241$).

Deformities

In the UVB treatment, median prevalence of deformities was 58.6% (95% CI: 26.7–92.9%), while in the control treatment, no individuals displayed deformities. This overall difference between treatments was highly significant ($Z = 4.0437$, $P = 0.0001$). According to the analysis of the results on the original scale, there was a non-significant trend toward higher mean prevalence of deformities in the UVB treatment compared with the control treatment within block 5 ($P = 0.0124$) and no differences between treatments for any of the other blocks (all $P \geq 0.0127$). Mean prevalence of deformities (± 1 SE) in the UVB treatment in block 5 was 80.0 ± 20.0. The analysis of the rank-transformed results found a somewhat different pattern, with median prevalence of deformities being significantly higher in the UVB treatment compared to the control treatment in block 5 (Fig. 1, $P = 0.0077$) and no differences between treatments for any of the other blocks (all $P \geq 0.0186$). Each deformed individual displayed one or more tail deformities consisting of lateral flexure, curling and/or fraying of the tail, except for one individual in the UVB treatment in block 2 with lateral flexure of the tail and a blister or edema on the side of the tail near the base and one individual in the UVB treatment in block 5 with abdominal edema.

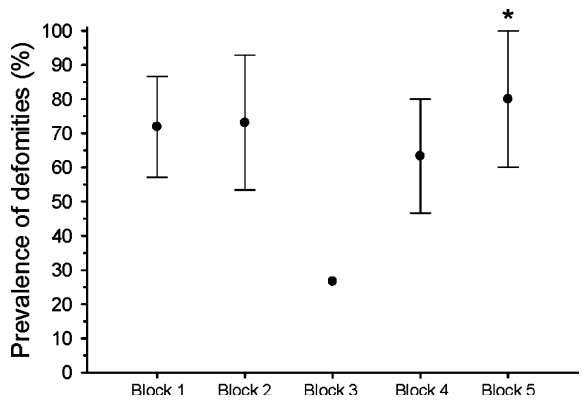

Fig. 1 Median prevalence of deformities of Cascades frog larvae in the UVB treatment in each of the five blocks. Error bars describe 95% confidence intervals. The *asterisk* denotes the increase in median prevalence of deformities in the UVB treatment compared to the control treatment within block 5

Final mass

Mean final mass was lower in the UVB treatment compared with the control treatment ($F_{1,10} = 32.4503$, $P = 0.0002$, Fig. 2). Mass was not different among different blocks ($F_{4,10} = 2.3179$, $P = 0.1281$) and there was no treatment–block interaction ($F_{4,10} = 1.0911$, $P = 0.4119$). In block 2, mass was lower in the UVB treatment compared with the control treatment ($q_{10,10} = 6.5622$, $0.01 < P < 0.025$). Mass was also lower in the UVB treatment–block 1 combination compared to the control treatment–block 2 combination ($q_{10,10} = 5.7902$, $0.025 < P < 0.05$), lower in the UVB treatment–block 1 combination compared to the control treatment–block 4 combination ($q_{10,10} = 5.6615$, $0.025 < P < 0.05$), and lower in the UVB treatment–block 2 combination compared to the control treatment–block 4 combination ($q_{10,10} = 6.4336$, $0.01 < P < 0.025$). No other pair-wise differences between treatment–block combinations were significant (all $P > 0.05$).

Development

All larvae were at Gosner (1960) developmental stage 25 (no hindlimb buds visible) at the end of the experiment.

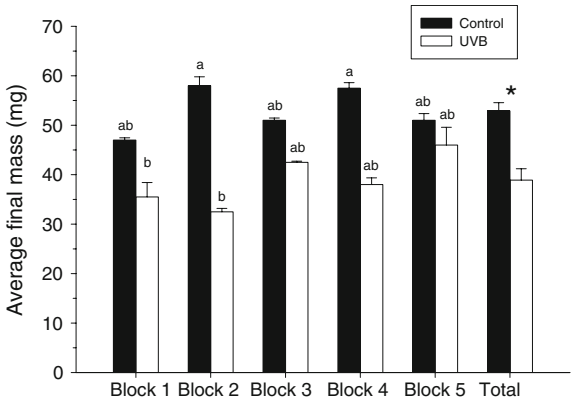

Fig. 2 Average final mass +1 SE of Cascades frog larvae according to treatment. Among the 10 different combinations of treatment and block, combinations that do not share a letter are statistically different (Tukey test, $P < 0.05$). The *asterisk* denotes the overall decrease in final mass between all experimental units in the UVB treatment compared with all the experimental units in the control treatment

Predation trial

Survival in the predation trial was generally lower in UVB treatment compared to the control treatment (Fig. 3). However the overall effect of UVB exposure on hazard of predation across all five blocks of larvae was not significant, although there was an effect of block (Table 3). Importantly, there was a block–treatment interaction, indicating that there was an effect of UVB and it depended on block. UVB increased hazard of predation in blocks 1 and 5 (Fig. 4, Table 3). In contrast, UVB had no significant influence on hazard of predation in blocks 2–4, although in block 3 there was a non-significant trend of higher hazard of predation in the UVB treatment compared with the control treatment. In the UVB treatment, the only treatment in which deformities were observed, prevalence of deformities in larvae used in the trial was not correlated with median time to predation (Fig. 5, positive correlation: $(r_s)_c = -0.0428$, d.f. $= 10$, $P > 0.5$, negative correlation: $(r_s)_c = 0.0795$, d.f. $= 10$, $P > 0.5$).

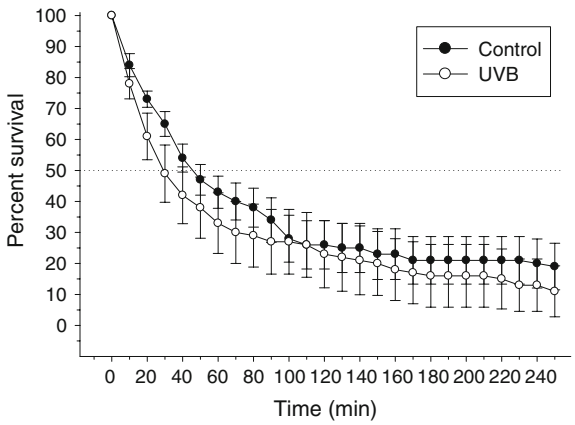

Fig. 3 Mean percent survival ± 1 SE of Cascades frog larvae in the predation trial according to treatment

Discussion

Survival prior to the predation trial was not different in UVB-exposed larvae compared to controls. However, previous work has shown that UVB can kill Cascades frog larvae. Hatch and Blaustein (2000) found that three weeks of exposure to 9–11 μW/cm^2 of artificial UVB killed larvae of this species at Gosner (1960) stages 23–24. Similarly, Belden et al. (2003) found that 6 weeks of exposure to ambient UVB, measured at 5.2–13.2 μW/cm^2 between 1000 and 1100 h, killed Cascades frog larvae. The lack of a direct effect of UVB on survival in this study may be due to the relatively short period of exposure employed (11 days). Also, additional nutrients from the fish flakes fed to larvae in this study but not those in Hatch and Blaustein (2000) or Belden et al. (2003) may have allowed increased resistance to UVB. It is also possible that the lack of a direct effect of UVB on survival in our study was due to the combination of a short exposure period and increased nutrition. Water depth, dimensions of containers, site of collection of Cascades frogs, number of larvae per container, and UVB exposure regimes also differed among these three studies. However, none of these factors seem likely to have contributed to the pattern of results regarding the direct effects of UVB on survival. For example, UVB irradiance at the surface of the water appeared to be, in general, slightly higher in our study, which did not show a direct effect of UVB on survival, than in the studies of Hatch and Blaustein (2000) and Belden et al. (2003), which did show direct negative effects of UVB on survival.

In addition, we found no evidence that rate of development was influenced by UVB exposure, perhaps because of the shortness of the exposure period. An effect on rate of development may require a

Table 3 Results of Cox Proportional Hazards models of hazard of predation during the predation trial for all blocks and for each individual block

Model	Factor	d.f.	X_1^2	P*	Relative risk
All blocks	Block	4	46.6643	<0.0001	–
	Treatment	1	<0.0001	1	1.6315
	Block × treatment	4	23.3568	0.0001	–
	Unit (within cells)	10	72.2985	<0.0001	–
	Arena (within units)	20	89.9517	<0.0001	–
	Error (within arenas)	80			
Block 1	Treatment	1	13.8212	0.0002	4.6830
	Unit (within cells)	2	11.8969	0.0026	–
	Arena (within units)	4	7.0268	0.1345	–
	Error (within arenas)	8			
Block 2	Treatment	1	5.2747	0.0216	0.0181
	Unit (within cells)	2	31.2166	<0.0001	–
	Arena (within units)	4	24.2164	<0.0001	–
	Error (within arenas)	8			
Block 3	Treatment	1	5.9011	0.0151	39.3826
	Unit (within cells)	2	4.0500	0.1320	–
	Arena (within units)	4	24.3483	<0.0001	–
	Error (within arenas)	8			
Block 4	Treatment	1	0.0058	0.9394	0.9725
	Unit (within cells)	2	12.9057	0.0016	–
	Arena (within units)	4	16.2687	0.0027	–
	Error (within arenas)	8			
Block 5	Treatment	1	10.2184	0.0014	3.4440
	Unit (within cells)	2	3.7899	0.1503	–
	Arena (within units)	4	6.1228	0.1902	–
	Error (within arenas)	8			

Relative risk values are for the risk of predation in the UVB treatment relative to the risk of predation in the control treatment

* The P-value for rejection of null hypotheses was 0.05 for the model with all blocks and 0.01 for each model for a single block

longer period of exposure to be detected. However, larvae exposed to UVB had smaller masses than those in the control treatment, indicating that UVB reduced growth. In our study, no larvae appeared to be large enough to reach a size refuge in which they are too large to be eaten by newts, which are gape-limited predators. However, in nature, reduced growth may prevent or delay amphibian larvae from reaching such size refugia from newts or other predators, potentially increasing the risk of predation. Furthermore, reduced growth during the larval stage may lead to smaller size at metamorphosis, which may delay sexual maturity (Smith, 1987; Semlitsch et al., 1988).

Our results also suggest that UVB increased the prevalence of deformities in Cascades frog larvae. Almost all of these deformities involved the tail, which impaired swimming ability in the predation trial. Each deformed tail exhibited lateral flexure, curling and/or fraying of the tail. Similarly, lateral flexure of the tail accounted for many of the deformities produced in long-toed salamander (*Ambystoma macrodactylum*) embryos exposed to ambient UVB (Blaustein et al., 1997).

The results of the predation trial suggest that UVB caused increased susceptibility to predation by newts. However, this effect depended on block. UVB increased susceptibility to predation in two blocks but had no significant influence on susceptibility to predation in the other three blocks. Differences between blocks in the effect of UVB on survival in the presence of predatory newts may have been due

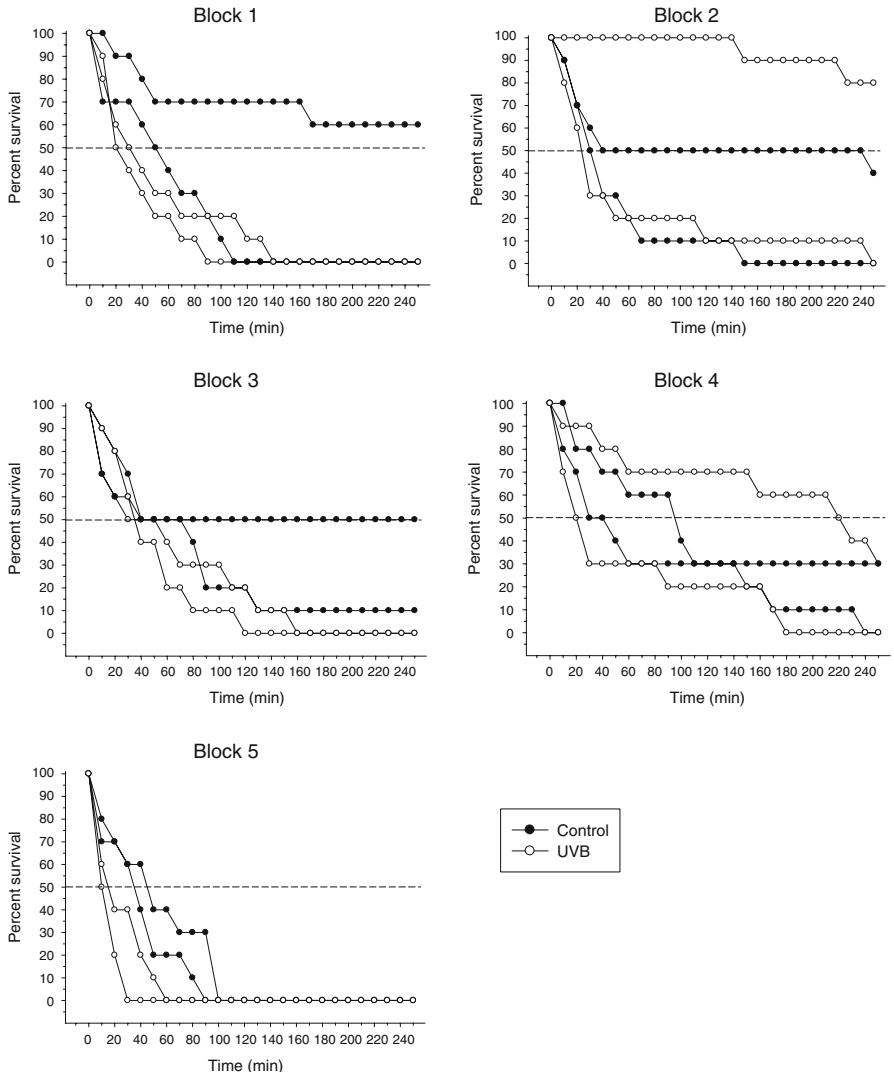

Fig. 4 Percent survival during the predation trial in each block. Each line represents a single experimental unit

to genetic differences between the different portions of the communal embryos mass from which the different blocks were derived. There is some evidence consistent with genetic differences influencing the effects of UVB exposure on amphibians (Belden and Blaustein, 2002c; Weyrauch & Grubb 2006). However, because each block was housed individually (in its own aquarium) prior to the start of the experiment, we cannot rule out the possibility that differences between aquaria (environmental conditions) contributed to these results.

Our study was not designed to test any mechanisms by which UVB might increase susceptibility to predatory newts. However, we were able to evaluate the possible role of UVB-induced deformities. It is likely that deformed larvae had reduced swimming ability relative to non-deformed larvae, since all deformed larvae had a deformed tail. However, we found no correlation between deformities and time to predation. Thus, it is unlikely that deformities caused or contributed to increased susceptibility to predation, despite any reduction in swimming ability they may have caused. Swimming ability of larvae may be irrelevant to interactions between Cascades frog larvae and newts, perhaps because differences in swimming ability between Cascades frog larvae do not alter their detectability to newts and, once detected by a newt, Cascades frog larvae have little chance of escaping, no matter how well they swim. Thus, other possible mechanisms besides reduced

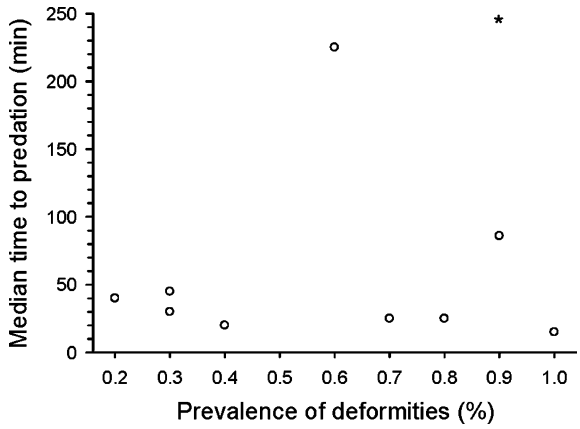

Fig. 5 Median time to predation in the predation trial versus prevalence of deformities in larvae used in the trial. Only experimental units in the UVB treatment are shown. Each *circle* represents a single experimental unit. The *asterisk* denotes an experimental unit in which median time to predation was undefined due to only two larvae being eaten during the predation trial

swimming ability from UVB-induced deformities are implicated. UVB-induced impairment of anti-predator behavior has already been demonstrated in Cascades frog larvae (Kats et al., 2000). In addition, the UVB-induced reduction in growth we observed may have contributed to the faster depletion of larvae in the UVB compared to the control treatment if smaller larvae require less handling time than larger larvae.

Our study suggests that UVB exposure increases susceptibility to predation in larvae of at least one amphibian species, Cascades frogs. If UVB increases susceptibility of Cascades frog larvae to predators in nature, this effect could eventually lead to effects at the population level. This is especially so if sublethal effects of UVB on Cascades frog larvae cause newts to concentrate on hunting this species rather than other prey. Indeed, mathematical modeling studies suggest that larval survival may have a substantial influence on population size in some amphibian species (Biek et al., 2002; Vonesh & De la Cruz, 2002).

Decreases in amphibian populations could force predators to alter their diets, which could lead to population declines in alternate prey species. There is evidence that declines in prey populations may lead to population declines or reduced reproduction in alternate prey (e.g., Drost & McCluskey, 1992; Beukema, 1993; Estes et al., 1998; Summers et al., 1998; Norrdahl & Korpimaki, 2000). If sufficient numbers of alternate prey do not exist, predator populations may decline. For amphibians, even if reductions in larval survival of prey species do not lead directly to declines at the level of the prey population, predators or alternative prey may still be affected by a reduced number of post-larval individuals. Thus UVB-induced alteration of predator–prey interactions may have important effects on food webs and could potentially lead to ecosystem-level changes in energy flow and nutrient cycling.

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