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Exposure of red-legged frog embryos to ambient UV-B radiation in the field negatively affects larval growth and development

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Abstract Exposure to ultraviolet-B radiation (UV-B; 280–320 nm) has a wide array of effects on aquatic organisms, including amphibians, and has been implicated as a possible factor contributing to global declines and range reductions in amphibian populations. Both lethal and sublethal effects of UV-B exposure have been documented for many amphibian species at various life-history stages. Some species, such as red legged frogs, *Rana aurora*, appear to be resistant to current ambient levels of UV-B, at least at the embryonic and larval stages, despite the fact that they have experienced range reductions in the Willamette Valley of Oregon, USA. However, UV-B is lethal to embryonic and larval *R. aurora* at levels slightly above those currently experienced during development. Therefore, we predicted that exposure of embryos to ambient UV-B radiation would result in sublethal effects on larval growth and development. We tested this by exposing *R. aurora* embryos to ambient UV-B in the field and then raising individuals in the laboratory for 1 month after hatching. Larvae that were exposed to UV-B as embryos were smaller and less developed than the non-exposed individuals 1 month post-hatching. These types of sublethal effects of UV-B exposure indicate that current levels of UV-B could already be influencing amphibian development.

Keywords Amphibians · *Rana aurora* · Sublethal effects · Ultraviolet radiation · UV-B

Introduction

Ultraviolet-B radiation (UV-B; 280–320 nm) as an important abiotic factor for both terrestrial and aquatic organisms has received more attention with predictions of increasing UV-B at the Earth's surface due to stratospheric ozone depletion (Tevini 1993; Hader et al. 1995; Hader 1997). Indeed, exposure to UV-B has been suggested as contributing to at least two major biodiversity crises in recent decades: bleaching events of coral reefs (e.g. Shick et al. 1996; Lyons et al. 1998) and worldwide amphibian population declines (e.g. Blaustein et al. 1998; Alford and Richards 1999). For amphibians, this has prompted research on the effects of UV-B exposure on embryonic and larval amphibians (Blaustein et al. 1998). As would be expected for any abiotic factor, tests on embryonic amphibians demonstrate that species vary in their sensitivity to UV-B. Even within a given geographic location, embryos of some species experience increased mortality in response to UV-B exposure, while others appear unaffected (e.g. Blaustein et al. 1994; Anzalone et al. 1998; Lizana and Pedraza 1998; Langhelle et al. 1999; Broomhall et al. 2000).

In Oregon, USA, red-legged frogs, *Rana aurora*, have disappeared over much of their historic range (see Kiesecker and Blaustein 1998), but *R. aurora* embryos and larvae do not experience increased mortality in the presence of ambient UV-B (Blaustein et al. 1996; Ovaska et al. 1997). However, Ovaska et al. (1997) observed decreased embryonic and larval survivorship at slightly enhanced UV-B levels. Mortality at enhanced levels implies that there is a specific physiological UV-B tolerance limit for this species. Even though current ambient levels are not sufficient to induce mortality, it could be energetically costly for *R. aurora* embryos exposed to UV-B to resist or repair potential cellular damage.

Because *R. aurora* egg masses are often laid at the surface of the water in direct sunlight, the embryos may receive relatively high doses of UV-B, compared to larvae or adults which may move away from sunlight. In addition, *R. aurora* embryonic development tends to be prolonged because *R. aurora* breeds in winter at the Oregon coast, when water temperatures are low. (Early *R. aurora* embryos have the lowest known temperature tolerance of the North American ranid frogs (4–21°C), Nussbaum et al. 1983) Thus, embryos can potentially be

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exposed to higher cumulative doses of UV-B than species with short times to hatching.

As UV-B is lethal to embryonic and larval *R. aurora* at levels slightly above ambient (Ovaska et al. 1997), we hypothesized that exposure of embryos to ambient levels of UV-B radiation would result in larvae that were less developed than the non-exposed individuals. These types of sublethal effects, which cross life-history stages, have not been well investigated and could impact the long-term survival of many amphibian populations. We tested our hypothesis by exposing *R. aurora* embryos to ambient UV-B radiation in the field and then rearing individual tadpoles for 1 month after hatching in the laboratory.

Materials and methods

In December 1999, we collected six fresh *R. aurora* egg masses from a pond 10 km south of Waldport, Oregon. Later that day, we set up 8 containers in the laboratory with 5 eggs from each of 6 of the masses (30 eggs/container). All eggs were at Gosner stages 2–6 (Gosner 1960). The containers were left in the laboratory overnight and the following morning were transported to outdoor mesocosms located in an open field at the Salmon Disease Laboratory of Oregon State University. Mesocosms consisted of 8 large plastic tubs (110 cm diameter, 25 cm deep) filled with well water. Within each mesocosm, we placed the eggs in a wood framed enclosure [80 cm×80 cm×10 cm (depth)] with mesh sides and bottom. Four of these were randomly assigned to a UV-B blocking regime (mylar filter) and the other four received an acetate filter, which allows approximately 80% of the UV-B to pass (Blaustein et al. 1994). No adverse effects of enclosure materials (e.g. mylar, acetate) have been observed in previous field experiments (e.g. Blaustein et al. 1994, 1996; Kiesecker et al. 2001). Mylar and acetate filters were placed over the appropriate wooden enclosures and were stapled to the edges of the frame, such that filters were 3 cm above the surface of the water. We measured temperatures in all 8 enclosures at noon on 5 separate days during the experiment. In addition, temperature data loggers (Hobo loggers, Onset Computer, Bourne, Mass., USA) that recorded water temperature every hour for the duration of the experiment were placed in a single acetate and a single mylar enclosure. Eggs were checked for mortality and were counted every 1–2 days, and always following freezing night-time temperatures. If ice was present on the enclosures, it was broken up and removed. UV-B readings were taken at the site between 1200 and 1300 hours on 15 different days with differing weather conditions to gain an estimate of the range of exposure these larvae were receiving. These were done both in the air to obtain an ambient measurement and under all 8 filters. All measurements of UV-B were done using a hand-held Solar Light meter with a UV-B probe (meter model PMA2100; UV-B detector model PMA2102; Solar Light, Philadelphia, Pa., USA). The detector provides output that is weighted for biological effect based on the human erythemal ation spectra, with an irradiance peak at 297 nm.

After 6 weeks of exposure, when all embryos were nearing hatching (Gosner stages 19–21, Gosner 1960), all of them were collected and returned to the laboratory. They were set up by mesocosm (8 groups) in plastic tubs and the total number hatched was recorded each day. All embryos had hatched (or were dead) within 5 days of being brought into the laboratory. Three days after they were all hatched, 15 individuals from each group were randomly selected and placed in individual 550 ml plastic containers filled with 350 ml of dechlorinated tap water. Containers were placed in random order on a lab bench in a 10×12 container grid. Every day, we removed waste products and uneaten food from all 120 containers and changed half of the water. Tadpoles were fed a 3:1 mixture of ground rabbit chow:Tetramin fish food daily, so that food was always available to them. After 2 weeks, all containers were completely cleaned and refilled with dechlorinated tap water. After 1 month, we recorded mass and developmental stage (per Gosner 1960) for all 120 individuals.

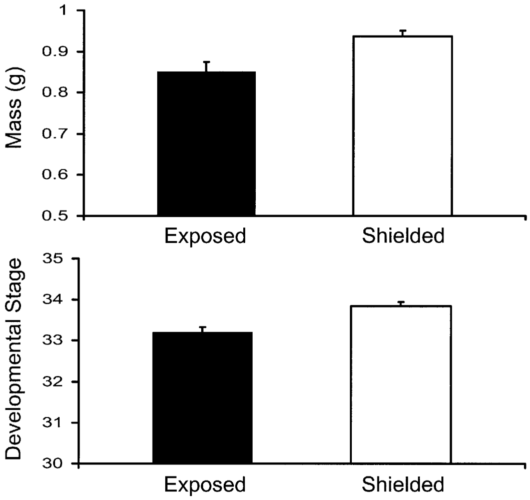

Fig. 1 Mean (±SE) mass (g) and developmental stage (Gosner 1960) of *Rana aurora* larvae 1 month after embryonic UV-B exposure in the field. *Black bars* represent exposed larvae. *White bars* represent larvae that were shielded from UV-B as embryos

Table 1 Results of MANOVA for overall effects of embryonic UV-B exposure on stage and mass of *Rana aurora* tadpoles 1 month after hatching, and ANOVAs for each response variable

	F	*df*	*P*
MANOVA			
Constant	310,175.3	2, 5	<0.001
UV treatment	6.546	2, 5	0.04
ANOVAs			
Stage	15.074	1, 6	0.008
Mass	10.352	1, 6	0.018

Analysis was done using MANOVA with the multivariate response of stage and mass on UV-B treatment. We used means from the original 8 rearing groups in our analysis. Mean temperatures in mylar versus acetate enclosures on our 5 days of temperature recording were compared using a paired *t*-test.

Results

Survival to hatching was high in all treatments (mylar= 93.3%, 96.7%, 100%, 100%; acetate=90%, 93.3%, 96.7%, 100%) with no difference between the two groups (Student's *t*-test; *P*=0.388). There was no mortality during the laboratory portion of the study. However, after 1 month, tadpoles that were not exposed to UV-B in the field as embryos were larger and more developed than individuals that were exposed as embryos (overall MANOVA for UV-B treatment effects, *P*=0.04; Table 1, Fig. 1). UV-B at the field site ranged from 0 μW/cm^2 while it was actively raining and overcast to 1.28–1.55 μW/cm^2 on clear sunny days**.** UV-B was undetectable under the mylar filters, and under acetate filters on

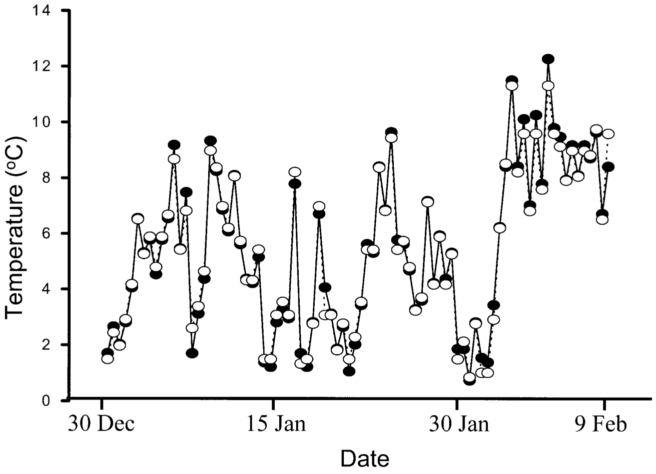

Fig. 2 Temperatures (°C) over the course of the field exposure for a single mylar (●) and acetate (○) enclosure. *Dots* represent temperatures every 12 h, starting at midnight of the first day. Statistical analysis for temperature was not performed on these data, but on means for the four acetate and four mylar enclosures taken at noon on 5 different days

clear days, levels ranged from 1.02 to 1.29 µW/cm². The maximum difference between the four acetate filters on any given day was 0.03 µW/cm². There were no temperature differences between the mylar and acetate enclosures (paired *t*-test; *P*=0.492; Fig. 2).

Discussion

Although *R. aurora* embryos have relatively high levels of the photorepair enzyme, photolyase (Blaustein et al. 1996), and do not experience greater mortality when exposed to ambient UV-B (Blaustein et al. 1996; Ovaska et al. 1997), our results suggest that there may be some energetic cost associated with UV-B exposure. In addition, our study demonstrates that UV-B exposure of embryos can have lasting effects on the larvae, at least up to 1 month post-hatching. Similar results have recently been documented for plains leopard frogs, *R. blairi* (Smith et al. 2000) and for common frogs, *R. temporaria* (Pahkala et al. 2001). Even though larvae may be able to behaviorally avoid UV-B (e.g. van de Mortel and Buttemer 1998) individuals exposed as embryos may already be at a disadvantage by the time they are able to escape from high UV-B environments. Indeed, size and rate of growth can be very important for larval anurans. Larger tadpoles may be better competitors (e.g. Travis 1980) may be more likely to attain the size threshold necessary for metamorphosis prior to pond drying (e.g. Wilbur and Collins 1973; Morey and Reznick 2000) and may be better able to avoid or ignore gape-limited predators (e.g. Puttlitz et al. 1999; Eklov 2000). In addition, larger larvae generally become larger metamorphic anurans which can have positive consequences for adult fitness (e.g. Smith 1987; Bervin 1990).

Other studies have documented growth effects on amphibians exposed to UV-B (e.g. Belden et al. 2000; Pahkala et al. 2000), but few have examined the effects of embryonic exposure on later stages. However, it is not surprising that the embryonic environment can have an influence on individuals at later life stages. This has been demonstrated for many animal groups, including mammals (e.g. Anisman et al. 1998), fish (e.g. McCormick 1998), reptiles (e.g. Shine et al. 1997) and amphibians (Watkins 2000).

In addition, various factors, such as the presence of predator cues in the environment (Sih and Moore 1993; Warkentin 1995), can alter the time to and size at hatching of embryonic amphibians. Changes in the size at hatching are likely to have effects similar to those that we observed. It may be the case that the changes we observed were already present at hatching and were still apparent 1 month later in the larvae. As amphibian embryos are generally exposed directly to the environment during development and lack a protective shell, there is a good chance that many abiotic factors could have a major influence on developmental traits.

As we have demonstrated, regulating or avoiding exposure to biologically damaging UV-B radiation can be important for amphibians. This is also true for other aquatic organisms, and may become increasingly important with predictions of escalating UV-B levels at the Earth's surface due to stratospheric ozone depletion. However, in addition to ozone depletion, which will result in increases in UV-B in both terrestrial and aquatic environments, there are other factors that will alter the levels of UV-B exposure for aquatic organisms. For example, acidification of lakes and ponds results in decreased dissolved organic carbons in the water and therefore increased penetrance of UV-B in the water column (Schindler et al. 1996; Yan et al. 1996). Also, changes in hydrologic cycles that may occur with global climate change can be expected to alter water depth and availability (e.g. Schindler et al. 1996; Yan et al. 1996; Pounds et al. 1999; Pienitz and Vincent 2000; Kiesecker et al. 2001), which could increase the UV-B exposure received by aquatic organisms. Factors such as these may be as important as ozone depletion for regulating UV-B exposure of aquatic organisms in the future (e.g. Schindler et al. 1996; Pienitz and Vincent 2000). However, our study demonstrates that even at current levels, UV-B can influence amphibian development and is potentially already shaping life histories of aquatic organisms.

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