Oecologia (2010) 162:237–245
DOI 10.1007/s00442-009-1440-8

GLOBAL CHANGE ECOLOGY - ORIGINAL PAPER

Experimental examination of the effects of ultraviolet-B radiation in combination with other stressors on frog larvae

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Received: 6 February 2009 / Accepted: 5 August 2009 / Published online: 29 August 2009

Abstract Ultraviolet-B radiation (UVB) is a ubiquitous stressor with negative effects on many aquatic organisms. In amphibians, ambient levels of UVB can result in impaired growth, slowed development, malformations, altered behavior and mortality. UVB can also interact with other environmental stressors to amplify these negative effects on individuals. In outdoor mesocosm and laboratory experiments we studied potential synergistic effects of UVB, a pathogenic fungus, *Batrachochytrium dendrobatidis* (Bd), and varying temperatures on larval Cascades frogs (*Rana cascadae*). First, we compared survivorship, growth and development in two mesocosm experiments with UVB- and Bd-exposure treatments. We then investigated the effects of UVB on larvae in the laboratory under two temperature regimes, monitoring survival and behavior. We found reduced survival of *R. cascadae* larvae with exposure to UVB radiation in all experiments. In the mesocosm experiments, growth and development were not affected in either treatment, and no effect of Bd was found. In the laboratory experiment, larvae exposed to UVB demonstrated decreased activity levels. We also found a trend towards reduced survival when UVB and cold temperatures were combined. Our results show that amphibian larvae can suffer both lethal and sublethal effects when exposed to UVB radiation.

Keywords *Rana cascadae* · *Batrachochytrium dendrobatidis* · Temperature · UVB

Communicated by Anssi Laurila.

Electronic supplementary material The online version of this article (doi:10.1007/s00442-009-1440-8) contains supplementary material, which is available to authorized users.

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Introduction

In the past 50 years, humans have altered ecosystems more rapidly than during any other time in history (Millennium Ecosystem Assessment 2005) creating environmental conditions that threaten many ecosystems. For example, recent anthropogenic emissions into the atmosphere have reduced stratospheric ozone from historic levels. This depletion of the ozone layer allows more harmful ultraviolet-B radiation (UVB; 280–315 nm) to reach the earth's surface. While the success of the 1987 Montreal Protocol on Substances that Deplete the Ozone Layer has greatly reduced the production of ozone-depleting chemicals (Solomon 2004), some estimates suggest that full recovery is not expected to occur until 2068 (Newman et al. 2006). Thus, the impacts of increased UVB exposure will continue for some time.

In aquatic systems, exposure to UVB can kill sensitive organisms or induce sublethal effects (Bancroft et al. 2007; Häder et al. 2007) that could potentially have large impacts

on populations. In addition to population effects, UVB can profoundly affect community structure in experimental aquatic systems (Chatila et al. 1999; Danilov and Ekelund 2000). For example, Danilov and Ekelund (2000) found that marine epilithic communities dominated by diatoms in microcosms quickly shifted to a community dominated by cyanobacteria after only 7 days' exposure to UVB radiation. Another example is that of Bothwell et al. (1994) who showed that greater amounts of algae accumulate in UV-exposed habitats than in UV-protected environments. UVB radiation inhibits algal consumers (Diptera: Chironomidae) since larval chironomids are more sensitive to UVB than sympatric algae. Differential sensitivity to UVB between algae and herbivores contributes to counterintuitive increases in algae in habitats exposed to UVB.

The ubiquitous nature of UVB radiation may result in wide-reaching effects on many organisms. Amphibians are one group of organisms for which there are well-documented negative effects of exposure to ambient levels of UVB radiation (reviewed in Croteau et al. 2008). UVB exposure in amphibians can reduce hatching success (e.g., Lizana and Pedraza 1998; Häkkinen et al. 2001), cause developmental and physiological deformities (e.g., Hays et al. 1996; Starnes et al. 2000; Pahkala et al. 2001; Ankley et al. 2002), alter growth rates (e.g., Smith et al. 2000; Pahkala et al. 2003a, b), increase pigmentation (Langhelle et al. 1999), alter behavior (e.g., Kats et al. 2000) and decrease survival (e.g., Nagl and Hofer 1997; Tietge et al. 2001; Blaustein et al. 2005a). However, these effects are species specific, vary with life history stage, and may be context dependent.

Many studies of the effects of UVB exposure have focused on amphibian embryos assuming them to be the most susceptible life stage. However, a recent meta-analysis by Bancroft et al. (2008a) demonstrates that amphibian larvae are more sensitive to UVB exposure than embryos. Limited data on the effects of UVB radiation on post-hatching stages of amphibians have restricted our ability to ascertain the overall importance of UVB on amphibian populations. Mortality at post-hatching stages may impact the population more significantly than mortality at the embryonic stage (Vonesh and De la Cruz 2002). Therefore, understanding the effects of UVB on amphibian larvae is an important step in understanding population-level processes.

Although exposure to UVB radiation alone can be harmful to amphibians, it may be especially harmful in combination with other stressors. Pollutants (e.g., Hatch and Blaustein 2003; Macías et al. 2007), pathogens (e.g., Kiesecker and Blaustein 1995), and low pH (e.g., Long et al. 1995; Pahkala et al. 2002), can enhance the toxic effects of UVB exposure. Additionally, UVB radiation may increase the negative effects of other stressors. For example, exposure to ambient UVB radiation can increase the occurrence and pathogenic effects of an oomycete (*Saprolegnia ferax*) in amphibian embryos (Kiesecker and Blaustein 1995). Complex interactions involving El Niño events and exposure to UVB radiation may influence the emergence of this pathogen (Kiesecker et al. 2001). Exposure to UVB radiation may make hosts more susceptible to pathogens by compromising immune systems or inducing other stress effects (Tevini 1993). While pathogens and UVB can interact to impact amphibian embryonic development and survival, no studies have examined this interaction in larvae. This is surprising because amphibian larvae are hosts to several emerging pathogens such as water molds (Kiesecker and Blaustein 1995), trematode parasites (Johnson et al. 2002) and the fungus, *Batrachochytrium dendrobatidis* (Bd: Longcore et al. 1999), and the forces stimulating the emergence of these pathogens are unknown.

Bd is an emerging infectious pathogen associated with mass mortality events of amphibians around the world (Lips et al. 2006; Rachowicz et al. 2006; Skerratt et al. 2007). It affects both larval and metamorphosed amphibians by infecting their mouthparts and keratinized epidermal cells, respectively (Longcore et al. 1999; Piotrowski et al. 2004). Infection by Bd can kill larvae and post-metamorphic stages of amphibians (Berger et al. 2005; Blaustein et al. 2005b; Carey et al. 2006) and can cause sublethal damage (Parris and Cornelius 2004). Susceptibility to Bd may be context dependent where changes in temperature (Andre et al. 2008), exposure to water contaminants (Parris and Baud 2004), climate change (Pounds et al. 2006; Bosch et al. 2007), differences in host species (Blaustein et al. 2005b) and differences in Bd strain (Berger et al. 2005) may alter host-pathogen dynamics. Thus, the combined detrimental effects of both Bd and UVB radiation on amphibian larvae may not be as straightforward as the effects of either stressor acting alone. There have been no investigations of the combined effects of UVB and Bd on larval amphibians which are important in understanding disease dynamics in amphibian populations.

UVB effects on amphibians can also be influenced by abiotic factors such as temperature. Van Uitregt et al. (2007) show an increase in the negative effects of UVB when the embryos and larvae of one species of amphibian are exposed to colder temperatures. If this occurs in other species, larvae in colder habitats or larvae that choose colder temperatures may be at greater risk than those in warmer temperatures. However, many amphibians seek warmer temperatures (Lucas and Reynolds 1967; Brattstrom 1979; Dupré and Petranka 1985; Wollmuth et al. 1987) to speed their development. Warmer temperatures preferred by amphibian larvae are often located in shallow areas of aquatic habitats where levels of UVB are higher (Kirk 1994; Bancroft et al. 2008b). Thus, larval preference to choose warmer temperatures may also expose them to higher levels of UVB, and this

temperature choice may influence the degree to which they are affected.

We conducted a series of experiments to examine the combined effects of UVB radiation and Bd infection, and the combined effects of UVB and temperature on the larvae of Cascades frogs (*Rana cascadae*). *R. cascadae* are found in the Cascade Mountains of the Pacific Northwest, USA, where they serve an important ecological role as herbivores at the larval stage and carnivores as adults (Jones et al. 2005). Population declines of *R. cascadae* have been observed in some parts of their range (Jones et al. 2005). In nature, *R. cascadae* are continually exposed to a combination of UVB radiation (Blaustein et al. 1994), pathogens (Kiesecker and Blaustein 1995; Fellers et al. 2008) and other biotic and abiotic stressors. Exposure of *R. cascadae* to UVB radiation as embryos decreased hatching success (Blaustein et al. 1994), and as larvae reduced survival (Belden et al. 2003), decreased growth, and increased deformities (Romansic et al. 2009). In laboratory experiments, juvenile *R. cascadae* show reduced survival when exposed to Bd (Garcia et al. 2006). In the only experimental study of the effects of Bd on larval *R. cascadae*, Bd-exposed individuals had malformed mouthparts but did not show greater mortality than control larvae (Blaustein et al. 2005b). These stressors have been examined in isolation, but it is unclear if they have synergistic effects on *R. cascadae*.

Because of the sensitivity of *R. cascadae* to both UVB radiation and Bd, we hypothesized that Bd and UVB may act synergistically to harm *R. cascadae* larvae. To test this hypothesis, we performed experiments in outdoor mesocosms. Outdoor mesocosms allow animals to be exposed to natural changes in light and temperature to best mimic natural conditions. In addition, we performed a laboratory experiment where we artificially controlled temperature and UVB to determine how temperature interacts with UVB to affect survival and activity in *R. cascadae* larvae.

Materials and methods

Animal husbandry

We collected *R. cascadae* as larvae (mesocosm experiment 1) or embryos (mesocosm experiment 2 and the laboratory experiment) and reared them in the laboratory in 37.8-l aquaria filled with dechlorinated water. As embryos, animals were maintained with one egg mass per aquarium. Larvae were kept at a density of approximately 200 animals per aquarium and fed a mixture of ground rabbit chow and Tetramin fish food (3:1 ratio by volume). Animals were given weekly water changes in a controlled laboratory environment with a 12:12-h light:dark photoperiod at 14.5–15.5°C.

Mesocosm experiment 1

This experiment was conducted from 12 September to 19 November. It consisted of a 2×3 design with larvae exposed to two levels of UVB radiation [shielded and exposed (ambient)] and three levels of Bd (strain JEL197, strain JEL215, and unexposed control) conducted in outdoor mesocosms near Corvallis (Benton County, Oregon; elevation: 71 m). Each treatment was replicated in five randomly assigned mesocosms. We filled 30 mesocosms (1.9 m in diameter) with water to a depth of 52 cm to contain ~368 l of water. Into each mesocosm, we added dried leaves, phytoplankton, rabbit chow, and zooplankton to mimic a natural environment. Mesocosms were left undisturbed for 2 weeks prior to the addition of larvae and application of treatments to allow phytoplankton and zooplankton to establish. At the beginning of the experiment, we measured nitrate (<2 mg/l), dissolved oxygen (6.4–8.4 mg/l), and pH (6.2–6.8) of the mesocosm water.

R. cascadae larvae were collected from Potholes wetland (Deschutes County, Oregon; elevation: 2,300 m) in August 2000. Eight larvae [Gosner (1960) stages 29–39] were randomly assigned and added to each of the 30 mesocosms. We controlled UVB exposure by covering mesocosms with transparent plastic filters that either shield or transmit UVB (mylar and acetate, respectively, 0.127 mm thickness; Hillcor Plastics). Mylar filters transmit 5% of ambient UVB radiation while acetate filters transmit 80% of ambient UVB radiation though both allow equal levels of other wavelengths to pass through (Blaustein et al. 1994). Filters were attached to the top of each mesocosm. Temperature loggers were placed in three UVB-shielded and three UVB-exposed mesocosms to record water temperature every 6 h for the duration of the experiment. Temperatures in the mesocosms ranged from 1 to 27°C during the experiment.

Bd was cultured in the laboratory using a standard protocol (Longcore et al. 1999). One day after adding larvae into the mesocosms, we added a single 10-cm Petri dish containing JEL197, JEL215 or a Bd-free control of nutrient agar to each mesocosm. Upon visual confirmation of zoospores using a light microscope, each Petri dish was flooded with 3 ml of mesocosm water and allowed to sit for 3 min to allow zoospores to discharge from zoosporangia (Longcore et al. 1999). After 3 min, 0.5 ml of the zoospore suspension was collected and analyzed under a hemocytometer to estimate zoospore density of each dish. Petri dishes were then placed in mesh bags approximately 2 cm beneath the surface of the mesocosm water and attached to the sides of the mesocosms with metal clips. Water samples were collected from the mesocosms for the following 2 days and observed under a light microscope to estimate zoospore densities in the mesocosms.

Three weeks after the addition of larvae to the mesocosms, we counted the number of larvae in each mesocosm to determine larval mortality (percent survival at week 3). We also collected a subsample of three larvae from each mesocosm, which were sent to the Zoological Society of San Diego to determine presence of Bd infection in mouthparts through histological examination.

During the experiment, any larva reaching metamorphosis [Gosner (1960) stage 43] was removed and recorded (time to metamorphosis). To determine if treatments had a carry-over effect into post-metamorphic stages, we fed and maintained these animals in the laboratory for 30 days post-metamorphosis to observe survivorship. These animals were placed in individual 15-cm Petri dishes with moistened paper towels at 14.5–15.5°C. Paper towels were changed once a week and animals were fed pinhead crickets approximately twice a week. After 30 days in the laboratory, animals were euthanized in MS-222 and preserved in 70% ethanol.

Survival to week 3, time to metamorphosis, and survival at 30 days past metamorphosis were analyzed with a generalized linear mixed model implemented in R statistical software environment. Mortality data were analyzed using a logit link while time to metamorphosis used an identity link. We nested individuals by mesocosm and tested UVB effects, Bd effects and the interaction of the two variables.

Mesocosm experiment 2

This experiment was conducted from 14 July to 31 July. Mesocosm experiment 2 differs from experiment 1 by using a different strain of Bd (JEL274), modifying the inoculation method, and increasing our sample size (in the number of mesocosms and the number of larvae per mesocosm). This experiment is also shorter in duration than mesocosm experiment 1 to determine the short-term effects of UVB and Bd exposure. We used a 2 × 2 design with two levels of UVB [shielded and exposed (ambient)] and two levels of Bd (strain JEL274, and unexposed control). Each treatment was replicated in six randomly chosen mesocosms. Mesocosms were filled as described for experiment 1 and left for 3 weeks before initiation of the experiment. We placed a temperature logger into three UVB-shielded and three UVB-exposed mesocosms to record temperature every hour. Temperatures in the mesocosm ranged from 19.1 to 34.2°C.

We created UVB-exposed and UVB-shielded treatments using filters as described in experiment 1. UVB levels were measured using a hand-held Solar Light meter with a UVB attachment (PMA21100 meter, 2102 probe; Solar Light, Philadelphia, Pa.) every 5 days between 1100 and 1300 hours. Ambient UVB levels at midday ranged from 16.6 to 19.4 μW/cm^2. Mesocosms covered with mylar had UVB levels from 1.3 to 1.9 μW/cm^2 at the top of mesocosm water, attenuating to 0.1–0.5 μW/cm^2 at 10 cm below the water's surface. Mesocosms covered with acetate had UVB levels ranging from 9.6 to 13.5 μW/cm^2 at the top of mesocosm water, attenuating to 0.3–2.0 μW/cm^2 at 10 cm below the water's surface.

In June 2006, three egg masses of *R. cascadae* and three egg masses of Pacific treefrogs (*Psuedacris regilla)* were collected from Susan's pond (Deschutes County, Oregon; elevation: 1,954 m). We applied Bd treatments by placing either Bd-exposed or Bd-control *P. regilla* larvae into the mesocosms. Therefore, *P. regilla* acted as our stimulus animals while *R. cascadae* remained our focal animals. To inoculate *P. regilla* larvae, we divided them into six 37.8-l aquaria with approximately 80 animals per aquarium and each aquarium was randomly assigned to Bd or control treatments. We used eight Petri dishes containing Bd strain JEL274 to inoculate the *P. regilla* in each Bd aquaria and eight sterile agar dishes for each control aquaria. Each dish was flooded with 15 ml distilled water for 20 min and this wash was then poured into the aquaria. Three additional Bd dishes were flooded in the same manner and wash was used to determine zoospore densities on a hemocytometer. Stimulus Bd treatment *P. regilla* larvae were exposed to an average of 1,020 zoospores of Bd per liter in their exposure aquaria. Similar methods have been shown to successfully infect both larval amphibians and adults (Berger et al. 2005; Blaustein et al. 2005b; Carey et al. 2006; Han et al. 2008). We allowed Bd infection to develop for 3 weeks after inoculation before using the *P. regilla* in our experiment.

On 13 July, we added ten *R. cascadae* larvae [Gosner (1960) stage 24–25] into each mesocosm. The following day, 12 *P. regilla* larvae [Gosner (1960) stage 25–27] confined within mesh bags were added to each mesocosm. Control mesocosms were given 12 unexposed *P. regilla* while Bd mesocosms were given 12 Bd-exposed *P. regilla*. Because Bd is transmitted through water (Longcore et al. 1999), infected *P. regilla* can shed zoospores that pass out of the bags to infect the *R. cascadae*. After 18 days, all animals were euthanized in MS-222 and preserved in 70% ethanol. Length (snout-vent) and weight were measured for each animal. To test for Bd infection, we randomly selected three *R. cascadae* from each mesocosm (if three animals were available) to analyze with quantitative-polymerase chain reaction (qPCR). Additionally, six randomly chosen *P. regilla* were also tested for Bd infection using qPCR. Mouthparts were removed from each animal and DNA was extracted using Prepman Ultra (Applied Biosystems) methods described by Boyle et al. (2004). Extractions were diluted to a 10% solution. Using the methods described by Boyle et al. (2004), we performed qPCR on each sample using an ABI PRISM 7500 sequence detection system.

Each sample was run in triplicate and any value above 0.1 genome equivalents was considered to show infection.

Length, weight, and survival data were analyzed as described in mesocosm experiment 1. In one UVB-shielded, Bd-exposed mesocosm, *P. regilla* larvae escaped from their mesh bags so this mesocosm was excluded from analysis.

Laboratory experiment

To examine the combined effect of UVB radiation and temperature on larvae, we used a 2 × 2 design in a controlled laboratory environment with two levels of UVB (shielded and exposed) and two levels of temperature (hot and cold). Twenty water baths were created and randomly assigned a hot or cold treatment. An aquarium heater was placed into the center of each hot treatment bath and turned on at 0900 hours and off at 1700 hours each day. This created an increase in water temperature during the middle of the day in the hot treatments, and a return to similar temperatures as the cold treatments at night. Into each water bath, we placed six 1-l containers filled with 800 ml of dechlorinated water to a depth of 11.5 cm. A digital thermometer (Traceable Thermometer; Fisher Scientific) was used to measure temperature every 2 h at the center of the water bath. Containers in the cold water baths averaged low temperatures of 15.2°C (SD = 0.46) and high temperatures of 16.0°C (SD = 0.60) throughout the day. Containers in the hot treatments averaged low temperatures of 15.6°C (SD = 0.36) and high temperatures of 30.5°C (SD = 1.87). Temperatures within the water baths are shown in Fig. S1 (electronic supplementary material).

Animals from six egg masses of *R. cascadae* were collected from Parish pond (Linn County, Oregon; elevation: 1,130 m) in April, 2007. One larva [Gosner (1960) stages 25–26] was added to each container. UV-B treatments were applied by placing larvae under a mixture of UVB (Q-Panel UVB 313; Q-Panel, Cleveland, Ohio) and full-spectrum bulbs (Vita-Light; Durotest, Fairfield, N.J.). Lights were turned on from 0800 to 1800 hours each day. In each water bath, three animals were randomly assigned a UVB-exposure treatment and three were assigned a UVB-shielded treatment. Treatments were applied by covering each container with either a mylar or acetate filter as in the previous experiments. UVB levels at the top of the water below acetate filters ranged from 11.8 to 15.7 μW/cm^2, whereas UVB levels below mylar filters ranged from 0.2 to 0.7 μW/cm^2. These are within UVB levels found in the Cascade Mountains, where *R. cascadae* naturally occur (Bancroft et al. 2008b).

Treatments were applied for 5 consecutive days. Each day, the containers within each water bath were randomly rearranged to remove possible effects of orientation. At 1800 hours on day 5, survival was recorded for all individuals. Fourteen surviving individuals from each treatment were randomly chosen as a subsample of animals for a behavior trial measuring activity levels. Activity levels are closely associated with growth rates, which are extremely important for development to metamorphosis (Werner and Anholt 1993). Thus, if UVB or temperature treatments influence larval activity, this could have profound effects on individuals. The behavior trial was conducted from 0900 to 1300 hours on day 6 after allowing treatments to acclimate overnight to the behavioral arena's temperature of 15°C. Each larva was placed into a plastic container (20 cm long by 30 cm wide) filled with water to a depth of 5 cm over a grid containing 5 × 5-cm squares. Containers were placed randomly within an observation arena surrounded by black sheets with small openings to allow observers to record tadpole movement without disturbing the animals. After a 1-h acclimation period in the new containers, activity levels were recorded by counting the number of lines crossed by each animal over a total of 4 min. Two observers recorded activity levels of each larva 8 times for 30 s with 30 min between observations.

We analyzed both survival and behavior using generalized linear mixed models to determine the effects of UVB, temperature and the interaction of the two variables. Survival was analyzed with a logit link function while activity levels used an identity link. Individuals were nested by water bath.

Results

Mesocosm experiment 1

After 3 weeks, larvae exposed to UVB had lower survival rates than shielded larvae ($\chi^2 = 9.27$, $df = 1$, $P = 0.002$; Fig. 1). On average, percent survival to 3 weeks in UVB-exposed mesocosms was 90.0% (SE = 3.0) while those shielded from UVB had an average survival rate of 99.2% (SE = 0.8; Fig. 1). There was no difference in survival at 3 weeks between Bd treatments ($\chi^2 = 0.19$, $df = 1$, $P = 0.663$) and no interaction between the two variables ($\chi^2 = 1.99$, $df = 1$, $P = 0.158$). Time to metamorphosis and survival to 30 days post-metamorphosis were not affected by either treatment. Active zoospores were seen on all plates when placed into the mesocosm. Few zoospores were seen in water collected from mesocosms on day 2, and none were found on day 3. Histological examination of larval mouthparts (by A. Pessier) indicated that larvae did not develop visible infections in our mesocosms.

Mesocosm experiment 2

Exposure to UVB reduced survival in *R. cascadae* larvae ($\chi^2 = 7.76$, $df = 1$, $P = 0.005$; Fig. 2). On average, 20.0% of animals survived in UVB-exposed mesocosms (SE = 9.2)

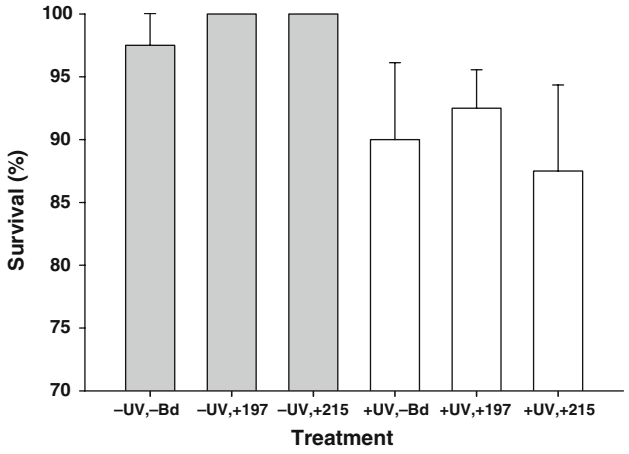

Fig. 1 Percent survival per mesocosm of *Rana cascadae* larvae in mesocosm experiment 1 (mean + SE) shielded from ultraviolet-B (UVB) (*−UV*; *gray bars*) or exposed to UVB (*+UV*; *white bars*). *Batrachochytrium dendrobatidis* (Bd) treatments are indicated as control (*−Bd*), JEL strain 197 (*+197*) or JEL strain 215 (*+215*). In treatments −UV, +197 and −UV, +215, all mesocosms experienced 100% survival

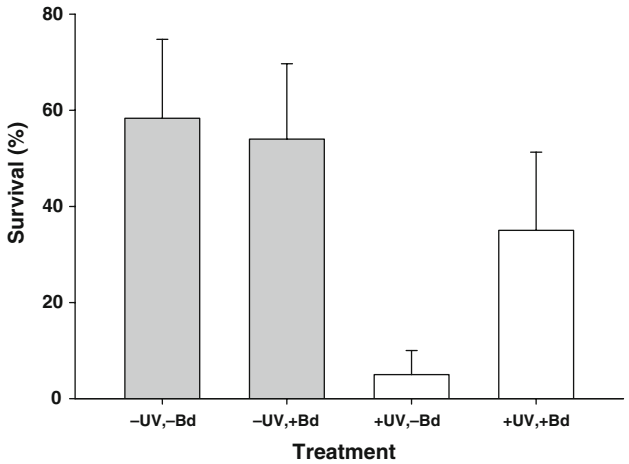

Fig. 2 Percent survival per mesocosm of *R. cascadae* larvae in mesocosm experiment 2 (mean + SE), −UV (*gray bars*), or +UV (*white bars*). Bd treatments are *−Bd*, or exposed with JEL strain 274 (*+Bd*). For other abbreviations, see Fig. 1

while 56.4% of animals shielded from UVB survived (SE = 10.9). There was no effect of Bd on survival ($\chi^2 = 0.96$, $df = 1$, $P = 0.327$) and there was no interaction between Bd and UVB ($\chi^2 = 2.47$, $df = 1$, $P = 0.116$). Of the animals that survived to the end of the experiment, growth and development were not affected by either treatment, or the interaction between treatments. In our qPCR analysis, all animals tested below the threshold for positive Bd infection.

Laboratory experiment

Exposure to UVB reduced survival in *R. cascadae* larvae ($\chi^2 = 27.99$, $df = 1$, $P < 0.001$; Fig. 3a). Additionally,

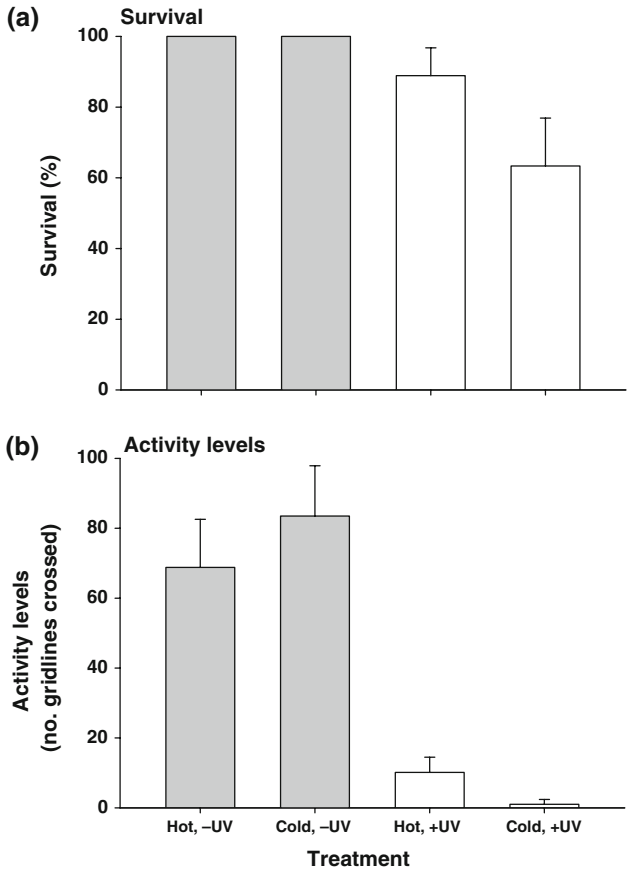

Fig. 3 **a** Percent survival per water bath treatment of *R. cascadae* larvae after 5 days −UV (*gray bars*) or +UV (*white bars*) in hot or cold water in the laboratory (mean + SE). Survival was 100% in the –UV treatments. **b** Activity levels from behavior trials after 5 days −UV (*gray bars*) or +UV (*white bars*) in hot (*Hot*) or cold water (*Cold*) in the laboratory (mean + SE). Animals were monitored for 30-s intervals 8 times. The total number of gridlines each animal crossed during the 4-min observation time equals its activity level. For other abbreviations, see Fig. 1

individuals exposed to UVB experienced reduced activity levels ($F_{1,16} = 41.64$, $P < 0.001$; Fig. 3b). Temperature did not have a significant effect on survival or activity levels alone, but there was a trend for an interaction between UVB and cold temperatures on survival ($\chi^2 = 3.31$, $df = 1$, $P = 0.069$).

Discussion

Exposure to ambient levels of UVB radiation reduced survival in larval *R. cascadae*. In all experiments, *R. cascadae* exposed to UVB radiation had significantly lower survival than those shielded from UVB. This is one of the few experimental studies to unequivocally demonstrate mortality of amphibian larvae when exposed to ambient levels of UVB radiation (Nagl and Hofer 1997; Tietge et al. 2001; Belden et al. 2003).

Furthermore, in our mesocosm experiments, larvae were exposed to ambient levels of UVB radiation in a close approximation of natural habitat (O'Hara and Blaustein 1985; Hokit and Blaustein 1997). Reduced survival in these conditions suggests that UVB exposure can cause mortality of amphibian larvae in the wild. In mesocosms, as in many natural habitats, larvae had the opportunity to behaviorally reduce their UVB exposure by choosing deeper waters or hiding under leaves. While we did not directly record behavior, we often observed *R. cascadae* larvae near the surface of the mesocosm water where UVB levels were highest. Additionally, mortality in the UVB-exposed treatments suggests that animals did not behaviorally limit their exposure to UVB radiation. This adds to the growing evidence that some species of larval amphibians do not behaviorally avoid UVB radiation, even when it causes them harm (Wollmuth et al. 1987; van de Mortel and Buttemer 1998; Belden et al. 2003; Bancroft et al. 2008b).

Direct comparisons between the mesocosm experiments cannot be made; however, overall survival in mesocosm experiment 1 was much greater than survival in mesocosm experiment 2. These differences could be explained in a number of ways. For example, the experiments were conducted in different years, different months, for different durations and with different protocols. Also, larvae in mesocosm experiment 2 were exposed at earlier developmental stages than those in the first mesocosm experiment. However, UVB exposure increased mortality in both mesocosm experiments.

Although we exposed our animals to Bd in mesocosm experiments, no effect of Bd was found. In experiment 1, animals did not show signs of Bd infection, even though appropriate levels for infection (Carey et al. 2006) were found inside the mesocosm water. It is possible that under our conditions the virulence of Bd was compromised. This could be due to a number of factors including competition with other microorganisms in the mesocosm, changes in temperature or the relative resistance of the host species. Another possibility is that larvae may have carried low levels of Bd infection that were undetectable by histological examination in mesocosm experiment 1. In experiment 2, temperatures in the mesocosms reached over 30°C for 8 consecutive days (experimental days 7–14) which is high enough to kill Bd in culture (Piotrowski et al. 2004). Therefore, our negative qPCR results may demonstrate the ability of high temperatures to cure Bd infection in aquatic systems (Woodhams et al. 2003). *R. cascadae* were exposed to Bd in the first 6 days of the experiment, but previous studies by Blaustein et al. (2005b) found no mortality of *R. cascadae* larvae with exposure to Bd, so this species may not be susceptible to Bd infection at this life stage. In a study on juvenile *R. cascadae*, Garcia et al. (2006) tested the combined effects of UVB radiation and Bd, and found increased mortality with exposure to Bd, but no UVB effect or interaction between treatments. However, Bd infection can vary greatly between life stages due to changes in keratinized structures. While larval amphibians are only infected in their mouthparts, juveniles have keratinized epidermal tissue which makes them susceptible to infection over most of their skin. Varying responses to the separate and combined effects of UVB and Bd infection between life stages of *R. cascadae* highlights the complexity underlying amphibian population declines.

In the laboratory, we found both lethal and sublethal effects on *R. cascadae* when exposed to UVB radiation. While lethal effects can dramatically alter populations, sublethal effects such as reduced activity can also have a substantial impact on larvae in the wild. Reduced activity levels can decrease feeding rates and lead to smaller size at metamorphosis (Skelly and Werner 1990). Lower activity levels may prevent predators from finding larvae (Werner and Anholt 1993), but if pursued by a predator, lethargic larvae may be unable to escape. Therefore, these sublethal effects of UVB may indirectly reduce larval survival and thus reduce population size.

Additionally, we found reduced survival of animals exposed to UVB radiation in colder temperatures. This complements the findings of van Uitregt et al. (2007) who observed similar trends with reduced survival, growth and performance when striped marsh frog (*Limnodynastes peronii*) larvae were exposed to UVB at colder temperatures. Cold temperatures could increase the negative effects of UVB by physiologically reducing a larva's ability to prevent damage caused by UVB. For example, repair enzymes are essential in fixing DNA damage caused by UVB radiation (Pang and Hays 1991). These enzymes work faster at warmer temperatures, so cold conditions may slow an organism's ability to recover after exposure to UVB. Additionally, changes in temperature may influence larval behavior and therefore alter their exposure to UVB radiation. Amphibian larvae at high elevations are exposed to high levels of UVB and colder conditions compared to those at lower elevations. This combination of environmental factors may act together and could contribute to amphibian population declines at high elevations. UVB can cause mortality in cold temperatures, but even surviving animals may have a decreased ability to cope with additional stressors. Therefore, any UVB-sensitive species occurring in cold climates may be at risk. However, within a habitat, many larval amphibians choose regions with warmer temperatures. While larval preference for warmer temperatures may help mitigate the effects of UVB, warmer regions of water bodies often have higher levels of UVB. Therefore, larval amphibians are forced to choose between high levels of UVB in warm conditions where they are more able to prevent negative effects of UVB, or lower levels of UVB in

cold regions where they are less able to cope with UVB. This complicated pattern makes it difficult to predict the threat of UVB in larval amphibians in the wild.

Our study shows that larval amphibians can suffer both lethal and sublethal negative effects of UVB radiation. Future work should continue to investigate the impacts of UVB on amphibian populations by studying all life stages to fully understand its effects on populations. While we did not directly study the effects of UVB at the population level, our results suggest that UVB radiation can impact *R. cascadae* at a life stage that is critical to amphibian populations (Vonesh and De la Cruz 2002). Therefore, investigating only the embryonic or adult stages may underestimate the negative effects of UVB. The cumulative effects of embryonic, larval and adult mortality from UVB could have larger impacts on populations than predicted by studying one stage alone. The ubiquitous increase of UVB radiation across the globe makes it a potentially major factor contributing to amphibian declines. Therefore, future work should focus on the effects of UVB in order to better understand how degradation of the ozone layer has affected, and continues to affect, aquatic organisms.

Acknowledgments We would like to thank A. Pessier, J. Spatafora, J. Romansic, J. Longcore, T. Raffel, P. Bradley, A. Searle, J. Carey, and M. Christie for their assistance. This work was supported by National Science Foundation Integrated Research Challenges in Environmental Biology (NSF IRCEB) Program (DEB0213851 and IBN9977063). Suggestions by two anonymous reviewers greatly contributed to this paper. These experiments comply with the current laws of the United States and with Oregon State University animal care regulations. Animals were collected according to Oregon Department of Fish and Wildlife regulations.

References

Andre SE, Parker J, Briggs CJ (2008) Effect of temperature on host response to *Batrachochytrium dendrobatidis* infection in the mountain yellow-legged frog (*Rana muscosa*). J Wildl Dis 44:716–720

Ankley GT, Diamond SA, Tietge JE, Holcombe GW, Jensen KM, Defoe DL, Peterson R (2002) Assessment of the risk of solar ultraviolet radiation to amphibians. I. Dose-dependent induction of hindlimb malformations in the northern leopard frog (*Rana pipiens*). Environ Sci Technol 36:2853–2858

Bancroft BA, Baker NJ, Blaustein AR (2007) Effects of UVB radiation on marine and freshwater organisms: a synthesis through meta-analysis. Ecol Lett 10:332–345

Bancroft BA, Baker NJ, Blaustein AR (2008a) A meta-analysis of the effects of ultraviolet B radiation and its synergistic interactions with pH, contaminants, and disease on amphibian survival. Conserv Biol 22:987–996

Bancroft BA, Baker NJ, Searle CL, Garcia TS, Blaustein AR (2008b) Larval amphibians seek warm temperatures and do not avoid harmful UVB radiation. Behav Ecol 19:879–886

Belden LK, Moore IT, Mason RT, Wingfield JC, Blaustein AR (2003) Survival, the hormonal stress response and UV-B avoidance in Cascades frog tadpoles (*Rana cascadae*) exposed to UV-B radiation. Funct Ecol 17:409–416

Berger L, Marantelli G, Skerratt LF, Speare R (2005) Virulence of the amphibian chytrid fungus *Batrachochytrium dendrobatidis* varies with the strain. Dis Aquat Organ 68:47–50

Blaustein AR, Hoffman PD, Hokit DG, Kiesecker JM, Walls SC, Hays JB (1994) UV repair and resistance to solar UV-B in amphibian eggs: a link to population declines? Proc Natl Acad Sci USA 91:1791–1795

Blaustein AR, Romansic JM, Scheessele EA (2005a) Ambient levels of ultraviolet-B radiation cause mortality in juvenile western toads, *Bufo boreas*. Am Midl Nat 154:375–382

Blaustein AR, Romansic JM, Scheessele EA, Han BA, Pessier AP, Longcore JE (2005b) Interspecific variation in susceptibility of frog tadpoles to the pathogenic fungus *Batrachochytrium dendrobatidis*. Conserv Biol 19:1460–1468

Bosch J, Carrascal LM, Durán L, Walker S, Fisher MC (2007) Climate change and outbreaks of amphibian chytridiomycosis in a montane area of Central Spain; is there a link? Proc R Soc B Biol Sci 274:253–260

Bothwell M, Sherbot DMJ, Pollock SM (1994) Ecosystem response to solar ultraviolet-B radiation: influence of trophic-level interactions. Science 265:97–100

Boyle DG, Boyle DB, Olsen V, Morgan JAT, Hyatt AD (2004) Rapid quantitative detection of chytridiomycosis (*Batrachochytrium dendrobatidis*) in amphibian samples using real-time Taqman PCR assay. Dis Aquat Organ 60:141–148

Brattstrom BH (1979) Amphibian temperature regulation studies in the field and laboratory. Am Zool 19:345–356

Carey C, Bruzgul JE, Livo LJ, Walling ML, Kuehl KA, Dixon BF, Pessier AP, Alford RA, Rogers KB (2006) Experimental exposures of boreal toads (*Bufo boreas*) to a pathogenic chytrid fungus (*Batrachochytrium dendrobatidis*). EcoHealth 3:5–21

Chatila K, Demers S, Mostajir B, Gosselin M, Chanut JP, Monfort P (1999) Bacterivory of a natural heterotrophic protozoan community exposed to different intensities of ultraviolet-B radiation. Aquat Microb Ecol 20:59–74

Croteau MC, Davidson MA, Lean DRS, Trudeau VL (2008) Global increases in ultraviolet B radiation: potential impacts on amphibian development and metamorphosis. Physiol Biochem Zool 81:743–761

Danilov RA, Ekelund RGA (2000) Effects of different levels of UV-B radiation on marine epilithic communities: a short-term microcosm study. Sci Mar 64:363–368

Dupré RK, Petranka JW (1985) Ontogeny of temperature selection in larval amphibians. Copeia 1985:462–467

Fellers GM, Pope KL, Stead JE, Koo MS, Welsh HH (2008) Turning population trend monitoring into active conservation: can we save the Cascade frog (*Rana cascadae*) in the Lassen region of California? Herpetol Conserv Biol 3:28–39

Garcia TS, Romansic JM, Blaustein AR (2006) Survival of three species of anuran metamorphs exposed to UV-B radiation and the pathogenic fungus *Batrachochytrium dendrobatidis*. Dis Aquat Organ 72:163–169

Gosner KL (1960) A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologica 16:183–190

Häder DP, Kumar HD, Smith RC, Worrest RC (2007) Effects of solar UV radiation on aquatic ecosystems and interactions with climate change. Photochem Photobiol Sci 6:267–285

Häkkinen J, Pasanen S, Kukkonen JVK (2001) The effects of solar UV-B radiation on embryonic mortality and development in three boreal anurans (*Rana temporaria, Rana arvalis* and *Bufo bufo*). Chemosphere 44:441–446

Han BA, Bradley PW, Blaustein AR (2008) Ancient behaviors of larval amphibians in response to an emerging fungal pathogen, *Batrachochytrium dendrobatidis*. Behav Ecol Sociobiol 63:241–250

Hatch AC, Blaustein AR (2003) Combined effects of UV-B radiation and nitrate fertilizer on larval amphibians. Ecol Appl 13:1083–1093

Hays JB, Blaustein AR, Kiesecker JM, Hoffman PD, Pandelova I, Coyle D, Richardson T (1996) Developmental responses of amphibians to solar and artificial UVB sources: a comparative study. Photochem Photobiol 64:449–456

Hokit DG, Blaustein AR (1997) The effects of kinship on interactions between tadpoles of *Rana cascadae*. Ecology 78:1722–1735

Johnson PT, Lunde KB, Thurman EM, Ritchie EG, Wray SN, Sutherland DR, Kapfer JM, Frest TJ, Bowerman J, Blaustein AR (2002) Parasite (*Ribeiroia ondatrae*) infection linked to amphibian malformations in the western United States. Ecol Monogr 72:151–168

Jones LLC, Leonard WP, Olson DH (2005) Amphibians of the Pacific Northwest. Seattle, 1st edn. Audubon Society, Seattle

Kats LB, Kiesecker JM, Chivers DP, Blaustein AR (2000) Effects of UV-B radiation on anti-predator behavior in three species of amphibians. Ethology 106:921–931

Kiesecker JM, Blaustein AR (1995) Synergism between UV-B radiation and a pathogen magnifies amphibian embryo mortality in nature. Proc Natl Acad Sci USA 92:11049–11052

Kiesecker JM, Blaustein AR, Belden LK (2001) Complex causes of amphibian population declines. Nature 410:681–684

Kirk JTO (1994) Optics of UV-B radiation in natural waters. Arch Fur Hydrobiol Beiheft 43:1–16

Langhelle A, Lindell MJ, Nyström P (1999) Effects of ultraviolet radiation on amphibian embryonic and larval development. J Herpetol 3:449–456

Lips KR, Brem F, Brenes R, Reeve JD, Alford RA, Voyles J, Carey C, Livo L, Pessier AP, Collins JP (2006) Emerging infectious disease and the loss of biodiversity in a Neotropical amphibian community. Proc Natl Acad Sci USA 103:3165–3170

Lizana M, Pedraza EM (1998) The effects of UV-B radiation on toad mortality in mountainous areas of central Spain. Conserv Biol 12:703–707

Long LE, Saylor LS, Soulé ME (1995) A pH/UV-B synergism in amphibians. Conserv Biol 9:1301–1303

Longcore JE, Pessier AP, Nicols DK (1999) *Batrachochytrium dendrobatidis* gen. et. sp. nov., a chytrid pathogenic to amphibians. Mycologia 91:219–227

Lucas EA, Reynolds WA (1967) Temperature selection by amphibian larvae. Physiol Zool 40:159–171

Macías G, Marco A, Blaustein AR (2007) Combined exposure to ambient UVB radiation and nitrite negatively affects survival of amphibian early life stages. Sci Total Environ 385:55–65

Millennium Ecosystem Assessment (2005) Ecosystems and human well-being: biodiversity synthesis. Island Press, Washington, DC

Nagl AM, Hofer R (1997) Effects of ultraviolet radiation on early larval stages of the Alpine newt, *Triturus alpestris*, under natural and laboratory conditions. Oecologia 110:514–519

Newman PA, Nash ER, Kawa SR, Montzka SA, Schauffler SM (2006) When will the Antarctic ozone hole recover? Geophys Res Lett 33:L12814. doi:10.1029/2005GL025232

O'Hara RK, Blaustein AR (1985) *Rana cascadae* tadpoles aggregate with siblings: an experimental field study. Oecologia 67:44–51

Pahkala M, Laurila A, Merilä J (2001) Carry-over effects of ultraviolet-B radiation on larval fitness in *Rana temporaria*. Proc R Soc Lond B 268:1699–1706

Pahkala M, Räsänen K, Laurila A, Johanson U, Björn LO, Merilä J (2002) Lethal and sublethal effects of UV-B/pH synergism on common frog embryos. Conserv Biol 16:1063–1073

Pahkala M, Laurila A, Merilä J (2003a) Effects of ultraviolet-B radiation on behavior and growth of three species of amphibian larvae. Chemosphere 51:197–204

Pahkala M, Merilä J, Ots I, Laurila A (2003b) Effects of ultraviolet-B radiation on metamorphic traits in the common frog *Rana temporaria*. J Zool Lond 259:57–62

Pang Q, Hays JB (1991) UV-B-inducible and temperature-sensitive photoreactivation of cyclobutane pyrimidine dimmers in *Arabidopsis thaliana*. Plant Physiol 95:536–543

Parris MJ, Baud DR (2004) Interactive effects of a heavy metal and chytridiomycosis on gray treefrog larvae (*Hyla chrysoscelis*). Copeia 2:344–350

Parris MJ, Cornelius TO (2004) Fungal pathogen causes competitive and developmental stress in larval amphibian communities. Ecology 85:3385–3395

Piotrowski JS, Annis SL, Longcore JE (2004) Physiology of *Batrachochytrium dendrobatidis*, a chytrid pathogen of amphibians. Mycologia 96:9–15

Pounds JA, Bustamante MR, Coloma LA, Consuegra JA, Fogden MPL, Foster PN, LaMarca E, Masters KL, Merino-Viteri A, Puschendorf R, Ron SR, Sánchez-Azofeifa GA, Still CJ, Young BE (2006) Widespread amphibian extinctions from epidemic disease driven by global warming. Nature 439:161–167

Rachowicz LJ, Knapp RA, Morgan JAT, Stice MJ, Vredenburg VT, Parker JM, Briggs CJ (2006) Emerging infectious disease as a proximate cause of amphibian mass mortality. Ecology 87:1671–1683

Romansic JM, Waggener AA, Bancroft BA, Blaustein AR (2009) Influence of ultraviolet-B radiation on growth, prevalence of deformities, and susceptibility to predation in Cascades frog (*Rana cascadae*) larvae. Hydrobiologia 624:219–233

Skelly K, Werner E (1990) Behavioral and life-historical responses of larval American toads to an odonate predator. Ecology 71:2313–2322

Skerratt LF, Berger L, Speare R, Cashins S, McDonald KR, Phillott AD, Hines HB, Kenyon N (2007) Spread of chytridiomycosis has caused rapid global decline and extinction of frogs. EcoHealth 4:125–134

Smith GR, Waters MA, Rettig JE (2000) Consequences of embryonic UV-B exposure for embryos and tadpoles of the Plains leopard frog. Conserv Biol 14:1903–1907

Solomon S (2004) The hole truth: what's news (and what's not) about the ozone hole. Nature 427:289–291

Starnes SM, Kennedy CA, Petranka JW (2000) Sensitivity of embryos of southern Appalachian amphibians to ambient solar UV-B radiation. Conserv Biol 14:277–282

Tevini M (1993) UV-B radiation and ozone depletion: effects on humans, animals, plants, microorganisms, and materials, 1st edn. Lewis, Boca Raton

Tietge JE, Diamond SA, Ankley GT, DeFoe DL, Holcombe GW, Jensen KM, Degitz SJ, Elonen GE, Hammer E (2001) Ambient solar UV radiation causes mortality in larvae of three species of *Rana* under controlled exposure conditions. Photochem Photobiol 74:261–268

van de Mortel TF, Buttemer WA (1998) Avoidance of ultraviolet-B radiation in frogs and tadpoles of the species *Litoria aurea*, *L. dentata*, and *L. peronii*. Proc Linn Soc N S W 119:173–179

van Uitregt VO, Wilson RS, Franklin CE (2007) Cooler temperatures increase sensitivity to ultraviolet B radiation in embryos and larvae of the frog *Limnodynastes peronii*. Glob Change Biol 13:1114–1121

Vonesh JR, De la Cruz O (2002) Complex life cycles and density dependence: assessing the contribution of egg mortality to amphibian declines. Oecologia 133:325–333

Werner EE, Anholt BR (1993) Ecological consequences of the trade-off between growth and mortality rates mediated by foraging activity. Am Nat 142:242–272

Wollmuth LP, Crawshaw LI, Forbes RB, Grahn DA (1987) Temperature selection during development in a montane anuran species, *Rana cascadae*. Physiol Zool 60:472–480

Woodhams DC, Alford RA, Marantelli G (2003) Emerging disease of amphibians cured by elevated body temperature. Dis Aquat Organ 55:65–67