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Survival, the hormonal stress response and UV-B avoidance in Cascades Frog tadpoles (*Rana cascadae*) exposed to UV-B radiation

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Summary

1. Despite the increasing occurrence of global environmental changes, including increases in ultraviolet-B radiation (UV-B; 280–320 nm), little is known about how factors such as UV-B affect animals physiologically. Amphibians provide a good model for examining physiological effects of UV-B exposure because studies documenting both lethal and sub-lethal effects have been completed on many species.

2. This study examines survival and the hormonal stress response, as measured by whole body levels of the glucocorticoid hormone corticosterone, of Cascades Frog, *Rana cascadae*, tadpoles exposed to ambient UV-B in the field for either 7 or 42 days.

3. There were no differences in corticosterone levels between UV-B exposed and non-exposed tadpoles at either time point. However, after 42 days, survival was significantly higher in tadpoles shielded from UV-B.

4. A stress response profile performed in the field demonstrated that young *R. cascadae* tadpoles are able to respond to confinement stress with increasing corticosterone. In addition, results from UV-B avoidance tests suggest that *R. cascadae* tadpoles do not avoid UV-B.

5. Thus, *R. cascadae* tadpoles may not perceive UV-B radiation, which would explain why they do not respond hormonally to exposure, despite the potential for exposure to result in mortality.

Key-words: Amphibian declines, corticosterone, ultraviolet-B radiation

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Introduction

Animals may respond physiologically to anthropogenic changes in habitat quality. For example, toads (*Bufo terrestris*) in habitats polluted by coal combustion waste have higher levels of circulating corticosterone and testosterone than toads in habitats without coal combustion waste (Hopkins, Mendonca & Congdon 1997). Bullfrog tadpoles (*Rana catesbeiana*) from polluted areas have higher resting metabolic rates than tadpoles from unpolluted sites (Rowe *et al*. 1998), and Brown Trout (*Salmo trutta*) from contaminated rivers have a suppressed hormonal response to acute stress (Norris *et al*. 1999).

However, while responses to specific pollutants have been examined, fewer studies have examined physiological responses of animals to global scale environmental changes. Ozone depletion, which is an example of this type of global change, will result in increasing levels of UV-B radiation at the surface of the Earth (e.g. Madronich *et al*. 1998). For aquatic organisms, such as fish and amphibian larvae, additional factors regulated by climate change, such as acidification and changes in water depth driven by local precipitation patterns, may also result in increased UV-B exposure (Schindler *et al*. 1996; Yan *et al*. 1996; Pienitz & Vincent 2000; Kiesecker, Blaustein & Belden 2001). Despite this, we know very little about how aquatic vertebrates respond physiologically to UV-B exposure. For a single fish species, the Roach (*Rutilus rutilus*), it has been suggested that UV-B exposure results in increased levels of circulating glucocorticoid hormones (Jokinen *et al*. 2000), but to our knowledge this is the only species that has been tested.

Amphibians provide a good model for the investigation of such responses because both lethal and sub-lethal effects of UV-B exposure have been documented for a variety of species at different life-history stages (e.g. Blaustein *et al*. 1994; Hays *et al*. 1996; Nagl &

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Hofer 1997; Anzalone, Kats & Gordon 1998; Fite *et al*. 1998; van de Mortel & Buttemer 1998; Langhelle, Lindell & Nyström 1999; Belden, Wildy & Blaustein 2000; Blaustein *et al*. 2000, 2001). The majority of studies have examined direct lethal effects on amphibian embryos exposed to natural levels of UV-B in the field (Blaustein *et al*. 1998). These studies have demonstrated variation between species in embryonic sensitivity. Less work has been completed on larval and adult life-history stages. However, effects on behaviour, growth and development, and survival have been documented for some species at these stages. For instance, following UV-B exposure, adult Roughskin Newts (*Taricha granulosa*) increase locomotion (Blaustein *et al*. 2000), larval Alpine Newts (*Triturus alpestris*) swim erratically (Nagl & Hofer 1997), and Cascade Frog tadpoles (*R. cascadae*) and juvenile Western Toads (*Bufo boreas*) decrease their responses to predator cues (Kats *et al*. 2000). In addition, larval Long-Toed and North-Western Salamanders (*Ambystoma macrodactylum* and *A. gracile*, respectively) experience decreased growth when exposed to UV-B (Belden *et al*. 2000; Belden & Blaustein 2002b) and several studies have demonstrated that embryonic UV-B exposure can result in sub-lethal effects on growth during the larval or metamorphic stages (Smith, Waters & Rettig 2000; Pahkala, Laurila & Merila 2001; Belden & Blaustein 2002a). Little work has been done to understand the physiological mechanisms driving these sub-lethal responses. However, one study suggested that increased activity in response to UV-B exposure may be indicative of a hormonal stress response involving glucocorticoid hormones (Blaustein *et al*. 2000).

Glucocorticoid hormones are released from the adrenal cortex by activation of the hypothalamic–pituitary–adrenal (HPA) axis, in response to stressful stimuli. Corticosterone, the main glucocorticoid hormone in amphibians (Idler 1972), acts to mobilize energy stores and suppress non-vital physiological processes until the stressful stimulus passes (Wingfield & Romero 2001; Romero 2002). In the short term, this can be beneficial to the organism, as immediate survival is promoted. However, chronic elevation of glucocorticoids in vertebrates can have deleterious effects, including decreased growth, depressed immune response and decreased reproductive output (Sapolsky 1993).

Both glucocorticoids and thyroid hormones are also critical in orchestrating the complex changes that amphibians undergo during metamorphosis (Hayes & Wu 1995). As such, many studies have examined basal levels of these hormones during that transition, and they are often reported as part of a control treatment (e.g. Krug *et al*. 1983; Denver 1997; Kloas, Reinecke & Hanke 1997). In addition, the larvae of at least three species of amphibians have a functional stress response during development: Leopard Frogs, *Rana pipiens* (Glennemeier & Denver 2002a, 2002b), African Clawed-Frogs, *Xenopus laevis* (Glennemeier & Denver 2002b) and Western Toads, *Bufo boreas* (Hayes 1997). A confinement/capture stress protocol (as in Glennemeier & Denver 2002b) is often used to examine the functioning of the stress response in free-living vertebrates (Wingfield *et al*. 1997). Individuals of a fourth species, Western Spadefoot Toads, *Scaphiopus hammondii*, are thought to rely on environmental cues from pond drying to initiate metamorphosis via the neuroendocrine stress pathway (Denver 1995, 1997).

Here, we present results of several experiments on Cascades Frog tadpoles (*Rana cascadae*) that begin to explore hormonal responses to UV-B exposure in larval amphibians. *Rana cascadae* is native to the Cascade Mountains of the Pacific North-west, USA. They have disappeared almost completely from the southern end of their range in northern California (Fellers & Drost 1993). *Rana cascadae* embryos experience increased mortality when experimentally exposed to ambient levels of UV-B radiation in the field (Blaustein *et al*. 1994). In this study, we addressed three hypotheses concerning *R. cascadae* tadpoles: (1) confinement stress over time results in increased levels of corticosterone; (2) exposure to UV-B radiation results in increased levels of corticosterone; and (3) tadpoles avoid UV-B when given a choice of regions with or without UV-B.

Materials and methods

CONFINEMENT STRESS

To determine whether tadpoles early in development (stage 25, Gosner 1960) are capable of responding to stress with increasing corticosterone, we completed a confinement stress response profile with tadpoles in the field. Tests were completed on a single day in May 2001, on free-living tadpoles at Parish Lake, a natural oviposition site for *R. cascadae* in the Cascade Mountains of Oregon, USA. The confinement stress protocol involved placing individual tadpoles in 800-ml plastic cups with mesh sides for 0, 30 or 60 min ($n = 8$ tadpoles/treatment). The three treatments were interspersed over the time period of testing to ensure that any changes seen were not simply an artefact of the time of day that sampling occurred. The containers were placed in the pond so that they were filled with 4 cm depth of water and were shaken every 3 min over the course of confinement. Water temperatures in the testing cups were identical to surrounding water temperatures during the trials. All tadpoles were rapidly frozen in the field. Tadpoles at the 0-min time point were netted in the field and immediately transferred to a 1·5-ml microcentrifuge tube. Tubes were then dipped into a dry ice/ethanol slurry for 60 s, so that freezing occurred within 2 min of initial capture. In pilot trials in the laboratory, 60 s was adequate for completely solidifying larval amphibians with masses up to 0·7 g. Tadpoles at the other time points were frozen in the same manner, with transfer to a 1·5-ml microcentrifuge

tube occurring immediately following their confinement period. Collection tubes were stored on dry ice until return to the laboratory, where they were stored at −70 °C until the radioimmunoassay (RIA) was completed. The mass (g) of each individual was recorded prior to RIA. Mean mass (± SD) of tested individuals was 0·07 ± 0·016 g. We compared the mean corticosterone levels in the three groups using an ANOVA.

1-WEEK FIELD UV-B EXPOSURE OF TADPOLES

The 1-week field study was completed at a natural oviposition site for *R. cascadae* in the Cascade Mountains in Linn County, Oregon, USA. In April 1998, we placed part of four *R. cascadae* egg masses in each of two field enclosures. This was done to ensure that after hatching, tadpoles could be readily collected for the experiments at the proper stage and with little handling time involved. Initial enclosures were constructed of a wooden frame measuring 100 × 100 × 75 cm^3 with fibreglass mesh (~1·5 mm squares) sides and bottom that allowed for water flow. When eggs were added, several handfuls of aquatic vegetation were also added to each enclosure to provide food and cover for hatching larvae.

After all eggs hatched and tadpoles were at Gosner (1960) stage 25, we randomly selected 24 tadpoles from the enclosures and moved them into individual containers. Individual containers were 800-ml plastic cups with mesh sides and bottoms attached to 46 cm long, 2·5 × 5 cm^2 boards. Mesh sides allowed for water flow and the mesh bottom ensured that waste products did not accumulate in the containers. Twelve containers were then randomly assigned a Mylar filter and the remainder were covered with an acetate filter. The Mylar filter blocks 90% of UV-B radiation and the acetate filter, which serves as a control for using a filter, transmits 80% of the UV-B radiation (Blaustein *et al*. 1994). Natural food (phytoplankton) in each container was supplemented with ground rabbit chow at the beginning of the experiment and again on day 4. Ambient levels of UV-B and the levels under the Mylar and acetate filters were measured between 1130 and 1230 h at the site on days 1, 4 and 7 using a hand-held Solar Light meter (model PMA2100; Solar Light Co., Philadelphia, PA) with a UV-B probe. The UV-B detector provides output that is weighted for biological effect based on the human erythemal action spectrum (irradiance peak = 297 nm).

On day 7, all experimental animals were collected from the field for the corticosterone RIA, with the exception of one individual that was missing from the acetate group. Collection involved freezing each individual within 2 min of capture by placing vials containing the animals in a dry ice/ethanol slurry for 60 s. After freezing in the field, larvae were transferred to a cooler containing dry ice, returned to the laboratory and stored at −70 °C until the RIA could be performed. The mass (g) of each individual was recorded immediately before the assay was performed. Mean mass (± SD) at collection was 0·27 (± 0·13) g. Whole body corticosterone levels between the treatment groups were compared using a non-parametric rank sum test.

6-WEEK FIELD EXPOSURE OF TADPOLES

The 6-week field experiment was conducted at Parish Lake, a natural oviposition site for *R. cascadae* in the Cascade Mountains (Linn County) of Oregon, USA. In spring, 1999, we added parts of five freshly laid *R. cascadae* egg masses (approximately 70 eggs from each mass) to a single large field enclosure (100 × 100 × 75 cm^3 (depth)) as described in the previous experiment. Several handfuls of aquatic vegetation were also added to the enclosure at that time to provide food and cover for hatching larvae. Shortly after hatching, when tadpoles were at Gosner (1960) stage 25, groups of 12 tadpoles were randomly selected from the initial enclosure and placed in each of 24 smaller experimental enclosures (total of 298 tadpoles). The experimental enclosures were constructed of two wooden hoops (diameter = 27 cm) placed 32 cm apart to form a cylinder with mesh sides and bottom. These provided maximal water exchange with the pond, allowed waste material to exit from the bottom and allowed for food (algae/diatoms) to enter. A single 11 × 11 cm^2 ceramic tile was placed in the bottom to weigh down the enclosures and they were each attached to two wooden dowels that were sunk into the substrate for stability. Water depth in the 24 enclosures at the beginning of the experiment ranged from 20 to 26 cm.

After tadpoles were added, 12 enclosures were randomly assigned to a UV-B treatment and covered with an acetate filter, while the other 12 were assigned to a no-UV-B treatment and were covered with Mylar filters (Blaustein *et al*. 1994). A single handful of aquatic vegetation was added to each enclosure as a substrate for algae and diatoms, and this was supplemented twice a week with ground rabbit chow. Tadpoles in each enclosure were counted once a week for the duration of the experiment. Ambient UV-B levels were recorded once a week as well, between 1000 and 1100 h (earlier in the day than in the 1-week experiment), using the hand-held Solar Light meter with a UV-B probe as in the 1-week experiment. Water temperatures within all enclosures were recorded on three days (beginning, middle and end) during the experiment. After 6 weeks, all remaining tadpoles were counted and from the six enclosures in each treatment group that contained the most tadpoles, three tadpoles were frozen for corticosterone analysis. We used the enclosures with the most remaining tadpoles to avoid confounding effects due to varying densities. Freezing was done by placing the tadpoles in individual vials and submersing the vials in a dry ice/ethanol slurry for 60 s. All tadpoles were completely frozen within 2 min of capture. Two enclosures had completely dried by the

6-week point, one UV-B (acetate) and one no UV-B (mylar), and these were not included in the analyses.

Mass was recorded immediately prior to RIA. Mean (± SD) mass of individuals was 0·16 ± 0·09 g. Survival differences between the treatments were analysed using a rank sum test. Corticosterone differences were analysed using a rank sum test on mean corticosterone level for each of the six enclosures in each treatment group from which tadpoles were collected.

UV-B AVOIDANCE TESTS

Field

For field tests of UV-B avoidance, approximately 75 *R. cascadae* eggs were collected from each of five different clutches at a natural oviposition site in the Cascade Mountains of Oregon and returned to the laboratory. Embryos were housed in the laboratory by clutch in 38-l aquaria filled with dechlorinated tap water. Large windows in the laboratory provided a natural photoperiod and the temperature was maintained at 16 °C. After hatching, larvae were reared in the same tanks and were fed ground rabbit chow *ad libitum* every other day. Complete water changes were completed once per week. When tadpoles were 2 months old, we tested for UV-B avoidance behaviour utilizing natural sunlight at EE Wilson Wildlife Refuge, 22 km N of Corvallis, Oregon. Tests were done in plastic tubs (45 L × 24 W × 15D cm^3), filled with 4 cm of dechlorinated tap water that was transported to the field site from the laboratory. The containers were set up in a 5 × 8 randomized block design in an open field, with the clutch (1–5) utilized as a block effect and eight replicates for each clutch. Five tadpoles from the appropriate clutch were placed into each tub. All tadpoles were preforelimb emergence (prestage 41, Gosner 1960). After the tadpoles were added to the tubs, the tubs were covered with $^1/_2$ Mylar (blocks UV-B) and $^1/_2$ acetate (allows UV-B to pass). Mylar and acetate filters were stapled to wooden boards (2·5 × 5 cm^2) that were the length of the tubs. The filters were draped over the edges of the tubs and were held in place by the weight of the boards. The side of the container receiving the Mylar filter was randomly assigned. After the filters were in place, we waited for half an hour before beginning to record observations. Then we recorded the number of tadpoles (out of five) on each side of the container every $^1/_2$ hour for $2^1/_2$ h (a total of five observation periods). Following each reading, we rotated the containers 180° to avoid preferences based on geographic orientation. We did the tests during midday (1000–1230 hours) to minimize shading created in the containers by the angle of the sun. To address that issue, we aligned the tubs initially so that any shading that did occur as the sun moved across the sky would be equal on both the Mylar and acetate sides of the tubs. UV-B measurements and water temperatures were taken in five randomly selected tubs under both filters at the beginning and end of the experiment. To record UV-B levels, we used the same hand-held Solar Light meter with UV-B probe that was used in the field experiments described above.

Data were summarized as the mean proportion of tadpoles on the Mylar (no UV-B) side of the container during the five sampling periods. We initially tested to see whether clutch had an effect on the mean proportion of tadpoles on the no UV-B side, but it did not (ANOVA; $F_{4,35} = 2{\cdot}131$; $P = 0{\cdot}098$), so we excluded it from further analyses. We then tested the hypothesis that the mean proportion of tadpoles on the no UV-B side of the containers was equal to 0·5, which would indicate no preference for either side. If larvae were consistently avoiding UV-B, we would have expected more larvae to be on the Mylar side and the mean proportion to be significantly higher than 0·5.

Laboratory

For laboratory trials of UV-B avoidance we randomly collected 100 tadpoles (stages 26–30, Gosner 1960) from a pond in the Cascade Mountains of Oregon. Larvae were returned to the laboratory and housed in four 38-l aquaria filled with dechlorinated tap water. The same day, they were fed ground rabbit chow. Natural photoperiod was provided in the laboratory by large windows and the temperature was maintained at 16 °C. The following day, all larvae were pooled into one tank and then randomly placed in groups of five into 15 rectangular plastic containers (32 × 18 × 8 cm^3) filled to a depth of 3 cm with dechlorinated tap water. These were moved into the room with UV lighting in the evening, when the lights were off. Mylar (blocks UV-B) and acetate (allows UV-B to pass) filters were utilized, as in the field experiment, to divide each container into UV-B exposed and non-exposed halves. Temperature in the room with the UV lighting was maintained at 14 °C. UV lighting was provided by a parallel array of lights, consisting of four UV-B lights (Q-Panel, UVB313; Q-Panel Inc., Cleveland, OH), alternated with four fluorescent full-spectrum lights (Vita Lite; Durotest Corporation, Fairfield, NJ). UV-B levels were recorded under Mylar and acetate filters from five randomly selected containers at the end of the experiment. The morning after larvae were moved into the room with UV lighting, avoidance trials were completed $^1/_2$ hour after the lights came on in the room (0930 h). For these trials, we recorded the number of tadpoles on the Mylar (no UV-B) side of the container every 10 min for 50 min (five time points). Data were summarized as the mean proportion of tadpoles on the no UV-B side of the container. As for the field experiment, we then tested the hypothesis that the mean proportion of tadpoles on the no UV-B side of the containers was equal to 0·5, which would indicate no preference for either side. The proportion would be significantly higher than 0·5 if larvae were avoiding UV-B.

RADIOIMMUNOASSAY

Whole body levels of corticosterone were measured by RIA following the procedures of Moore *et al*. (2000) and Ball & Wingfield (1987) with modifications. Each of the three experiments was run in a separate assay. Briefly, whole body homogenates were used for each assay. Each tadpole was weighed and homogenized with a mass adjusted amount of distilled water (mass × 10 ml + 0·5 ml rinse; minimum 1·5 ml and maximum 4 ml). For individual recovery determination, each sample was equilibrated overnight with 2000 cpm of tritiated corticosterone. Each sample was then extracted in 5 ml of dichloromethane (except for the 1-week exposure samples, which were extracted twice in 2 ml of anhydrous ethyl ether). To break the emulsion each sample was centrifuged at 2000 r.p.m. on a clinical centrifuge. The organic phase was then removed and dried in a warm water bath, under a stream of nitrogen gas. The extracts were then resuspended in 10% ethyl acetate in isooctane. The samples were chromatographed through individual celite columns to separate the steroid fractions and neutral lipids. The fractions were eluted using stepwise increasing proportions of ethyl acetate in isooctane. The purified eluates were dried and resuspended in buffer (phosphate buffered saline with 0·1% gelatin) for the assay. For the assay, individual sample recoveries were determined from 100 µl of the sample while 200 µl of the sample was allocated in duplicate for the assay. Serial dilutions for the standard curves were performed in duplicate. All samples, including serial dilutions and total bound, were incubated overnight with 100 µl of antibody and 100 µl of tritiated steroid. Unbound steroid was separated using dextran-coated charcoal and the bound steroid decanted into scintillation vials. Intraassay variation was 14% and interassay variation was 24%. Average recoveries were 68·8%.

Results

CONFINEMENT STRESS

Sixty minutes of confinement resulted in significant elevation of corticosterone in *R. cascadae* tadpoles in the field (overall ANOVA, $F_{2,21} = 9{\cdot}676$; $P = 0{\cdot}001$; *post hoc* Tukey test, 0 *vs* 30 min, NS; 0 *vs* 60 min, $P = 0{\cdot}001$; Fig. 1). Sixty minutes of confinement resulted in mean (± SD) whole body corticosterone levels of 1·21 (± 0·39) ng/g, while baseline individuals had a mean of 0·42 (± 0·26) ng/g.

1-WEEK FIELD EXPOSURE OF TADPOLES

No tadpoles died during the 1-week field experiment (one tadpole escaped; Fig. 2). There was not a significant difference between corticosterone levels in the two treatment groups (rank sum test; $P = 0{\cdot}16$). The median corticosterone level in the shielded group was 0·26 ng g^{-1} and in the UV-B exposed group the median was 0·42 ng g^{-1} (Fig. 1). Ambient levels of UV-B at the field site on the three days of measurement ranged from 14·5 to 16·6 µW cm^{-2}. Values under the Mylar filters ranged from 0·9 to 1·2 µW cm^{-2}, and under acetate ranged from 10·0 to 12·1 µW cm^{-2}. Water would attenuate this further (e.g. Schindler *et al*. 1996 and references therein), so that actual exposures of the larvae would be lower.

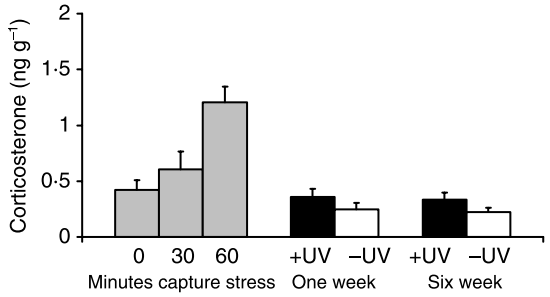

Fig. 1. Mean (± SE) whole body corticosterone levels for confinement stress individuals (0, 30 and 60 min) and for individuals exposed to UV-B or shielded from UV-B for 1 or 6 weeks.

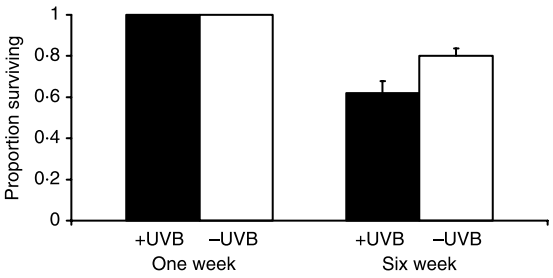

Fig. 2. Mean (± SE) proportion of larvae surviving in exposed to UV-B and shielded from UV-B treatments in the 1 and 6-week field experiments. There was 100% survival in the 1-week experiment.

6-WEEK FIELD EXPOSURE OF TADPOLES

Survival after 6 weeks in the field was significantly lower in the UV-B exposed enclosures than in those shielded from UV-B (rank sum test; $P = 0{\cdot}025$; Fig. 2). However, corticosterone levels in larvae from UV-B exposed treatments were not significantly higher than those shielded from UV-B (rank sum test; $P = 0{\cdot}310$). The median corticosterone level in the UV-B exposed enclosures was 0·31 ng g^{-1} *vs* 0·22 ng g^{-1} in the enclosures shielded from UV-B (Fig. 1). Ambient UV-B measurements during this experiment ranged from 5·2 to 13·2 µW cm^{-2}.

UV-B AVOIDANCE TESTS

Field

There was no detectable avoidance of the UV-B exposed sides of the containers (one-way *t*-test;

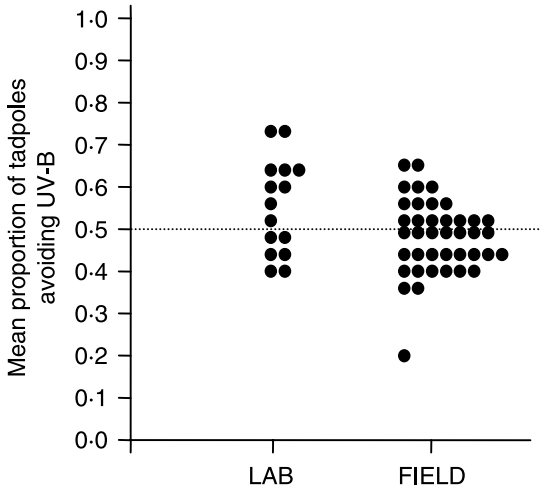

Fig. 3. Proportion of tadpoles on the side of the container shielded from UV-B in laboratory and field trials. Each dot represents the mean for a single container.

$H_0 = 0{\cdot}5$ on the no UV-B side; $P = 0{\cdot}112$; Fig. 3). Mean (± SD) UV-B levels at the beginning of the field avoidance trials were $10{\cdot}0 \pm 0{\cdot}53$ μW/cm^2 under acetate filters and $1{\cdot}1 \pm 0{\cdot}07$ μW cm^{-2} under Mylar filters. At the end of the trials, mean (± SD) UV-B levels were $15{\cdot}5 \pm 0{\cdot}37$ μW cm^{-2} under Mylar filters and $1{\cdot}3 \pm 0{\cdot}08$ μW cm^{-2} under acetate filters. Mean water temperatures (± SD) in the tubs at the beginning of the experiment were $25{\cdot}2 \pm 0{\cdot}3$ °C under Mylar and $25{\cdot}1 \pm 0{\cdot}3$ °C under acetate. At the end of the experiment, temperatures were $33{\cdot}1 \pm 0{\cdot}7$ °C under Mylar and $33{\cdot}0 \pm 0{\cdot}9$ °C under acetate.

Laboratory

As in the field trials, there was no detectable avoidance of the UV-B exposed side of the container in the laboratory (one-way *t*-test; $H_0 = 0{\cdot}5$ on the no UV-B side; $P = 0{\cdot}097$; Fig. 3). Mean (± SD) UV-B levels in the laboratory under Mylar filters were $0{\cdot}32 \pm 0{\cdot}6$ μW cm^{-2} and under acetate filters were $3{\cdot}7 \pm 0{\cdot}4$ μW cm^{-2}.

Discussion

In our study, we utilized confinement stress to demonstrate that free-living *R. cascadae* tadpoles have a functional HPA axis. Whole body levels of corticosterone in *R. cascadae* were significantly elevated after 60 min of confinement. These results indicate that larvae can respond to environmental stress with increases in corticosterone during early development.

However, we did not see increased corticosterone levels in response to UV-B exposure during either the 1 or 6-week exposures. It is possible that there was an initial increase and we did not see it due to the timing of our sampling (1 and 6-week UV-B exposure *vs* only 60 min of confinement stress). However, we were interested in the longer-term changes that chronic exposure to a stressor might have on basal corticosterone levels. The confinement stress results do tell us that the larvae are physiologically capable of activating the HPA axis in response to a stressor, but tadpoles did not do so following 1 or 6-week exposures to UV-B. The levels of corticosterone in both UV-B exposed and non-exposed groups in both field experiments were within the range of the baseline animals (time = 0) in the stress response profile (mean ± SD = $0{\cdot}42 \pm 0{\cdot}257$ ng g^{-1} corticosterone).

The lack of a hormonal response is somewhat surprising, as during the 6-week exposure, we saw decreased survival in UV-B exposed larvae. However, we suspect, based on the results of our choice experiments, that *R. cascadae* larvae may not be able to perceive UV-B radiation and therefore are not able to respond physiologically to exposure. Species such as *R. cascadae*, that utilize high elevation temporary ponds, may actually face a trade-off between exposing themselves to UV-B and selecting warm microhabitats that speed developmental rates. Developmental rate is important in temporary habitats because larvae must reach a minimum size threshold to metamorphose (Wilbur & Collins 1973). However, in these mountain ponds, the warmest regions of the ponds are the margins that also receive the most incident UV radiation.

Few studies have examined the potentially lethal effects of UV-B on larval amphibians, although mortality, similar to that observed in our study, has been observed for some species. For example, Nagl & Hofer (1997) found that UV-B was lethal to Alpine Newt larvae, *Triturus alpestris*, in the laboratory. However, in the field, high levels of dissolved organic matter in the ponds probably shield these larvae, and no UV-B induced mortality was observed. UV-B induced mortality has also been observed in larvae of another European newt, *T. cristatus*, but in the same study larvae of four other European amphibian species were not affected (Langhelle *et al.* 1999). In Canada, larval mortality caused by UV-B enhanced 23–30% above current ambient levels has been documented for Pacific Treefrogs, *Hyla regilla*, and Red-Legged Frogs, *Rana aurora* (Ovaska, Davis & Flamarique 1997, 1998).

Larvae of a few amphibian species do appear to be capable of recognizing and avoiding UV-B light (Nagl & Hofer 1997; van de Mortel & Buttemer 1998). However, the results from our UV-B avoidance tests in both the laboratory and the field suggest that *R. cascadae* larvae may not actively avoid high UV-B areas, despite the potential lethal effects of longer-term exposures in the field. We have seen a similar lack of UV-B avoidance in Long-Toed Salamanders, *Ambystoma macrodactylum* (Belden *et al.* 2000). One possible explanation is that *R. cascadae* larvae are not able to perceive light in the UV-B range. This could explain why larvae do not actively avoid UV-B and could also potentially explain why larvae do not respond hormonally to UV-B exposure as an environmental stressor. While at least one amphibian is known to have UV

photoreceptors, they are not known to be activated by radiation in the UV-B range (Deutschlander & Phillips 1995). Species differences in the ability to detect and avoid UV-B exposure could potentially have an impact on species composition in amphibian communities in the future.

UV-B as a potential source of environmental stress for amphibians and other organisms is a topic worthy of continued research. Levels of UV-B reaching the surface of the Earth will continue to increase with ozone depletion (e.g. Kerr & McElroy 1993; Zerefos *et al*. 1998; Middleton *et al*. 2001), and for aquatic organisms, decreases in water levels and/or dissolved organic matter can further increase UV-B exposure (e.g. Schindler *et al*. 1996; Pienitz & Vincent 2000; Kiesecker *et al*. 2001). Therefore, the likely scenario for most organisms is one of increasing exposure and increasing potential for UV-B induced damage to physiological systems. Exposure to UV-B has been linked to eye damage (Fite *et al*. 1998), cellular damage (e.g. Blaustein *et al*. 1994), developmental abnormalities (e.g. Hays *et al*. 1996; Blaustein *et al*. 1997) and decreased growth (e.g. Belden *et al*. 2000; Smith *et al*. 2000; Pahkala *et al*. 2001) in amphibians, and may increase circulating glucocorticoid levels in at least one fish species (Jokinen *et al*. 2000). Therefore, there is good reason to believe that UV-B exposure could be physiologically stressful for amphibians.

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