AN ABSTRACT OF THE DISSERTATION OF

Betsy A. Bancroft for the degree of Doctor of Philosophy in Zoology presented on May 10, 2007.

Title: Ultraviolet Radiation as an Environmental Stressor of Amphibians

Abstract approved:

Andrew R. Blaustein

My thesis explored the effects of and potential mediating mechanisms for an important environmental stressor, ultraviolet-B (UVB) radiation. UVB radiation has negative effects on organisms in both terrestrial and aquatic systems. I used meta-analysis to quantify the effects of UVB radiation on a diversity of aquatic organisms (Chapter 2). UVB negatively affects aquatic organisms by reducing both survival and growth. In particular, UVB reduces growth of embryos more than any other life history stage. Some taxonomic groups may be more affected by UVB radiation than others. In our analysis, the growth of members of the kingdom Protozoa was suppressed by UVB radiation to a greater degree than any other kingdom. These analyses suggest that UVB is an important stressor in both freshwater and marine systems.

Amphibians are a common component of freshwater systems and are experiencing world-wide population declines. These declines may be due to a number of causes including habitat loss, introduced species, global climate change, disease, toxic chemicals and UVB radiation. I used meta-analytic techniques to quantify the effects of UVB radiation on amphibians. By synthesizing the results of 41 articles on

the effects of UVB radiation on amphibians (Chapter 3), I found a nearly 2-fold reduction in survival of amphibians exposed to UVB radiation. Salamanders (caudates) appear to be more susceptible to damage from UVB than frogs or toads (anurans). Moreover, survival of larvae was much lower than survival of embryos or metamorphic individuals under UVB radiation. In addition, I used factorial meta-analytic techniques to explore the interaction between UVB radiation and other stressors in amphibian habitats. UVB radiation acted synergistically with other stressors to reduce survival of amphibians.

Behavioral avoidance of UVB radiation may help mediate the negative effects of UVB radiation on amphibians. In aquatic systems, behavioral avoidance usually requires movement out of shallow water, where UVB levels can be high, into deeper waters with lower UVB transmittance. However, these two microhabitats have very different thermal profiles, creating a trade-off between exploiting warm waters with high UVB levels and avoiding UVB by seeking cooler, deeper regions of ponds. I explored the microhabitat use of larvae of four species through a series of laboratory experiments, field experiments, and observational field transects at three different amphibian habitats (Chapter 4). Larvae did not avoid UVB radiation in either the laboratory or field experiments. Larvae in thermal gradients selected relatively high temperatures regardless of the UVB exposure at these temperatures. In field transects, salamander larvae were most common in deeper, cooler waters where UVB levels were lower. In contrast, anuran larvae were frequently observed in the warmer and shallower regions of each habitat. These regions also had the highest UVB levels,

suggesting that anuran larvae are exposed to high levels of UVB due to thermoregulatory behavior.

Behavioral avoidance of UVB radiation is not the only mechanism amphibians may use to prevent damage from UVB. Pigments such as melanin may allow larvae to exploit warm shallow waters by absorbing harmful UVB radiation before it causes cellular damage. I tested the efficacy of melanin as a photoprotective pigment in the larvae of two species, *Rana cascadae* and *Pseudacris regilla* (Chapter 5). I found no evidence of a photoprotective function for melanin in these larvae. In contrast, lighter colored tadpoles grew more under UVB radiation compared to darker colored tadpoles. Overall, exposure to UVB reduced survival of *P. regilla* larvae and reduced growth of *R. cascadae* larvae. Larvae of both of these species were frequently observed in very shallow water with intense solar radiation.

This thesis emphasizes the importance of UVB radiation as an environmental stressor in aquatic habitats. Many aquatic organisms are negatively affected by UVB exposure. My thesis work quantitatively demonstrated that UVB radiation is one factor that reduces survival of amphibians and suggests that some species are exposed to high levels of UVB radiation in natural habitats. While UVB radiation is not the sole cause of amphibian population declines, my work suggests that UVB radiation is an important stressor for amphibians that should not be overlooked. In addition, UVB radiation is clearly an important stressor for many other aquatic organisms. Future work should consider the effects of UVB in aquatic systems, particularly the effects of UVB radiation on community structure and ecosystem function.

Ultraviolet Radiation as an Environmental Stressor of Amphibians

by
Betsy A. Bancroft

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APPROVED:

Major Professor, representing Zoology

Chair of the Department of Zoology

Dean of the Graduate School

I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

Betsy A. Bancroft, Author

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CONTRIBUTION OF AUTHORS

Nick J. Baker assisted with many aspects of this dissertation. He performed literature searches, extracted data and helped analyze data for Chapters 2 & 3. Nick also helped collect data both in the lab and in the field for Chapter 4. Catherine Searle helped collect field data for Chapter 4. Tiffany Garcia helped in the formulation of the idea, assisted in thermal gradient design, collected laboratory data, and provided housing for parts of Chapter 4.

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DEDICATION

This dissertation is dedicated to the memory of my uncle, Bruce Stewart.

I miss him every day.

Ultraviolet Radiation as an Environmental Stressor of Amphibians

CHAPTER 1: GENERAL INTRODUCTION

Anthropogenic changes to the environment have altered the habitat of many organisms. Habitats have been altered with the addition of contaminants (Fleeger *et al.*, 2003), introduction of exotic organisms (Sakai *et al*., 2001), alteration of flood (Gergel *et al.*, 2005) and fire regimes (Allen *et al*., 2002), and increases in temperature (IPCC, 2007). In addition, reduction in the stratospheric ozone layer has resulted in increases in ultraviolet-B (UVB) radiation reaching the earth's surface (Kerr & McElroy, 1993; Madronich, 1993; Madronich *et al*., 1998; Solomon, 1999). Stratospheric ozone depletion and the concomitant increase in ultraviolet-B (UVB) radiation pose an important threat to ecological systems.

Ultraviolet-B radiation negatively impacts organisms in both terrestrial and aquatic systems (Caldwell *et al*., 1998; Häder *et al.*, 1998). At the organismal level, UVB damages DNA, proteins and lipids. This can result in increased mortality and effects on growth, development, photosynthesis and immunity (Tevini, 1993; Caldwell *et al.*, 1998). These effects have been reported in many organisms including viruses and bacteria (Karentz *et al*., 1994), phytoplankton and algae (Häder *et al.*, 2003), amphibians and fish (Blaustein *et al.*, 1998; Hessen, 2003), crops and forests (Caldwell *et al.*, 1998), crustaceans (Hessen, 2003) and humans (van der Leun & de Gruijl, 1993).

The effects of UVB radiation on organisms in aquatic habitats are of particular concern for a number of reasons (Häder, 1993). UVB negatively affects many

freshwater and marine organisms (de Mora *et al*., 2000; Helbling & Zagarese, 2003). UVB causes mortality in zooplankton, amphibians and fish (Siebeck *et al*., 1994; de Mora *et al*., 2000; Blaustein & Belden, 2003; Hessen, 2003). Sublethal effects in aquatic organisms, such as reduced growth, behavioral changes and increased susceptibility to disease have also been reported after exposure to UVB radiation (e.g., Williamson *et al.*, 1997; Belden *et al.*, 2000; Salo *et al.*, 2000). These effects may scale up to the population or community level, causing changes in community structure and function (Bothwell *et al*., 1994; Mostajir *et al.,* 1999; Marinone *et al.,* 2006; but see Wahl *et al.*, 2004).

Habitat alteration has negatively affected many aquatic organisms, including amphibians. Amphibians are of particular conservation concern as amphibian populations are declining more rapidly than either birds or mammals (Stuart *et al*., 2004) with perhaps as many as 122 species becoming extinct since 1980 (Mendelson *et al*., 2006). Many factors appear to be contributing to amphibian population declines. These include habitat loss, pathogens, contaminants, climate change, and increases in UVB radiation (Blaustein & Kiesecker, 2002; Collins & Storfer, 2003).

The effects of UVB radiation on amphibians include increased mortality, reduced growth, developmental abnormalities, increased susceptibility to disease and behavioural changes (reviewed in Blaustein *et al.*, 1998; Blaustein & Kiesecker, 2002). In addition, the magnitude of the effect may vary among species or among different populations or life history stages of the same species (e.g., Belden & Blaustein, 2002a). For example, survival of moor frog (*Rana arvalis*) embryos was

higher when UVB radiation was filtered out compared with embryos exposed to UVB radiation (Häkkinen *et al*., 2001). However, survival of larval moor frogs was not affected by UVB exposure (Häkkinen *et al*., 2001). In contrast, survival of embryonic common toads (*Bufo bufo*) was not affected when exposed to UVB, but survival was lower in larvae exposed to UVB compared with those shielded from UVB (Häkkinen *et al*., 2001). Many studies suggest that UVB may interact synergistically with other stressors such as contaminants, climatic factors or pathogens (Blaustein *et al.*, 2001, 2003). For example, western toad (*Bufo boreas*) embryos are susceptible to a complex interaction between UVB radiation, a pathogenic water mould (*Saprolegnia* sp.), and changes in precipitation (Kiesecker *et al.*, 2001). Thus, mortality in western toad embryos increases when they are infected with *Saprolegnia* in the presence of increasing UVB radiation during years of lower precipitation when the UVB shielding property of the water is diminished. Climate cycles such as El Niño and La Niña, and the associated changes in precipitation, affect this dynamic (Kiesecker *et al*., 2001). UVB negatively affects amphibians both alone and in conjunction with other environmental factors.

Amphibians have several defense mechanisms that may mediate the negative effects of UVB. Generally, amphibians either repair damage after it occurs or use various mechanisms to avoid or lessen exposure to UVB. Amphibians, like most organisms, use photorepair mechanisms (e.g., photolyase) to repair cyclobutane pyrimidine dimers in DNA induced by UVB exposure (Sancar & Sancar, 1988, Blaustein *et al*., 1994, Hays *et al*., 1996; Smith *et al*., 2002). However, there are

differences in the ability to repair UV-induced DNA damage. For example, in amphibians there are large differences in photolyase activity between species (Blaustein *et al*., 1994). Species with high levels of photolyase activity are usually more resistant to the harmful effects of UV radiation (Hays *et al*., 1996; Smith *et al*., 2002).

Amphibians mitigate damage from UVB via two strategies: 1) behaviorally avoiding UVB radiation or 2) using photoprotective compounds such as the pigment melanin (Blaustein & Belden, 2003). Amphibians may avoid high UVB levels by seeking deeper waters (Belden *et al*., 2000; Blaustein & Belden, 2003; Licht, 2003). Thus, in choice experiments, larvae and adults of some species prefer areas with lower UV irradiance (Nagl & Hofer, 1997; van de Mortel & Buttemer, 1998; Belden *et al*., 2000; Garcia *et al*., 2004; Han *et al*., 2007). However, some species are frequently observed in warm shallow waters or basking in sunlight (e.g., Brattstrom & Warren, 1955; Lillywhite, 1970; O'Hara, 1981; Bradford, 1984; Wollmuth *et al*., 1987), suggesting that these species do not avoid UVB radiation. Some species of larval amphibians darken in response to UVB (Belden & Blaustein, 2002b; Garcia *et al*., 2004). Darkening via photoprotective compounds such as melanin may allow certain species to exploit the resource-rich, warm shallows despite intense solar radiation and high levels of UVB present in these microhabitats. Mammals with darker skin are less prone to UV-induced skin damage than those with lighter skin (Kollias *et al*., 1991). However, darker skin does not appear to fully protect all amphibian species from lethal and sublethal UVB damage (Lesser *et al*., 2001; Belden & Blaustein, 2002b).

Thesis organization

Many papers have been published on the effects of UVB on aquatic organisms, including several dozen on amphibians. There is a need for quantitative synthesis of the available information, both to resolve existing controversy and to generate further research directions. In Chapter 2, I used meta-analysis to quantitatively summarize the effect of UVB radiation on survival and growth in all aquatic organisms. This synthesis brings together two systems that have previously been considered separately in reviews and books: marine and freshwater systems. This broad analysis suggests that UVB reduces survival and growth in aquatic organisms. Although this analysis highlights the negative effect of UVB on aquatic organisms, the suggestion that UVB negatively affects amphibians has been controversial (e.g., Licht, 2003). In Chapter 3, I conducted a meta-analysis on the effects of UVB radiation on survival of amphibians. In addition, I used factorial meta-analysis techniques to explore the effects of UVB in conjunction with additional stressors on survival of amphibians. The two analyses in Chapter 3 reaffirm the large negative effect of UVB on survival of amphibians, and quantify the interaction between UVB and other common environmental stressors such as contaminants, pathogens, and pH.

The strong negative effect of UVB detected in the meta-analyses suggests that UVB radiation is harmful to amphibians. Therefore, it is reasonable to predict that amphibians avoid exposure to UVB radiation in natural systems. Chapter 4 tests this

hypothesis using laboratory and field approaches. I tested microhabitat choice in a laboratory thermal gradient experiment and in a field UVB choice experiment, and then quantified habitat use in the field using transect surveys at three field sites. The results from these three approaches suggest that larval amphibians do not generally avoid solar radiation and exploit microhabitats where they are exposed to high levels of UVB.

Amphibians exposed to high levels of UVB due to habitat use may be protected from UVB by photoprotective pigments such as epidermal melanin. If melanin acts as a photoprotective pigment, darker individuals should exhibit increased survival under UVB radiation compared to lighter individuals. I tested this hypothesis in Chapter 5 by manipulating larval amphibian skin color and exposure to UVB in the laboratory. The results from this chapter suggest that melanin is not an effective photoprotective pigment in all amphibian species.

Chapter 2

Effects of UVB radiation in marine and freshwater organisms: a synthesis through meta-analysis

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Abstract

Ultraviolet-B radiation (UVB) is a global stressor with potentially far-reaching ecological impacts. In the first quantitative analysis on the effects of UVB on aquatic organisms, we used meta-analytic techniques to explore the effects of UVB on survival and growth in freshwater and marine systems. Based on the large body of literature on the effects of UVB in aquatic systems, we predicted that UVB would have different effects in different habitats, experimental venues, trophic groups and life history stages. Contrary to our predictions, we found an overall negative effect of UVB on both survival and growth that crossed life histories, trophic groups, habitats and experimental venues. UVB had larger negative effects on growth in embryos compared with later life history stages. Despite the overall negative effect of UVB, effect sizes varied widely. In the survival analyses, no relationship between mean effect size and taxonomic groups or levels of exposure to UVB was detected. In the growth analyses, a larger negative effect on protozoans was observed. Our analyses suggest that the effects of UVB in aquatic systems are large and negative but highly variable between organisms. Variation in susceptibility may have important implications for population and community structure.

Introduction

Stratospheric ozone depletion and the concomitant increase in ultraviolet-B (UVB) radiation pose an important threat to ecological systems. The work of Molina and Rowland (1974) on ozone-degrading compounds and the discovery of the Antarctic ozone hole (Farman *et al.*, 1985) were influential in the formulation and signing of the Montreal Protocol on Substances that Deplete the Ozone Layer in 1987. This landmark international treaty was designed to halt the production and use of chlorofluorocarbons (CFCs) to avoid further degradation of the ozone layer. Despite the success of the Montreal Protocol (Blaustein *et al.*, 2003; Solomon, 2004), the ozone layer remains damaged. This damage results in increasing levels of UVB radiation reaching the earth's surface (Kerr & McElroy, 1993; Madronich, 1993, 1994; Madronich *et al*., 1998; Solomon, 1999).

Ultraviolet-B radiation negatively impacts organisms in both terrestrial and aquatic systems (Caldwell *et al*., 1998; Häder *et al.*, 1998). Within living cells, nucleic acids, proteins and lipids are the primary targets of UVB damage (Buma *et al.*, 2003). Cyclobutane pyrimidine dimers (CPDs) in DNA are the most common lesion induced by UVB exposure. These dimers as well as other types of UV-induced DNA damage can inhibit transcription and replication (Buma *et al.*, 2003). Damage to proteins can interfere with normal cellular processes, while damage to lipids can disrupt cell membranes. Many organisms are able to repair damage caused by UVB radiation. Of these repair mechanisms, DNA repair is the most studied process. Organisms use different types of DNA repair mechanisms including photorepair,

excision repair and post-replication repair (Tevini, 1993). Photorepair is mediated by a group of enzymes called photolyases and relies on radiant energy in the UVA and photosynthetically active radiation (PAR) bands (Kelner, 1949; Sancar & Sancar, 1988). Photolyases primarily remove CPDs. Excision repair and post-replication repair are independent of radiant energy but are less efficient at repairing CPDs. Repair efficiencies can differ between species (e.g., Blaustein *et al.*, 1994). Many experiments on the effects of UVB have been conducted in the laboratory (e.g., Damkaer *et al.*, 1981; Charron *et al.*, 2000; Roleda *et al.*, 2004a). Although these experiments use ambient levels of UVB, researchers rarely replicate the full spectral environment found in natural systems. Because photorepair relies on wavelengths spanning UVA and PAR, laboratory experiments that do not carefully modulate the ratio of UVB to UVA and PAR may overemphasize the effects of UVB (Day & Neale, 2004).

At the organismal level, damage to DNA, proteins and lipids may result in a variety of lethal and sublethal effects. These effects have been reported in many organisms including viruses and bacteria (Karentz *et al*., 1994), phytoplankton and algae (Häder *et al.*, 2003), amphibians and fish (Blaustein *et al.*, 1998; Hessen, 2003), crops and forests (Caldwell *et al.*, 1998), crustaceans (Hessen, 2003) and humans (van der Leun & de Gruijl, 1993). The effects of UVB radiation on organisms in aquatic habitats are of particular concern (Häder, 1993).

The effects of UVB on aquatic organisms depend in part on the dose of harmful radiation to which an individual organism is exposed. UVB dose is affected

by both organismal behaviour/location within the water column and the optical characteristics (i.e., UVB transmittance) of the water body. UVB penetration in aquatic habitats is modulated by factors including dissolved organic carbon (DOC), suspended particles (including phytoplankton), and surface reflection (Díaz *et al.*, 2000; Hargreaves, 2003). In most aquatic habitats, UV attenuation is controlled primarily by absorption of UVB energy by DOC (Scully & Lean, 1994). As the majority of DOC is derived from terrestrial sources, freshwater habitats generally show higher UVB attenuation (and therefore lower levels of UVB in the water column) compared to marine waters (Kirk, 1994). Thus, marine organisms and freshwater organisms inhabiting clear alpine lakes may be most at risk to damage caused by recently increased UVB radiation.

In some communities, organisms at lower levels in a food web tend to be more susceptible to environmental stress (Rafaelli, 2004). Aquatic autotrophs, including those inhabiting the shallow benthos, are generally exposed to some level of UVB. Several studies have shown negative effects of UVB on photosynthetic rates in aquatic systems (Litchman & Neale, 2005; Roleda *et al.*, 2004a; Han *et al.*, 2003). Unlike benthic autotrophs, phytoplankton are found throughout the water column and can be exposed to high levels of UVB (Villafañe *et al.*, 2003). In addition, phytoplankton tend to be small in size and therefore have short pathlengths, allowing UVB penetration deep into the cells (Day & Neale, 2002). These observations have led to the speculation that phytoplankton may be particularly susceptible to damage from UVB radiation (Häder, 1993; Day & Neale, 2002; but see Halac *et al.*, 1997). While

photosynthetic organisms must inhabit the photoactive zone, consumers (particularly mobile consumers) may behaviourally avoid regions of high UVB within the water column by using refugia or seeking deeper waters (reviewed in Leech & Johnsen, 2003). However, other constraints such as foraging behavior, avoiding predation, or thermal requirements could force consumers into areas with high UVB exposure (Leech & Johnsen, 2003). Consumers at these higher trophic levels in aquatic systems experience direct negative effects from UVB radiation (Siebeck *et al.*, 1994; Williamson, 1995; Blaustein *et al.*, 1998; Hessen, 2003). UVB causes mortality in some species of zooplankton, amphibians and fish (Siebeck *et al*., 1994; Blaustein & Belden, 2003; Hessen, 2003). Sublethal effects such as reduced growth, behavioral changes and increased susceptibility to disease have also been reported (e.g., Williamson *et al.*, 1997; Belden *et al.*, 2000; Salo *et al.*, 2000).

The effects of UVB can vary over life-history stage in both consumers and primary producers. Some life history stages may be less adept at repair or have habitat requirements that place the organism in areas with high UVB exposure (e.g., Siebeck *et al.*, 1994; Epel *et al.*, 1999; McNamara & Hill, 1999). For example, brine shrimp (*Artemia franciscana*) are more susceptible to damage from UVB in naupliar stages compared to the adult stage (Dattilio *et al.*, 2005). Early life history stages of autotrophic organisms can also be more susceptible to damage from UVB. For example, early life history stages of some macroalgae are more susceptible to damage than the later stages (e.g., Roleda *et al.*, 2004b). Embryos may be particularly

susceptible to damage from UVB due to reduced capacity for repair and limited mobility (Epel *et al.*, 1999).

Lethal and sublethal effects of UVB on both primary producers and consumers can cause shifts in community structure and function and thus can impact overall ecological processes in both aquatic and terrestrial systems (see reviews in de Mora *et al.*, 2000; Hessen, 2003). For example, one half of all photosynthetic production is due to the activities of phytoplankton in aquatic systems (Houghton & Woodwell, 1989; Zepp, 2003). UVB radiation may alter community composition, size distribution, productivity, or nutritional quality of algae and photosynthetic bacteria (Karentz *et al.*, 1994). Reductions in the biomass and photosynthetic yield of phytoplankton may reduce the available carbon sink in the oceans, resulting in higher atmospheric CO_2 concentrations (Zepp, 2003). Shifts in phytoplankton community composition, size distribution and nutritional quality could reduce the resources available to higher trophic levels.

The potential of UVB to act as a stressor in aquatic environments has led to a number of reviews of the effects of UVB in both freshwater and marine systems (e.g., Häder, 1993; Karentz *et al.*, 1994; Siebeck *et al.*, 1994; Häder *et al.*, 1998; de Mora *et al.*, 2000; Helbling & Zagarese, 2003; Häder *et al.*, 2003). However, organisms vary widely in their responses to UVB: some species are highly susceptible while others appear relatively resilient. Moreover, even within a species, susceptibility may differ between populations and in different life history stages. Many reviews focus on the

observed variation in susceptibility between organisms. A synthetic analysis of the overall effects of UVB is lacking.

Previous reviews on the effects of UVB in aquatic systems have been qualitative. In general, these reviews present lists of effects and use the reported statistical significance of each study to assess the magnitude of the effects of UVB radiation. However, assessing the strength of an effect or importance of a stressor by counting the proportion of studies reporting a significant result (i.e., 'vote-counting') has poor statistical power (Rosenberg *et al.*, 2000). Meta-analysis techniques avoid the problems of conventional vote-counts and the subjectivity of traditional reviews. Meta-analyses have been used to identify broad trends in several aspects of global change (e.g., Root *et al.*, 2003).

We used meta-analytic techniques to test several hypotheses regarding the effects of UVB radiation in aquatic systems. For each hypothesis, we tested the effects of UVB on survival and growth separately. We hypothesized that UVB radiation has a negative effect on survival and growth of aquatic organisms. Furthermore, we hypothesized that the effect of UVB would be larger 1) in marine systems compared to freshwater habitats, 2) in the laboratory compared to field studies, 3) in primary producers compared to consumers, and 4) in earlier life history stages compared to later stages. Our paper is the first quantitative review of the effects of UVB in aquatic systems using rigorous statistical procedures. Our analyses reveal a large negative effect of UVB radiation on both survival and growth across all habitats, experimental venues, trophic groups, and life-history stages.

Materials and methods:

Data selection

We used six electronic databases (BIOSIS, Web of Science, Aquatic Sciences & Fisheries Abstracts, Fish & Fisheries Worldwide, Wildlife & Ecology Studies Worldwide, and Biological & Agricultural Index) to identify the studies used in our analyses. Within these databases, we searched for all combinations of the terms: *ultraviolet, UV, UVB* with *survival, growth,* and *mortality* to find primary literature on the effects of UVB radiation in aquatic organisms. We limited our search to experimental manipulations of UVB radiation (i.e., not UVA or UVA combined with UVB) where the investigators used the standard technique of applying plastic filters that differentially transmitted or filtered out UVB radiation. To explore both the lethal and the sublethal effects of UVB, we selected mortality and growth as response variables. Other sublethal response variables are possible (i.e., reproduction and life-stage duration) but growth is commonly measured for many organisms and can be assessed for multiple life stages. To avoid potential biases in the selection of studies, we established criteria for the inclusion of a study in the meta-analyses *a priori* (see Appendix A). Any data points within an article that met the criteria were considered for inclusion. To avoid personal bias, we made no attempt to judge study quality.

Several articles included more than one species, location (e.g., lakes with different spectral transmission properties), UVB irradiance (or dose), or sampling period. All species and locations from a given article were included in our analyses if the overall criteria for including the study were met. Although including all species or

locations from one study might decrease the independence among some data points, the inclusion of all available species and environments allowed us to more fully explore the effects of UVB radiation in these systems (Gurevitch *et al.*, 1992; Searles *et al.*, 2001). However, if more than one ambient UVB irradiance or dose were used in the original article we randomly selected only one irradiance level or dose for inclusion. If the article reported survival or growth over a time series, we selected the final measurement for these analyses. When articles quantified growth using several response variables (i.e., length and mass), we randomly selected one variable for inclusion.

All data were obtained from primary research articles. When necessary, data were extracted from published figures using TechDig V.2.0 software (Jones, 1998). We used the ITIS Catalogue of Life: 2005 Annual Checklist (http://annual.sp2000.org/2005/search.php) for taxonomic information. We followed the classification given by the authors for life-history stage; thus some categories may include photosynthetic organisms and non-photosynthetic organisms (e.g., "embryo") while other categories may be specific to certain taxa (e.g., "sporophyte").

Effect sizes

Our primary goal was to calculate an overall measure, including magnitude and direction (positive or negative), of the effect of UVB radiation on survival and growth in aquatic organisms. We used Hedges' *d* as our metric of standardized effect size (Hedges & Olkin, 1985). Hedges' *d* is an unbiased weighted measure of the difference between the means of the control and experimental groups divided by the

pooled standard deviation and multiplied by a correction term to adjust for small sample sizes. Convention dictates that a value of *d* greater than or equal to 0.8 is a large effect, *d* equal to 0.5 is a moderate effect, and *d* equal to 0.2 is a small effect (Cohen, 1969; Gurevitch *et al.*, 1992). We defined the control group as the group shielded from UVB radiation; therefore, a negative value of *d* indicates a negative effect of UVB on survival or growth. We also calculated a log response ratio (lnR) as a measure of effect size, but because the results were qualitatively similar using both measures, we report only those based on *d*. The only differences between the two metrics are a larger effect of UVB on growth in primary producers compared to consumers, a larger (although not significantly larger) effect of UVB on growth in adult stages compared to embryos and larvae, and a nonsignificant effect of UVB on growth in field studies and on the larval life history stage (Appendix B and Appendix C). We used MetaWin Version 2.0 (Rosenberg *et al.*, 2000) for all statistical procedures.

We identified 115 articles with primary data on the effects of UVB radiation on survival. Of those, only 46 met our criteria, generating 87 total comparisons of 61 species (Table 2.1). Of the 87 comparisons, a significant difference in survival between UVB exposed and UVB shielded organisms was reported in 39 comparisons. We found 71 studies on the effects of UVB on growth in our searches. Only 27 of these met our criteria. The articles used in the analysis yielded 46 comparisons of 32 different species (Table 2.2). Out of the 46 comparisons, a significant difference in

growth between UVB-exposed organisms and UVB-shielded organisms was reported in 29 comparisons (Table 2.2).

Full models

We selected our response variables with the intention of quantifying both lethal and sublethal effects. As such, we used all survival data in one analysis and all growth data in a separate analysis. We used a random effects model to calculate the grand mean effect size for each analysis. Although fixed effects models are more typical in meta-analyses, we expected the true effect size to vary among studies due to the broad taxonomic scope of these analyses: thus, a random effects model was necessary (Gurevitch & Hedges, 1999). Random effects models use the pooled standard deviation to estimate the distribution of effect sizes within the population. Therefore, using a random effects model allows effect size estimates to vary not only due to sampling error, but due to real biological or environmental differences between organisms and studies.

The output of each statistical test consisted of the grand mean effect size for the analysis with an accompanying bias-corrected bootstrapped 95% confidence interval (Adams *et al.*, 1997) and a total heterogeneity statistic (Q). The mean effect size is significantly different from zero if the confidence intervals do not overlap with zero. The heterogeneity statistic is a weighted sum of squares and is tested against a chi-square distribution with $n-1$ degrees of freedom. A significant value of Q in a random effects model indicates that the variation among effect sizes is greater than expected from sampling error and the random component included in the model,

suggesting that all effect sizes may not come from the same population (Rosenberg *et al.*, 2000).

Exploratory analyses

Our secondary goal was to examine the similarity in effect size among *a priori* selected groups including trophic group, habitat, life history stages, or experimental venue. We performed separate exploratory analyses testing the heterogeneity of mean effect sizes between groups using mixed effects models. This method of analysis is not ideal and is problematic in most cases. Performing multiple analyses on the same dataset increased the chance of Type I error. However, the purpose of these analyses was to quantify patterns in the literature, not to explain or partition heterogeneity. This distinction is subtle but important: future analyses should not use these methods to partition variance, but rather use hierarchical analytic methods (below, under *Sources of heterogeneity*). Due to low sample sizes in some groups, performing multiple analyses was the only way to explore the patterns in the literature. In addition, as groups with fewer than four comparisons were removed from the analyses, two of the four exploratory analyses used a subset of the data (trophic groups and developmental stages). We compared the mean effect size between marine and freshwater organisms, between studies conducted in the laboratory (and therefore under artificial UVB radiation) and studies conducted in the field (here defined as any experiment with natural solar radiation), between trophic groups, and between developmental stages. A mean effect size and bias-corrected bootstrapped 95% confidence interval were calculated for each group in the exploratory analyses. We

report parametric 95% confidence intervals when group sample size is small (i.e., <10) because parametric CIs provide a more conservative error estimate. When significant differences in mean effect size between three or more groups were observed in these exploratory analyses, we adjusted α using the Bonferroni method prior to conducting multiple two-way tests to determine where differences occurred (Gotelli & Ellison, 2004). Heterogeneity statistics were calculated to quantify both within-group (Q_W) and between-group (Q_B) variation. The interpretation of significant values of Q in mixed effects models is similar to random effects models. However, mixed effects models also incorporate group differences. In mixed effects models, significant values of Q suggest that observed variation is greater than expected due to sampling error, real variation in effect sizes (random component of model), and group differences (Gurevitch & Hedges, 1999). Although using only additive models may obscure some relationships (e.g., if embryos in freshwater habitats are more susceptible to damage from UVB compared to embryos in marine habitats our models would not detect the difference), current factorial meta-analytic techniques are only appropriate for use when the original studies are factorial in structure (Gurevitch *et al.*, 2000).

Sources of heterogeneity within groups

We assumed that variation in effect size could be due to taxonomic grouping (i.e., closely related organisms may be more similar in effect size compared with more distant groups) or due to dose-rate for each experiment. We used a step-wise partitioning technique *sensu* Gurevitch *et al.* (1992) to identify sources of within-group variation (Qw) using taxonomic information. Therefore, we used a mixed

effects model to compare mean effect sizes between taxonomic groups when enough comparisons (≥ 4) were available. We used a continuous random effects model to explore the relationship between effect size and hours of UVB radiation per day, total hours of UVB for each experiment, and dose when available. Several weighting functions are commonly used to assess irradiance and dosage levels. Weighting functions are calculated according to which specific wavelengths in the UVB band are most damaging to particular organisms. These weighting functions include erythemal (McKinlay & Diffey, 1987), DNA (Setlow, 1974), and plant (Caldwell, 1971) action spectra. As dose estimates with different weighting functions are not comparable, each weighting function was considered separately.

Sensitivity analysis

For each analysis, including exploratory analyses, we used a form of sensitivity analysis to assess the influence of unusually large effect sizes on the analyses. We ranked each comparison by magnitude of effect size, removed each unusually large comparison step-wise, and re-ran each analysis. This procedure tests the influence of these large effect sizes on the conclusions of the analyses and the heterogeneity statistics.

Publication bias

For each analysis, we used several standard methods to identify potential publication bias ("file drawer problem;" Rosenthal, 1979). We generated normal quantile plots of standardized effect size against the standard normal distribution to visually assess bias (Wang & Bushman, 1998; Rosenberg *et al.*, 2000). We used

Spearman's rank correlation test to formally test for publication bias. In addition, we calculated Rosenberg's failsafe number (Rosenberg, 2005) to quantitatively assess the importance of potential publication bias to the outcome of our analyses. Rosenberg's failsafe number is the number of studies with an effect size of precisely zero necessary to change the results of an analysis from significant to non-significant. No evidence of publication bias was detected in normal quantile plots of standardized effect size (*d*) in either the survival analysis or the growth analysis, as the plots were relatively linear and all points fall within the confidence intervals (Figure 2.1). Spearman's rank correlation tests were nonsignificant for both survival ($R = 0.087$; $p = 0.42$) and growth ($R = -0.04$; $p = 0.81$), indicating no significant correlation between standardized effect size and sample size. Rosenberg's failsafe number was large for both the survival (227 comparisons) and growth (76 comparisons) analyses.

Results

Effect of UVB radiation on survival

UVB radiation had a large negative effect on survival in aquatic organisms (Figure 2.2a). However, there were no differences in mean effect size between marine and freshwater organisms (Figure 2.2b), field and laboratory experiments (Figure 2.2c), trophic levels (Figure 2.2d), or life history stages (Figure 2.2e). With the exception of primary producers, UVB radiation had a large negative effect ($d_+ > -0.8$) in all groups (all effect size estimates were significantly different from zero). UVB

radiation had a moderate effect (d_+ = -0.6) on primary producers that was not different from zero.

In all models, within-group heterogeneity was observed (Table 2.3). Therefore, we used hierarchical structure (taxonomic groups) and dose rate in an attempt to partition variance. Our analyses were limited by the small number of taxonomic groups with more than four comparisons; however, no differences between taxonomic groups were detected and significant within-group heterogeneity persisted through all levels of taxonomic grouping (Table 2.4). Similarly, significant residual error was found in each model examining dose-rate variables (Table 2.5). Surprisingly, the relationship between dose-rate variables and effect size was negative in only two models (hours of UVB radiation per day and DNA-weighted dose estimates).

Effect of UVB radiation on growth

UVB radiation had a large negative effect on growth in aquatic organisms (Figure 2.3a). No differences in growth were detected between habitats (Figure 2.3b) or experimental venue (Figure 2.3c). UVB radiation had a larger negative effect on primary producers than on consumers, but this trend was only marginally significant (Figure 2.3d; p = 0.057; Table 2.6). However, the effects of UVB radiation on growth varied by life history stage (Figure 2.3e; p = 0.002; Table 2.6). The effects of UVB radiation were larger in embryos than in larvae (p < 0.0001). No differences were detected between embryos and adults (p = 0.19) or between adults and larvae (p = 0.019; nonsignificant at α = 0.017 after Bonferroni adjustment). The analysis

included only four studies of growth in embryos. The wide confidence intervals around the effect size estimate for embryos are a direct result of one large negative effect size (-27.55). A difference between these groups remained after removal of this large value ($p = 0.02$). As in the survival analysis, all mean effect sizes in the growth analysis were moderate to large and negative ($d_+ > -0.7$). Moreover, the mean effect size for every group was significantly different from zero.

Although the between-group heterogeneity was frequently nonsignificant, the within-group heterogeneity was large in every model (Table 2.6). Similar to the survival analysis, we used taxonomic grouping and dose-rate variables to partition variance. UVB radiation had a larger effect on members of the kingdom Protozoa than any other kingdom in this analysis (Figure 2.4). However, heterogeneity within groups was still detected at the level of kingdom (Table 2.7). Further partitioning was limited by the number of comparisons within groups. Within-group heterogeneity persisted through all levels of taxonomic grouping (Table 2.7). The relationship between effect size and dose-rate variables was nonsignificant in all models except days of exposure (Table 2.8). Moreover, significant residual error persisted in all models except between dose and mean effect size (Table 2.8). Unfortunately, few studies reported dose and those that did used one of several possible weighting functions. Therefore, we are unable to fit different slopes using analysis of covariance models to assess the contribution of different taxonomic or other grouping variables on the dose-effect size relationship.

Sensitivity analyses

Weighted histograms of effect size are left-skewed due to some extreme negative values (Figure 2.5). These values do not affect the overall normality of the data in the normal quantile plots (Figure 2.1). These two types of plots reveal different types of patterns in the data. In a weighted histogram, the height of each bar reflects the combined weight of the studies within that class (Rosenberg *et al.*, 2000). If the effect sizes are plotted as a simple frequency histogram, the left skew is greatly reduced (data not shown). Therefore, the apparent conflict between the normal quantile plots and the weighted histograms is a direct result of the weighting function. The unusually large effect sizes have very low weights compared to the smaller effect sizes, even if their frequency of occurrence is similar.

To test the robustness of our analyses against these extreme values, we removed each comparison with a large negative effect step-wise and re-ran each analysis. We began with the highest ranked effect size in each analysis and continued to remove the next largest effect size until Q was non-significant. Ten and eight comparisons were removed from the survival and growth analyses, respectively. After removal of these values, the weighted histograms were essentially unimodal with the highest frequency around zero effect size (Figure 2.6). Removing extreme values had no effect on the overall conclusions of the analyses: UVB radiation still had a large effect on survival and growth (d_{++} = -0.88, -1.22), respectively. In addition, heterogeneity in the full model was reduced to non-significance after removal of these effect sizes (Q = 85.33, df = 73, p = 0.15; Q = 50.22, df = 37, p = 0.07) in the survival and growth analyses, respectively.

Removal of the largest values step-wise had few effects on the conclusions of the exploratory analyses. Removal of the 10 largest effect sizes had no effect on the conclusions of the survival analyses. Heterogeneity was reduced to non-significance in all survival analyses (data not shown). In the growth analyses, removal of the 8 largest effect sizes had no effect on the conclusions of the venue, habitat, or life history stage exploratory analyses. The difference in growth between trophic groups moved from marginally significant to non-significant when the largest effect size was removed from the analysis. Heterogeneity was reduced to non-significance in all but the analysis of the effects of growth between life history stages.

Discussion

UVB radiation had a large negative effect on both survival and growth in our analyses. Traditional reviews and syntheses of the effects of UVB on aquatic organisms generally suggest that the effects of UV vary widely among organisms (Siebeck *et al.*, 1994; de Mora *et al*., 2000; Häder *et al*., 2003; Helbling & Zagarese, 2003). This assertion reflects the patterns in the literature. A conventional 'vote-count' of the comparisons included in the analysis would conclude that UVB radiation has a limited effect on survival. Less than half (45%) of the original comparisons observed a significant effect of UVB radiation on survival. A vote-count of the effects of UVB radiation on growth would detect a negative effect, as approximately 60% of studies reported a significant effect on growth. These analyses demonstrate the potential for meta-analytic techniques to identify broad trends that may be obscured by

variation and poor statistical power. Significance level depends on both the size of the measured effect and the sample size of each treatment (Gurevitch *et al.*, 1992). Thus, two studies measuring the same effect may have different statistical outcomes simply due to different sample sizes.

Due to the large heterogeneity statistics in both the survival and growth analyses, it may be incorrect to conclude that the grand mean effect size plus the 95% confidence interval is an accurate estimate of the true distribution of effects of UVB radiation in aquatic organisms (Hedges & Olkin, 1985; Gurevitch *et al.*, 1992). However, when we removed the largest effect sizes, the heterogeneity statistic moved to non-significance. Thus, it is possible that there were two distinct populations of effect sizes that we did not isolate using taxonomic groupings or dose-response variables.

To explore the heterogeneity further, we examined the details of each comparison with unusually large effect sizes to find commonalities between these comparisons that may indicate the source of the large effects. In the survival analyses, the large effect sizes were almost equally distributed across the groups (Table 2.1). All of the unusually large effect sizes were members of the kingdom Animalia (and therefore also classified as consumers in the trophic analysis), but this alone is not surprising given the paucity of comparisons in the other kingdoms (Table 2.1). The only striking similarity between these comparisons is that 9 of the 10 with the largest effect sizes also have extremely large variance (Table 2.1). In the growth analyses, 7 of the 8 unusually large effect sizes were laboratory studies. However, given that 40

of the 46 comparisons were laboratory studies, this was expected. All 8 of these comparisons were primary producers. These 8 comparisons represented members of all three phototrophic kingdoms (Table 2.2), but half of them were from the kingdom Protozoa. Similar to the survival analysis, all 8 comparisons removed from the growth analyses had extremely large variance (Table 2.2). Thus, the majority of the comparisons with unusually large effect sizes in each analysis also had large variance, and therefore may have low precision. Our estimates of variance were calculated using both sample size and the value of *d* for each comparison (Rosenberg *et al.*, 2000). Samples with very large effect sizes and small sample sizes will therefore have large estimates of variance. However, small sample size alone did not always lead to a large effect size or a large variance.

Considering the general lack of commonality between these comparisons we could not isolate the potential cause of the heterogeneity. Moreover, we could not justify removing these comparisons from the analysis. Therefore, below we discuss the results from the full analyses (with the very large effects included), but cautiously interpret results that were altered by the removal of the large effect sizes. We believe that for the majority of the comparisons in these analyses, the estimate of mean effect size and the associated 95% confidence interval is a reasonable approximation of the effect of UVB radiation on survival and growth in these organisms. Moreover, it is important to realize that the large heterogeneity observed in these models is driven by extremely large negative effects that may lie outside the expected distribution of effect sizes. These large effect sizes may be due to extreme experimental conditions or

alternatively, may indicate that these organisms are particularly susceptible to damage from UVB radiation. More research on these organisms with larger sample sizes is necessary to explore the basis for the large effect sizes observed in these comparisons. However, the large negative effect of UVB radiation on survival and growth persists with or without these unusually large effect size estimates.

Our analyses suggest that the magnitude of the effect varies among organisms, but the effect tends to be large and negative. The negative effect of UVB on both survival and growth was detected despite different habitat types, life history stages, trophic levels and diverse taxonomic groups.

No differences in survival or growth between marine and freshwater systems were detected, suggesting that the magnitude of the effect of UVB radiation cannot be predicted by habitat type. The optical qualities of marine waters can be very different from freshwater habitats. The depth at which 10% of surface UVB can be detected can vary by more than two orders of magnitude between temperate lakes and clear ocean waters, with much higher attenuation in freshwater habitats (Díaz *et al*., 2000). Despite the potential difference in UVB penetration between these systems, we did not observe a difference in effect size between freshwater and marine habitats. It is possible that the UVB penetration in these two habitat types was similar since the majority of marine studies were conducted in coastal environments where terrestrial run-off and estuarine contributions increase the levels of dissolved organic carbon in near-shore waters (Kirk, 1994).

Exposure of organisms to UVB in the laboratory rarely approximates natural environments. Of specific concern is the ratio of UVB to both UVA and PAR necessary for photorepair of DNA. In most laboratory experiments the UVB dose is closely monitored and applied, but the dose of UVA and PAR tends to be much lower than in natural systems (e.g., Ankley *et al.*, 2000). Surprisingly, no difference in mean effect size between laboratory and field exposures was found in either the survival or growth analyses. We do not believe that the ratio of UVB to UVA and PAR is unimportant; rather, our analyses suggest that the overall effect of UVB is negative regardless of the spectral quantity or quality of available light.

The effects of UVB radiation may vary over developmental stages (e.g., Häkkinen *et al.*, 2001; Altamirano *et al.*, 2003a; Hessen, 2003). However, variation in survival at different life history stages may have little impact at the population or community levels. For example, some amphibian species may be especially sensitive to UVB in early life stages but this may not affect them at the population level (Vonesh & De La Cruz, 2002). Regardless, in our analysis, survival under UVB radiation was lower than shielded controls in all life-history stages. Furthermore, embryos tend to grow more slowly when exposed to UVB radiation as illustrated in our analysis. Hampered growth in embryos could be due to several factors. Most importantly, the rate of cellular division is generally higher in embryos compared with other life history stages and DNA repair may be limited during rapid divisions within an embryo (Epel *et al.*, 1999). Thus, development could be slowed by a damaged genome. Alternatively, development may be delayed by time-intensive repair

mechanisms (Epel *et al.*, 1999). However, this result is ambiguous because of the relatively few (4) comparisons of growth in embryos. More research is needed on the effects of UVB on growth of embryos to clarify the relationship between life history stage and reduced growth due to UVB exposure. Regardless of the differences in inherent susceptibility between life history stages, embryos are generally non-motile, so behavioural avoidance of UVB in natural systems is unlikely unless oviposition occurs in a shielded environment. Therefore, embryos may be exposed to high levels of UVB during development. In contrast, motile stages and mobile species may prevent damage by behaviourally avoiding UVB radiation (Banaszak, 2003; Blaustein & Belden, 2003).

Later life history stages of animals and many species of phytoplankton actively select microhabitats that may reduce exposure to UVB radiation (Häder, 1993). However, there may be a trade-off between exposure to warm sunlit areas with higher levels of PAR (optimal for photosynthesis and thermoregulation) and avoidance of areas with harmful levels of UVB (Hutchison & Duprè, 1992; Häder, 1993). Negative effects of reduced growth during early life history stages on lifetime fitness have been demonstrated in several taxa including insects (Mouer & Istock, 1980), birds (Sedinger *et al.*, 1995) and amphibians (Semlitsch *et al.*, 1988). These delayed life history effects are rarely explicitly incorporated into theoretical models of population and community dynamics, but are important to our understanding of how early environments and conditions affect population fluctuations in natural systems (Beckerman *et al.*, 2002).

Previous reviews have suggested that primary producers, particularly phytoplankton, may be especially sensitive to UVB radiation (e.g., Häder, 1993; Day & Neale, 2002). In our analysis, no differences in survival were observed between trophic groups. In the survival analysis, the mean effect size in primary producers was smaller than in consumers and not different from zero. Few studies have examined survival in primary producers and the wide confidence interval may reflect the small sample size (N = 6) in our analysis. Clearly, more research on the effects of UVB on survival of primary producers is necessary to determine the importance of UVB on trophic interactions.

Our growth analysis suggests that UVB radiation may affect primary producers more than consumers, although the trend was only marginally significant and disappeared when the very large effect sizes were removed from the analysis. This trend may be driven by the large negative effect of UVB on protozoans. However, more research on the effect of UVB radiation on growth in primary producers is needed to clarify this trend. In particular, more work on the effects of UVB radiation on protozoans is necessary. Of the nine comparisons in the kingdom Protozoa, seven were of dinoflagellates and eight of the nine were the work of one laboratory (Ekelund, 1990; Ekelund, 1991; Ekelund, 1993). In mesocosm experiments with plankton communities, several studies reported a larger negative effect of UVB on phytoflagellates (protozoans) compared to diatoms (Villafañe *et al.*, 1995; Hernando *et al.*, 2006) but in other experiments the opposite trend was observed (Wängberg *et*

al., 1996). Reduced growth of primary producers may lead to bottom-up control of these systems due to diminished food resources.

Previous reviews highlight the variation in susceptibility to UVB between organisms (de Mora *et al.*, 2000; Helbling & Zagarese, 2003). Our analyses demonstrate this variation in the distribution of effect sizes represented by mean and 95% confidence intervals. Effect sizes in these analyses ranged from -27.5 to 5.20 (Tables 2.1 and 2.2). Although the overall effect was large and negative, individual species may be more susceptible to damage from UVB. Our random effects model allowed for a distribution of effect sizes and the estimates of pooled SD were relatively large (1.8262 and 2.6332 in the survival and growth analyses, respectively).

Varying resistance to damage from UVB may lead to shifts in diversity or richness in both freshwater and marine phytoplankton populations, as has been observed in zooplankton communities (Marinone *et al.*, 2006). Community composition may shift to favour a microbial web over a heterotrophic web (e.g., Mostajir *et al.*, 1999). These shifts in community composition, diversity, or species richness in addition to the effects of UVB on dissolved carbon may alter the carbon dynamics in the oceans (Mostajir *et al.*, 2000). The majority of experiments on community-level effects of UVB focus on one component of a natural community (i.e., phytoplankton community). Including more community components may reveal shifts in community structure that are not predicted based on sensitivity to UVB alone. For example, Bothwell *et al.* (1994) observed indirect positive effects of UVB radiation on algae due to a reduction in herbivory. The effects of UVB on

communities may be transient. A recent study by Wahl *et al.* (2004) found no difference in diversity or biomass between marine benthic communities exposed to UVB and communities shielded from UVB after 12 weeks. More long term experiments on communities are necessary to fully understand the effects of UVB radiation on diversity, richness and ecological function.

Although we predicted that the effects of UVB would vary along taxonomic groupings, significant heterogeneity persisted through all taxonomic levels. Partitioning variance using the level of kingdom was impossible in the survival analysis as the vast majority of comparisons in this analysis focused on members of the kingdom Animalia (80 out of 86). Even within the kingdom Animalia, our attempts to partition variance through taxonomic structure were unsuccessful and heterogeneity persisted in each model. Similarly, heterogeneity persisted in every model in the growth analysis. This variation in effect size most likely reflects both intra- and interspecies variation in susceptibility to UVB radiation in addition to variation due to experimental conditions such as optical characteristics of water, timing of UVB exposure and dose rate.

In an attempt to quantify the relationship between experimental conditions and effect size we used dose and dose-rate variables that included hours of UVB per day, total hours of UVB, days of UVB exposure, and daily dose. In the survival analysis we were also able to include total erythemal dose (erythemal dose per day summed across all exposure days). We could not use optical characteristics in our analysis as most authors do not report these types of data (e.g., extinction coefficients). We

predicted a relationship between dose and effect size, as many organisms respond to UVB with a dose-response curve (Damkaer, 1981; McNamara & Hill, 1999; Ankley *et al*., 2002; Browman *et al.*, 2003; Hessen, 2003). In all cases the fit to the model was nonsignificant, suggesting that the relationship between dose-rate variables and effect size was weak in the survival analysis. In the growth analysis, the regression term was nonsignificant in all models except days of UVB exposure. Although the regression term was significant in the days of exposure regression model, the residual error term was highly significant; thus, the overall fit to the model was poor. These analyses did not detect a strong relationship between dose-rate variables and effect size. Interspecific variation in conjunction with experimental variation may obscure the dose/effect size relationship in our analyses.

Broader impacts and conservation implications

To our knowledge, these analyses are the first quantitative evidence of the overall negative impact of UVB radiation on aquatic organisms. Traditional reviews of the effects of UVB radiation are unable to detect the broad patterns revealed by our meta-analyses. These reviews emphasize the variation between organisms, habitats, life-history stages, and trophic levels (e.g., Siebeck *et al.*, 1994; de Mora *et al*., 2000; Häder *et al*., 2003; Helbling & Zagarese, 2003). Our analyses captured this variation through the distribution of effect sizes, but also reveal a strong negative effect of UVB despite this variation. The dynamics of UVB exposure and resulting organismal damage is complex in natural systems. The effects of UVB in both freshwater and marine systems are modulated by many factors including seasonality of UVB dose,

total ozone concentration in the stratosphere, cloudiness, local topography, DOC, organismal behaviour and repair mechanisms. These factors may vary widely between studies, habitats, developmental stage, or species; however, these analyses emphasize the commonality of a negative effect of UVB radiation. The most striking and important result of these analyses is the consistency of the effect of UV regardless of other moderating variables within each study. These variables may have a large impact within a study, but when all the data were combined, the majority of comparisons showed a negative effect of UVB that was within the expected distribution of effect sizes. Moreover, those studies that did not fall within the expected distribution had larger (more negative) effect sizes. Our analyses highlight the importance of UVB radiation in both marine and freshwater organisms.

The response variables selected for these analyses are only two of the many possible effects of UVB radiation; therefore, it is likely that these analyses underestimate the potential effects of UVB in natural systems. For example, effects such as reduced photosynthetic rates, tissue damage, and behavioural changes have been documented in many species (reviewed in Tevini, 1993; de Mora *et al.*, 2000; Helbling & Zagarese, 2003). If UVB radiation has a negative effect on all of these variables, the overall influence of UVB could be high in these systems. Moreover, predicted increases in acidification may reduce the DOC levels in freshwater systems, resulting in higher UVB exposure in these systems (Vinebrooke *et al.*, 2004).

As a consequence of global environmental change, stressors such as UVB, chemical contaminants, drought, disease, and acidification are increasingly common in

natural systems. We did not include additional stressors in these analyses, but it is unlikely that a system would be exposed to only one stressor at a time. Environmental stressors such as UVB may interact with other environmental or biotic stressors and result in non-additive responses that are larger than predicted by each stressor individually (Vinebrooke *et al.*, 2004). For example, UVB radiation acts synergistically with other stressors such as contaminants, disease, and extreme thermal events (Kiesecker & Blaustein, 1995; Häder *et al.*, 2003; Pelletier *et al.*, 2006). Alternatively, one stressor may have an antagonistic effect on other stressors, such that exposure to two stressors is less than additive (Christensen *et al.*, 2006).

At the community level, differences in susceptibility to environmental stressors may vary between organisms, leading to unforeseen interactions between stressors on the community as a whole. For example, one species may be more susceptible to chemical contaminants and less susceptible to UVB radiation, while another species is less susceptible to chemical contaminants but more susceptible to UVB radiation. Because these two stressors may be found in the same habitat, the overall effect of the stressors may be greater than predicted considering each stressor alone. Exposure to multiple stressors may shift communities towards dominance by a few hardy species (Christensen *et al.*, 2006). Synergisms among stressors are increasingly important in the face of global environmental change and must not be ignored when considering both the effects of UVB on a single species and the effects of UVB on entire communities and systems.

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Table 2.1. Summary information for each comparison included in the survival analysis. M = marine, FW = freshwater, FD = field, LB = lab, P = primary producer, C = consumer, E = embryo, L = larvae, A = adult, N = naupli, CP = copepodid, S = spore, J = juvenile.

Species	Habitat	Venue	Trophic Level	Life-history stage	Effect size (*d*)	Variance of *d*	Source
Acartia ornorii	M	LB	C	E	-20.04*	34.14	Lacuna & Uye 2001
Acropora palmata	M	FD	C	L	-0.57*	0.060	Wellington & Fitt 2003
Agaricia agaricites	M	FD	C	L	-1.94*	0.74	Gleason & Wellington 1995
Ambystoma gracile	FW	FD	C	E	-4.58*	1.81	Blaustein *et al.* 1995
Ambystoma maculatum	FW	FD	C	E	-0.40	0.20	Starnes *et al.* 2000
Boeckella brevicaudata	FW	FD	C	A	-0.05	0.40	Zagarese *et al.* 1997
Boeckella gibbosa	FW	FD	C	A	-0.01	0.40	Zagarese *et al.* 1997
Boeckella gracilipes	FW	FD	C	A	-1.47*	0.51	Zagarese *et al.* 1997
Boeckella gracilipes	FW	FD	C	N	0.91	1.10	Cabrera *et al.* 1997
Boeckella gracilipes	FW	FD	C	CP	-0.73	1.07	Cabrera *et al.* 1997
Boeckella gracilipes	FW	FD	C	A	-0.90	1.10	Cabrera *et al.* 1997

Boeckella gracilipes	FW	FD	C	A	-2.25*	1.09	de los Ríos & Soto 2005
Brachydanio rerio	FW	LB	C	E	-3.67	1.34	Charron *et al.* 2000
Brachydanio rerio	FW	LB	C	L	-12.21*	9.82	Charron *et al.* 2000
Bufo americanus	FW	LB	C	L	-0.96	0.56	Grant & Licht 1995
Bufo boreas	FW	LB	C	J	-3.85*	0.57	Blaustein *et al.* 2005
Bufo boreas	FW	FD	C	E	-0.24	0.14	Corn 1998
Bufo boreas	FW	FD	C	E	0.22	0.14	Corn 1998
Bufo bufo	FW	FD	C	E	0.06	0.50	Häkkinen *et al*. 2001
Bufo bufo	FW	FD	C	L	-8.50*	5.01	Häkkinen *et al*. 2001
Calanus finmarchicus	M	FD	C	E	0.63	0.30	Browman *et al.* 2000
Cancer magister	M	LB	C	L	-10.14*	5.54	Damkaer *et al.* 1980
Cancer oregonesis	M	LB	C	L	-0.70	0.42	Damkaer *et al.* 1980
Chaetoceros brevis	M	LB	P	A	0.68	0.71	van de Poll *et al.* 2005
Chaetoceros brevis	M	FD	P	A	-0.71	1.06	van de Poll *et al.* 2005
Chondrus crispus	M	LB	P	S	-0.79*	0.72	Roleda *et al.* 2004

Coregonus albula	M	LB	C	L	-0.05	0.50	Häkkinen *et al.* 2004
Coregonus lavaretus	M	LB	C	L	0.84	0.55	Häkkinen *et al.* 2004
Coregonus lavaretus	M	LB	C	L	-0.67	0.53	Ylönen & Karjalainen 2004
Corella inflata	M	FD	C	J	-2.29	0.83	Bingham & Reitzel 2000
Daphnia magna	FW	LB	C	A	-1.34*	0.41	Borgeraas & Hessen 2000
Daphnia pulex	FW	FD	C	A	-3.48*	1.68	de los Ríos & Soto 2005
Daphnia pulicaria	FW	LB	C	A	-1.55	1.30	Williamson *et al.* 2001
Daphnia pulicaria	FW	FD	C	A	-3.32*	2.38	Zagarese *et al.* 1994
Esox lucius	FW	FD	C	L	0	0.5	Häkkinen & Oikari 2004
Gadus morhua	M	FD	C	E	-2.44*	0.50	Beland *et al.* 1999
Gadus morhua	M	FD	C	E	-17.52*	11.25	Browman *et al.* 2000
Heterocapsa triquetra	M	LB	P	A	-0.49	0.69	Wängberg *et al.* 1997
Homarus americanus	M	LB	C	L	0.38	1.02	Rodriguez *et al.* 2000
Hyla cadaverina	FW	FD	C	E	-2.42*	0.43	Anzalone *et al.* 1998
Hyla chrysoscelis	FW	FD	C	E	-0.24	0.20	Starnes *et al.* 2000

Hyla regilla	FW	FD	C	E	-0.13	0.25	Anzalone *et al.* 1998
Keratella cochlearis	M	FD	C	A	-1.55*	0.43	Vinebrooke & Leavitt 1999
Keratella quadrata	M	FD	C	A	-2.67*	0.63	Vinebrooke & Leavitt 1999
Lepadella sp.	M	FD	C	A	-1.24*	0.40	Vinebrooke & Leavitt 1999
Lepomis macrochirus	FW	FD	C	E	-1.30*	0.61	Gutiérrez-Rodríguez & Williamson 1999
Lepomis macrochirus	FW	FD	C	E	-1.14*	0.58	Gutiérrez-Rodríguez & Williamson 1999
Litoria aurea	FW	FD	C	E	-1.09	0.57	van de Mortel & Buttemer 1996
Litoria aurea	FW	FD	C	E	0.14	0.50	van de Mortel & Buttemer 1996
Litoria dentata	FW	FD	C	E	-0.38	0.51	van de Mortel & Buttemer 1996
Litoria dentata	FW	FD	C	E	-0.20	0.50	van de Mortel & Buttemer 1996
Litoria peronii	FW	FD	C	E	-0.36	0.51	van de Mortel & Buttemer 1996
Litoria peronii	FW	FD	C	E	-0.98	0.56	van de Mortel & Buttemer 1996
Mastocarpus stellatus	M	LB	P	S	-2.02*	1.01	Roleda *et al.* 2004
Montastraea annularis	M	FD	C	L	-8.24*	0.54	Wellington & Fitt 2003

Montastraea franksi	M	FD	C	L	-2.42*	0.10	Wellington & Fitt 2003
Notholca sp.	M	FD	C	A	-0.63	0.35	Vinebrooke & Leavitt 1999
Oncorhynchus apache	FW	LB	C	L	5.20*	2.92	Little & Fabacher 1994
Oncorhynchus clarki henshawi	FW	LB	C	L	-4.40*	2.28	Little & Fabacher 1994
Pandalus hypsinotus	M	LB	C	L	-1.54*	0.52	Damkaer *et al.* 1980
Pandalus platyceros	M	LB	C	L	-0.58*	0.42	Damkaer *et al.* 1981
Psuedacris crucifer	FW	LB	C	L	-4.18*	1.59	Baud & Beck 2005
Psuedacris triseriata	FW	FD	C	E	-0.67	0.42	Starnes *et al.* 2000
Rana arvalis	FW	FD	C	E	-2.94*	1.04	Häkkinen *et al*. 2001
Rana arvalis	FW	FD	C	L	-1.92	0.73	Häkkinen *et al*. 2001
Rana arvalis	FW	FD	C	E	0.43	0.09	Pahkala *et al.* 2001
Rana arvalis	FW	LB	C	E	-2.66	0.24	Pahkala *et al.* 2001
Rana blairi	FW	FD	C	E	-0.10	0.15	Smith *et al.* 2000
Rana cascadae	FW	FD	C	L	-1.13*	0.19	Belden *et al.* 2003

Rana cascadae	FW	LB	C	L	0.41	0.51	Hatch & Blaustein 2002
Rana clamitans	FW	FD	C	L	-9.32*	11.87	Tietge *et al.* 2001
Rana luteiventris	FW	FD	C	E	-0.07	0.50	Blaustein *et al.* 1999
Rana pipiens	FW	LB	C	L	2.11	1.56	Ankley *et al.* 2000
Rana pipiens	FW	FD	C	L	-6.07*	5.60	Ankley *et al.* 2000
Rana pipiens	FW	FD	C	L	-10.69*	15.29	Tietge *et al.* 2001
Rana pretiosa	FW	FD	C	E	0.18	0.50	Blaustein *et al.* 1999
Rana septentrionalis	FW	FD	C	L	-23.80*	71.83	Tietge *et al.* 2001
Rana sylvatica	FW	LB	C	L	-0.34	0.59	Grant & Licht 1995
Rana temporaria	FW	FD	C	E	-0.38	0.51	Häkkinen *et al*. 2001
Rana temporaria	FW	FD	C	L	-0.02	0.50	Häkkinen *et al*. 2001
Rana temporaria	FW	LB	C	E	-0.02	0.10	Pahkala *et al.* 2001
Rana temporaria	FW	FD	C	E	0.12	0.10	Merilä *et al.* 2000
Rana temporaria	FW	FD	C	E	0	0.1	Pahkala *et al.* 2000
Sinocalanus tenellus	M	LB	C	E	-14.43*	18.02	Lacuna & Uye 2000

Taricha torosa	FW	FD	C	E	-2.97*	0.25	Anzalone *et al.* 1998
Thalassiosira sp	M	FD	P	A	-0.57	0.69	Hernando *et al.* 2002
Thysanoessa raschii	M	LB	C	L	-1.45*	0.51	Damkaer *et al.* 1980

* Significant effect reported in original article

Table 2.2. Summary information for each comparison included in the growth analysis. M = marine, FW = freshwater, FD = field, LB = lab, P = primary producer, C = consumer, E = embryo, L = larvae, A = adult, SP = sporophyte, GM = gametophyte, J = juvenile.

Species	Habitat	Venue	Trophic Level	Life-history stage	Effect size (*d*)	Variance of *d*	Source
Ambystoma gracile	FW	LB	C	L	-4.07*	0.88	Belden & Blaustein 2002a
Ambystoma macrodactylum	FW	LB	C	L	-1.29*	0.35	Belden & Blaustein 2002b
Ambystoma macrodactylum	FW	LB	C	L	-0.55*	0.05	Belden & Blaustein 2002c
Ambystoma macrodactylum	FW	LB	C	L	-0.42*	0.04	Belden & Blaustein 2002c
Amphistegina gibbosa	M	LB	C	A	0.09	0.67	Williams & Hallock 2004
Bufo americanus	FW	LB	C	L	-1.94	0.74	Grant & Licht 1995
Chondrus crispus	M	LB	P	GM	-1.41	0.50	Roleda *et al.* 2004a
Coregonus albula	M	LB	C	L	0.08	0.50	Häkkinen *et al.* 2002
Coregonus lavaretus	M	LB	C	L	-0.76	0.54	Ylönen & Karjalainen 2004

Coregonus lavaretus	M	LB	C	L	0.11	0.50	Häkkinen *et al.* 2002
Cylindrotheca closterium	M	LB	P	A	-2.47*	0.88	Rijstenbil 2003
Ditylum brightwellii	M	LB	P	A	-0.88*	0.73	Ekelund 1990
Ditylum brightwellii	M	LB	P	A	0.62*	0.70	Ekelund 1990
Enteromorpha intestinalis	M	LB	P	SP	-11.24*	6.72	Cordi *et al.* 2001
Enteromorpha intestinalis	M	LB	P	GM	-0.91*	0.44	Cordi *et al.* 2001
Euglena gracilis	FW	LB	P	A	-11.65*	3.27	Ekelund 1993
Fucus gardneri	M	LB	P	E	-27.55*	47.93	Henry & Van Alstyne 2004
Fucus gardneri	M	LB	P	J	-0.35	0.51	Henry & Van Alstyne 2004
Fucus serratus	M	LB	P	E	-3.48*	1.68	Altamirano *et al.* 2003a
Fucus serratus	M	LB	P	GM	1.43	0.84	Altamirano *et al.* 2003b
Fucus spiralis	M	LB	P	GM	-4.55*	2.40	Altamirano *et al.* 2003b
Fucus vesiculosus	M	LB	P	GM	-10.00*	9.00	Altamirano *et al.* 2003b
Gyrodinium aureolum	M	LB	P	A	-9.08*	7.54	Ekelund 1990

Gyrodinium aureolum	M	LB	P	A	-5.68*	3.36	Ekelund 1990
Gyrodinium aureolum	M	LB	P	A	-1.79*	0.93	Ekelund 1991
Heterocapsa triquetra	M	LB	P	A	-2.53*	1.20	Ekelund 1991
Hyla versicolor	FW	LB	C	L	-0.11	1.00	Grant & Licht 1995
Laminaria ochroleuca	M	LB	P	SP	-0.51	0.41	Roleda *et al.* 2004b
Mastocarpus stellatus	M	LB	P	GM	0.32	0.41	Roleda *et al.* 2004a
Nannochloropsis gaditana	M	LB	P	A	-3.81*	2.81	Sobrino *et al.* 2004
Phaeodactylum tricornutum	M	LB	P	A	0.73	0.71	Ekelund 1990
Phaeodactylum tricornutum	M	FD	P	A	1.63*	0.67	Behrenfeld *et al.* 1992
Prorocentrum minimum	M	LB	P	A	-18.77*	30.02	Ekelund 1990
Prorocentrum minimum	M	LB	P	A	-7.36*	5.18	Ekelund 1991
Prorocentrum minimum	M	LB	P	A	-3.04*	1.44	Ekelund 1990
Pseudonitzschia seriata	M	FD	P	A	-6.06*	2.24	Nilawati *et al.* 1997
Rana arvalis	FW	FD	C	E	-3.91	0.27	Pahkala *et al.* 2001a

Rana pipiens	FW	LB	C	L	-1.58*	0.66	Crump *et al.* 2002
Rana pipiens	FW	LB	C	L	-1.28	1.20	Ankley *et al.* 2000
Rana sylvatica	FW	LB	C	L	1.83*	0.71	Grant & Licht 1995
Rana temporaria	FW	LB	C	E	-3.55	0.26	Pahkala *et al.* 2001b
Selenastrum capricornutum	FW	LB	P	A	-3.42*	0.55	West *et al*. 1999
Selenastrum capricornutum	FW	LB	P	A	-0.75*	0.36	West *et al*. 1999
Thalassiosira sp.	M	FD	P	A	-3.78*	1.86	Hernando *et al.* 2002
Ulva expansa	M	FD	P	A	-0.28	0.20	Grobe & Murphy 1998
Ulva rigida	M	FD	P	A	-1.21*	1.18	Altamirano *et al.* 2000

* Significant effect reported in original article

Table 2.3.Heterogeneity statistics for each model in the survival analysis. Separate analyses were conducted to compare similarity in effect size between each group.

Statistical model	df	Q	Probability
Full model (no structure)	86	169.66	<0.0001
Habitat type			
Between groups	1	2.01	0.16
Within groups	85	169.57	<0.0001
Experimental venue			
Between groups	1	0.001	0.97
Within groups	85	168.23	<0.0001
Trophic group			
Between groups	1	0.90	0.34
Within groups	85	168.16	<0.0001
Developmental stage			
Between groups	2	2.45	0.29
Within groups	78	164.35	<0.0001

Table 2.4. Results from variance partitioning using taxonomic group in the survival analysis. Variance between mean effect size estimates for each group was compared with other members of the preceding hierarchical level only (i.e., only classes within a phylum were compared to each other).

Statistical model	df	Q	Probability	Representative groups
Phylum (within Animalia)				
Between groups	3	6.88	0.08	Arthropoda (20), Chordata (53)
Within groups	77	158.07	<0.0001	Cnidaria (4), Rotifera (4)
Class (within Arthropoda)				
Between groups	2	1.20	0.55	Branchiopoda (4), Copepoda (9)
Within groups	16	35.15	0.004	Malacostraca (6)
Class (within Chordata)				
Between groups	1	0.64	0.42	Actinopterygii (12), Amphibia (40)
Within groups	50	112.08	<0.0001	
Family (within Anura)				
Between groups	2	0.89	0.64	Bufonidae (6), Hylidae (11)
Within groups	34	62.85	0.002	Ranidae (20)

Table 2.5. Relationship between mean effect size estimates and dose-rate variables in the survival analysis.

Parameter	Slope	Source	df	*Q*	*P*
Hours of UVB per day	-0.0343	Regression	1	0.20	0.65
		Residual	25	58.10	<0.001
Total hours of UVB	0.0074	Regression	1	1.25	0.26
		Residual	23	53.15	<0.001
Days of exposure	0.0125	Regression	1	0.40	0.53
		Residual	61	135.55	<0.001
Dose (DNA)	-0.5317	Regression	1	0.30	0.59
		Residual	5	4.42	0.49
Dose (Erythemal)	2.3127	Regression	1	3.65	0.06
		Residual	12	27.64	0.006
Cumulative dose (Erythemal)	0.1168	Regression	1	1.92	0.17
		Residual	12	31.49	0.002

Table 2.6. Heterogeneity statistics for each model in the growth analysis. Separate analyses were conducted to compare similarity in effect size between each group.

Statistical model	df	Q	Probability
Full model (no structure)	45	104.42	<0.0001
Habitat type			
Between groups	1	0.17	0.68
Within groups	44	100.74	<0.0001
Experimental venue			
Between groups	1	0.039	0.84
Within groups	44	102.98	<0.0001
Trophic group			
Between groups	1	3.61	0.057
Within groups	44	97.67	<0.0001
Developmental stage			
Between groups	2	12.22	0.002
Within groups	34	79.82	<0.0001

Table 2.7. Results from variance partitioning using taxonomic group in the growth analysis. Variance between mean effect size estimates for each group was compared with other members of the preceding hierarchical level only (i.e., only classes within a phylum were compared to each other).

Statistical model	df	*Q*	*P*	Representative groups
Kingdom				
Between groups	3	12.1981	0.007	Animalia (14)
Within groups	42	90.1467	<0.0001	Chromista (11) Plantae (12) Protozoa (9)
Phylum (within Plantae)				
Between groups	1	0.34	0.56	Bacillariophyta (4)
Within groups	8	17.12	0.03	Chlorophyta (6)
Order (within Amphibia)				
Between groups	1	0.20	0.65	Anura (7)
Within groups	9	16.67	0.054	Urodela (4)

Table 2.8. Relationship between mean effect size estimates and dose-rate variables in the growth analysis.

Parameter	Slope	Source	df	*Q*	*P*
Hours of UVB per day	0.0582	Regression	1	0.89	0.35
		Residual	35	82.30	<0.0001
Total hours of UVB	0.0023	Regression	1	0.34	0.53
		Residual	33	84.30	<0.0001
Days of exposure	0.0328	Regression	1	3.71	0.05
		Residual	41	102.98	<0.0001
Dose (DNA)	-0.381	Regression	1	1.21	0.27
		Residual	4	3.20	0.53
Dose (Erythemal)	0.669	Regression	1	1.56	0.21
		Residual	5	9.60	0.09

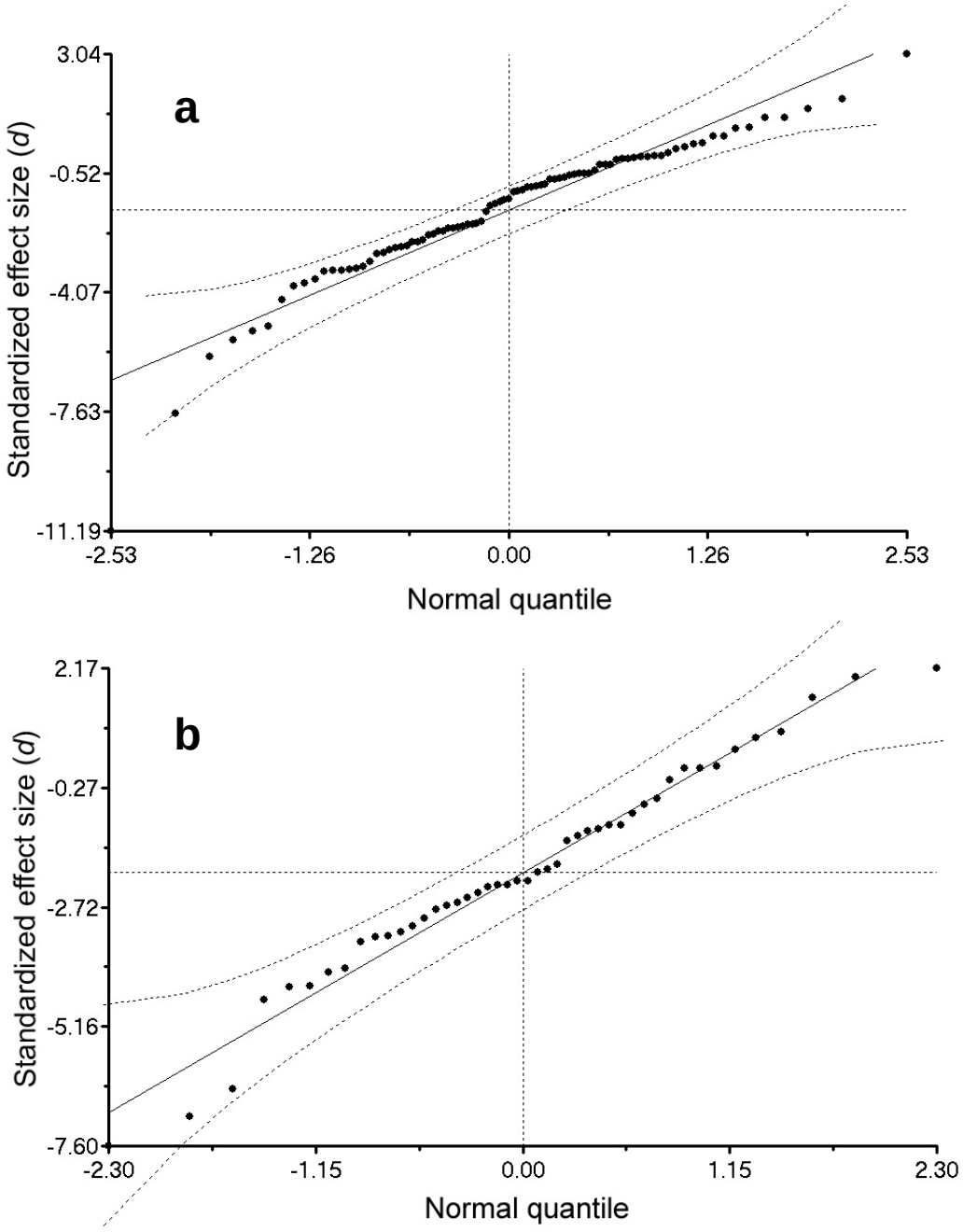

Figure 2.1.Normal quantile plots of effect size for survival (a) and growth (b) analyses. Dashed lines indicate 95% confidence intervals. Each point represents the effect size calculated for one comparison in the analysis. The distribution of effect sizes is linear and within the bounds of the 95% confidence interval in both plots, suggesting the data are normally distributed.

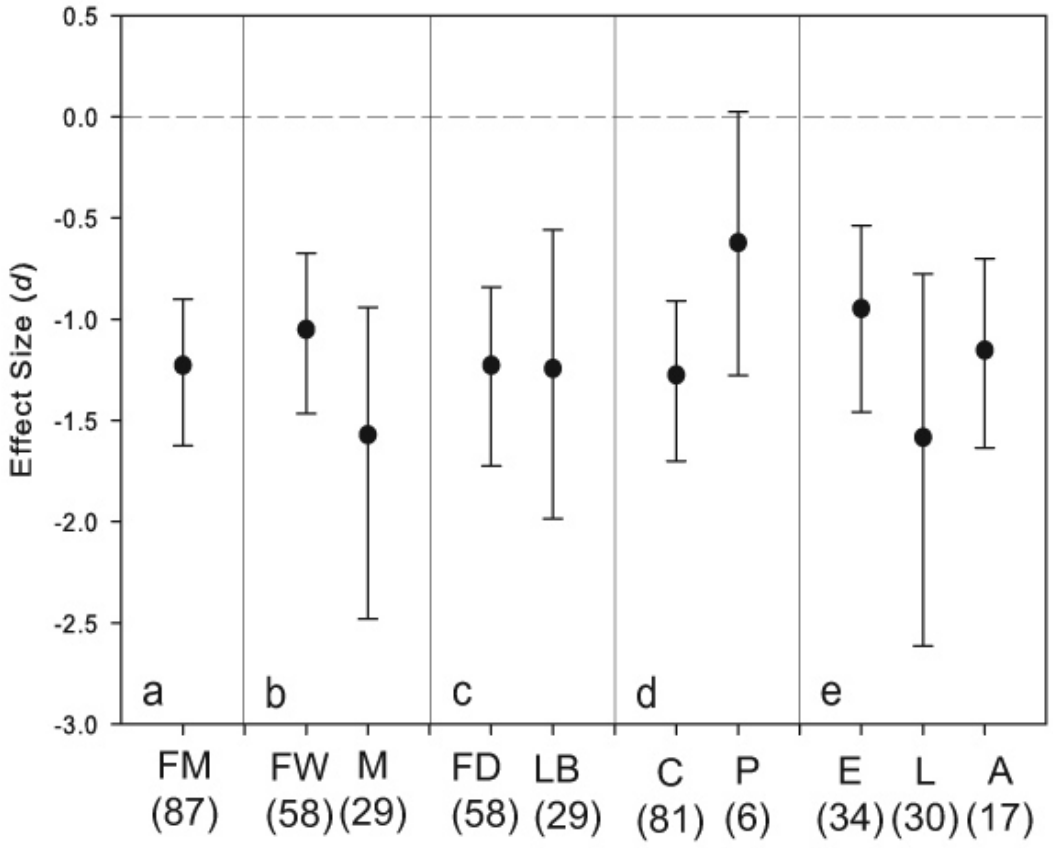

Figure 2.2.The effect of UVB radiation on survival. The mean and 95% confidence interval is shown for each analysis. The number of comparisons used to calculate each mean is shown in parentheses. Confidence intervals that overlap the dashed line at zero are not significantly different from zero. FM = full model, FW = freshwater, M = marine, FD = field, LB = laboratory, C = consumer, P = primary producer, E = embryo, L = larva, A = adult.

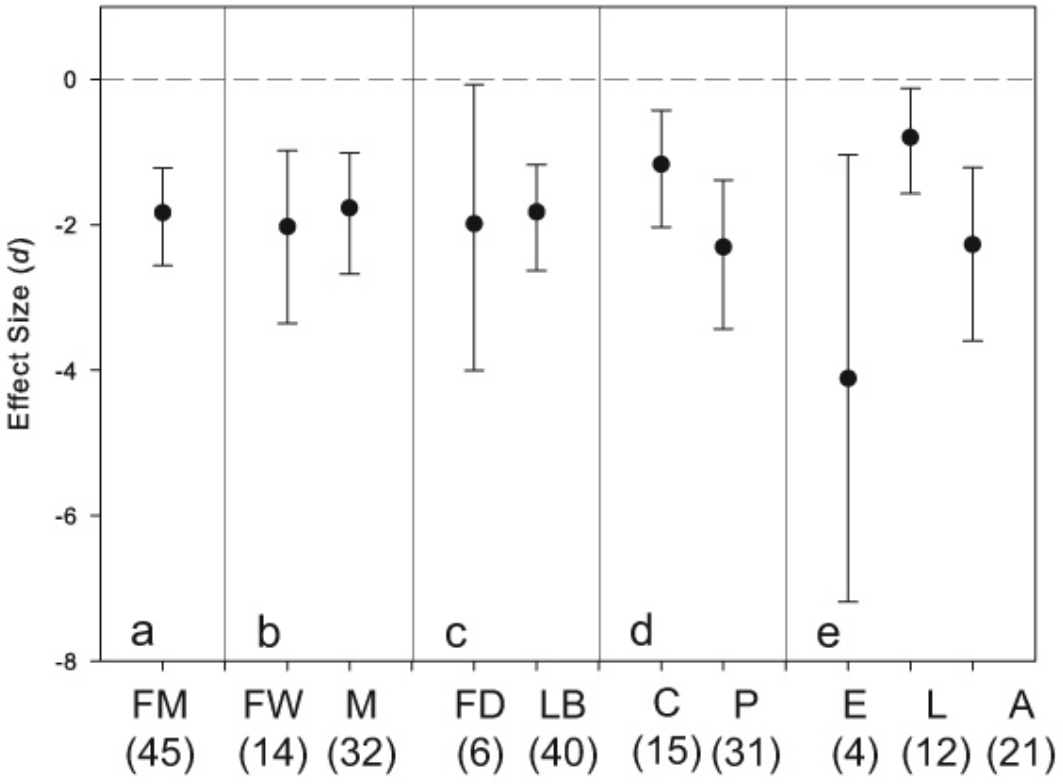

Figure 2.3.Effect of UVB radiation on growth. The mean and 95% confidence interval is shown for each analysis. The number of comparisons used to calculate each mean is shown in parentheses. All means are significantly different from zero (95% confidence intervals do not overlap with the dashed line at zero). FM = full model, FW = freshwater, M = marine, FD = field, LB = laboratory, C = consumer, P = primary producer, E = embryo, L = larva, A = adult.

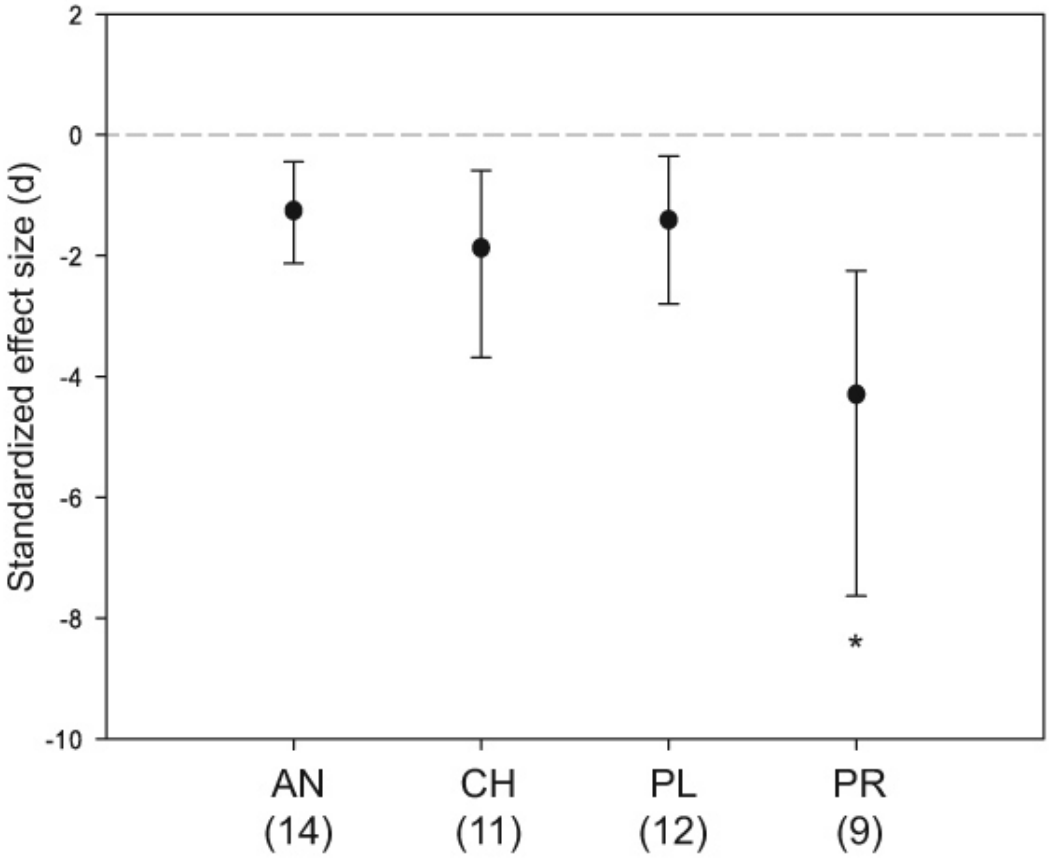

Figure 2.4. Effect of UVB radiation on growth in each kingdom. The mean and 95% confidence interval are shown for each kingdom. The means are all significantly different from zero as none of the 95% confidence intervals overlap zero. UVB radiation has a significantly larger negative effect on members of the kingdom Protozoa (asterisk). AN = Animalia, CH = Chromista, PL = Plantae, PR = Protozoa.

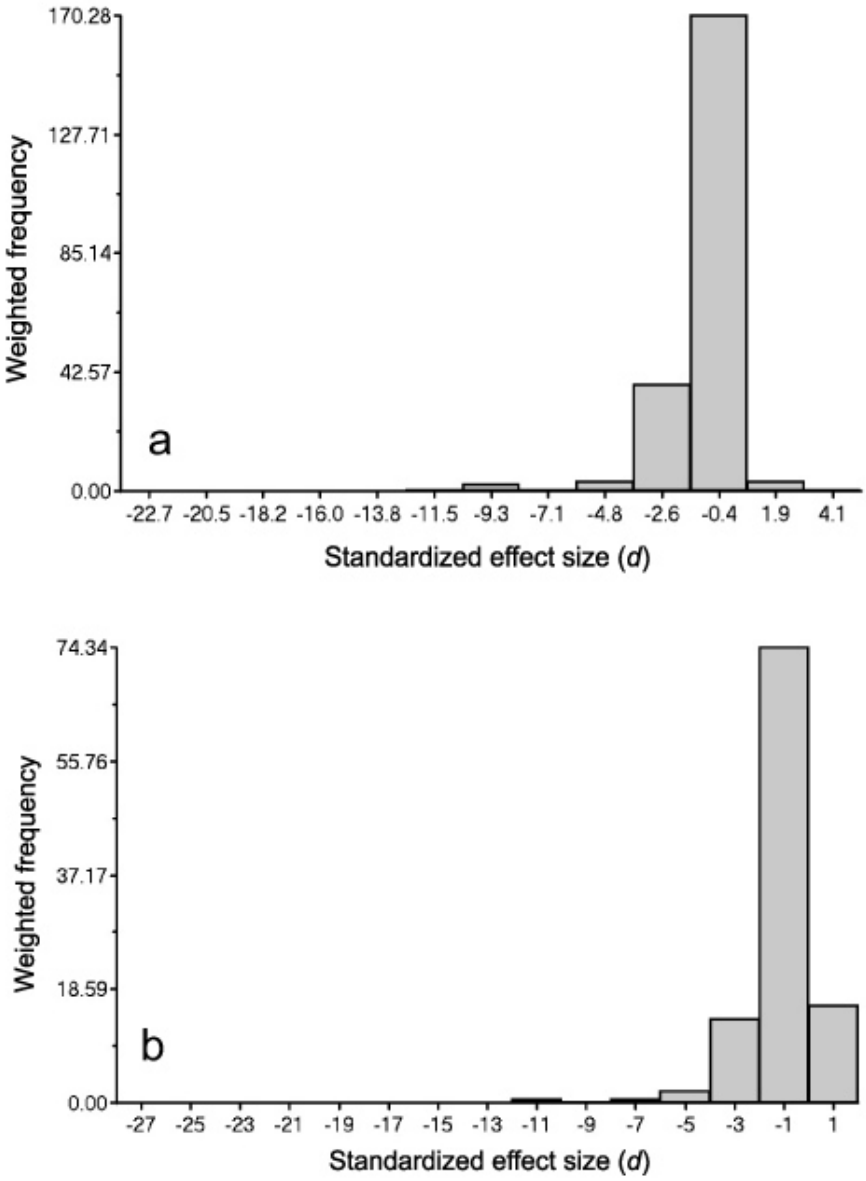

Figure 2.5. Weighted histogram of effect sizes for all comparisons in the survival analysis (a) and the growth analysis (b). The height of each bar indicates the combined weight of effect sizes in each class. The distribution is left-skewed due to several extreme negative values with low weight.

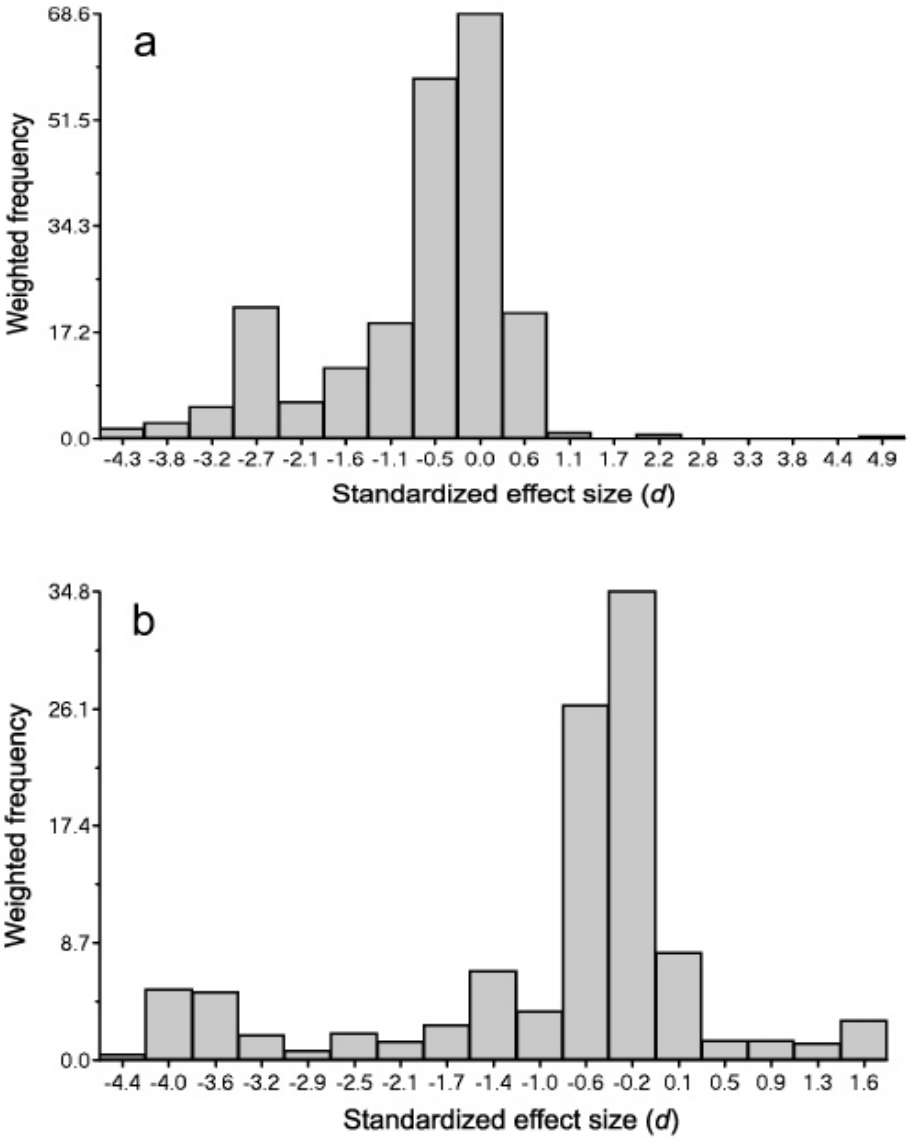

Figure 2.6. Weighted histogram of effect sizes after removal of effect sizes greater than 1 standard deviation from zero. The height of each bar indicates the combined weight of effect sizes in each class. The distribution of weighted effect sizes for the survival analysis (a) and the growth analysis (b) are normal and show no evidence of publication bias.

Chapter 3

A meta-analysis of the effects of ultraviolet B radiation and other stressors on survival in amphibians

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Abstract

Human alterations to natural systems have resulted in a loss of biological diversity in many species and locations around the world, including amphibian populations. Hypotheses for the decline of amphibian populations include habitat loss, introduction of exotic species, pathogens, pollution of habitats, and global environmental changes such as increases in ultraviolet-B (UVB) radiation reaching the earth's surface. UVB negatively affects many amphibian species and can cause mortality and sublethal effects, such as reduced growth and increased susceptibility to disease. In addition, UVB radiation can act synergistically with natural and anthropogenic stressors in amphibian habitats, resulting in greater than additive effects. However, the effect of UVB varies widely among species, and can also vary within populations of the same species or at different life history stages. This variation, in combination with a lack of significant effects of UVB in much of the published literature, has resulted in controversy regarding the importance of UVB as a stressor for amphibians. We used meta-analysis techniques to explore the overall effects of UVB radiation on survival in amphibians. We also used recently developed factorial meta-analytic techniques to quantify potential interactions between UVB radiation and other stressors on amphibians. UVB radiation reduced survival of amphibians by 1.9-fold compared to shielded controls. Days of exposure, life-history stage, and taxonomic order accounted for the majority of variation in effect size in multiple linear regression. Larvae are more susceptible to damage from UVB radiation compared to embryos, and caudates are more susceptible compared to anurans. Furthermore, UVB radiation

interacts synergistically with other environmental stressors and results in greater than additive effects on survival when two stressors are present. Our results suggest that UVB radiation is an important stressor in amphibians, particularly in light of potential synergisms between UVB and other stressors in amphibian habitats.

Introduction

Anthropogenic changes to the environment have altered the abiotic and biotic habitat of many organisms. These changes include the addition of contaminants (Fleeger *et al.*, 2003), introduction of exotic organisms (Sakai *et al*., 2001), alteration of flood (Gergel *et al.*, 2005) and fire regimes (Allen *et al*., 2002), and increases in mean temperatures (IPCC, 2007). In addition, reduction in the stratospheric ozone layer has resulted in increases in ultraviolet-B (UVB) radiation reaching the earth's surface (Kerr & McElroy, 1993; Madronich, 1993; Madronich *et al*., 1998; Solomon, 1999). UVB negatively affects a wide variety of freshwater and marine organisms (Bancroft *et al.*, 2007). The effects of UVB range from increased mortality to sublethal effects on growth, development, photosynthesis and immunity (Tevini, 1993; Caldwell *et al.*, 1998). These effects may scale up to the population or community level, causing changes in community structure and function (Bothwell *et al*., 1994; Mostajir *et al.,* 1999; Marinone *et al.,* 2006; but see Wahl *et al.*, 2004).

Both abiotic and biotic alterations of habitat have negatively affected many organisms, including amphibians. Amphibians are of particular conservation concern, as amphibian populations are declining more rapidly than either birds or mammals

(Stuart *et al*., 2004) with perhaps as many as 122 species becoming extinct since 1980 (Mendelson *et al*., 2006). Many factors appear to be contributing to amphibian population declines. These include habitat loss, introduced species, pathogens, contaminants, climate change, and increases in UVB radiation (Blaustein & Kiesecker, 2002; Collins & Storfer, 2003). The diversity of locations where amphibian populations have declined prompted consideration of atmospheric factors—e.g., increased terrestrial UVB irradiance associated with stratospheric ozone depletion (Blaustein *et al*., 1994).

The effects of UVB radiation on amphibians include increased mortality, reduced growth, developmental abnormalities, increased susceptibility to disease and behavioural changes (reviewed in Blaustein *et al*., 1998; Blaustein & Kiesecker, 2002). In addition, the magnitude of the effect may vary between species or between different populations or life history stages of the same species (e.g., Blaustein *et al*., 1998). For example, survival of moor frog (*Rana arvalis*) embryos was higher when UVB radiation was filtered out compared with embryos exposed to UVB radiation (Häkkinen *et al*., 2001). However, survival of larval moor frogs was not affected by UVB exposure (Häkkinen *et al*., 2001). In contrast, survival of embryonic common toads (*Bufo bufo*) was not affected when exposed to UVB, but survival was lower in larvae exposed to UVB compared with those shielded from UVB (Häkkinen *et al*., 2001). Numerous factors may influence the results and interpretation of experimental studies reporting the effects of UVB radiation on living organisms (Bancroft *et al*., 2007). These factors may be aspects of the environment (e.g., altitude, climate,

latitude) or even the methods used by the investigators. For example, venue (laboratory or field) and the duration of an experiment often vary between studies, which may make it difficult to compare results between studies. Venue may be particularly important, as the quality and quantity of UVA (315-400 nm) and photosynthetically active radiation (PAR) in laboratory experiments rarely approximates conditions in the field (e.g., Ankley *et al*., 2000). These wavelengths are necessary for effective DNA repair after exposure to UVB radiation (Sancar & Sancar, 1988). Lack of efficient repair or exposure to high doses of UVB radiation that overwhelm repair mechanisms may be responsible for the observed negative effects of UVB in amphibians.

Many studies suggest that UVB may interact synergistically with other stressors such as contaminants, climatic factors or pathogens (Blaustein *et al.*, 2001, 2003). For example, western toad (*Bufo boreas*) embryos are susceptible to a complex interaction between UVB radiation, a pathogenic water mould (*Saprolegnia sp.*), and changes in precipitation (Kiesecker *et al.*, 2001). Thus, mortality in western toad embryos increases when they are infected with *Saprolegnia* in the presence of higher UVB radiation which occurs during years of lower precipitation when water levels are low and the UVB shielding property of the water is diminished. Climate cycles such as El Niño and La Niña, with associated changes in precipitation, affect this dynamic (Kiesecker *et al*., 2001).

Several studies have reported no effects of UVB alone or in conjunction with other stressors on amphibians (e.g., Blaustein *et al*., 1996; Starnes *et al*., 2000; Merilä

et al., 2000; Pahkala *et al.*, 2001). The lack of a UVB effect on amphibians reported by many studies has led to controversy regarding the importance of UVB as an environmental stressor in habitats where amphibians live. This controversy stems from using formal or informal 'vote count' summaries of the literature, where the numbers of studies reporting significant effects are summed and a conclusion about the overall importance of a factor is estimated (e.g., Licht, 2003). However, this method suffers from low statistical power and is biased towards finding no effect (Rosenberg *et al*., 2000). In contrast, meta-analytic techniques are designed to avoid these issues. Meta-analysis techniques are less biased and have been used to explore the effects of UVB radiation and other global environmental factors (e.g., Root *et al*., 2003; Parmesan & Yohe, 2003; Bancroft *et al*., 2007). Furthermore, recent advances in meta-analytic techniques now allow for assessing the potential interaction among factors in fully factorial experiments (Gurevitch *et al*., 2000; Hawkes & Sullivan, 2001).

We used meta-analytic techniques to quantify the overall effect of UVB radiation on survival of amphibians. We then used multiple linear regression to explore the sources of variation in estimates of effect size and to identify important predictors of effect size in published studies. Finally, we used factorial meta-analysis to quantify the interaction between UVB and additional stressors. Our results suggest that UVB is indeed an important stressor for amphibians, as it is for many other aquatic organisms (Bancroft *et al*., 2007).

Materials and Methods

Effects of UVB on survival

We searched six electronic databases (BIOSIS, Web of Science, Aquatic Sciences and Fisheries Abstracts, Fish and Fisheries Worldwide, Wildlife and Ecology Studies Worldwide, and Biological and Agricultural Index) to identify papers used in these analyses. We searched using all possible combinations of the terms *ultraviolet*, *UV*, *UVB* with *survival* and *amphibian*, *frog*, *toad*, *salamander* or *newt* and identified 41 peer-reviewed articles that provided a measure of amphibian survival after exposure to ambient levels of UVB. We limited our search to include only experimental manipulations of UVB, using the standard technique of applying plastic filters that differentially transmitted or filtered UVB radiation. We included all study locations and species within each article. When multiple sampling dates were reported, we selected the final sampling day. If multiple doses of UVB radiation were used, we randomly selected only one dose from each study. One half of these articles were used in a previous analysis (Bancroft *et al*., 2007). The 41 articles generated 89 comparisons and included two of the three amphibian orders (anura and caudata), eight genera, and 32 individual species (Table 3.1). We extracted data from figures using TechDig 2.0 (Jones, 1998).

We calculated the log-response ratio (lnR) as a measure of effect size for each study. The log-response ratio is calculated as the natural log of the ratio of survival with and without UVB radiation within each comparison (Hedges *et al*., 1999). We selected the lnR because of the clear biological interpretation (proportional survival of

experimental organisms compared to controls) and good statistical properties (Osenberg *et al*., 1997; Shurin *et al*., 2002). We calculated the grand mean effect size for the full model and generated bias-corrected bootstrapped 95% confidence intervals (Adams *et al.*, 1997). We calculated the grand mean effect size using unweighted effect sizes because approximately half of the comparisons did not include a measure of variance. Effect size calculations and summary analyses were conducted in MetaWin 2.0 (Rosenberg *et al*., 2000). Using unweighted effect size estimates decreases the probability of detecting differences among groups; however, restricting our analyses to comparisons with estimates of precision would drastically reduce our sample size and could introduce bias into our analyses (Englund *et al*., 1999). We explored the variables contributing to residual variance in our model using multiple linear regression (Borer *et al*., 2005). The variables examined included biological, geographical and methodological factors reported in each study (Table 3.2). However, some studies did not include enough information to estimate study duration, days of UVB exposure, or altitude of study location. Therefore we conducted separate analyses using all variables, effectively excluding the comparisons without all variables reported, and then on the subset of variables reported by every study. We used backwards selection to identify the best fit model. Multiple linear regressions were conducted in JMP V.6 (SAS Institute Incorporated, Cary, NC, USA). We generated mean effect sizes and bias-corrected bootstrapped 95% confidence intervals (CIs) for groups with a significant term in our regression model. When fewer than 10 comparisons were available within a group, we used parametric 95% CIs as a more

conservative estimate of the CI. Mean effect sizes were considered significantly different from zero if the 95% CI did not overlap with zero.

Effects of UVB and additional stressors on survival

To explore the possible interaction between UVB and additional environmental stressors, we used factorial meta-analysis (Gurevitch *et al*., 2000). This type of meta-analysis allowed the examination of main effects (i.e., UVB and the other stressor alone), plus the interaction between the two stressors. However, only studies that were originally factorial in design were used (Gurevitch *et al*., 2000; Hawkes & Sullivan, 2001). We conducted a separate search in the same six databases (see above) for studies testing the effects of UVB and an additional stressor on survival alone and in combination (Table 3.3).

We used the log-response ratio as our effect size metric. We calculated the main effects of each stressor and the interaction between the stressors, in addition to the individual effect sizes for each treatment (Figure 3.1). A negative value of the main effects indicates that the stressor had a negative effect on survival. A negative value of the interaction term indicates a synergistic (more than additive) effect of the two stressors together. Effect size estimates were considered significantly different from each other if the 95% CIs did not overlap. Furthermore, effect size estimates were considered significantly different from zero if the 95% CIs did not overlap with zero. Two comparisons were removed from the analysis due to mathematical incompatibility (zero survival in all treatments except controls).

Results

Effects of UVB on survival

Exposure to UVB resulted in a 1.85-fold decrease in survival compared to controls (Figure 3.2a). When all explanatory variables were included in the regression analysis, days of UVB exposure, life history stage and taxonomic order accounted for 41.4% of the variation in survival (Table 3.4). When all comparisons were included in the analysis, and therefore fewer explanatory variables, life history stage and taxonomic order explained 18.2 % of the variation in survival after exposure to UVB (Table 3.4). Surprisingly, the correlation coefficient for days of UVB exposure was positive (0.012), suggesting that amphibian survival was higher with longer exposure times. Larvae were more sensitive to UVB than embryos (Figure 3.2b), and caudates were more susceptible to UVB than anurans (Figure 3.2c).

Nearly one third (28%) of the comparisons in these analyses were the work of A.R. Blaustein and colleagues. To explore the possibility of bias, we removed all comparisons generated by this laboratory and re-ran the analyses. The final regression models were qualitatively the same without studies conducted by the Blaustein laboratory group (Table 3.5). Without the Blaustein laboratory comparisons in the analysis, UVB reduced survival by 1.9-fold. The only change is a shift of the mean effect size for metamorphic individuals from negative (-0.22, 95% CI: -1.8154 to 1.3670) to positive (0.08, 95% CI: -2.4085 to 2.5598).

Effect of UVB and additional stressors on survival

In the factorial analysis, the main effects (UVB and additional stressors) were different from zero, but not different from each other (Figure 3.3a). There was evidence of a synergistic (more than additive) effect of UVB and additional stressors, as the interaction effect size was negative and different from zero (Figure 3.3a). UVB alone had the smallest effect size estimate but was different from zero (Figure 3.3b). The combination of an additional stressor and UVB radiation had the largest effect size estimate and was also different from zero (Figure 3.3b).

Discussion

UVB radiation alone and in combination with other factors is an important stressor in natural systems. The interaction between stressors can lead to "ecological surprises" even when one stressor is not considered to be a major stressor in the system (Christensen *et al*., 2004).

Effect of UVB alone

Exposure to UVB radiation reduced survival of amphibians by approximately 1.9-fold. This result was surprising because only 32 of the original 89 comparisons (35%) reported a significant reduction in survivorship under UVB radiation. This analysis clearly illustrates the power of meta-analysis to reveal patterns in published literature that are otherwise obscured by low statistical power. The majority of the variation in effect size was explained by life history stage, taxonomic order and the number of days of exposure.

The average effect size was much smaller for embryos than larvae, suggesting that UVB radiation has a larger effect on survival of larvae. Only four studies on the effects of UVB on metamorphic amphibians were included. Clearly, more research is necessary on the effects of UVB at this important life history stage. Embryos may have been less susceptible to UVB radiation because they were exposed to UVB for fewer days on average (embryos, 12.5 days; larvae, 34.5 days), most likely due to the shorter period spent in this life history stage. In addition, embryos may be better protected from UVB by the jelly envelope, pigmentation, or cellular repair mechanisms such as photolyase (Epel *et al*., 1999; Blaustein & Belden, 2003).

Larvae may be less defended from UVB radiation because they have the potential to behaviourally avoid areas with high UVB levels in natural systems. The experimental design of the comparisons in this analysis did not allow larvae to seek refuge from UVB. Larvae of some species appear to avoid regions with high UVB exposure (Nagl & Hofer, 1997; van de Mortel & Buttemer, 1998; Garcia *et al*., 2004). In contrast, larvae of other species do not avoid UVB and are frequently observed in very shallow water (O'Hara, 1981; Wollmuth *et al.*, 1987; van de Mortel & Buttemer, 1998; Belden *et al*., 2000; Belden *et al.*, 2003). Larger effects of UVB on survival of larvae may have important implications for population persistence. A recent demographic model suggests that reduction in post-embryonic survival results in decreases in equilibrium density in the adult portion of the population (Vonesh & De la Cruz, 2002). Therefore, reduction in larval survival due to UVB may result in

reductions in the adult population over time, particularly in habitats with high transmission of UVB and in species that do not avoid UVB radiation.

The average effect size estimate was much larger for caudates compared to anurans, suggesting that UVB has a much larger effect on survival in salamanders and newts compared to frogs and toads. This result is in accordance with previous work on relative photolyase activity in embryos. Photolyases are a group of enzymes responsible for the majority of DNA repair after exposure to UVB radiation (Sancar & Sancar, 1988). In general, salamander and newt species have much lower levels of photolyase compared to frogs and toads (Blaustein *et al*., 1994; Smith *et al*., 2002).

These differences in photolyase levels and the observed difference in effect size between caudates and anurans may reflect the differences in expected exposure to UVB in natural systems, particularly at the egg stage (Blaustein *et al.*, 1994). For example, oviposition occurred at deeper depths on average for two salamander species compared to five frog species (Smith *et al*., 2002). However, this relationship is not always observed and some caudates lay their eggs in shallow water. Oviposition depth varies between species and even between individuals of the same population (Palen *et al.*, 2005). Mobile larvae, however, may be exposed to varying levels of UVB due to habitat use. If larvae do not avoid UVB radiation, other factors influencing habitat use may lead to increased UVB exposure. Temperature is one important predictor of habitat use in amphibians, including caudates (Lucas & Reynolds, 1967; Keen & Schroeder, 1975; Dupré & Petranka, 1985; Hutchison & Dupré, 1992). Temperature preferences vary by species and life-history stage, but

many amphibians select warmer temperatures where growth and development are maximized (Hutchison & Dupré, 1992). Seeking warm, shallow water for thermoregulation also may expose larvae to high levels of UVB during development. Thus, caudate larvae may be exposed to similar UVB levels as anuran larvae, and these UVB levels may result in larger effects on survival in caudates.

We expected the relationship between days of exposure and effect size to be negative, as the effects of UVB on an organism are closely related to total dose of UVB (e.g., Ankley *et al*. 2002). However, we observed the opposite trend in this analysis. It is possible that organisms surviving the first day of UVB exposure could develop a "UV-hardening" response, similar to the temperature hardening response seen in many organisms (Hutchison & Maness, 1979; Nobel, 1982; Lee *et al*., 1987). UV hardening in amphibians may involve the use of melanin as a sunscreen pigment (Jablonski, 1998; Blaustein & Belden, 2003) or rely on up-regulation of cellular mechanisms such as photolyases, heat shock proteins or antioxidant enzymes (e.g., superoxide dismutase) (Feder, 1999; Lesser *et al*., 2001; Blaustein & Belden, 2003). Alternatively, *a priori* estimates of UV tolerance could result in longer exposure times for species or stages with higher tolerance for UVB. Interestingly, a negative correlation between days of UVB exposure and effect size was observed for embryos (r = -0.38, p = 0.013), while a positive correlation was observed for larvae (r = 0.57, p = 0.006). Thus, the relationship between effect size and days of UVB exposure is in the expected direction for embryos but not larvae. This difference in the relationship between exposure days and effect size suggests differences may exist in physiological

tolerance for UVB exposure between life history stages. Embryos may be more likely to accrue damage from longer term exposure, while larvae may be more likely to exhibit UV hardening or acclimation. More research on the physiological mechanisms of UV tolerance at the larval life history stage is necessary.

Effect of UVB and additional stressors on survival

Our results suggest that the combination of UVB radiation and an additional stressor leads to a synergistic interaction and greater than additive mortality in amphibians. These additional stressors were diverse and ranged from pathogens to agricultural contaminants and low pH. The effect of these stressors was large and significantly different from zero when amphibians were exposed to the stressor alone. However, amphibians in natural systems are frequently exposed to more than one stressor and these stressors commonly interact synergistically (Sih *et al*., 2004). The finding that UVB radiation interacts with other stressors is particularly important, as many amphibian habitats in temperate regions are exposed to some degree of UVB (Diamond *et al*., 2005). Levels of UVB radiation vary widely both between years and habitats and at smaller spatial and temporal scales. Thus, the effects of these stressors in natural populations could vary on similar spatial and temporal scales.

The potential for interaction with other stressors suggests that UVB is an important factor even in species that are relatively resistant to UVB radiation. For example, Pacific treefrogs (*Pseudacris regilla*) are generally considered resistant to damage from UVB radiation. However, when Pacific treefrog embryos were exposed to UVB and nitrate, survival was drastically reduced (Hatch & Blaustein, 2003).

Experiments on the same species found no interaction between UVB and a pathogen, *Saprolegnia ferax* (Kiesecker & Blaustein, 1995). Thus, the strength of the interaction may vary within a species depending on the stressors involved. Common frogs (*Rana temporaria*) are also considered relatively resistant to damage from UVB, but when both UVB and low pH were present, survival was reduced (Pahkala *et al*., 2002). The results of this analysis support the observation that amphibian population declines are likely due to multiple causes that may interact (Blaustein & Kiesecker, 2002).

The main effect of UVB was smaller in the factorial analysis compared to the analysis of the effects of UVB alone. Fewer comparisons were included in the factorial analysis, as only fully factorial studies were included. However, none of the included studies in the factorial analysis were on caudates. In the analysis of UVB alone, UVB had a much larger negative effect on caudates compared to anurans. Furthermore, only four of the nineteen comparisons in the factorial analysis were on larvae. The rest of the comparisons were on embryos, the least susceptible life-history stage. Thus, the factorial analysis may underestimate the strength of the interaction between UVB and additional stressors. More research on caudates and later life-history stages is necessary to understand the potential for non-additive effects in amphibians exposed to UVB radiation.

Conclusions

These analyses suggest that UVB radiation is an important stressor for amphibians. A negative effect of UVB radiation on survival was detected despite the apparent lack of significant UVB effects in much of the literature. Moreover, UVB

radiation interacts synergistically with many common stressors in natural systems. Future work should focus on under-represented life-history stages and should explore the potential for interactions among stressors in natural systems. These types of data are necessary for including stressors in demographic models of amphibian populations. Understanding the effects of various stressors on multiple species of amphibians at multiple life-history stages is vital to clarifying causes of amphibian population declines.

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Table 3.1. Amphibian species, experimental variables and biological variables used in multiple linear regression analysis of the effect of UVB alone on survival. Study duration was defined as "short" for experiments ≤ 7 days, or "long" for experiments > 8 days in duration.

Species	Latitude	Study duration	Days UVB	Elevation (m)	Venue	Life history stage	Taxonomic order	Reference
Ambystoma macrodactylum	44	Long	28	75	Laboratory	Larva	Caudata	Belden *et al.* 2000
Ambystoma macrodactylum	44	Long	28	1939	Laboratory	Larva	Caudata	Belden *et al.* 2000
Ambystoma gracile	44	Long	27	183	Field	Embryo	Caudata	Blaustein *et al.* 1995
Ambystoma macrodactylum	44	Long	13	2000	Field	Embryo	Caudata	Blaustein *et al.* 1997
Ambystoma maculatum	45	Long	17		Laboratory	Embryo	Caudata	Lesser *et al.* 2001
Ambystoma maculatum	38	Short	1	441	Laboratory	Embryo	Caudata	Calfee *et al.* 2006
Ambystoma maculatum	38	Long	14	441	Laboratory	Larva	Caudata	Calfee *et al.* 2006

Ambystoma maculatum	35	Long	15	660	Field	Embryo	Caudata	Starnes *et al.* 2000
Ambystoma talpoideum	38	Short	7	14	Laboratory	Larvae	Caudata	Calfee *et al.* 2006
Bufo americanus	43.4	Long	63		Laboratory	Larvae	Anura	Grant & Licht 1995
Bufo americanus	43.4	Long	14		Laboratory	Metamorph	Anura	Grant & Licht 1995
Bufo boreas	44			2000	Field	Embryo	Anura	Kiesecker & Blaustein 1995
Bufo boreas	44			1220	Field	Embryo	Anura	Kiesecker & Blaustein 1995
Bufo boreas	44			2000	Field	Embryo	Anura	Blaustein *et al.* 1994
Bufo boreas	44			1200	Field	Embryo	Anura	Blaustein *et al.* 1994
Bufo boreas	40.3	Long	9	3266	Field	Embryo	Anura	Corn 1998
Bufo boreas	40.3	Long	13	2810	Field	Embryo	Anura	Corn 1998

Bufo boreas	44	Short	7	1220	Laboratory	Metamorph	Anura	Blaustein *et al.* 2005
Bufo boreas	39	Long	70	3222	Laboratory	Larva	Anura	Little *et al.* 2003
Bufo boreas	44	Long	14	1220	Field	Embryo	Anura	Kiesecker *et al.* 2001
Bufo boreas	44	Long			Laboratory	Larvae	Anura	Worrest & Kimeldorf 1976
Bufo boreas	44	Long			Laboratory	Larvae	Anura	Worrest & Kimeldorf 1975
Bufo bufo	62	Long	18	91	Field	Embryo	Anura	Häkkinen *et al.* 2001
Bufo bufo	62	Long	18	91	Field	Larva	Anura	Häkkinen *et al.* 2001
Bufo bufo	39			1920	Field	Embryo	Anura	Lizana & Pedraza 1998
Bufo calamita	39			1920	Field	Embryo	Anura	Lizana & Pedraza 1998
Bufo woodhousii	39	Long	70	1500	Laboratory	Larva	Anura	Little *et al.* 2003

Hyla cadaverina	34	Long	10	290	Field	Embryo	Anura	Anzalone *et al.* 1998
Hyla chrysoscelis	35	Short	1	660	Field	Embryo	Anura	Starnes *et al.* 2000
Hyla chrysoscelis	35	Short	5	300	Field	Embryo	Anura	Bruner *et al.* 2002
Hyla (Pseudacris) regilla	44			2000	Field	Embryo	Anura	Kiesecker & Blaustein 1995
Hyla (Pseudacris) regilla	44			1190	Field	Embryo	Anura	Kiesecker & Blaustein 1995
Hyla (Pseudacris) regilla	34	Short	7	290	Field	Embryo	Anura	Anzalone *et al.* 1998
Hyla (Pseudacris) regilla	44			2000	Field	Embryo	Anura	Blaustein *et al.* 1994
Hyla (Pseudacris) regilla	44			1655	Field	Embryo	Anura	Blaustein *et al.* 1994
Hyla (Pseudacris) regilla	44	Long	21	1017	Field	Embryo	Anura	Hatch & Blaustein 2003

Hyla versicolor		Short	4	214	Laboratory	Embryo	Anura	Zaga *et al.* 1998
Hyla versicolor		Short	4	214	Laboratory	Larva	Anura	Zaga *et al.* 1998
Litoria aurea	34	Short	4	13.5	Field	Embryo	Anura	van de Mortel & Buttemer 1996
Litoria aurea	34	Short	4	575	Field	Embryo	Anura	van de Mortel & Buttemer 1996
Litoria dentata	34	Long	9	13.5	Field	Embryo	Anura	van de Mortel & Buttemer 1996
Litoria dentata	34	Long	9	575	Field	Embryo	Anura	van de Mortel & Buttemer 1996
Litoria peronii	34	Long	8	13.5	Field	Embryo	Anura	van de Mortel & Buttemer 1996
Litoria peronii	34	Long	8	575	Field	Embryo	Anura	van de Mortel & Buttemer 1996

Pseudacris crucifer	35	Short	7	100	Laboratory	Larva	Anura	Baud & Beck 2005
Pseudacris triseriata	35	Long	8	660	Field	Embryo	Anura	Starnes *et al*. 2000
Rana arvalis	62	Long	18	91	Field	Embryo	Anura	Häkkinen *et al.* 2001
Rana arvalis	62	Long	18	91	Field	Larva	Anura	Häkkinen *et al.* 2001
Rana arvalis	63	Long		50	Laboratory	Embryo	Anura	Pahkala *et al.* 2001
Rana arvalis	63	Long		50	Field	Embryo	Anura	Pahkala *et al.* 2001
Rana aurora	44	Long	21	76	Field	Embryo	Anura	Blaustein *et al.* 1996
Rana blairi	39	Long	8	270	Field	Embryo	Anura	Smith *et al.* 2000
Rana blairi	35	Short	5	300	Field	Embryo	Anura	Bruner *et al.* 2002
Rana blairi	35	Long	8	300	Field	Embryo	Anura	Bruner *et al.* 2002

Rana cascadae	44			1190	Field	Embryo	Anura	Kiesecker & Blaustein 1995
Rana cascadae	44			2000	Field	Embryo	Anura	Kiesecker & Blaustein 1995
Rana cascadae	44			1190	Field	Embryo	Anura	Blaustein *et al.* 1994
Rana cascadae	44			1190	Field	Embryo	Anura	Blaustein *et al.* 1994
Rana cascadae	44	Long	42	1017	Field	Embryo	Anura	Belden *et al.* 2003
Rana cascadae	44	Long	21	1017	Laboratory	Larvae	Anura	Hatch & Blaustein 2002
Rana clamitans	45	Long	36	184	Field	Larvae	Anura	Tietge *et al.* 2001
Rana clamitans	43	Long	14		Laboratory	Metamorph	Anura	Grant & Licht 1995
Rana luteiventris	48	Short	7	1679	Field	Embryo	Anura	Blaustein *et al.* 1999

Rana pipiens	49	Long	35	184	Field	Larva	Anura	Ankley *et al.* 2000
Rana pipiens	49	Long	57	184	Laboratory	Larva	Anura	Ankley *et al.* 2000
Rana pipiens	46	Long	125	184	Laboratory	Larva	Anura	Ankley *et al.* 2002
Rana pipiens	45	Long	49	184	Field	Larva	Anura	Tietge *et al.* 2001
Rana pipiens	37	Long	12	250	Field	Embryo	Anura	Long *et al.* 1995
Rana pipiens	45	Long	24	184	Laboratory	Larva	Anura	Ankley *et al.* 1998
Rana pretiosa	46	Long	11	596	Field	Embryo	Anura	Blaustein *et al.* 1999
Rana pretiosa	47	Long	14	37	Field	Embryo	Anura	Blaustein *et al.* 1999
Rana septentrionalis	45	Long	9	184	Field	Larva	Anura	Tietge *et al.* 2001
Rana sylvatica	43	Short	2		Laboratory	Embryo	Anura	Grant & Licht 1995

Rana sylvatica	43	Long	66		Laboratory	Larva	Anura	Grant & Licht 1995
Rana sylvatica	43	Long	14		Laboratory	Larva	Anura	Grant & Licht 1995
Rana temporaria	62	Long	18	91	Field	Embryo	Anura	Häkkinen *et al.* 2001
Rana temporaria	62	Long	18	91	Field	Larva	Anura	Häkkinen *et al.* 2001
Rana temporaria	55	Long		22	Laboratory	Embryo	Anura	Pahkala *et al.* 2001
Rana temporaria	69	Long	14	485	Field	Embryo	Anura	Merilä *et al.* 2000
Rana temporaria	59	Long	26	50	Field	Embryo	Anura	Pahkala *et al.* 2000
Rana temporaria	66	Long	30	401	Field	Embryo	Anura	Pahkala *et al.* 2000
Rana temporaria	63.5	Long	10	64	Laboratory	Embryo	Anura	Pahkala *et al.* 2002a
Rana temporaria	55	Long	11	19	Laboratory	Embryo	Anura	Pahkala *et al.* 2002a

Rana temporaria	62.4	Long	20	119	Laboratory	Larva	Anura	Koponen & Kukkonen 2002
Rana temporaria	60	Long			Field	Embryo	Anura	Pahkala *et al.* 2002b
Taricha torosa	34	Long	21	290	Field	Embryo	Caudata	Anzalone *et al.* 1998
Triturus alpestris	47	Short	2	1950	Laboratory	Larva	Caudata	Nagl & Hofer 1997
Xenopus laevis		Short	4	214	Laboratory	Embryo	Anura	Zaga *et al.* 1998
Xenopus laevis		Short	4	214	Laboratory	Larva	Anura	Zaga *et al.* 1998

Table 3.2. Biological and methodological explanatory variables used in regression analysis of the effects of UVB on survival in amphibians. The asterisk denotes variables available for all studies.

Variable	Possible values	Number of comparisons including the variable
Latitude	Continuous variable	88
Duration of study	Long (> 7 days)	75
	Short (≤ 7 days)	
Days of UVB exposure	Continuous variable	70
Elevation	Continuous variable	79
Venue*	Laboratory	89 (all)
	Field	
Life history stage*	Embryo	89 (all)
	Larva	
	Metamorphic	
Taxonomic order*	Anura	89 (all)
	Caudata	

Table 3.3. Additional stressors used in factorial meta-analysis

Additional stressor	Life history stage	Species	Reference
Saprolegnia (pathogenic water mould)	Embryo	*Bufo boreas*	Kiesecker & Blaustein 1995
		Pseudacris regilla	
		Rana cascadae	
pH	Embryo	*R. arvalis*	Pahkala *et al.* 2001
		R. pipiens	Long *et al.* 1995
		R. temporaria	Pahkala *et al.* 2002b
	Larva	*R. cascadae*	Hatch & Blaustein 2002
Nitrate	Embryo	*P. regilla*	Hatch & Blaustein 2003
	Larva	*R. cascadae*	Hatch & Blaustein 2002
Landfill leachate	Embryo	*R. blairi*	Bruner *et al*. 2002
Copper	Larva	*P. crucifer*	Baud & Beck 2005
Methoprene	Larva	*R. pipiens*	Ankley *et al*. 1998
Carbaryl	Embryo	*Xenopus laevis*	Zaga *et al*. 1998
		Hyla versicolor	
	Larva	*X. laevis*	
		H. versicolor	
Bisphenol A	Larvae	*R. temporaria*	Koponen & Kukkonen 2002

Table 3.4. Final regression model after backwards selection. Variables were iteratively removed until only significant terms remained in the model. All explanatory variables were included (excluding studies that did not report all information in Table 3.1) or all studies were included (using a subset of variables indicated by an asterisk in Table 3.1).

Factor	df	Type II SS	*F*	*P*
All explanatory variables included				
Overall model (n = 58, residual df = 54, model r^2 = 0.414)				
Days of UVB	1	3.07	4.10	0.048
Life history stage	1	12.89	17.22	0.0001
Taxonomic order	1	11.94	15.95	0.0002
All studies included				
Overall model (n = 89, residual df = 86, model r^2 = 0.182)				
Life history stage	1	12.82	9.26	0.0031
Taxonomic order	1	12.75	9.21	0.0032

Table 3.5 Final regression models after excluding Blaustein laboratory studies. Variables were iteratively removed until only significant terms remained in the model.

Factor	df	Type II SS	*F*	*P*
All explanatory variables included				
Overall model (*n* = 45, residual df = 41, model r^2 = 0.5760)				
Days of UVB	1	5.75	8.96	0.0047
Life history stage	1	21.52	33.55	<0.0001
Taxonomic order	1	7.28	11.40	0.0016
All studies included				
Overall model (*n* = 64, residual df = 61, model r^2 = 0.1862)				
Life history stage	1	14.84	8.23	.0056
Taxonomic order	1	10.69	5.93	0.018

		UVB treatments (ln) +	UVB treatments (ln) -
Other stressor treatment (ln)	+	**1** +UV, +stressor	**2** -UV, +stressor
	-	**3** +UV, -stressor	**4** -UV, -stressor

Calculations:

Mean effects
UVB exposure: (1 + 3) – (2 + 4)
Other stressor: (1 + 2) – (3 + 4)
Interaction: (1 – 3) – (2 – 4)

Individual effects
UVB alone: (3-4)
UVB in the presence of an additional stressor: (1-2)
Stressor alone: (2-4)
Stressor in the presence of UVB: (1-3)

Figure 3.1. Conceptual framework for factorial meta-analysis. The value for the boxes (1-4) was the natural log of survival reported from each comparison. The mean effects were calculated as in Hawkes and Sullivan, 2001.

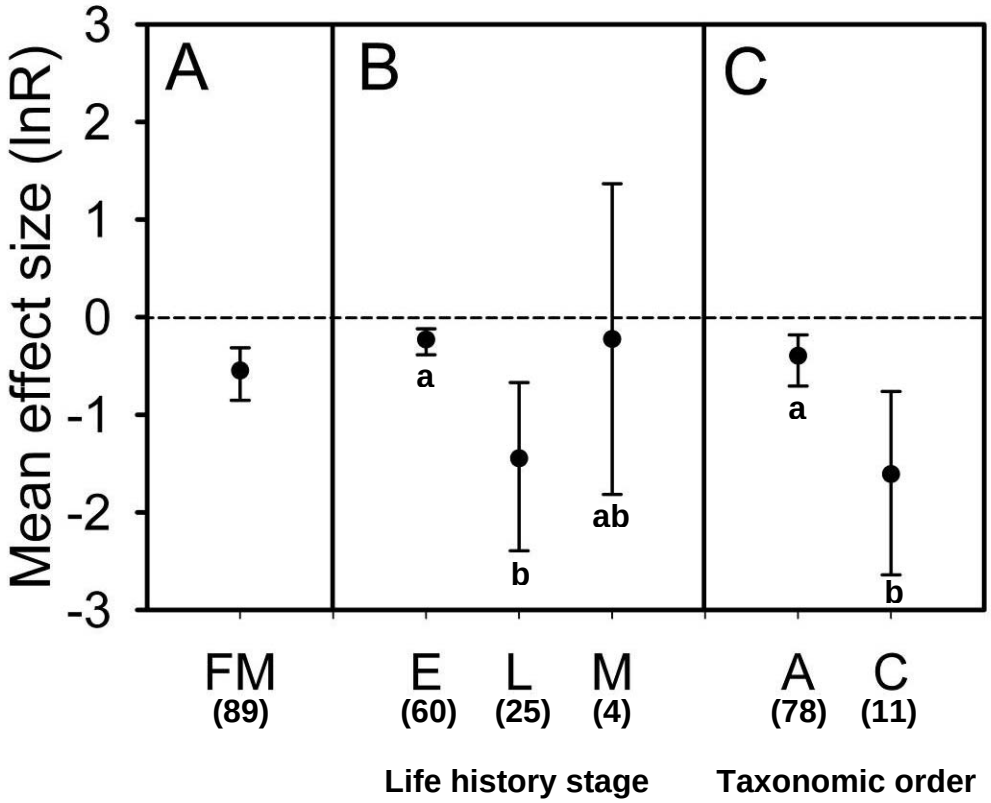

Figure 3.2. Mean effect sizes (log response ratio) for all studies included in the analysis of the effect of UVB on survival alone. All effect sizes are significantly different from zero except the metamorphic life history stage (M). Numbers in parentheses indicate sample size for each estimate. FM = full model, E = embryo, L = larva, M = metamorph, A = anura, C = caudata. Data are means and bias-corrected bootstrapped confidence intervals, except the confidence interval for M is a 95% parametric confidence interval. Means that share a common lowercase letter are not significantly different.

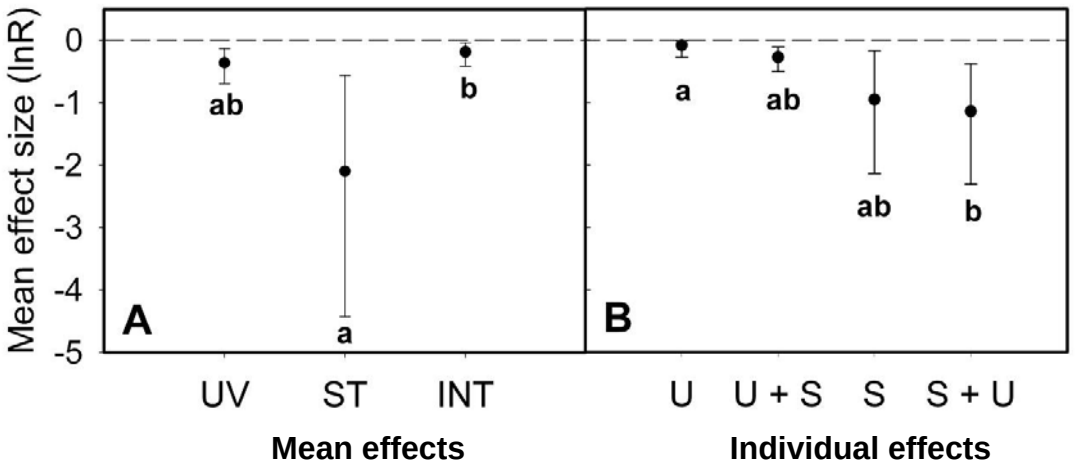

Figure 3.3. Mean effect size estimates for the factorial meta-analysis. Estimates are calculated as in Figure 3.1. Data are means and bias-corrected 95% confidence intervals. All effect estimates are significantly different from zero. U = UVB present, S = additional stressor present. Means that share a common lowercase letter are not significantly different.

Chapter 4

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

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Abstract

Habitat use by animals often reflects the balance between conflicting demands such as foraging and avoiding predation. Environmental stressors such as temperature can also affect habitat use in many organisms, particularly in ectothermic animals. Amphibian larvae in ephemeral ponds must grow and develop before their habitat dries or freezes. Warm, shallow thermal regimes in ponds can optimize growth and developmental rate but may also expose larvae to potentially harmful levels of ultraviolet-B (UVB) radiation. Exposure to UVB radiation may lead to mortality or result in sublethal effects, including physiological changes, anatomical deformities, and delayed growth. Thus, optimally, amphibians seeking sunlight for thermoregulation must balance this behavior with limiting their exposure to harmful UVB radiation. We conducted a series of laboratory and field experiments to test the hypothesis that larval amphibians avoid UVB by selecting microhabitats with lower exposure to UVB. We then quantified habitat use of the larvae of four amphibian species using field transects in three ponds with different UVB transmission. Larval *Pseudacris regilla* and *Rana cascadae* did not avoid UVB radiation in laboratory or field experiments and preferred warmer temperatures in laboratory thermal gradients, regardless of UVB exposure. In field surveys, the majority of anuran larvae were observed in water less than 10-15 cm deep, whereas salamander larvae were most often observed in deeper, cooler water. The similarity in habitat use across different

sites and the lack of evidence of UVB avoidance in choice tests suggests that larval anuran amphibians may be exposed to high levels of UVB radiation due to habitat use. The observed habitat use may be the result of other pressures, such as thermoregulation, foraging, or predator avoidance.

Introduction

An individual's use of habitat can occur on a large scale (macrohabitat) or a small scale (microhabitat). Generally, the selection and use of habitat is assumed to have fitness consequences, as selecting and using advantageous or optimal habitats will increase the fitness of organisms within a population (Fretwell & Lucas, 1970; Jaenike & Holt, 1991). These habitat use decisions are most likely based on some set of environmental criteria. Both abiotic and biotic factors are responsible for the distribution of a population throughout a habitat and the resulting fitness outcomes to the organisms. Abiotic parameters such as light intensity, temperature, soil moisture and nutrient availability can influence habitat use and selection in many species (e.g., Huk & Kühne, 1999). Biotic factors such as food resources (prey), competition, facilitation, and predation can also influence the distribution of animals (Fretwell & Lucas, 1970; Kats *et al*., 1988; Rosenzweig, 1991). These abiotic and biotic factors can act as selection pressures, resulting in trade-offs between different habitat types or patches (Sih, 1980; Lima, 1998). Variation in both the abiotic and biotic environment can interact, resulting in trade-offs between physiological requirements (e.g., thermal

optima) and biological pressures (e.g., competition, predation) which often affect both micro- and macro-habitat use.

Trade-offs between abiotic and biotic factors are especially important in ectothermic animals. Temperature greatly influences the behavior and ecology of ectotherms (Hutchison & Dupré, 1992), and many ectotherms can actively thermoregulate, using microhabitat variation to control body temperature (Huey & Slatkin, 1976, Hutchison & Dupré, 1992). However, thermoregulatory demands must be balanced with biological demands such as foraging, avoiding predation, and reproduction (Holomuzki, 1986; Downes & Shine, 1998; Martín, 2001; Martín & López, 2003). These trade-offs can result in complex spatial and temporal patterns of habitat use in ectothermic organisms.

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

amphibians in ephemeral ponds are therefore exposed to a host of conflicting abiotic and biotic selection pressures.

Amphibian populations are declining world-wide (Stuart *et al*., 2004), and a number of hypotheses have been advanced to explain the declines (Blaustein & Kiesecker, 2002; Collins & Storfer, 2003). One factor that may contribute to some of these population declines is increasing ultraviolet-B (UVB) radiation, which causes a variety of lethal and sublethal effects in many aquatic organisms (Bancroft *et al*., 2007), including amphibians (e.g., Blaustein *et al*., 1998; Pahkala *et al*., 2000; Häkkinen *et al*., 2001; Tietge *et al*., 2001; Blaustein *et al*., 2005). Sensitivity to UVB radiation may vary interspecifically, between life stages within a species, between populations and with environmental conditions (Blaustein & Belden, 2003). Exposure to ambient levels of UVB can increase mortality (Blaustein *et al*., 1998), reduce growth (Belden *et al*., 2000), alter behavior (Kats *et al*., 2000), and increase susceptibility to disease (Kiesecker & Blaustein, 1995; Kiesecker *et al*., 2001).

Amphibians may avoid high UVB levels by seeking deeper waters (Belden *et al*., 2000; Blaustein & Belden, 2003; Licht, 2003). In choice experiments, larvae and adults of some species prefer areas with lower UV irradiance (Nagl & Hofer, 1997; van de Mortel & Buttemer, 1998; Belden *et al*., 2000; Garcia *et al*., 2004; Han *et al*., in press). However, avoiding UVB by seeking cooler, deeper water may reduce growth and create a conflict between thermal requirements and avoiding exposure to harmful levels of UVB.

Because of the accumulating evidence that UVB is harmful to amphibians, we conducted a series of experiments to test the hypothesis that larval amphibians avoid UVB radiation by seeking microhabitats with low UVB exposure. Thus, we 1) measured the preferred temperature of larvae in thermal gradients with a simultaneous UVB choice test, 2) conducted UVB choice tests in isothermic field enclosures to explore avoidance behaviors in the absence of a trade-off between UVB and temperature and 3) quantified habitat use of amphibian larvae by conducting transect surveys in different amphibian habitats.

Materials and Methods

Study sites:

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

UVB avoidance in thermal gradients:

These experiments tested if larval amphibians alter thermoregulatory behavior to avoid UVB radiation. *Pseudacris regilla* (Pacific treefrog) larvae were collected from the Potholes on 17 July 2005 and *Bufo boreas* (western toad) larvae were collected from Todd Lake on 22 July 2005. Larvae were returned to the laboratory and kept in 38 L glass aquaria filled with treated dechlorinated tap water. Animals

were fed a 3:1 mix of alfalfa pellets and fish flakes (TetraMin, Melle, Germany) *ad libitum*. To prevent acclimation to a constant temperature in the laboratory, all animals were kept at 15ºC from 2000 - 0800hr and warmed to 22ºC during daylight hours. All trials were completed within 10 days of field collection.

Experimental lanes were created in 1.2 m aluminum rain gutters painted with Pratt & Lambert PalGard™ epoxy paint (Sherwin-Williams Company, Cleveland, Ohio, USA) and filled to a depth of 3.5 cm with dechlorinated tap water. Thermal gradients were created by filling a small metal pocket at one end of each lane with dry ice while the opposite end was placed on a hotplate. This method allowed the establishment of a ~10ºC difference between the two ends. Temperature was recorded throughout each trial with iButton temperature loggers (Maxim Integrated Products, Sunnyvale, California, USA) placed every 27 cm along each experimental lane for a total of 6 iButtons per lane. In addition, thermometers were placed at each end to directly monitor temperatures throughout the trials. Each lane was covered by two filters, one which blocks the passage of UVB radiation (Mylar) and one which permits the passage of UVB radiation (acetate). Each filter covered half the length of the lanes and two filters were placed such that the entire lane was covered by filters. During each trial, 4 lanes had the blocking filter placed over the warm end with the transmitting filter over the cool end, while the remaining 4 lanes had the transmitting filter over the warm end and the blocking filter over the cool end. Experimental lanes were placed under an array of UVB (Q-Panel UVB 313; Q-Panel, Cleveland, Ohio, USA) and full-spectrum bulbs (Vita-Light; Durotest Corporation, Fairfield, New

Jersey, USA), such that each lane was illuminated by both types of lights. UVB radiation was measured using a hand-held Solar Light meter with a UVB probe (meter model PMA2100, probe model 2102; Solar Light Company, Philadelphia, Pennsylvania, USA). In each experiment, UVB under acetate filters was approximately 10 $\mu W/cm^2$, while UVB under Mylar filters was virtually undetectable ($\leq 0.05\ \mu W/cm^2$). These levels are within the range of ambient UVB levels in the Oregon Cascades (Belden *et al*., 2000; Kiesecker *et al*., 2001).

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

Field UVB choice experiments:

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

Field transects

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

All transects were in place by 1000 hr. We allowed sediment to settle for 30 minutes prior to walking transects. For each observation, two observers slowly walked either side of each transect, counting the number of larvae of any observed amphibian species within his or her half of the square meter. Care was taken not to disturb animals or sediment. We measured UVB radiation at the surface of the water and at every depth from 10 cm to 50 cm (or the bottom of the pond) before each

observation period using a hand-held light meter with a UVB probe (see model listed under "*UVB avoidance in thermal gradients*"). Our goal was to capture larval movement due to diel thermal fluctuations. At Susan's Pond and Todd Lake, we walked each transect three times a day for two consecutive days. We walked the transects at Susan's Pond and Todd Lake in the morning (0900-1100 hr), afternoon (1400-1500 hr) and in the evening (1800-1900 hr). Transects at the Potholes were in place for one day. We walked the transects at the Potholes twice, once in the afternoon (1200 hr) and once in the evening (1700 hr).

The number of tadpoles observed in each transect varied widely across transects and at each sampling time. We standardized the data for each transect by using the total number of each species observed in each transect per sampling time to calculate the percent of each species observed in each square meter along each transect at each sampling time. We used Spearman's rank order correlation statistic to examine the relationship between the percentage of animals per square meter and the average depth of each square meter at each sampling time. Each day and time was analyzed separately.

Results

UVB avoidance in thermal gradients:

Thermal gradients ranged from 20-31ºC in the Pacific treefrog trials and from 21-31ºC in the western toad trials. No difference in average temperature selected was detected between treatments in either *P. regilla* ($p = 0.885$) or *B. boreas* larvae ($p =$

0.293; Mann-Whitney *U* test; Table 4.1). Both species selected relatively warm temperatures (Table 4.1). Because we did not detect a difference in mean temperature selection, we used equivalency tests and calculated the least significant value using power analysis. The equivalency test and power analysis support our findings of no difference between mean temperatures selected in the two UVB treatments (Table 4.1).

Field UVB choice experiments:

We found no evidence for UVB avoidance in larvae of the three species in these experiments (binomial test; *P. regilla*, p = 0.16; *B. boreas*, p = 0.15; *R. cascadae*, p = 0.14). Three *B. boreas* larvae escaped during the trials and were excluded from the analysis. Several tadpoles from each species exhibited no choice between sides (i.e., equal number of observations on each side of the container; 5 *B. boreas*, 5 *R. cascadae*, 3 *P. regilla*) and were thus excluded from the analysis. No difference in temperature was observed between sides in any trial (Wilcoxon sign-rank test; *B. boreas*: p = 0.99, df = 5; *R. cascadae*: p = 0.13, df = 4; *P. regilla*: p = 0.99, df = 5).

Field transects:

Susan's Pond:

Over the two days of observations in Susan's Pond, we counted 2151 *A. macrodactylum*, 984 *R. cascadae* larvae and 215 *P. regilla* larvae. These values do not necessarily reflect independent larvae; our methods did not include marking larvae and it is possible that the same larva could have been counted more than once.

However, within a transect and sampling time, we avoided counting individual larvae more than once.

Water depth influenced both UVB penetration and temperature. The transects spanned a half-meter depth gradient (minimum of 3.1 cm to a maximum 51.3 cm). UVB penetration was relatively low in Susan's Pond: only 5.2% of surface UVB was detected at 10 cm (Figure 4.1). A negative correlation between depth and temperature was detected at all sampling times (Table 4.2). Greater thermal stratification was present during mid-day compared to morning or evening observations (Appendix F). Larvae were exposed to a maximum thermal gradient of 12ºC during mid-day (18º-30ºC).

Within the transects, water depth and temperature were correlated with species distribution. The relationship between water depth and larval distribution was different for each species (Table 4.3). The distribution of *A. macrodactylum* was positively correlated with depth at every sampling time except for the afternoon and evening of 19 July. At mid-day on both sampling days, the majority of *A. macrodactylum* larvae were observed in deeper water in temperatures at least 5ºC cooler than the maximum temperature (Figure 4.2a). In contrast, a negative correlation between the distribution of *R. cascadae* larvae and depth was detected during mid-day (1415 hr and 1430 hr). The relationship between *R. cascadae* distribution and depth was non-significant at all other sampling periods. Few *P. regilla* larvae were observed at 1015 hr on 20 July and 1800 hr on both days. Thus, we removed them from the analysis at these sampling times. A negative correlation between *P. regilla*

distribution and depth was detected at 1030 hr and 1415 hr. A marginally significant negative relationship was detected at 1430 hr. However, at 1430 hr, more than 80% of the larvae were found in water <5 cm deep, and the rest of the observed *P. regilla* larvae were nearly evenly dispersed across other depths. The majority of both *R. cascadae* and *P. regilla* larvae were observed in shallow water (<15 cm, *R. cascadae*; <10 cm, *P. regilla*) at temperatures within 2 ºC of the maximum temperature (Figure 4.2 b,c).

Todd Lake:

During the two-day survey at Todd Lake, we counted 3954 *B. boreas*, 185 *R. cascadae* larvae and only 3 *P. regilla* larvae. Because we observed very few *P. regilla*, we removed them from the analyses. At mid-day on both observation days, the majority of *R. cascadae* larvae (> 65%) were observed in water < 10 cm deep. *R. cascadae* were excluded from further analyses due to low sample sizes in each transect.

As in Susan's Pond, water depth again influenced both UV penetration and temperature. Within the 7 m transect, water depth increased from a minimum of 3.3 cm to a maximum of 107.9 cm. UVB penetration was much greater in Todd Lake compared to Susan's Pond (Figure 4.1). More than 60% of surface UVB was detected at 10cm. Strong diel fluctuations in thermal profiles were observed (Figure 4.3). In the early morning, a positive relationship between temperature and depth was detected, with deeper locations warmer than shallow regions (0800-0900 hr, Spearman's *rho* = 0.47, p = 0.0023). Between 0900-1000 hr, no relationship between

temperature and water depth was observed (Spearman's *rho* = 0.21, p = 0.15). After 1100 hr, shallow regions were warmer than deeper regions (1100-1200 hr, Spearman's *rho* = -0.34, p = 0.002; 1400-1500, Spearman's *rho* = -0.46, p < 0.0001). This relationship persisted until 1800 hr, when no relationship between depth and temperature was detected (1800-1900 hr, Spearman's *rho* = -0.057, p = 0.66). After 2000 hr, the shallow regions were again cooler than deeper regions (2000-2100 hr, Spearman's *rho* = 0.47, p = 0.003). The maximum thermal gradient was observed at mid-day (1300-1400 hr) with a difference of 8.5 ºC between the shallowest and deepest regions (17-25.5 ºC).

The distribution of *B. boreas* tadpoles within transects was correlated with temperature and depth. The direction of this relationship varied across sampling times and mirrored diel temperature fluctuations (Table 4.4). At the earliest observation (0945 hr, 10 August), more tadpoles were observed in deeper water (Figure 4.3a). At 1045 hr on 9 August, more animals were observed in shallow water (Table 4.4). More animals were also observed in shallow water during mid-day sampling on both days (Table 4.4, Figure 4.3). Indeed, at mid-day on the sunniest day (9 August), over 88% of animals observed were located in water less than 10 cm deep. More tadpoles were observed in deeper water during the evening on both sampling days (Table 4.4). On 10 August, we observed a large mass of tadpoles swimming towards the shore from 1000-1030 hr. The tadpoles formed a thick band at the edge of the pond and appeared to forage in the shallows, occasionally stirring up sediment on the bottom of the lake

(Figure 4.4a). The tadpole aggregation was so dense that individual tadpoles had portions of their bodies out of the water (Figure 4.4b).

Potholes:

UVB transmission was greater in Ponds A and B than in Ponds C and D. In Ponds A and B, 30 % of surface UVB was detected at 10 cm, while in Ponds C and D, 8% of surface UVB was detected at 10 cm (Figure 4.1). The thermal environment in the Potholes was also different from the thermal regimes in Susan's Pond and Todd Lake. Larvae were exposed to a maximum thermal gradient of 5ºC (Pond B; Appendix G) or 3ºC (Pond C; Appendix G).

In addition to structural differences between ponds, the ponds at Potholes varied biologically. Ponds A and D contained *A. macrodactylum* and *P. regilla* larvae. We observed *A. macrodactylum*, *P. regilla*, and *R. cascadae* larvae in Pond B, while Pond C contained larval *P. regilla* and *R. cascadae*.

The physical differences between the ponds combined with the inability to run replicate transects in each pond made statistical comparisons difficult. The depth choices available to each larva were different between ponds, and the depth distribution of each species varied between ponds (Figure 4.5). To facilitate statistical comparisons, we ranked the depth classes (5 cm intervals) in each pond, such that the shallowest depth class was assigned a rank value of "1", the next depth class was assigned a rank of "2", and so forth and then used Spearman's *rho* to look for correlations at each observation time. Although this method obscured the details of depth within each transect, it allowed us to look for patterns in depth choice at the

scale of the whole site rather than individual pond scale. No correlations were detected between depth rank and percentage of animals observed for either *A. macrodactylum* or *R. cascadae*. However, a negative correlation between depth rank and percentage of *P. regilla* larvae was observed at noon (Spearman's $\rho = -0.52$; $p = 0.016$), suggesting that larval *P. regilla* were more likely to be found in shallower regions of each pond.

Discussion:

UVB radiation causes negative effects in all four species examined in this study. This includes increased mortality and a variety of sublethal effects including reduced growth (e.g., Blaustein *et al*., 1994; Blaustein *et al*., 1997; Belden *et al*., 2000; Blaustein *et al*., 2005). Therefore, the hypothesis that these species avoid UVB is reasonable. Accordingly, we used a combination of approaches to explore habitat use in amphibians, with specific reference to temperature and UVB radiation.

In laboratory thermal gradients, larval *P. regilla* (Pacific treefrog) and *B. boreas* (western toad) did not avoid UVB by selecting cooler temperatures with lower UVB levels. Rather, larvae preferred relatively high temperatures, regardless of UVB exposure. In the field, larvae of these two species, in addition to *R. cascadae* larvae, showed no preference for low UVB areas in choice tests. Using similar methods, Belden *et al*. (2000) found that larval *A. macrodactylum* preferred shade to full sun but did not discriminate between differences in UVB levels manipulated by plastic filters. The results of the two experiments presented here, in conjunction with previous

research, suggest that the larvae of the four species in this study do not avoid UVB. These results were further supported by our field transects. If amphibian larvae avoid UVB in the field, we expect to see fewer larvae in shallow water at Todd Lake due to the relatively high UVB transmittance at this lake. Yet, we observed large numbers of tadpoles in shallow water (< 10 cm), despite high levels of UVB at these depths in Todd Lake.

The distribution of larvae varied across sites, but anuran larvae (*P. regilla* and *R. cascadae*) were generally observed in shallow regions, especially during peak UV hours. In contrast, *A. macrodactylum* larvae were usually located in deeper regions, which is consistent with Belden *et al*. (2000). No pattern in depth choice was observed for either Cascades frog or long-toed salamander larvae at the Potholes. Pacific treefrog larvae were observed more often in shallow regions at the Potholes at noon. However, compared to the other two sites, the ponds at the Potholes were relatively isothermic at each sampling. Moreover, the distribution of larvae in the only Pothole with a shallow margin (Pond C) was similar to the distribution of larvae at Susan's Pond and Todd Lake: more than 70% of larvae were observed in less than 5 cm of water. Thus, larvae at these sites, particularly anurans, do not avoid sunlight and are exposed to relatively high levels of UVB radiation.

The dose of UVB received by larvae is a function of many factors, including habitat use and the UVB transmittance of the habitat. The three sites used in this study vary widely in UVB transmittance. A recent study of 136 amphibian breeding habitats estimated a mean 40.3% percent transmittance of UVB at 10 cm for habitats

containing three of the four species observed in the current study (*R. cascadae*, *B. boreas*, *A. macrodactylum*; Palen *et al*., 2002). Both Susan's Pond (5.2%) and the four ponds at the Potholes site (29.6%, 26.0%, 7.9% and 3.1%) have much lower UVB transmittance at 10 cm than average. However, anuran larvae in our study were commonly observed in water < 10 cm deep, regardless of the UVB transmittance of each site. The sites in our study were not randomly selected but were selected to represent a range of amphibian habitats. Therefore, we cannot conclude that these sites are representative of all amphibian breeding habitats in the P acific Northwest. However, the similarity in habitat use among sites despite widely varying UVB environments suggests that, at the landscape scale, larval amphibians may be exposed to high levels of UVB radiation due to habitat use. Moreover, UVB did not influence habitat use in these sites. The observed habitat use may be due to other pressures such as thermal requirements, resource use, or predation risk.

Behaviors such as thermoregulation, foraging, and predator avoidance commonly affect habitat use. Temperature is frequently the dominant physical factor affecting physiology and behavior of larval amphibians (Ultsch *et al*., 1999). The diel movements of *B. boreas* tadpoles at Todd Lake closely followed diel temperature fluctuations, suggesting a strong relationship between temperature and habitat use. Similar diel movements have been observed in the larvae of other species (Brattstrom, 1962; Beiswenger, 1977). Previous work on habitat use in *B. boreas, R. cascadae* and *P. regilla* suggests that temperature is the single most important habitat variable for predicting tadpole density (Brattstrom, 1962; O'Hara, 1981). Wollmuth *et al*.

(1987) found the highest densities of Cascades frog tadpoles in the warmest (and shallowest) regions of a pond, and tadpole aggregations moved throughout the afternoon to track the warmest temperatures. Hokit and Blaustein (1997) observed aggregations of Cascades frog larvae in the warm shallow regions over potential food sources. Observations of larval Pacific treefrog aggregations suggest that they orient their bodies such that the maximal dorsal surface area is exposed to the sun, presumably for thermoregulation (Brattstrom & Warren, 1955). The importance of temperature as a cue guiding habitat use is not surprising given the influence of temperature on growth rate in amphibians (Atlas, 1935; Ryan, 1941; Álvarez & Nicieza, 2002).

We did not quantify the distribution of potential predators or food resources in this study. Compared with the other species, habitat use in western toads is probably least likely to be affected by predators, as these toads have potent toxins in their skin (Arnold & Wassersug, 1978). Conversely, large salamander larvae and a variety of invertebrates eat Pacific treefrog and Cascades frog larvae (Peterson & Blaustein, 1992; Wildy *et al*., 1998) and these predators may have an impact on habitat use in these species. For example, the temperature where Pacific treefrog tadpoles were most dense at Susan's Pond was higher than the preferred temperature of this species in our laboratory experiments, suggesting that habitat use may be influenced by other factors such as the presence of predators as well as the thermal regime. The large long-toed salamander larvae were most common in the cooler deeper water in Susan's Pond; therefore, Pacific treefrog larvae could avoid predation by seeking warmer,

shallower water. In contrast, long-toed salamander larvae were not large enough to consume tadpoles in the Potholes, where little segregation between Pacific treefrogs and long-toed salamanders was observed. Habitat use in these larvae could be influenced by a number of factors not measured in the current study. However, UVB radiation does not appear to influence habitat use, despite the potential for significant damage caused by exposure to UVB.

The effects of UVB radiation depend on the dose received by an individual organism, which is a direct consequence of habitat characteristics (i.e., UVB penetration, topography) and individual behavior. Some studies have suggested that habitat characteristics such as dissolved organic carbon may fully protect amphibians from damage caused by UVB radiation (Adams *et al*., 2001; Palen *et al*., 2002). However, these studies did not consider temporal and spatial habitat use within a pond and the potential for other selection pressures to affect exposure to UVB. Our results suggest that larval amphibians are exposed to potentially damaging doses of UVB as a consequence of thermoregulatory behaviors. Including the interplay between habitat characteristics and habitat use is vital to our understanding of how environmental stressors and conflicting selection pressures may affect organisms.

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M. Kavanaugh, L. E. Petes and R. H. Bancroft provided assistance in the field. Conversations with L. Crawshaw were essential to the design of the thermal gradients. B. A. Menge and E. Seabloom provided statistical advice. C. Baker provided housing

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Table 4.1. Results from Mann-Whitney *U* test, equivalency test and power analysis in thermal gradient laboratory trials. Treatment indicates the UVB exposure of the warm end of the gradient.

Species	Treatment	Average temp selected	df	Z value	P-value	Equivalence p-value*	Least significant value
Bufo boreas	UV shielded	28.3	1	1.052	0.293	0.0286	1.34
	UV exposed	27.6					
Pseudacris regilla	UV shielded	25.20	1	-0.134	0.885	0.0020	1.26
	UV exposed	25.09					

*Practical difference = 2ºC

Table 4.2. Depth and temperature correlations at Susan's Pond, Oregon on 19 July 2006 at each observation time.

Time	Spearman's *rho*	p-value
10:00	-0.5585	<0.0001
14:00	-0.5605	<0.0001
18:00	-0.5198	<0.0001

Table 4.3. Correlation between water depth and mean percent animals observed for each species at Susan's Pond. Oregon. For *P. regilla*, fewer than 20 animals were observed at the observation times not shown.

		Ambystoma macrodactylum		*Rana cascadae*		*Pseudacris regilla*	
Time (hr)	Date	Spearman's *rho*	p	Spearman's *rho*	p	Spearman's *rho*	p
1015	7/20/2006	0.5959	0.0005	-0.3463	0.1347		
1030	7/19/2006	0.6250	0.0002	-0.3326	0.1519	-0.4499	0.0126
1415	7/20/2006	0.7357	0.0001	-0.5359	0.0149	-0.4597	0.0414
1430	7/19/2006	0.2858	0.1257	-0.6281	0.0002	-0.4005	0.0802
1800	7/20/2006	0.6254	0.0002	0.1045	0.5825		
1800	7/19/2006	0.2308	0.2198	-0.0543	0.8201		

Table 4.4. Correlation between water depth and mean percent *Bufo boreas* observed at each sampling time in Todd Lake, Oregon.

Date	Time	Spearman's rho	p-value
10 August	9:45	0.6096	0.0033
9 August	10:45	-0.4390	0.0465
9 August	14:00	-0.7992	<.0001
10 August	14:45	-0.7464	0.0001
9 August	17:50	0.4263	0.0540
10 August	18:30	0.6088	0.0034

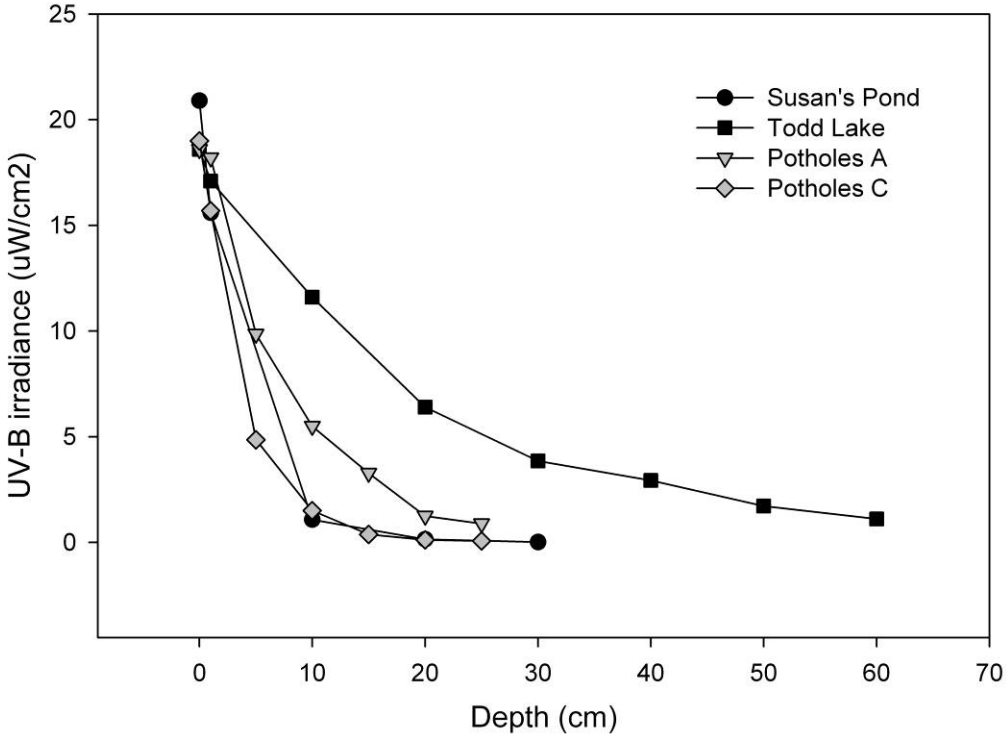

Figure 4.1. UVB profiles from four Oregon study sites during mid-day. Todd Lake had the highest UVB transmittance. Only Ponds A and C are shown from the Potholes for clarity. The transmittance of Pond B was nearly identical to Pond A, while Pond D was similar to Pond C.

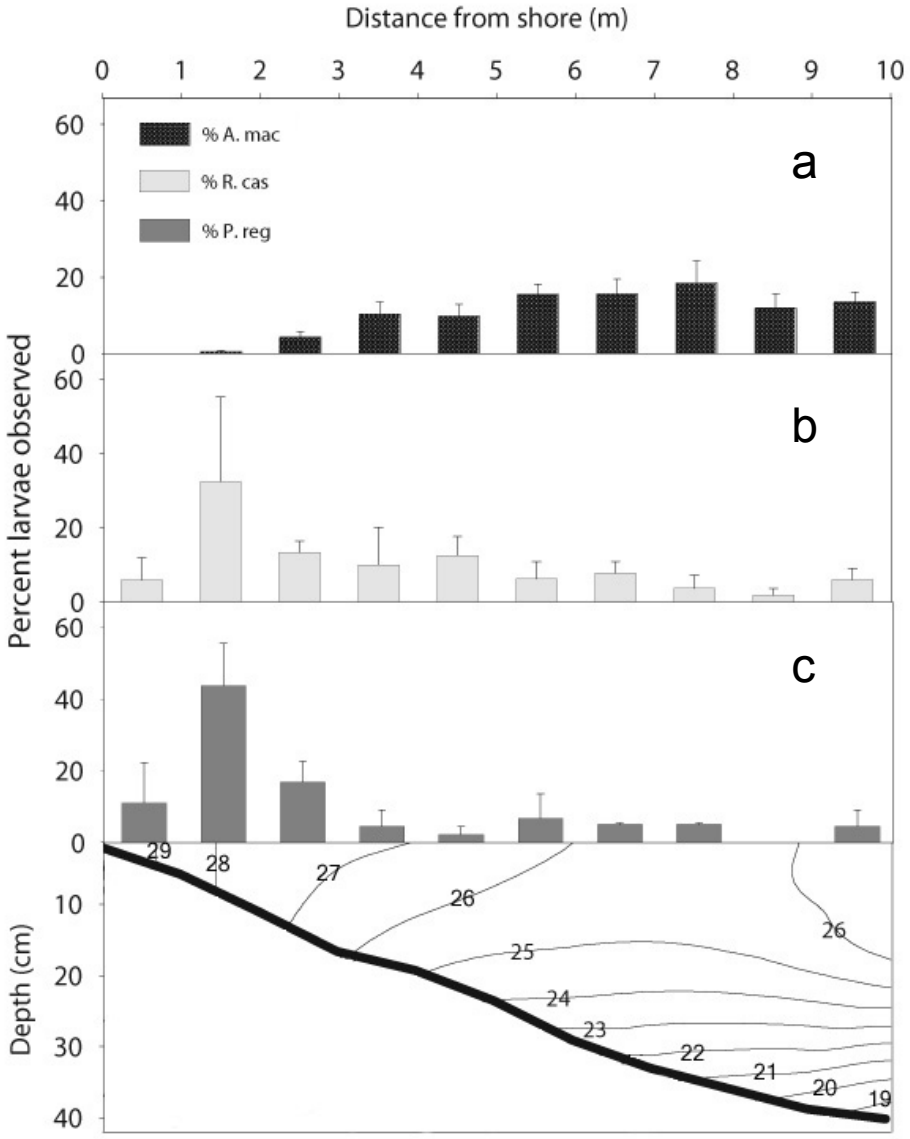

Fig. 4.2. Distribution of the larvae of three species at mid-day on 20 July, 2006 in Susan's Pond, Oregon. Thick black line in the bottom panel represents the bottom of the pond and the numbers are temperature in degrees Celsius. Bars represent the mean percent larvae of each species observed at each depth $\pm$ standard error (mean of three transects). The majority of anuran larvae (*R. cascadae* and *P. regilla*) were observed closer to shore in water < 15cm deep. Most salamander larvae (*A. macrodactylum*) were observed in water > 20cm deep.

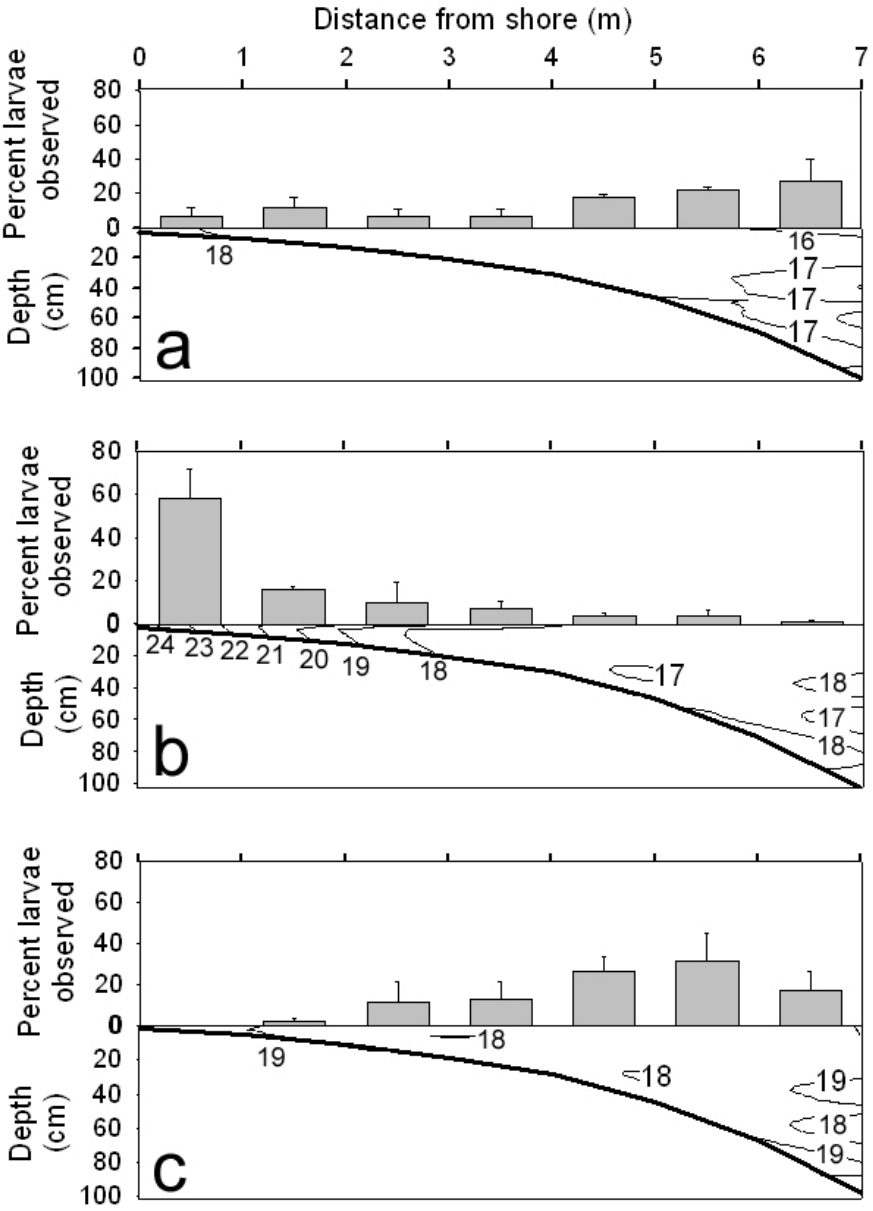

Fig. 4.3. Distribution of *Bufo boreas* larvae in Todd Lake, Oregon, on 10 August 2006. The thick black line indicates the bottom of the lake. Contour lines represent thermal stratification in degrees Celsius. Bars indicate the mean percent (± SE) *B. boreas* tadpoles observed in three transects in the morning (A), mid-day (B), and evening (C).

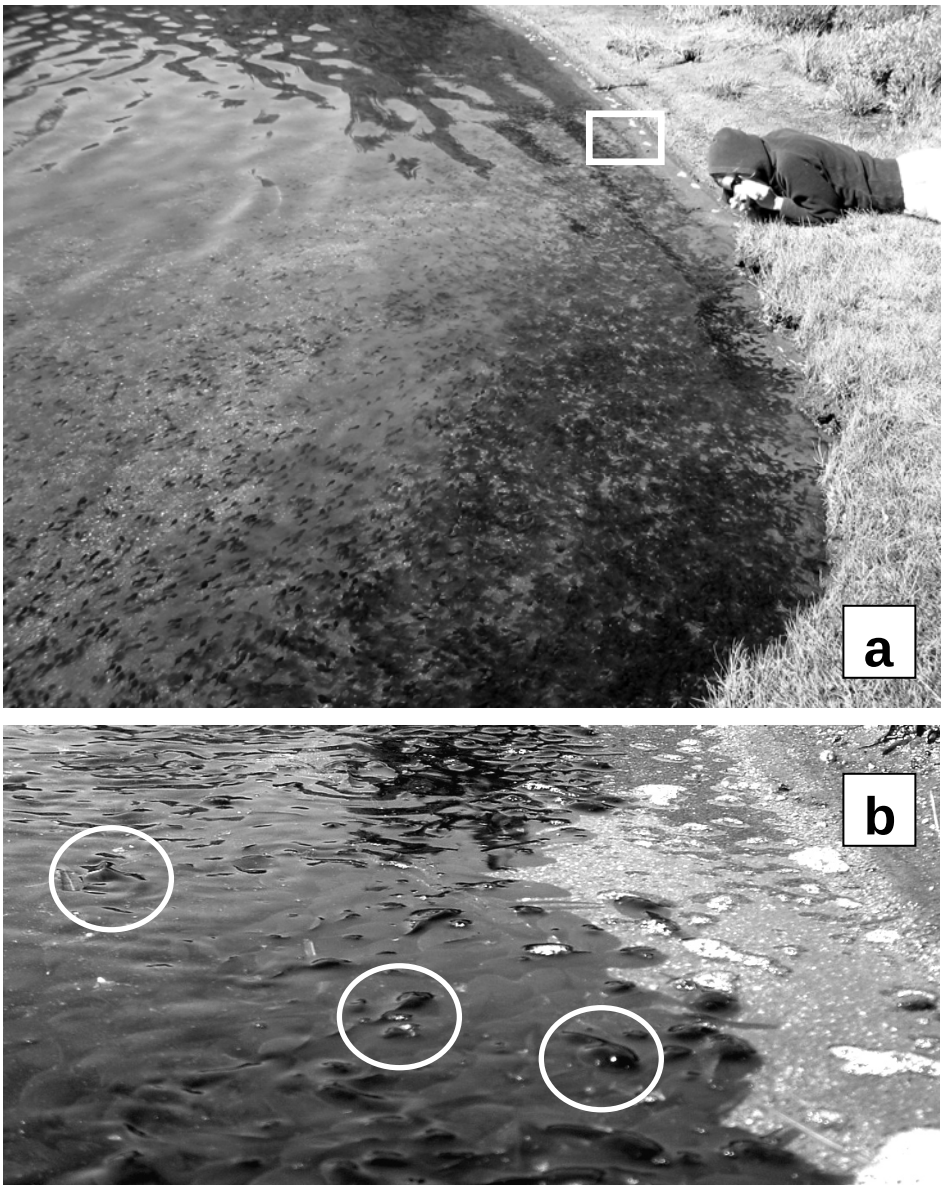

Figure 4.4. Toad tadpoles at Todd Lake, Oregon on 10 August 2006 (A). Panel B is a close-up view of the region indicated by the white rectangle in Panel A. White circles in panel B indicate tadpoles with at least a portion of their body out of water.

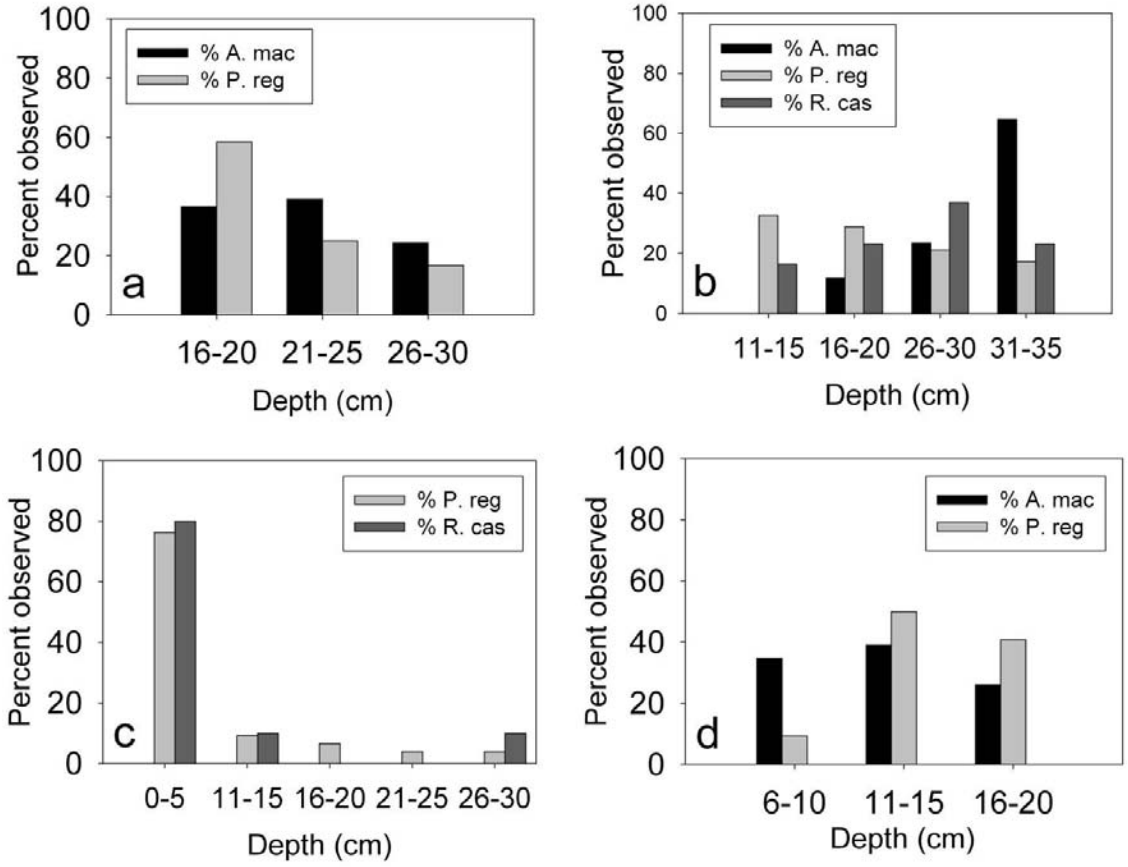

Figure 4.5. Distribution of larvae at mid-day on 10 August 2006 at the Potholes, Oregon. Individual potholes (A-D) are shown separately. Bars indicate percent of each species observed within each depth class.

Chapter 5

Effects of skin color and UVB radiation on survival and growth of amphibian larvae

Betsy A. Bancroft and Andrew R. Blaustein

Abstract

Environmental stressors such as ultraviolet-B (UVB) radiation are increasing due to anthropogenic influences in natural systems. UVB radiation negatively affects many aquatic organisms such as phytoplankton, crustaceans, fish and amphibians. UVB radiation has been suggested as one factor contributing to world-wide declines in amphibian populations. In amphibians, exposure to UVB radiation increases mortality, reduces growth, alters behavior and increases susceptibility to disease. However, UVB has been present throughout evolutionary history and amphibians have a number of strategies to avoid or mediate damage caused by UVB. One defense strategy is the use of photoprotective compounds and pigments. Melanin is a photoprotective pigment that can be induced upon exposure to UVB. In addition, tadpoles use melanin to adjust body color in response to substrate color. We raised tadpoles of two species (*Pseudacris regilla* and *Rana cascadae*) on dark or light backgrounds and exposed them to UVB in the laboratory for three weeks. We hypothesized that darker tadpoles would have higher survival and growth rates than light tadpoles when exposed to UVB. Exposure to UVB reduced survival of *P. regilla* and reduced growth of *R. cascadae* regardless of background color. In addition, we observed a negative correlation between larval coloration and growth under UVB, indicating that lighter colored larvae grew more over three weeks than dark-skinned larvae. No correlation between larval color and growth was detected in the absence of UVB radiation. Our results suggest that melanin does not prevent negative effects of UVB radiation in

larval anurans. Further, a trade-off may exist between investing resources in melanin synthesis and investing resources in photorepair and growth.

Introduction

Over evolutionary history, organisms of all types have experienced many stressors in natural systems. However, recent anthropogenic changes to many habitats have resulted in an increase in the frequency, intensity, and number of stressors in these habitats. For example, ultraviolet-B radiation (UVB; 280-320 nm) is one stressor that has recently increased in intensity due to stratospheric ozone depletion (Madronich *et al*., 1998; Cockell & Blaustein, 2001). UVB radiation negatively affects many organisms in terrestrial and aquatic systems (reviewed in Tevini, 1993; de Mora *et al*., 2000; Hessen, 2002; Helbling & Zagarese, 2003). A recent meta-analysis suggests that UVB radiation has a large negative effect on aquatic organisms, including amphibians (Bancroft *et al.*, 2007). Moreover, amphibian populations are currently experiencing widespread declines (Stuart *et al*., 2001) and are potentially more threatened than either birds or mammals (Mendelson *et al*., 2006). Environmental stressors, including UVB radiation, may be contributing to many of these population declines (Blaustein & Kiesecker, 2002; Collins & Storfer, 2003).

UVB radiation has negative effects on amphibians (Bancroft, Chapter 3; reviewed in Blaustein & Kiesecker, 2002). Larval and embryonic stages of amphibian species differ in sensitivity to UVB radiation (Blaustein & Belden, 2003). Within a species, sensitivity to UVB radiation may change with ontogeny. Embryos and larvae

of some species experience direct mortality, while other species show subtle sublethal effects (Blaustein & Belden, 2003). For example, exposure of red-legged frogs (*Rana aurora*) embryos to ambient UVB results in reduced growth and increased incidence of malformations in the larval stage (Belden & Blaustein, 2002a). However, much less is known about how amphibians protect themselves from UVB radiation.

Amphibians employ two defense strategies against damage from UVB radiation: repairing damage once it occurs or by preemptively avoiding damage (Blaustein & Belden, 2003). In amphibians, the most well studied mechanism for repairing molecular-level UVB damage occurs via the action of the DNA repair enzyme photolyase (e.g., Blaustein *et al*., 1994; Hays *et al*., 1996). One enzyme, CPD-photolyase, uses visible light energy (1300-500 nm) to remove the most frequent UV-induced lesion in DNA, cyclobutane pyrimidine dimers (CPDs) (Friedberg *et al*., 1995). A second related enzyme [6-4]-photolyase, similarly uses light energy to reverse pyrimidine-[6-4']-pyrimidone photoproducts ([6-4] photoproducts). Moreover, multi-protein broad specificity excision repair processes can remove CPDs and [6-4] photoproducts. Both mechanisms may be used simultaneously, but excision repair is typically more efficient for [6-4] photoproducts than for CPDs. Thus, CPD-photolyase appears to be the first level of defense against CPDs for many organisms exposed to sunlight (Pang & Hays, 1991; Friedberg *et al*., 1995; Blaustein *et al*., 2001). Large differences in photolyase activity exist between species (Blaustein *et al*., 1994; Hays *et al*., 1996; Smith *et al*., 2002). Species with high levels of photolyase activity are usually those species that are resistant to UV radiation (Blaustein *et al*., 2001).

Because UVB radiation has been a stressor throughout evolutionary history (Cockell, 2001), amphibians may have developed behavioral strategies to avoid areas with high UVB radiation. Several amphibian species appear to avoid UVB radiation (Nagl & Hofer, 1997; van de Mortel & Buttemer, 1998; Belden *et al*., 2000; Garcia *et al*., 2004; Han *et al*., 2007). However, this avoidance behavior is not common to all species, as many amphibians are exposed to high levels of UVB as they bask in sunlight or in warm shallow waters for thermoregulation (Brattstrom & Warren, 1955; O'Hara, 1981; Wollmuth *et al.,* 1987; Fite *et al*., 1998; Bancroft *et al*., Chapter 4). Seeking warm temperatures speeds growth and development in larval amphibians (Atlas, 1935; Lillywhite *et al*., 1973). Therefore, amphibians may use photoprotective pigments or other compounds in their skin to allow behavioral thermoregulation while lowering the risk of damage from UVB exposure in shallow waters.

Melanin is a common pigment in amphibian skin and may function as a photoprotective strategy in embryos and larvae (Novales & Davis, 1969; Jablonski, 1998; Garcia *et al*., 2004). Some species of larval amphibians darken in response to UVB (Belden & Blaustein, 2002b; Garcia *et al*., 2004). Mammals with darker skin are less prone to UV-induced skin damage than those with lighter skin (Kollias *et al*., 1991). However, darker skin does not appear to increase survival or growth in salamanders (Lesser *et al*., 2001; Belden & Blaustein, 2002b), although only a few have been examined. It is possible that constant production of pigment is physiologically costly and may decrease growth or survival. It is also possible that

increased production of melanin does not substantially mitigate the negative effects of UVB.

In the laboratory, we tested the hypothesis that amphibian larvae with darker skin exhibit increased growth and survival under UVB radiation compared with lighter skinned larvae due to the protective qualities of increased skin pigmentation.

Materials and Methods

Animals

Larval *Pseudacris regilla* (Pacific treefrog) and *Rana cascadae* (Cascades frogs) at Gosner stage 25 (Gosner, 1960) were collected from an ephemeral pond in the Oregon Cascade Mountains, 40 km west of Bend, OR (elevation: 2300 m) on 5 July 2005. Animals were returned to the laboratory at Oregon State University and maintained in conditioned water in 38 L aquaria at 15º C. Animals were fed a mixture of alfalfa pellets and commercial fish flakes (3:1 ratio) *ad libitum*.

Experimental treatments

After five days, 72 animals (36 per species) were placed individually into 15 cm diameter plastic Petri dishes filled with 1 cm conditioned water and then randomly assigned to either a black or a white background. Background color can be used to manipulate larval amphibian skin color (Belden & Blaustein, 2002b; Garcia & Sih, 2003). Each animal was individually photographed under standardized lighting conditions using a Canon Powershot G3 digital camera prior to placement in a treatment (Garcia *et al*., 2004). One half of the larvae on each background color were

assigned to a UVB blocking filter (Mylar film), while the other half from each background color were assigned to a UVB transmitting filter (acetate film). Petri dishes were placed on plastic sheeting of the appropriate color and larvae were allowed to acclimate to background color for 4 days. Larvae were then were moved to a temperature controlled (16 º C) UV chamber. Each dish was placed on a black or white 20 cm^2 plastic square under an array of UVB (Q-Panel, UVB 313, Q-Panel Inc., Cleveland, Ohio, USA) and full-spectrum lights (Vita Lite, Durotest Corporation, Fairfield, New Jersey, USA) on a 12:12 photoperiod. UVB radiation under acetate filters was 9.0 $\mu W/cm^2$, while UVB under Mylar filters was 0.3 $\mu W/cm^2$. These levels are within the range of typical UVB levels in the Oregon Cascades (e.g., Kiesecker *et al*., 2001; Belden & Blaustein, 2002c). Filters were supported by wooden frames such that the filters did not touch the water surface.

Animals were checked daily for survival and fed *ad libitum*. Complete water changes were done every 5-7 days. On the final day of the trial (day 21), animals were again individually photographed under the same lighting conditions as above. Each animal was then sacrificed in 1.5 g/L MS-222 (Argent Laboratories, Redmond, WA) and flash frozen in liquid nitrogen before being stored at -80 ºC.

Digital image analysis

Digital images were analyzed for brightness using Adobe PhotoShop 7.0 (Garcia *et al*., 2004). Each file was converted to LAB color, which separates color into a brightness channel (0-100), a red/green channel and a blue/yellow channel. Brightness measurements were taken in three standardized locations per animal. We

then used principle component analysis to generate one measurement (PC1) for each animal at the beginning and at the end of the experiment. In addition, whole body length (including tail) was measured from the images using ImageJ 1.34s.

Results

Survival was lower in *P. regilla* larvae under UVB radiation (Fisher's Exact test; $p = 0.0002$, df = 1; Figure 5.1). No difference in survival of *R. cascadae* was detected (Fisher's Exact test; $p = 0.33$, df = 1; Figure 5.1). Only four *P. regilla* larvae in the UVB treatments survived the experiment; therefore, further statistical tests were impossible for *P. regilla* alone. We used analysis of variance (ANOVA) followed by Student's *t* tests to analyze differences in group means for growth and color. Growth was lower in *R. cascadae* larvae exposed to UVB ($t = -4.71$, $p = 0.001$, df = 3; Figure 5.2). No effect of background color or UVB treatment was detected on larval brightness after three weeks ($F = 0.36$, $p = 0.78$, df = 3; Figure 5.3). As no effect of background color was observed on larval brightness, we used Pearson's *r* to look for a correlation between larval brightness and growth in the two UV treatments. Lighter colored larvae exposed to UVB exhibited higher growth than darker colored larvae exposed to UVB (Figure 5.4a). No relationship was detected between larval color and growth in larvae shielded from UVB (Figure 5.4b).

Discussion

UVB radiation had negative effects on larvae of *Pseudacris regilla* and *Rana cascadae*. The levels of UVB in our experiment were lethal to *Pseudacris* and reduced growth in *Rana*. However, no evidence of darkening in response to either background color or UVB exposure was observed. The positive correlation between skin brightness and growth under UVB radiation suggests that a trade-off may exist between synthesis of melanin pigmentation and growth. Thus, melanin may not be an effective sunscreen method in these larvae.

Pseudacris regilla is commonly cited as a species that is relatively resistant to damage from UVB exposure, based on studies of embryos (e.g., Blaustein *et al.*, 1994; Ovaska *et al*., 1997; Anzalone *et al*., 1998). However, after prolonged exposure to UVB radiation at an early larval stage, we observed significant mortality in this species. No mortality was observed until after seven days of exposure; thus, repair mechanisms may have been "swamped" by continuous exposure to UVB radiation. It is important to note that the ratio of UVB to the wavelengths necessary for photorepair (UVA and visible light) was different from natural conditions. Much less UVA and visible light was available for photorepair in our laboratory experiment. However, two recent meta-analyses of the effects of UVB on aquatic organisms and the effects of UVB on amphibians found no difference between laboratory and field studies (Chapter 2; Chapter 3), suggesting that UVB has negative effects regardless of the efficiency of repair mechanisms. Thus, UVB radiation clearly has negative effects on

survival in larval *P. regilla*, but this effect may be tempered in the field by effective repair mechanisms.

UVB radiation decreases growth in early life history stages of several amphibian species (e.g., *Xenopus laevis*, Bruggeman *et al*., 1998; *R. temporaria*, Pahkala *et al*., 2000; *Ambystoma macrodactylum*, Belden *et al*., 2000; *R. aurora*, Belden & Blaustein, 2002a). Our results suggest that growth of larval *R. cascadae* is also reduced by exposure to UVB. Growth is important in larval amphibians, as size at metamorphosis is positively correlated with adult size (Werner, 1986), and adult size is positively correlated with fitness (Semlitsch *et al*., 1988, Altwegg & Reyer, 2003). In addition, a recent study of habitat use in *R. cascadae* suggests that these larvae are frequently found in water less than 15 cm deep and may be exposed to high levels of UVB radiation during the larval period (Bancroft, Chapter 4). Therefore, reduced growth at the larval stage due to UVB exposure may have implications for overall fitness in adult *R. cascadae*.

The hypothesis that melanin acts as a photoprotective pigment in larval amphibians predicts that darker individuals exposed to UVB radiation should exhibit increased survival and growth compared with brighter individuals. Surprisingly, we observed the opposite relationship between growth and brightness. This relationship is only correlative; therefore, we cautiously suggest that a trade-off may exist between pigment production and growth. If melanin is not an effective photoprotective strategy, allocating resources toward synthesizing and maintaining this pigment is not an optimal strategy. Rather, allocating resources towards repair mechanisms may be

more efficient. More research is necessary to explore this potential trade-off in larval amphibians. Investigation of oxidative stress indicators may elucidate the relationship between larval color, growth, and stress due to UV exposure.

Our results suggest that melanin may not be an effective strategy to avoid damage from UVB radiation. Therefore, amphibian larvae that exploit shallow waters for thermoregulation may be negatively affected by the high levels of UVB radiation in these microhabitats. These larvae may rely on photorepair mechanisms to mediate damage caused by UVB. However, photorepair efficiencies vary among species (Blaustein *et al*., 1994; Hays *et al*., 1996; Smith *et al*., 2002) and the variation between species may thus lead to differential survival in species that exploit shallow microhabitats or in habitats with high UVB transmission. This differential survival among species may be contributing to population declines in several species of amphibians.

Acknowledgements

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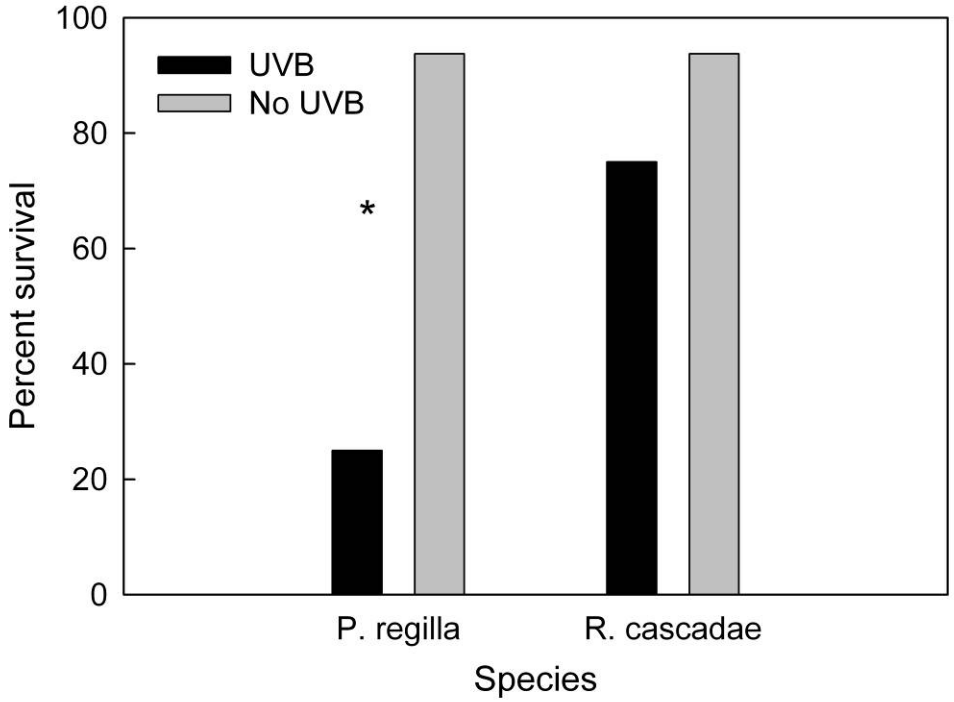


Figure 5.1. Survival of *Pseudacris regilla* and *Rana cascadae* larvae after exposure to UVB radiation. Colors were lumped for statistical analysis due to imbalanced sample sizes resulting from mortality. The asterisk denotes significant difference in survival at $\alpha = 0.05$.

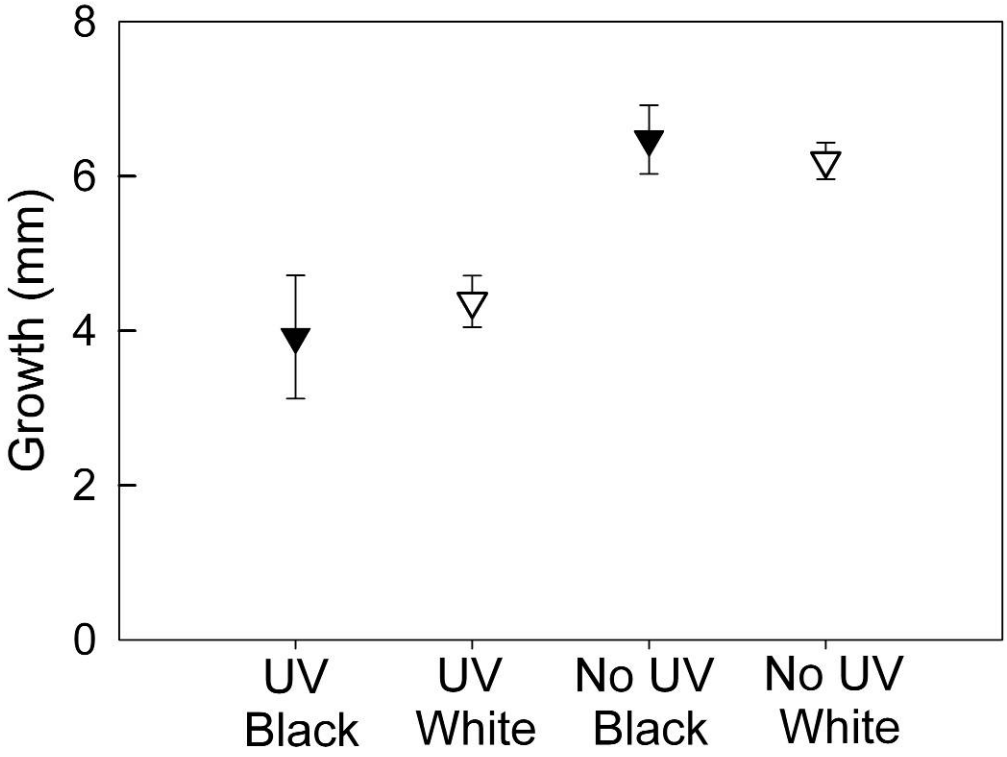


Figure 5.2. Growth (final size – initial size) of *R. cascadae* larvae in each treatment. No effect of background color was detected, but larvae under UVB radiation grew less than shielded larvae. Data are mean ± standard error.

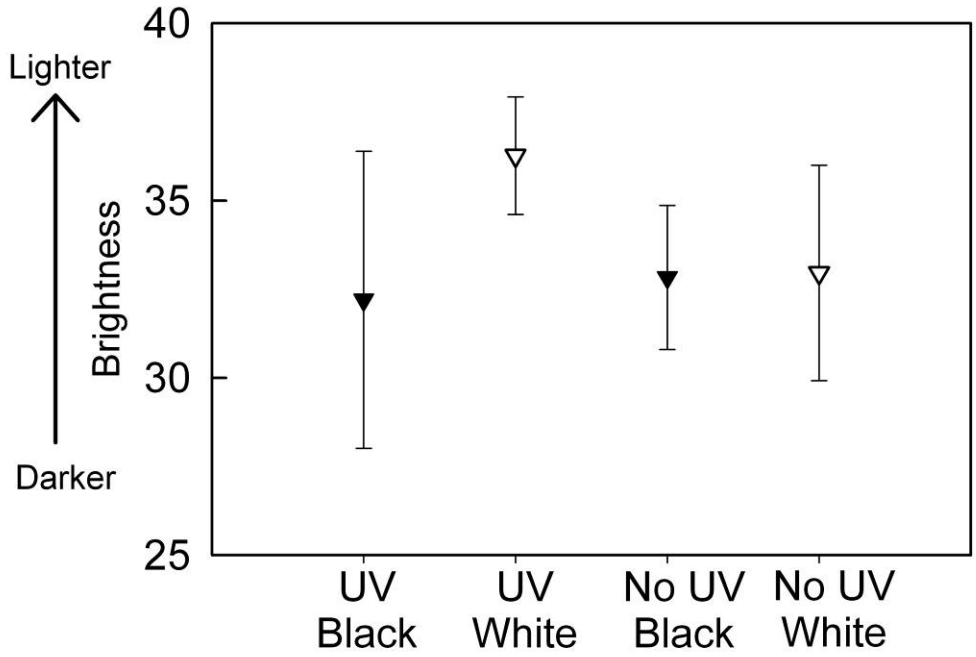


Figure 5.3. Average brightness of *R. cascadae* larvae in each treatment. No effect of any treatment on brightness was observed.

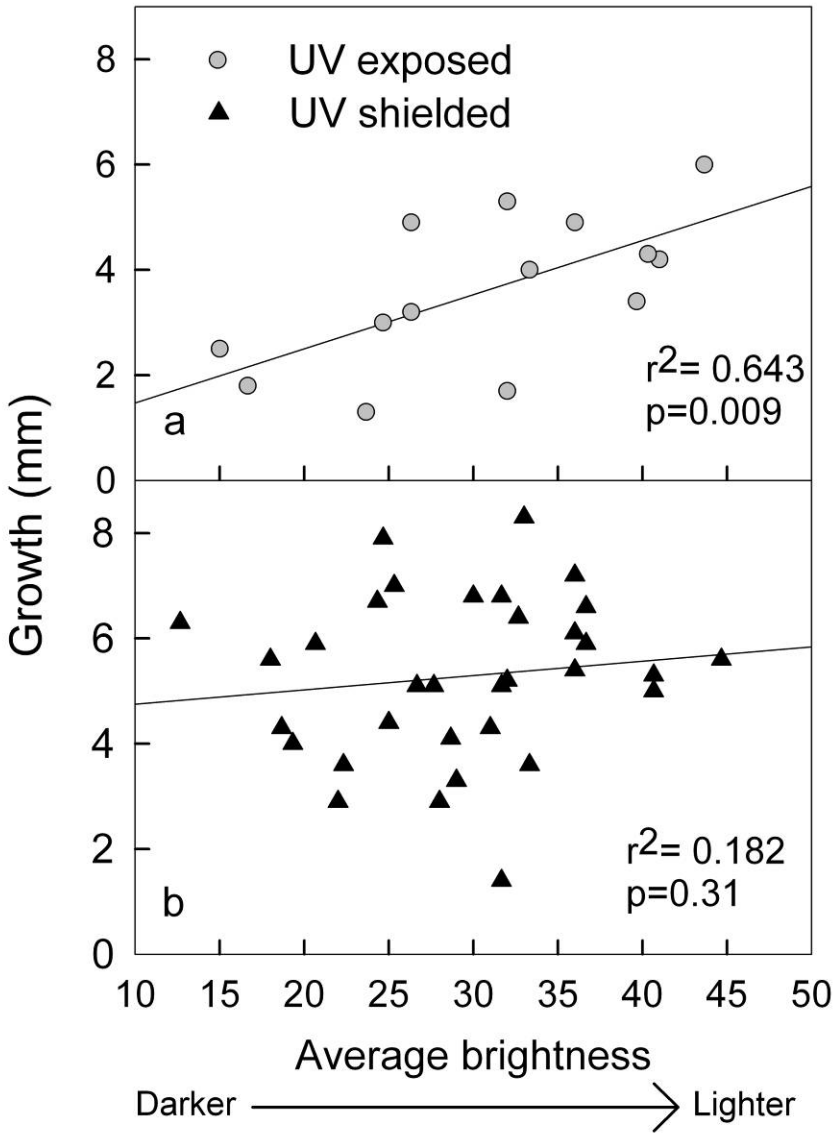


Figure 5.4. Relationship between growth (final-initial size) and brightness for all larvae in the two UVB treatments.

CHAPTER 6: CONCLUSIONS

My thesis explored the effects of and potential mediating mechanisms for an important environmental stressor, ultraviolet-B radiation. UVB radiation has negative effects on organisms in both terrestrial and aquatic systems (e.g., Tevini, 1993). This stressor negatively affects aquatic organisms by reducing survival and growth in many species (Chapter 2). In particular, UVB reduces growth of embryos more than any other life history stage. Some taxonomic groups may be more affected by UVB radiation than others. In our analysis, the growth of members of the kingdom Protozoa was suppressed by UVB radiation to a greater degree than any other kingdom. These analyses suggest that UVB is an important stressor in aquatic systems.

Amphibians are a common component of freshwater systems and are experiencing world-wide population declines (Blaustein & Kiesecker, 2002; Stuart *et al.*, 2004). No single factor is responsible for the observed population declines. These declines may be due to a number of causes including habitat loss, introduced species, global climate change, disease, toxic chemicals and UVB radiation (Blaustein & Kiesecker, 2002; Collins & Storfer, 2003). These factors may interact synergistically, resulting in larger effects together than each factor would alone. The global nature of the declines, in conjunction with declines in relatively pristine habitats, suggests that a global stressor may be involved. UVB radiation is a global stressor that negatively affects many aquatic organisms (Chapter 2); therefore, UVB is a likely stressor in amphibian habitats. Indeed, many species of amphibians exploit shallow freshwater

habitats at several life history stages. These shallow habitats can have high exposure to UVB radiation; thus, UVB may be a particularly important stressor for these species.

I used meta-analytic techniques to quantify the effects of UVB radiation on amphibians. In Chapter 3, I synthesized the results of 41 articles on the effects of UVB radiation on amphibians and found a nearly 2-fold reduction in survival of amphibians exposed to UVB radiation. Salamanders (caudates) appear to be more susceptible to damage from UVB than frogs or toads (anurans): survival of salamanders was reduced 4-fold under UVB radiation, compared to an approximately 1.5-fold reduction in anurans. Moreover, survival of larvae was much lower than survival of embryos or metamorphic individuals under UVB radiation. In addition, I used factorial meta-analytic techniques to explore the interaction between UVB radiation and other stressors in amphibian habitats. UVB radiation acted synergistically with other stressors to reduce survival of amphibians, suggesting that amphibians in contaminated habitats and amphibians exposed to *Saprolegnia ferax* may exhibit high mortality due to the interaction between stressors. Clearly, exposure to UVB radiation negatively affects many amphibian species.

UVB radiation, although recently increased by stratospheric ozone depletion, has been a stressor in amphibian habitats throughout evolutionary time (Cockell, 2001). Thus, it is important to consider the mechanisms by which amphibians can mediate damage caused by UV exposure. Amphibians can repair UVB-induced DNA damage through the use of photolyases (Blaustein *et al*., 1994, Hays *et al*., 1996;

Smith *et al*., 2002). Alternatively, amphibians can avoid damage through behavioral avoidance (e.g., van de Mortel & Buttemer, 1998) or through the use of sunscreen pigments such as melanin (Blaustein & Belden, 2003).

Behavioral avoidance of UVB radiation may help mediate the negative effects of UVB radiation on amphibians. In aquatic systems, behavioral avoidance usually requires movement out of shallow water, where UVB levels can be high, into deeper waters with lower UVB transmittance. However, these two microhabitats have very different thermal profiles; deeper waters are typically several degrees cooler than shallower waters. Water temperature is important for amphibian development as warmer temperatures speed developmental rate (Atlas, 1935; Ryan, 1941; Álvarez & Nicieza, 2002). This variation in microhabitats creates a trade-off between exploiting warm waters with high UVB levels and avoiding UVB by seeking cooler, deeper regions of ponds. I explored the microhabitat use of larvae of four species through a series of laboratory experiments, field experiments, and observational field transects at three different amphibian habitats (Chapter 4). Larvae did not avoid UVB radiation in either the laboratory or field experiments. Larvae in thermal gradients selected relatively high temperatures regardless of the UVB exposure at these temperatures. In field transects, salamander larvae were most common in deeper, cooler waters where UVB levels were lower. In contrast, anuran larvae were frequently observed in the warmer and shallower regions of each habitat. These regions also had the highest UVB levels, suggesting that anuran larvae are exposed to high levels of UVB due to thermoregulatory behavior.

Behavioral avoidance of UVB radiation is not the only mechanism amphibians may use to prevent damage from UVB. Pigments such as melanin may allow larvae to exploit warm shallow waters by absorbing harmful UVB radiation before it causes cellular damage. I tested the effectiveness of melanin as a photoprotective pigment in the larvae of two species, *Rana cascadae* and *Pseudacris regilla* (Chapter 5). I found no evidence of a photoprotective function for melanin in these larvae. In contrast, lighter colored tadpoles grew more under UVB radiation compared to darker colored tadpoles. Overall, exposure to UVB reduced survival of *P. regilla* larvae and reduced growth of *R. cascadae* larvae. Larvae of both of these species were frequently observed in very shallow water with intense solar radiation (Chapter 4).

My thesis work emphasizes the importance of UVB radiation as an environmental stressor in aquatic habitats. Many aquatic organisms are negatively affected by UVB exposure. In particular, amphibian survival is reduced by UVB exposure and the effects of UVB are intensified by other environmental stressors such as disease and contaminants. One of the most important conclusions of my research is that anuran larvae do not avoid UVB radiation; rather, anuran larvae actively seek out warm shallow waters where UVB radiation can be intense. Moreover, darker skin did not promote faster growth in larvae exposed to UVB radiation, suggesting that melanin may not act as a photoprotective pigment in these larvae.

Amphibian declines are a complex problem with many potentially interacting factors implicated as causes. My thesis work quantitatively demonstrates that UVB radiation is one factor that reduces survival of amphibians and suggests that some

species are exposed to high levels of UVB radiation in natural habitats. UVB radiation is not the sole cause of amphibian population declines. However, my work suggests that UVB radiation is an important stressor for amphibians that should not be overlooked. In addition, UVB radiation is clearly an important stressor for many other aquatic organisms and future work should consider the effects of UVB in aquatic systems, particularly the effects of UVB radiation on community structure and ecosystem function.

Bibliography

Adams, D.C., Gurevitch J. & Rosenberg, M.S. (1997). Resampling tests for meta-analysis of ecological data. *Ecology*, **78**, 1277-1283.

Adams, M.J., Schindler, D.E. & Bury, R.B. (2001) Association of amphibians with attenuation of ultraviolet-b radiation in montane ponds. *Oecologia*, **128**, 519-525.

Allen, C.D., Savage, M., Falk, D.A., Suckling, K.F., Swetnam, T.W., Schulke, T., Stacey, P.B., Morgan, P., Hoffman, M. & Klingel, J.T. (2002) Ecological restoration of Southwestern ponderosa pine ecosystems: A broad perspective. *Ecological Applications*, **12**, 1418-1433.

Altamirano, M., Flores-Moya, A. & Figueroa, F.L. (2000). Long-term effects of natural sunlight under various ultraviolet radiation conditions on growth and photosynthesis of intertidal *Ulva rigida* (Chlorophyceae) cultivated *in situ*. *Botanica Marina*, **43**, 119-126.

Altamirano, M., Flores-Moya, A., Kuhlenkamp, R. & Figueroa, F. (2003a). Stage-dependent sensitivity to ultraviolet radiation in zygotes of the brown alga *Fucus serratus*. *Zygote*, **11**, 101-106.

Altamirano, M., Flores-Moya, A. & Figueroa, F.L. (2003b). Effects of UV radiation and temperature on growth of germlings of three species of *Fucus* (Phaeophyceae). *Aquatic Botany*, **75**, 9-20.

Altwegg, R. & Reyer, H.-U. (2003) Patterns of natural selection on size at metamorphosis in water frogs. *Evolution*, **57**, 872-882.

Álvarez, D. & Nicieza, A.G. (2002) Effects of temperature and food quality on anuran larval growth and metamorphosis. *Functional Ecology*, **16**, 640-648.

Ankley, G.T., Diamond, S.A., Tietge, J.E., Holocombe, G.W., Jensen, K.M., DeFoe, D.L. & 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*). *Environmental Science Technology*, **36**, 2853-2858.

Ankley, G.T., Tietge, J.E., DeFoe, D.L., Jensen, K.M., Holocombe, G.W., Durhan, E.J. & Diamond, S.A. (1998) Effects of ultraviolet light and methoprene on survival and development of *Rana pipiens*. *Environmental Toxicology and Chemistry*, **17**, 2530-2542.

Ankley, G.T., Tietge, J.E., Holocombe, G.W., DeFoe, D.L., Diamond, S.A., Jensen, K.M. & Degitz, S.J. (2000) Effects of laboratory ultraviolet radiation and natural sunlight on survival and development of *Rana pipiens*. *Canadian Journal of Zoology*, **78**, 1092-1100.

Anzalone, C.R., Kats, L.B. & Gordon, M.S. (1998) Effects of solar UV-B radiation on embryonic development in *Hyla cadaverina, Hyla regilla,* and *Taricha torosa*. *Conservation Biology,* **12**, 646-653.

Arnold, S.A. & Wassersug, R.J. (1978) Differential predation on metamorphic anurans by garter snakes (*Thamnophis*): social behavior as a possible defense. *Ecology*, **59,** 1014-1022.

Atlas, M. (1935) The effect of temperature on the development of *Rana pipiens*. *Physiological Zoology*, **8,** 290.

Banaszak, A.T. (2003) Photoprotective physiological and biochemical responses of aquatic organisms. In: *UV Effects in Aquatic Organisms and Ecosystems* (eds. Helbling, E.R. & Zagarese, H.). Royal Society of Chemistry, Cambridge, UK, pp. 329-356.

Bancroft, B.A., Baker, N.J. & Blaustein, A.R. (2007) The effects of UVB radiation in marine and freshwater organisms: a synthesis through meta-analysis. *Ecology Letters*, **10**, 332-345.

Baud, D.R. & Beck, M.L. (2005) Interactive effects of UV-B and copper on spring peeper tadpoles (*Pseudacris crucifer*). *Southeastern Naturalist*, **4**, 15-22.

Beckerman, A., Benton, T.G., Ranta, E., Kaitala, V. & Lundberg, P. (2002) Population dynamic consequences of delayed life-history effects. *Trends in Ecology and Evolution,* **17**, 263-269.

Behrenfeld, M.J., Hardy, J.T. & Lee II, H (1992) Chronic effects of ultraviolet-B radiation on growth and cell volume of *Phaeodactylum tricornutum* (Bacillariophyceae). *Journal of Phycology,* **28**, 757-760.

Beiswenger, R.E. (1977) Diel patterns of aggregative behavior in tadpoles of *Bufo americanus*, in relation to light and temperature. *Ecology*, **58**, 98-108.

Beland, F., Browman, H.I., Rodriguez, C.A. & St-Pierre, J.F. (1999) Effect of solar ultraviolet radiation (280-400 nm) on the eggs and larvae of Atlantic cod (*Gadus morhua*). *Canadian Journal of Fisheries and Aquatic Sciences*, **56**, 1058-1067.

Belden, L.K. & Blaustein, A.R. (2002a) Exposure of red-legged frog embryos to ambient UV-B radiation in the field negatively affects larval growth and development. *Oecologia*, **130**, 551-554.

Belden, L.K. & Blaustein, A.R. (2002b) UV-B induced skin darkening in larval salamanders does not prevent sublethal effects of exposure on growth. *Copeia,* **2002**, 748-754.

Belden, L.K. & Blaustein, A.R. (2002c) Population differences in sensitivity to UV-B radiation for larval long-toed salamanders. *Ecology,* **83,** 1586-1590.

Belden, L.K., Moore, I.T., Mason, R.T, Wingfield, J.C. & Blaustein, A.R. (2003) Survival, the hormonal stress response and UV-B avoidance in Cascades Frog tadpoles (Rana cascadae) exposed to UV-B radiation. *Functional Ecology*, **17**, 409-416.

Belden, L.K., Wildy, E.K. & Blaustein, A.R. (2000) Growth, survival and behavior of larval long-toed salamanders (*Ambystoma macrodactylum*) exposed to ambient levels of UV-B radiation. *Journal Zoology London*, **251**, 473-479.

Bingham, B.L. & Reitzel, A.M. (2000) Solar damage to the solitary ascidian, *Corella inflata*. *Journal of the Marine Biological Association of the United Kingdom***,** **80**, 515-521.

Blaustein, A.R. & Belden, L.K. (2003) Amphibian defenses against ultraviolet-B radiation. *Evolution and Development*, **5**, 89-97.

Blaustein, A.R. & Kiesecker, J.M. (2002) Complexity in conservation: Lessons from the global decline of amphibian populations. *Ecology Letters*, **5**, 597-608.

Blaustein, A.R., Belden, L.K., Hatch, A.C., Kats, L.B., Hoffman, P.D., Hays, J.B., Marco, A., Chivers, D.P. and J. M. Kiesecker. 2001. Ultraviolet radiation and amphibians. In *Ecosystems and Ultraviolet Radiation* (Cockell, C.S. & Blaustein, A.R., eds). Springer Verlag Publishers, New York, pp. 63-79.

Blaustein, A.R., Edmond, B., Kiesecker, J.M., Beatty, J.J. & Hokit, D.G. (1995) Ambient ultraviolet radiation causes mortality in salamander eggs. *Ecological Applications,* **5**, 740-743.

Blaustein, A.R., Hatch, A.C., Belden, L.K., Scheessele, E. & Kiesecker, J.M. (2003) Global change: Challenges facing amphibians. In: *Amphibian Conservation* (ed. Semlitsch, R.D.). Smithsonian Books, Washington, D.C., pp 187-198.

Blaustein, A.R., Hays, J.B., Hoffman, P.D., Chivers, D.P., Kiesecker, J.M., Leonard, W.P., Marco, A., Olson, D.H., Reaser, J.K. & Anthony, R.G. (1999) DNA repair and resistance to UV-B radiation in western spotted frogs. *Ecological Applications*, **9**, 1100-1105.

Blaustein, A.R., Hoffman, P.D., Hokit, D.G., Kiesecker, J.M., Walls, S.C. & Hays, J.B. (1994) UV repair and resistance to solar UV-B in amphibian eggs: a link to population declines? *Proceedings of the National Academy of Science, USA*, **91**, 1791-1795.

Blaustein, A.R., Hoffman, P.D., Kiesecker, J.M. & Hays, J.M. (1996) DNA repair activity and resistance to solar UV-B radiation in eggs of the red-legged frog. *Conservation Biology*, **10**, 1398-1402.

Blaustein, A.R., Kiesecker, J.M., Chivers, D.P. & Anthony, R.G. (1997) Ambient UV-B radiation causes deformities in amphibian embryos. *Proceedings of the National Academy of Science, USA*, **94**, 13735-13737.

Blaustein, A.R., Kiesecker, J.M., Chivers, D.P., Hokit, D.G., Marco, A., Belden, L.K. & Hatch, A. (1998) Effects of ultraviolet radiation on amphibians: Field experiments. *American Zoologist*, **38**, 799-812.

Blaustein, A.R., Romansic, J.M., Kiesecker, J.M. & Hatch, A.C. (2003) Ultraviolet radiation, toxic chemicals and amphibian population declines. *Diversity and Distributions*, **9**, 123-140.

Blaustein, A.R., Romansic, J.M. & Scheessele, E.A. (2005) Ambient levels of ultraviolet-B radiation cause mortality in juvenile Western toads, *Bufo boreas*. *American Midland Naturalist*, **154**, 375-382.

Blaustein, A.R., Wildy, E.L., Belden, L.K. & Hatch, A. (2001) The influence of abiotic and biotic factors on amphibians in ephemeral ponds with special reference to long-toed salamanders (*Ambystoma macrodactylum*). *Israel Journal of Zoology*, **47,** 333-345.

Borer, E.T., Halpern, B.S. & Seabloom, E.W. (2006) Asymmetry in community regulation: effects of predators and primary productivity. *Ecology*, **87**, 2813-2820.

Borer, E.T., Seabloom, E.W., Shurin, J.B., Anderson, K.E., Blanchette, C.A., Broitman, B., Cooper, S.D. & Halpern, B.S. (2005) What determines the strength of a trophic cascade? *Ecology*, **86**, 528-537.

Borgeraas, J. & Hessen, D.O. (2000) UV-B induced mortality and antioxidant enzyme activities in *Daphnia magna* at different oxygen concentrations and temperatures. *Journal of Plankton Research*, **22**, 1167-1183.

Bothwell, M.L., Sherbort, D.M.J. & Pallock, C.M. (1994) Ecosystem response to solar ultraviolet-B radiation: influence of trophic level interactions. *Science*, **265**, 97-100.

Brattstrom, B.H. (1962) Thermal control of aggregation behavior in tadpoles. *Herpetologica*, **18,** 38-46.

Brattstrom, B.H. & Warren, J.W. (1955) Observations on the ecology and behavior of the Pacific treefrog, *Hyla regilla*. *Copeia*, **3**,181-191.

Browman, H.I., Rodriguez, C.A., Béland, F., Cullen, J.J., Davis, R.F., Kouwenberg, J.H.M., Kuhn, P.S., McArthur, B., Runge, J.A., St-Pierre, J.-F. & Vetter, R.D. (2000) Impact of ultraviolet radiation on marine crustacean zooplankton and ichthyoplankton: a synthesis of results from the estuary and Gulf of St. Lawrence, Canada. *Marine Ecology Progress Series,* **199**, 293-311.

Browman, H.I., St-Pierre, J.F. & Kuhn, P. (2003) Dose and dose-rate dependency in the mortality response of *Calanus finmarchicus* embryos exposed to ultraviolet radiation. *Marine Ecology Progress Series*, **247**, 297-302.

Bruggeman, D.J., Bantle, J.A. & Goad, C. (1998) Linking teratogenesis, growth, and DNA photodamage to artificial ultraviolet B radiation in *Xenopus laevis* larvae. *Environmental Toxicology and Chemistry*, **17**, 2114-2121.

Bruner MA, Shipman PA, Rao M. & Bantle, J.A. (2002) Developmental effects of ambient UV-B light and landfill leachate in *Rana blairi* and *Hyla chrysoscelis*. *Ecotoxicology and Environmental Safety*, **53**, 73-80.

Buma, A.G.J., Boelen, P. & Jeffery, W.H. (2003) UVR-induced DNA damage in aquatic organisms. In: *UV Effects in Aquatic Organisms and Ecosystems* (eds. Helbling, E.R. & Zagarese, H.). Royal Society of Chemistry, Cambridge, UK, pp. 291-327.

Cabrera, S., López, M. & Tartarotti, B. (1997) Phytoplankton and zooplankton response to ultraviolet radiation in a high-altitude Andean lake: short- versus long-term effects. *Journal of Plankton Research*, **19**, 1565-1582.

Caldwell, M.M. (1971) Solar UV irradiation and the growth and development of higher plants. *Photophysiology*, **6**, 131-177.

Caldwell, M.M., Björn, L.O., Bornman, J.F., Flint, S.D., Kulandaivelu, G., Teramura, A.H. & Tevini, M. (1998) Effects of increased solar ultraviolet radiation on terrestrial ecosystems. *Journal of Photochemistry and Photobiology B*, **46**, 40-52.

Calfee, R.D., Bridges, C.M. & Little, E.E. (2006) Sensitivity of two salamander (*Ambystoma*) species to ultraviolet radiation. *Journal of Herpetology*, **40**, 35-42.

Charron, R.A., Fenwick, J.C., Lean, D.R.S. & Moon, T.W. (2000) Ultraviolet-B radiation effects on antioxidant status and survival in the zebrafish, *Brachydanio rerio*. *Photochemistry and Photobiology*, **72**, 327-333.

Christensen, M.R., Graham, M.D., Vinebrooke, R.D., Findlay, D.L., Paterson, M.J. & Turner, M.A. (2006) Multiple anthropogenic stressors cause ecological surprises in boreal lakes. *Global Change Biology*, **12**, 2316-2322.

Cockell, C. S. 2001. A photobiological history of earth. In *Ecosystems, Evolution and Ultraviolet Radiation* (Cockell, C.S. & Blaustein, A.R. (eds.). Springer, New York, pp. 63–79.

Cockell, C.S. & Blaustein, A.R., eds (2001) *Ecosystems, Evolution and Ultraviolet Radiation*. Springer, New York, NY, USA.

Cohen, J. (1969) *Statistical Power Analysis for the Behavioral Sciences*. Academic Press Inc., New York, NY.

Collins, J.P. & Storfer, A. (2003) Global amphibian declines: Sorting the hypotheses. *Diversity and Distributions*, **9**, 89-98.

Cordi, B., Donkin, M.E., Peloquin, J., Price, D.N. & Depledge, M.H. (2001) The influence of UV-B radiation on the reproductive cells of the intertidal macroalga, *Entermorpha intestinalis*. *Aquatic Toxicology,* **56**, 1-11.

Corn, P.S. (1998) Effects of Ultraviolet Radiation on Boreal Toads in Colorado. *Ecological Applications,* **8**, 18-26.

Crump, D., Lean, D. & Trudeau, V.L. (2002) Octylphenol and UV-B radiation alter larval development and hypothalamic gene expression in the leopard frog (*Rana pipiens*). *Environmental Health Perspectives,* **110**, 277-284.

Damkaer, D.M., Dey, D.B. & Heron, G.A. (1981) Dose/dose-rate responses of shrimp larvae to UV-B radiation. *Oecologia*, **48**, 178-182.

Damkaer, D.M., Dey, D.B., Heron, G.A. & Prentice, E.F. (1980) Effects of UV-B radiation on near-surface zooplankton of Puget Sound. *Oecologia,* **44**, 149-158.

Dattilio, A., Bracchini, L., Carlini, L., Loiselle, S. & Rossi, C. (2005) Estimate of the effects of ultraviolet radiation on the mortality of Artemia franciscana in naupliar and adult stages. *International Journal of Biometeorology*, **49**, 388-395.

Day, T.A. & Neale, P.J. (2002) Effects of UV-B radiation on terrestrial and aquatic primary producers. *Annual Review of Ecology and Systematics*, **33**, 371-396.

de los Ríos, P. & Soto, D. (2005) Survival of two species of crustacean zooplankton under two chlorophyll concentrations and protection from or exposure to natural ultraviolet radiation. *Crustaceana,* **78**, 163-169.

de Mora, S., Demers, S. & Vernet, M., eds. (2000) The Effects of UV Radiation in the Marine Environment. Cambridge Environmental Chemistry Series: 10. Cambridge University Press, Cambridge, UK.

Diamond, S.A., Trenham, P.C., Adams, M.J., Hossack, B.R., Knapp, R.A., Stark, S.L., Bradford, D., Corn, P.S., Czarnowski, K., Brooks, P.D., Fagre, D., Breen, B., Detenbeck, N.E. & Tonnessen, K. (2005) Estimated ultraviolet radiation doses in wetlands in six national parks. *Ecosystems*, **8**, 462-477.

Díaz, S.B., Morrow, J.H. & Booth, C.R. (2000) UV physics and optics. In: *The Effects of UV Radiation in the Marine Environment* (eds. de Mora, S., Demers, S. & Vernet, M.). Cambridge University Press, Cambridge, UK, pp. 35-71.

Downes, S. & Shine, R. (1998) Heat, safety or solitude? Using habitat selection experiments to identify a lizard's priorities. *Animal Behaviour*, **55**,1387-1396.

Dupré, R.K. & Petranka, J.W. (1985) Ontogeny of temperature selection in larval amphibians. *Copeia*, **1985**, 462-467.

Ekelund, N.G.A. (1990) Effects of UV-B radiation on growth and motility of four phytoplankton species. *Physiologia Plantarum*, **78**, 590-594.

Ekelund, N.G.A. (1991) The effects of UV-B radiation on dinoflagellates. *Journal of Plant Physiology*, **138**, 274-278.

Ekelund, N.G.A. (1993) The effect of UV-B radiation and humic substances on growth and motility of the flagellate, *Euglena gracilis*. *Journal of Plankton Research*, **15**, 715-722.

Englund, G., Sarnelle, O. & Cooper, S.D. (1999) The importance of data-selection criteria: meta-analyses of stream predation experiments. *Ecology*, **80**, 1132-1141.

Epel, D., Hemela, K., Shick, M. & Patton, C. (1999) Development in the floating world: Defenses of eggs and embryos against damage from UV radiation. *American Zoologist*, **39**, 271-278.

Farman, J.C., Gardiner, B.G. & Shanklin J.D. (1985) Large losses of total ozone in Antarctica reveal seasonal ClO_x/NO_x interaction. *Nature*, **315**, 207-210.

Feder, M.E. (1999) Organismal, ecological, and evolutionary aspects of heat-shock proteins and the stress response: established conclusions and unresolved issues. *American Zoologist*, **39**, 857-864.

Fite, K.V., Blaustein, A., Bengston, L. & Hewitt, H.E. (1998) Evidence of retinal light damage in *Rana cascadae*: A declining amphibian species**.** *Copeia*, **1998**, 906-914.

Fleeger, J.W., Carman, K.R. & Nisbet, R.M. (2003) Indirect effects of contaminants in aquatic ecosystems. *The Science of the Total Environment*, **317**, 207-233.

Friedberg E. C., Walker G. C. and Siede W. 1995. *DNA Repair and Mutagenesis.* ASM Press, Washington D.C.

Fretwell, S.D. & Lucas, H.D. (1970) On territorial behavior and other factors influencing habitat distribution in birds. I. Theoretical development. *Acta Biotheoretica*, **19,** 16-36.

Garcia, T.S. & Sih, A. (2003) Effects of predation risk on color change and color-dependent behavior in salamander sister species *Ambystoma barbouri* and *Ambystoma texanum*. *Oecologia*, **137**, 131-139.

Garcia, T.S., Stacy, J. & Sih, A. (2004) Larval salamander response to UV radiation and predation risk: Color change and microhabitat use. *Ecological Applications*, **14**, 1055-1064.

Gergel, S.E., Carpenter, S.R. & Stanley, E.H. (2005) Do dams and levees impact nitrogen cycling? Simulating the effects of flood alterations on floodplain denitrification. *Global Change Biology*, **11**, 1352-1367.

Gleason, D.F. & Wellington, G.M. (1995) Variation in UVB sensitivity of planula larvae of the coral *Agaricia agaricites* along a depth gradient. *Marine Biology,* **123**, 693-703.

Gosner, K.L. (1960) A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* **16**, 183-190.

Grant, K.P. & Licht, L.E. (1995) Effects of ultraviolet radiation on life-history stages of anurans from Ontario, Canada. *Canadian Journal of Zoology,* **73**, 2292-2301.

Grobe, C.W. & Murphy, T.M. (1998) Solar ultraviolet-B radiation effects on growth and pigment composition of the intertidal alga *Ulva expansa* (Setch.) S. & G. (Chlorophyta). *Journal of Experimental Marine Biology and Ecology,* **225**, 39-51.

Gotelli, N.J. & Ellison, A.M. (2004) *A Primer of Ecological Statistics*. Sinauer Associates, Inc., Sunderland, MA.

Gurevitch, J. & Hedges, L.V. (1999) Statistical issues in ecological meta-analysis. *Ecology*, **80**, 1142-1149.

Gurevitch, J., Morrison, J.A. & Hedges, L.V. (2000) The interaction between competition and predation: A meta-analysis of field experiments. *American Naturalist,* **155**, 435-453.

Gurevitch, J., Morrow, L.L., Wallace, A. & Walsh, J.S. (1992) A meta-analysis of competition in field experiments. *American Naturalist,* **140**, 539-572.

Gutiérrez-Rodríguez, C. & Williamson, C.E. (1999) Influence of solar ultraviolet radiation on early life-history stages of the bluegill sunfish, *Lepomis macrochirus*. *Environmental Biology of Fishes,* **55**, 307-319.

Häder, D.-P. (1993) Effects of enhanced solar ultraviolet radiation on aquatic ecosystems. In: *UV-B Radiation and Ozone Depletion: Effects on Humans, Animals, Plants, Microorganisms, and Materials* (ed. Tevini, M.). Lewis Publishers, Boca Raton, FL, pp. 155-192.

Häder, D.-P., Kumar, H.D., Smith, R.C. & Worrest, R.C. (1998) Effects on aquatic ecosystems. *Journal of Photochemistry and Photobiology B.*, **46**, 53-68.

Häder, D.-P., Kumar, H.D., Smith, R.C. & Worrest, R.C. (2003) Aquatic ecosystems: effects of solar ultraviolet radiation and interactions with other climatic change factors. *Photochemical and Photobiological Sciences*, **2**, 39-50.

Häkkinen, J. & Oikari, A. (2004) A field methodology to study effects of UV radiation on fish larvae. *Water Research,* **38**, 2891-2897.

Häkkinen, J., Pasanen, S. & Kukkonen, J.V.K. (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.

Häkkinen, J., Vehniainen, E., Ylönen, O., Heikkila, J., Soimasuo, M.R., Kaurola, J. Oikari, A. & Karjalainen, J. (2002) The effects of increasing UV-B radiation on pigmentation, growth and survival of coregonid embryos and larvae. *Environmental Biology of Fishes,* **64**, 451-459.

Halac, S. Felip, M., Camarero, L., Sommaruga-Wögrath, S., Psenner, R., Catalan, J. & Sommaruga, R. (1997) An in situ enclosure experiment to test the solar UVB impact on plankton in a high-altitude mountain lake. I. Lack of effect on phytoplankton species composition and growth. *Journal of Plankton Research*, **19**, 1671-1686.

Han, T., Han, Y.-S., Kain, J.M & Häder, D.P. (2003) Thallus differentiation of photosynthesis, growth, reproduction, and UV-B sensitivity in the green alga *Ulva pertusa* (Chlorophyceae). *Journal of Phycology*, **39**, 712-721.

Han, B.A., Kats, L.B., Pommerening, R.C., Ferrer, R.P., Murry-Ewers, M. & Blaustein, A.R. (2007) Behavioral avoidance of ultraviolet-B radiation by two species of neotropical poison-dart frogs. *Biotropica*, **39**, 433-435.

Hargreaves, B.R. (2003) Water column optics and penetration of UVR. In: *UV Effects in Aquatic Organisms and Ecosystems* (eds. Helbling, E.R. & Zagarese, H.). Royal Society of Chemistry, Cambridge, UK, pp. 59-105.

Hatch, A.C. & Blaustein, A.R. (2002) Combined effects of UV-B, nitrate, and low pH reduce the survival and activity level of larval Cascades frogs (*Rana cascadae*). *Archives of Environmental Contamination and Toxicology,* **39**, 494-499.

Hatch, A.C. & Blaustein, A.R. (2003) Combined effects of UV-B radiation and nitrate fertilizer on larval amphibians. *Ecological Applications*, **13**, 1083-1093.

Hawkes, C.V. & Sullivan, J.J. (2001) The impact of herbivory on plants in different resource conditions: A meta-analysis. *Ecology*, **82**, 2045-2058.

Hays, J.B., Blaustein, A.R., Kiesecker, J.M., Hoffman, P.D., Pandelova, I., Coyle, D. & Richardson, T. (1996) Developmental responses of amphibians to solar and

artificial UVB sources: A comparative study. *Photochemistry and Photobiology*, **64**, 449-456.

Hedges, L.V. & Olkin, I. (1985) *Statistical Methods for Meta-Analysis*. Academic Press, Inc., Orlando, FL.

Hedges, L.V., Gurevitch, J. & Curtis, P.S. (1999) The meta-analysis of response ratios in experimental ecology. *Ecology*, **80**, 1150-1156.

Helbling, E.W. & Zagarese, H., eds. (2003) *UV Effects in Aquatic Organisms and Ecosystems*. Royal Society of Chemistry, Cambridge, UK.

Henry, B.E. & Van Alstyne, K.L. (2004) Effects of UV Radiation on Growth and Phlorotannins in *Fucus gardneri* (Phaeophyceae) Juveniles and Embryos. *Journal of Phycology*, **40**, 527-533.

Hernando, M., Carreto, J.I., Carignam, M.O., Ferreyra, G.A. & Gross, C. (2002) Effects of solar radiation on growth and mycosporine-like amino acids content in *Thalassiosira* sp, an Antarctic diatom. *Polar Biology,* **25**, 12-20.

Hernando, M., Schloss, I., Roy, S. & Ferreyra, G. (2006) Photoacclimation to long-term ultraviolet radiation exposure of natural sub-Antarctic phytoplankton communities: fixed surface incubations versus mixed mesocosms. *Photochemistry and Photobiology*, **82**, 923-935.

Hessen, D.O. ed (2002) *UV Radiation and Arctic Ecosystems*. Springer-Verlag Publishers, Berlin, Germany.

Hessen, D.O. (2003) UVR and pelagic metazoans. In: *UV Effects in Aquatic Organisms and Ecosystems* (eds. Helbling, E.R. & Zagarese, H.). Royal Society of Chemistry, Cambridge, UK, pp. 401-430.

Holomuzki, J.R. (1986) Predatory avoidance and diel patterns of microhabitat use by larval tiger salamanders. *Ecology* **, 67,** 737-748.

Houghton, R.A. & Woodwell, G.M. (1989) Global climatic change. *Scientific American*, **260**, 18-26.

Huey, R.B. & Slatkin, M. (1976) Cost and benefits of lizard thermoregulation. *The Quarterly Review of Biology***, 51,** 363-384.

Huk, T. & Kühne, B. (1999) Substrate selection by *Carabus clatratus* (Coleoptera, Caribidae) and its consequences for offspring development. *Oecologia*, **121,** 348-354.

Hutchison, V.H & Duprè, R.K. (1992) Thermoregulation. In: *Environmental Physiology of the Amphibia* (eds. Feder, M.E. & Burggren, W.W.). University of Chicago Press, Ltd, London, UK, pp. 206-249.

Hutchison, V.H. & Maness, J.D. (1979) The role of behavior in temperature acclimation and tolerance in ectotherms. *American Zoologist*, **19**, 367-384.

Intergovernmental Panel on Climate Change (2007) Climate Change 2007: The Physical Basis. Contribution of Working Group 1 to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Available at http://www.ipcc.ch/SPM2feb07.pdf

Jablonski, N.J. (1998) Ultraviolet light-induced neural tube defects in amphibian larvae and their implications for the evolution of melanized pigmentation and declines of amphibian populations. *Journal of Herpetology*, **32**, 455-457.

Jaenike, J. & Holt, R.D. (1991) Genetic variation for habitat preference: evidence and explanations. *The American Naturalist*, **137,** S67-S90.

Jones, R.B. (1998) TechDig 2.0 Software. Available at http://www.winsite.com/bin/Info?500000016137

Karentz, D., Bothwell, M.L., Coffin, R.B., Hanson, A., Herndl, G.J., Kilham, S.S., Lesser, M.P., Lindell, M., Moeller, R.E., Morris, D.P., Neale, P.J., Sanders, R.W., Weiler, C.S. & Wetzel, R.G. (1994) Impact of UV-B radiation on pelagic freshwater ecosystems: report of working group on bacteria and phytoplankton. *Archiv für Hydrobiologie Beiheft Ergebnisse der Limnologie*, **43**, 31-69.

Kats, L.B., Kiesecker, J.M., Chivers, D.P. & Blaustein, A.R. (2000) Effects of UV-B radiation on anti-predator behavior in three species of amphibians. *Ethology*, **106,** 921-931.

Kats, L.B., Petranka, J.W. & Sih, A. (1988) Antipredator defenses and the persistence of amphibian larvae with fishes. *Ecology*, **69,** 1865-1870.

Keen, W.H. & Schroeder, E.E. (1975) Temperature selection and tolerance in three species of Ambystoma larvae. *Copeia*, **1975**, 523-530.

Kelner, A. (1949) Photoreactivation of ultraviolet-irradiated *Escherichia coli*, with special reference to the dose-reduction principle and to ultraviolet-induced mutation. *Journal of Bacteriology*, **58**, 511-522.

Kerr, J.B. & McElroy, C.T. (1993) Evidence for large upward trends of ultraviolet-B radiation linked to ozone depletion. *Science*, **262**, 1032-1034.

Kiesecker, J.M. & Blaustein, A.R. (1995) Synergism between UV-B radiation and a pathogen magnifies amphibian embryo mortality in nature. *Proceedings of the National Academy of Science, USA*, **92**, 11049-11052.

Kiesecker, J.M., Belden, L.K. & Blaustein, A.R. (2001) Complex causes of amphibian population declines. *Nature*, **410**, 681-684.

Kirk, J.T.O. (1994) Optics of UV-B radiation in natural waters. *Archiv für Hydrobiologie Beiheft Ergebnisse der Limnologie*, **43**, 1-16.

Kollias, N., Sayre, R.M., Zeise, L. & Chedekel, M.R. (1991) Photoprotection by melanin. *Journal of Photochemistry and Photobiology B: Biology*, **9**, 135-160.

Koponen, P.S. & Kukkonen, J.V.K. (2002) Effects of bisphenol A and artificial UVB radiation on the early development of *Rana temporaria*. *Journal of Toxicology and Environmental Health, Part A*, **65**, 947-959.

Lacuna, D.G. & Uye, S.-i. (2000) Effect of UVB radiation on the survival, feeding, and egg production of the brackish-water copepod, *Sinocalanus tenellus*, with notes on photoreactivation. *Hydrobiologia*, **434**, 73-79.

Lacuna, D.G. & Uye, S.-i. (2001) Influence of mid-ultraviolet (UVB) radiation on the physiology of the marine planktonic copepod *Acartia omorii* and the potential role of photoreactivation. *Journal of Plankton Research,* **23**, 143-155.

Lee, R.E., Chen, C.-p. & Denlinger, D.L. (1987) A rapid cold-hardening process in insects. *Science*, **238**, 1415-1417.

Leech, D.M. & Johnsen, S. (2003) Behavioral responses—UR avoidance and vision. In: *UV Effects in Aquatic Organisms and Ecosystems* (eds. Helbling, E.R. & Zagarese, H.). Royal Society of Chemistry, Cambridge, UK, pp. 455-481..

Lesser, M.P., Turtle, S.L., Farrell, J.H. & Walker, C.W. (2001) Exposure to ultraviolet radiation (290-400 nm) causes oxidative stress, DNA damage, and expression of p53/p73 in laboratory experiments on embryos of the spotted salamander, *Ambystoma maculatum*. *Physiological and Biochemical Zoology*, **74**, 733-741.

Licht, L.E. (2003) Shedding light on ultraviolet radiation and amphibian embryos. *Bioscience*, **53**, 551-561.

Litchman, E. & Neale, P.J. (2005) UV effects on photosynthesis, growth and acclimation of an estuarine diatom and cryptomonad. *Marine Ecology Progress Series*, **300**, 53-62.

Lillywhite, H. B., Licht, P. & Chelgren, P. (1973) The role of behavioral thermoregulation in the growth energetics of the toad, *Bufo boreas*. *Ecology*, **54,** 375-383.

Lima, S. L. (1998) Nonlethal effects in the ecology of predator-prey interactions. *Bioscience*, **48,** 25-35.

Little, E.E., Calfee, R.D., Fabacher, D.L., Carey, C., Blazer, V.S. & Middleton, E.M. (2003) Effects of ultraviolet radiation on toad early life stages. *Environmental Science and Pollution Research*, **10**, 167-172.

Little, E.E. &. Fabacher, D.L. (1994) Comparative sensitivity of rainbow trout to two threatened salmonids, Apache trout and Lahontan cutthroat trout, to ultraviolet-B radiation. *Archiv für Hydrobiologie Beiheft,* **43**, 217-226.

Lizana, M. & Pedraza, E.M. (1998) The effects of UV-B radiation on toad mortality in mountainous areas of central Spain. *Conservation Biology*, **12**, 703-707.

Long, L.E., Saylor, L.S. & Soulé, M.E. (1995) A pH/UV-B synergism in amphibians. *Conservation Biology*, **9**, 1301-1303.

Lucas, E.A. & Reynolds, W.A. (1967) Temperature selection by amphibian larvae. *Physiological Zoology*, **40**, 159-171.

Madronich, S. (1993) UV radiation in the natural and perturbed atmosphere. In: *UV-B Radiation and Ozone Depletion: Effects on Humans, Animals, Plants, Microorganisms, and Materials* (ed. Tevini, M.). Lewis Publishers, Boca Raton, FL, pp.17-69.

Madronich, S. (1994) Increases in biologically damaging UV-B radiation due to stratospheric ozone reductions: a brief review. *Archiv für Hydrobiologie Beiheft Ergebnisse der Limnologie*, **43**, 17-30.

Madronich, S., McKenzie, R.L., Björn, L.O. & Caldwell, M.M. (1998) Changes in biologically active ultraviolet radiation reaching the Earth's surface. *Journal of Photochemistry and Photobiology, B*, **46**, 5-19.

Marinone, M.C., Marque, S.M., Suárez, D.A., Diéguez, M.d.C., Pérez, P., De Los Ríos, P., Soto, D. & Zagarese, H.E. (2006) UV radiation as a potential driving

force for zooplankton community structure in Patagonian lakes. *Photochemistry and Photobiology*, **82**, 962-971.

Martín, J. (2001) When hiding from predators is costly: Optimization of refuge use in lizards. *Etologia*, **9,** 9-13.

Martín, J. & López, P. (2003) Ontogenetic variation in antipredator behavior of Iberian rock lizards (*Lacerta monticola*): effects of body-size-dependent thermal-exchange rates and costs of refuge use. *Canadian Journal of Zoology*, **81,** 1131-1137.

McKinlay, A.F. & Diffey, B.L. (1987) A reference action spectrum for ultraviolet induced erythema in human skin. *CIE Journal*, **6**, 17-22.

McNamara, A.E. & Hill, W.R. (1999) Effects of UV-B dose and irradiance: comparison among grazers. *Journal of the North American Benthological Society*, **18**, 370-380.

Mendelson III, J.R., *et al*. (2006) *Science*, **313**, 48.

Merilä, J., Laurila, A. Pahkala, M. (2000) Effects of ambient UV-B radiation on early development of the common frog (*Rana temporaria*) embryos in the subarctic. *Annales Zoologici Fennici,* **37**, 51-58.

Moeur, J.E. & Istock, C.A. (1980) Ecology and evolution of the pitcher-plant mosquito. *Journal of Animal Ecology,* **49**, 775-792.

Molina, M.J. & Rowland, F.S. (1974) Stratospheric sink for Chlorofluromethanes: Chlorine atom-catalyzed destruction of ozone. *Nature*, **249**, 810-812.

Mostajir, B., Demers, S., de Mora, S., Belzile, C., Chanut, J.-P., Gosselin, M., Roy, S., Villegas, P.Z., Fauchot, J. & Bouchard, J. (1999) Experimental test of the effect of ultraviolet-B radiation in a planktonic community. *Limnology and Oceanography,* **44**, 586-596.

Mostajir, B.J., Demers, S., de Mora, S.J., Bukata, R.P. & Jerome, J.H. (2000) Implications of UV radiation for the food web structure and consequences on the carbon flow. In: *The Effects of UV Radiation in the Marine Environment* (eds. de Mora, S., Demers, S. & Vernet, M.). Cambridge University Press, Cambridge, UK, pp.310-320.

Nagl, A.M. & 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.

Nielsen, T., Björn, L.O. & Ekelund, N.G.A. (1995) Impact of natural and artificial UVB radiation on motility and growth rate of marine dinoflagellates. *Journal of Photochemistry and Photobiology, B,* **27**, 73-79.

Nilawati, J., Greenberg, B.M. & Smith, R.E.H. (1997) Influence of ultraviolet radiation on growth and photosynthesis of two cold ocean diatoms. *Journal of Phycology*, **33**, 215-224.

Nobel, P.S. (1982) Low-temperature tolerance and cold hardening of cacti. *Ecology*, **63**, 1650-1656.

Novales, R.R. & Davis, W.J. (1969) Cellular aspects of the control of physiological color changes in amphibians. *American Zoologist*, **9**, 479-88.

O'Hara, R.K. (1981) Habitat selection behavior in three species of anuran larvae: environmental cues, ontogeny, and adaptive significance. PhD thesis. Oregon State University.

Osenberg, C.W., Sarnelle, O. & Cooper, S.D. (1997) Effect size in ecological experiments: the application of biological models in meta-analysis. *The American Naturalist*, **150**, 798-812.

Ovaska, K., Davis, T.M. & Flamarique, I.N. (1997) Hatching success and larval survival of the frogs *Hyla regilla* and *Rana aurora* under ambient and artificially enhanced solar ultraviolet radiation. *Canadian Journal of Zoology* **75**, 1081-1088.

Pahkala, M., Laurila, A. & Merilä, J. (2000) Ambient ultraviolet-B radiation reduces hatchling size in the common frog *Rana temporaria*. *Ecography*, **23**, 531-538.

Pahkala, M., Laurila, A. & Merilä, J. (2001) Carry-over effects of ultraviolet-B radiation on larval fitness in *Rana temporaria*. *Proceedings of the Royal Society of London, B,* **268**, 1699-1706.

Pahkala, M., Laurila, A., Björn, L.O. & Merilä, J. (2001) Effects of ultraviolet-B radiation and pH on early development of the moor frog *Rana arvalis*. *Journal of Applied Ecology*, **38**, 628-636.

Pahkala M, Laurila A, Merilä J (2002a) Effects of ultraviolet-B radiation on common frog *Rana temporaria* embryos from along a latitudinal gradient. *Oecologia*, **133**, 458-465.

Pahkala M, Räsänen K, Laurila A *et al* (2002b) Lethal and sublethal effects of a UV-B/pH synergism on common frog embryos. *Conservation Biology*, **16**, 1063-1073.

Palen, W.J., Schindler, D.E., Adams, M.J., Pearl, C.A., Bury, R.B. & Diamond, S.A. (2002) Optical characteristics of natural waters protect amphibians from UV-B in the U.S. Pacific Northwest. *Ecology*, **83,** 2951-2957.

Palen, W.J., Williamson, C.E. & Clauser, A.A. (2005) Impact of UV-B exposure on amphibian embryos: linking species physiology and oviposition behaviour. *Proceedings of the Royal Society B*, **272**, 1227-1234.

Pang, Q. & Hays, J.B. (1991) UV-B-inducible and temperature-sensitive photoreactivation of cyclobutane pyrimidine dimers in *Arabidopsis thaliana*. *Plant Physiology*, **95**, 536-543.

Parmesan, C. & Yohe, G. (2003) A globally coherent fingerprint of climate change impacts across natural systems. *Nature*, **421**, 37-42.

Pelletier, E., Sargian, P., Payet, J. & Demers, S. (2006) Ecotoxicological effects of combined UVB and organic contaminants in coastal waters: a review. *Photochemistry and Photobiology,* **82**, 981-993.

Peterson, J.A. & Blaustein, A.R. (1992) Relative palatabilities of anuran larvae to natural aquatic insect predators. *Copeia*, **1992,** 577-584.

Rafaelli, D. (2004) How extinction patterns affect ecosystems. *Science*, **306**, 1141-1142.

Rijstenbil, J.W. (2003) Effects of UVB radiation and salt stress on growth, pigments and antioxidative defense of the marine diatom *Cylindrotheca closterium*. *Marine Ecology Progress Series,* **254**, 37-48.

Rodriguez, C.A., Browman, H.I. & St. Pierre, J.-F. (2000) High survival of neustonic zoea I larvae of American lobster *Homarus americanus* following short-term exposure to ultraviolet radiation (280-400 nm). *Marine Ecology Progress Series,* **193**, 305-309.

Roleda, M.Y., Hanelt, D., Kräbs, G. & Wiencke, C. (2004a) Morphology, growth, photosynthesis and pigments in *Laminaria ochroleuca* (Laminariales, Phaeophyta) under ultraviolet radiation. *Phycologia*, **43**, 603-613.

Roleda, M.Y., van de Poll, W.H., Hanelt, D. & Wiencke, C. (2004b) PAR and UVBR effects on photosynthesis, viability, growth and DNA in different life history stages of two coexisting Gigartinales: implications for recruitment and zonation pattern. *Marine Ecology Progress Series*, **281**, 37-50.

Root, T.L., Price, J.T., Hall, K.R., Schneider, S.H., Rosenzweig, C. & Pounds, J.A. (2003) Fingerprints of global warming on wild animals and plants. *Nature*, **421**, 57-60.

Rosenberg, M.S. (2005) The file-drawer problem revisited: a general weighted method for calculating fail-safe numbers in meta-analysis. *Evolution*, **59**, 464-468.

Rosenberg, M.S., Adams, D.C. & Gurevitch, J. (2000) *MetaWin: Statistical Software for Meta-Analysis*, Version 2.0. Sinauer Associates, Sunderland, MA.

Rosenthal, R. (1979) The "file drawer problem" and tolerance for null results. *Psychology Bulletin,* **86**, 638-641.

Rosenzweig, M. L. (1991) Habitat selection and population interactions: the search for mechanism. *The American Naturalist*, **137,** S5-S28.

Ryan, F. J. (1941) The time-temperature relation of different stages of development. *Biology Bulletin*, **81,** 432-440.

Sakai, A.K., Allendorf, F.W., Holt, J.S., Lodge, D., Molofsky, J., With, K., Buaughman, S., McCauley, D., O'Neil, P., Parker, I., Thompson, J. & Weller, S. (2001) The population biology of invasive species. *Annual Reviews in Ecology and Systematics*, **32**, 305-332.

Salo, H.M., Jokinen, I., Markkula, S.E. & Aaltonen, T.M. (2000) Ultraviolet B irradiation modulates the immune system of fish (*Rutilus rutilus*, Cyprinidae) II: blood. *Photochemistry and Photobiology*, **71**, 65-70.

Sancar, A. & Sancar, G.B. (1988) DNA repair enzymes. *Annual Review of Biochemistry*, **57**, 29-67.

Scully, N.M. & Lean, D.R.S. (1994) The attenuation of ultraviolet radiation in temperate lakes. *Archiv für Hydrobiologie Beiheft*, **43**, 135-144.

Searles, P.S., Flint, S.D., & Caldwell, M.M. (2001) A meta-analysis of plant field studies simulating stratospheric ozone depletion. *Oecologia,* **127**, 1-10.

Sedinger, J.S., Flint, P.L. & Lindberg, M.S. (1995) Environmental influence on life-history traits: growth, survival, and fecundity in Black Brandt (*Branta bernicla*). *Ecology*, **76**, 2404-2414.

Semlitsch, R.D., Scott, D.E. & Pechmann, J.H.K. (1988) Time and size at metamorphosis related to adult fitness in Ambystoma talpoideum. *Ecology*, **69**, 184-192.

Setlow, R.B. (1974) The wavelengths in sunlight effective in producing skin cancer: A theoretical analysis. *Proceedings of the National Academy of Science, USA*, **71**, 3363-3366.

Siebeck, O., Vail, T.L., Williamson, C.E., Vetter, R., Hessen, D., Zagarese, H., Little, E., Balseiro, E., Modenutti, B., Seva, J. & Shumate, A. (1994) Impact of UV-B radiation on zooplankton and fish in pelagic freshwater ecosystems. *Archiv für Hydrobiologie Beiheft Ergebnisse der Limnologie*, **43**, 101-114.

Sih, A. (1980) Optimal behavior: can foragers balance two conflicting demands? *Science*, **210,** 1041-1043.

Sih, A., Bell, A.M. & Kerby, J.L. (2004) Two stressors are far deadlier than one. *Trends in Ecology and Evolution*, **19**, 274-276.

Shurin, J.B., Borer, E.T., Seabloom, E.W., Anderson, K., Broitman, B., Cooper, S.D. & Halpern, B.S. (2002) A cross-ecosystem comparison of the strength of trophic cascades. *Ecology Letters*, **5**, 785-791.

Smith, M.A., Berrill, M. & Kapron, C.M. (2002) Photolyase activity of the embryo and the ultraviolet absorbance of embryo jelly for several Ontario amphibian species. *Canadian Journal of Zoology*, **80**, 1109-1116.

Smith, G.R., Waters, M.A. & Rettig, J.E. (2000) Consequences of Embryonic UV-B Exposure for Embryos and Tadpoles of the Plains Leopard Frog. *Conservation Biology,* **14**, 1903-1907.

Sobrino, C., Montero, O. & Lubián, L.M. (2004) UV-B radiation increases cell permeability and damages nitrogen incorporation mechanisms in *Nannochloropsis gaditana*. *Aquatic Sciences,* **66**, 421-429.

Solomon, S. (1999) Stratospheric ozone depletion: a review of concepts and history. *Reviews of Geophysics*, **37**, 275-316.

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

Starnes, S.M., Kennedy, C.A. & Petranka, J.W. (2000) Sensitivity of embryos of Southern Appalachian amphibians to ambient solar UV-B radiation. *Conservation Biology,* **14**, 277-282.

Stuart, S.N., Chanson, J.S., Cox, N.A., Young, B.E., Rodrigues, A.S.L., Fischman, D.L. & Waller, R.W. (2004) Status and trends of amphibian declines and extinctions worldwide. *Science*, **306,** 1783-1786.

Sype, W. E. (1974) Breeding habits, embryonic thermal requirements and embryonic and larval development of the Cascade frog, *Rana cascadae* Slater. PhD thesis, Oregon State University.

Tevini, M. (1993) Molecular biological effects of ultraviolet radiation. In: *UV-B Radiation and Ozone Depletion: Effects on Humans, Animals, Plants, Microorganisms, and Materials* (ed. Tevini, M.). Lewis Publishers, Boca Raton, FL, pp. 1-15.

Tietge, J.E., Diamond, S.A., Ankely, G.T., DeFoe, D.L., Holocombe, G.W., Jensen, K.M., Degitz, S.J., Elonen, G.E. & Hammer, E. (2001) Ambient solar UV radiation causes mortality in larvae of three species of Rana under controlled exposure conditions. *Photochemistry and Photobiology,* **74**, 261-268.

Ultsch, G.R., Bradford, D.F. & Freda, J. (1999) Physiology: coping with the environment. In: *Tadpoles: The Biology of Anuran Larvae* (R.W. McDiarmid & R. Altig, eds). University of Chicago Press, Chicago, IL, pp 189-214.

van de Mortel, T.F. & Buttemer. W.A. (1996) Are *Litoria aurea* eggs more sensitive to ultraviolet-B radiation than eggs of sympatric *L. peronii* or *L. dentata*? *Australian Zoologist,* **30**, 150-157.

van de Mortel, T.F. & Buttemer, W.A. (1998) Avoidance of ultraviolet-B radiation in frogs and tadpoles of the species *Litoria aurea, L. dentate,* and *L. peronii*. *Proceedings of the Linnean Society of New South Wales*, **119**, 173-179.

van de Poll, W., van Leeuwe M.A., Roggeveld, J. & Buma, A.G.J. (2005) Nutrient limitation and high irradiance acclimation reduce PAR and UV-induced viability loss in the Antarctic diatom *Chaetoceros brevis* (Bacillariophyceae). *Journal of Phycology*, **41**, 840-850.

van der Leun, J.C. & de Gruijl, F.R. (1993) Influences of ozone depletion on human and animal health. In: *UV-B Radiation and Ozone Depletion: Effects on Humans, Animals, Plants, Microorganisms, and Materials* (ed. Tevini, M.). Lewis Publishers, Boca Raton, FL, pp. 95-123.

Villafañe, V.E., Helbling, E.W., Holm-Hansen, O. & Chalker, B.E. (1995) Acclimatization of Antarctic natural phytoplankton assemblages when exposed to solar ultraviolet radiation. *Journal of Plankton Research,* **17**, 2295-2306.

Villafañe, V.E., Sundbäck, K., Figueroa, F.L. & Helbling, E.W. (2003) Photosynthesis in the aquatic environment as affected by UVR. In: *UV Effects in Aquatic Organisms and Ecosystems* (eds. Helbling, E.R. & Zagarese, H.). Royal Society of Chemistry, Cambridge, UK, pp. 357-397.

Vinebrooke, R.D. & Leavitt, P. R. (1999) Differential Responses of Littoral Communities to Ultraviolet Radiation in an Alpine Lake. *Ecology*, **89**, 223-237.

Vinebrooke, R.D., Cottingham, K.L., Norberg, J., Scheffer, M., Dodson, S.I., Maberly, S.C. & Sommer, U. (2004) Impacts of multiple stressors on biodiversity and ecosystem functioning: the role of species co-tolerance. *Oikos*, **104**, 451-457.

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

Wahl, M., Molis, M., Davis, A., Dobretsov, S., Dürr, S.T., Johansson, J., Kinley, J., Kirugara, D., Langer, M., Lotze, H.K., Thiel, M., Thomason, J.C., Worm, B. & Zeeviben-Yosef, D. (2004) UV effects that come and go: a global comparison of marine benthic community level impacts. *Global Change Biology,* **10**, 1962-1972.

Wang, M.C. & Bushman, B.J. (1998) Using the normal quantile plot to explore meta-analytic data sets. *Psychological Method*s, **3**, 46-54.

Wängberg, S.-Å., Persson, A. & Karlson, B. (1997) Effects of UV-B radiation on synthesis of mycosporine-like amino acid and growth in *Heterocapsa triquerta* (Dinophyceae). *Journal of Photochemistry and Photobiology, B,* **37**, 141-146.

Wängberg, S.-Å., Selmer, J.-S. & Gustavson, K. (1996) Effects of UV-B radiation on biomass and composition in marine phytoplankton communities. *Scientia Marina,* **60**, 81-88.

Wellington, G.M. & Fitt, W.K. (2003) Influence of UV radiation on the survival of larvae from broadcast-spawning reef corals. *Marine Biology,* **143**, 1185-1192.

Werner, E.E. (1986) Amphibian metamorphosis: growth rate, predation risk, and the optimal size at transformation. *American Naturalist*, **128,** 319-341.

West, L.J.A., Greenberg, B. M. & Smith, R.E.H. (1999) Ultraviolet radiation effects on a microscopic green alga and the protective effects of natural dissolved organic matter. *Photochemistry and Photobiology*, **69**, 536-544.

Wilbur, H.M. (1980) Complex life cycles. *Annual Review of Ecology and Systematics*, **11**, 67-93.

Wildy, E.L., Chivers, D.P., Kiesecker, J.M. & Blaustein, A.R. (1998) Cannibalism enhances growth in larval long-toed salamanders. *Journal of Herpetology*, **32**, 286-289.

Williams, D.E. & Hallock, P. (2004) Bleaching in *Amphistegina gibbosa* d'Orbigny (class Foraminifera): observations from laboratory experiments using visible and ultraviolet light. *Marine Biology*, **145**, 641-649.

Williamson, C.E. (1995) What role does UV-B radiation play in freshwater ecosystems? *Limnology and Oceanography*, **40**, 386-392.

Williamson, C.E., Metgar, S.L., Lovera, P.A. & Moeller, R.E. (1997) Solar ultraviolet radiation and the spawning habitat of Yellow Perch, *Perca flavescens*. *Ecological Applications*, **7**, 1017-1023.

Williamson, C.E., Neale, P.J., Grad, G., De Lange, H.J. & Hargreaves, B.R. (2001) Beneficial and detrimental effects of UV on aquatic organisms: implications of spectral variation. *Ecological Applications*, **11**, 1843-1857.

Wollmuth, L.P., Crawshaw, L.I., Forbes, R.B. & Grahn, D.A. (1987) Temperature selection during development in a montane anuran species, *Rana cascadae*. *Physiological Zoology*, **60**, 472-480.

Worrest, R.C. & Kimeldorf, D.J. (1975) Photoreactivation of potentially lethal, UV-induced damage to boreal toad (*Bufo boreas boreas*) tadpoles. *Life Sciences*, **17**, 1545-1550.

Worrest, R.C. & Kimeldorf, D.J. (1976) Distortions in amphibian development induced by ultraviolet-B enhancement (290-315 nm) of a simulated solar spectrum. *Photochemistry and Photobiology*, **24**, 377-382.

Ylönen, O. & Karjalainen, J. (2004) Growth and survival of European whitefish larvae under enhanced UV-B irradiance. *Journal of Fish Biology,* **65**, 869-875.

Zaga, A., Little, E.E., Rabeni, C.F. & Ellersieck, M.R. (1998) Photoenhanced toxicity of a carbamate insecticide to early life stage anuran amphibians. *Environmental Toxicology and Chemistry*, **17**, 2543-2553.

Zagarese, H.E., Feldman, M. & Williamson, C.E. (1997) UV-B-induced damage and photoreactivation in three species of *Boeckella* (Copepoda, Calanoida). *Journal of Plankton Research,* **19**, 357-367.

Zagarese, H.E., Williamson, C.E., Mislivets, M. & Orr, P.S. (1994) The vulnerability of *Daphnia* to UV-B radiation in the Northeastern United States. *Archiv für Hydrobiologie*, **43**, 207-216.

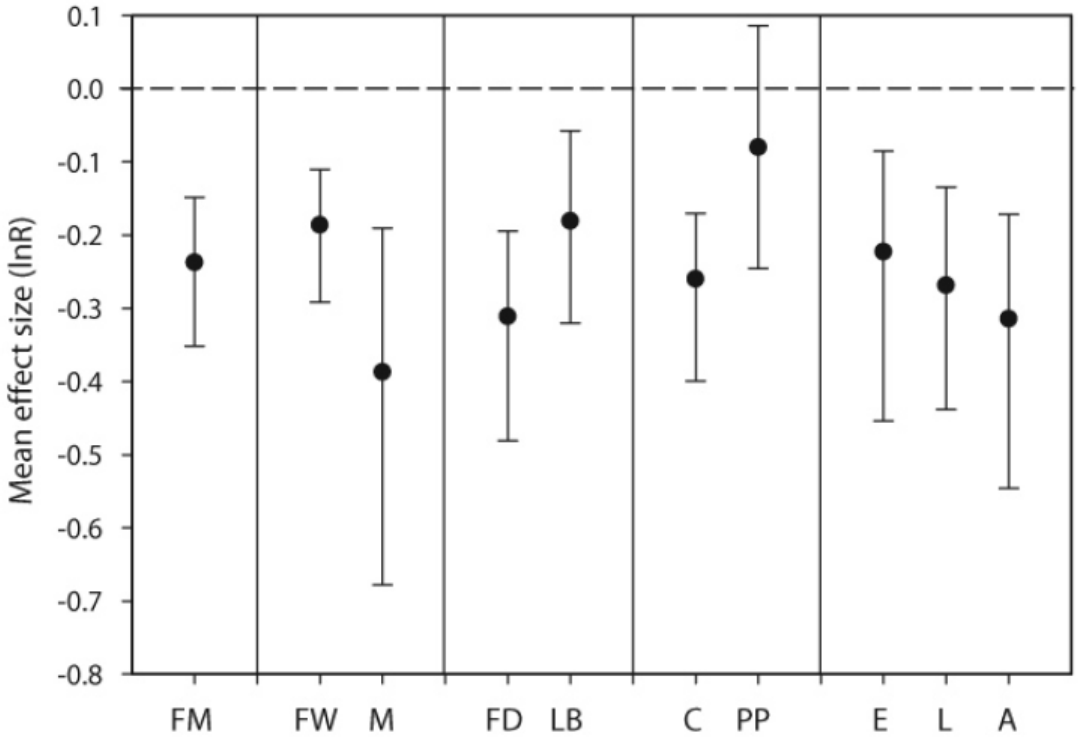
Zepp, R.G. (2003) Solar UVR and aquatic cycles. In: *UV Effects in Aquatic Organisms and Ecosystems* (eds. Helbling, E.R. & Zagarese, H.). Royal Society of Chemistry, Cambridge, UK, pp. 137-183.

APPENDICES

APPENDIX A

Criteria for inclusion in meta-analyses presented in Chapter 2:

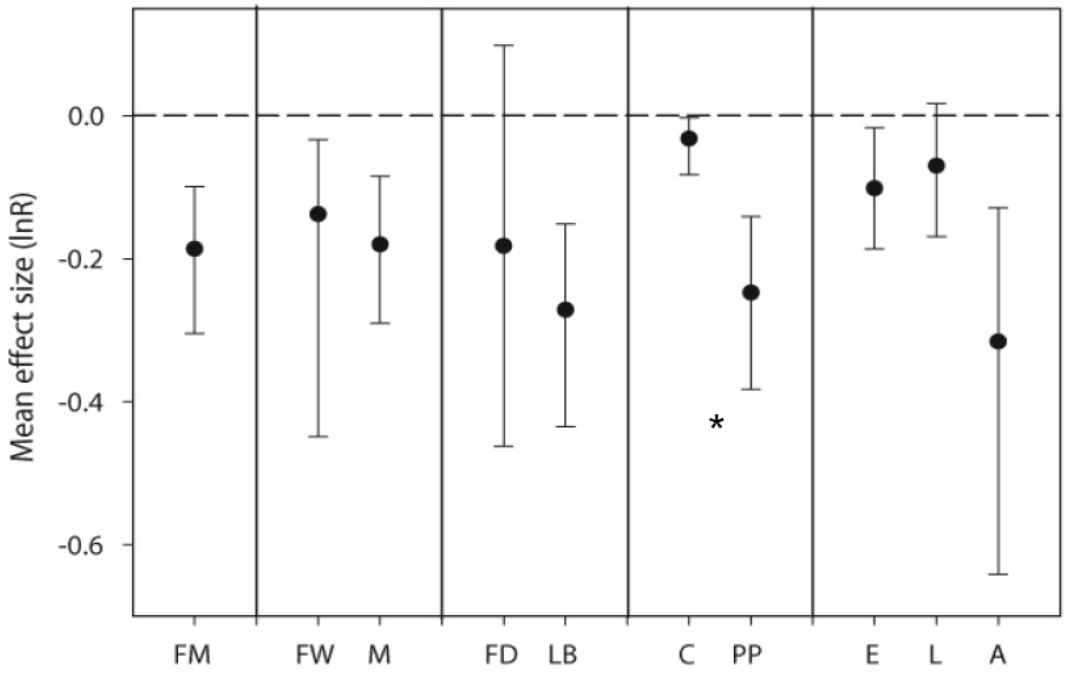
1) Each study must give the mean survival or growth for both an experimental group (UVB exposed) and an appropriate control group (UVB shielded).
2) Each study must give a measure of error and sample size for both the experimental group and control group.
3) The experimenter(s) must have directly manipulated UVB exposure through the use of UVB-specific filters (e.g., not shade).
4) Irradiance levels and/or dose must be within ambient levels for the location. If no values were given, the author(s) must state that the levels or dose was within ambient range.
5) The organisms under study can be assumed to receive some degree of exposure to UVB in natural conditions.
6) A response was measured in specific organism(s) that were identified at least to genus (e.g., experiments on "phytoplankton" would be excluded).



APPENDIX B

Appendix B: The effect of UVB radiation on survival (log response ratio [lnR]). Data are mean ± 95% confidence intervals. The mean effect size estimate is significantly different from zero if the 95% confidence intervals do not overlap with the dashed line at zero. No significant differences between any groups in the exploratory analyses were detected. FM = full model, FW = freshwater, M = marine, FD = field, LB = laboratory, C = consumers, PP = primary producers, E = embryos, L = larvae, A = adults.

APPENDIX C



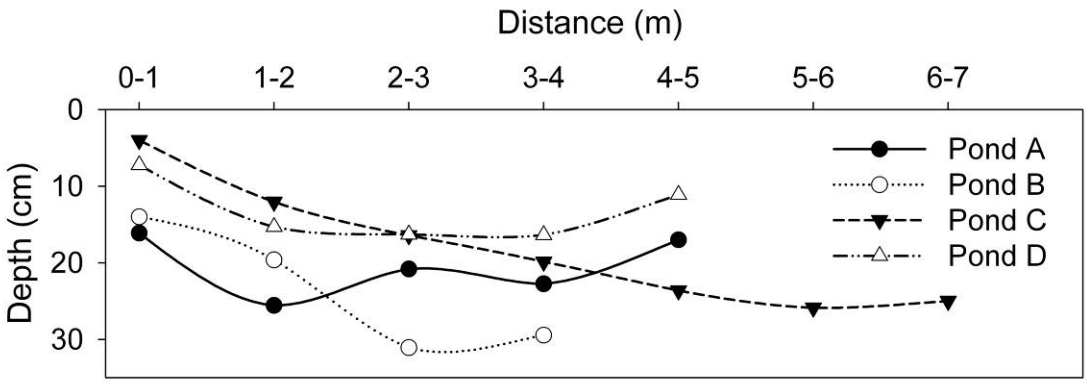
Appendix C: The effect of UVB radiation on growth (log response ratio [lnR]). Data are mean ± 95% confidence intervals. The mean effect size estimate is significantly different from zero if the 95% confidence intervals do not overlap with the dashed line at zero. A significant difference between consumers and primary producers is denoted with an asterisk. FM = full model, FW = freshwater, M = marine, FD = field, LB = laboratory, C = consumers, PP = primary producers, E = embryos, L = larvae, A = adults.

APPENDIX D

Appendix D. Amphibian species observed and transect information for each field site

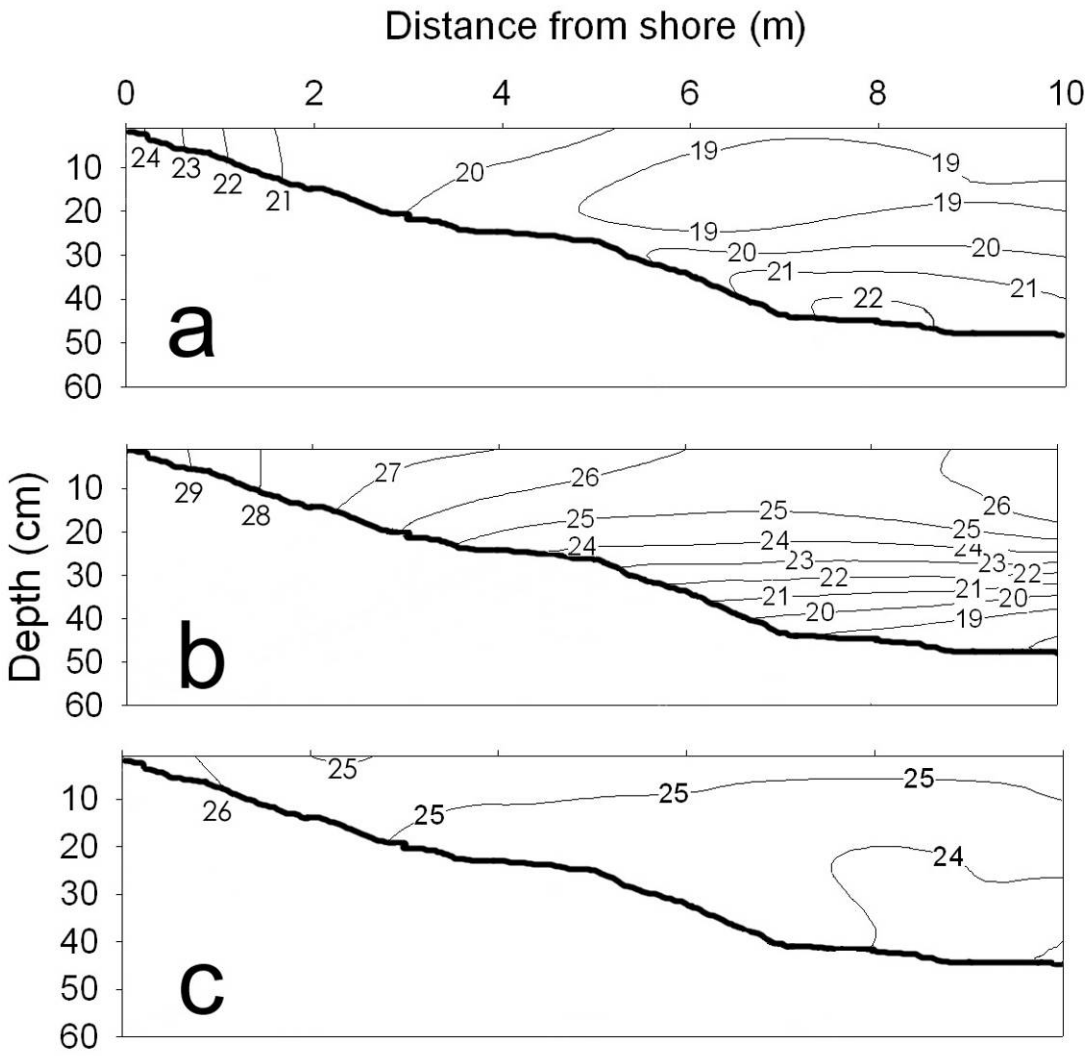
Site	Amphibian species observed	Date of transects	Number of transects	Length of transects (m)	Distance between transects (m)
Todd Lake	Western toad (*Bufo boreas*)	9-10 August 2006	3	7	35
	Cascades frog (*Rana cascadae*)				
	Pacific treefrog (*Pseudacris regilla*)				
Susan's Pond	Long-toed salamander (*Ambystoma macrodactylum*)	19-20 July 2006	3	10	20
	Cascades frog (*R. cascadae*)				
	Pacific treefrog (*P. regilla*)				
Potholes	Long-toed salamander (*A. macrodactylum*)	10 August 2006	4	4-7	--
	Cascades frog (*R. cascadae*)				
	Pacific treefrog (*P. regilla*)				

APPENDIX E



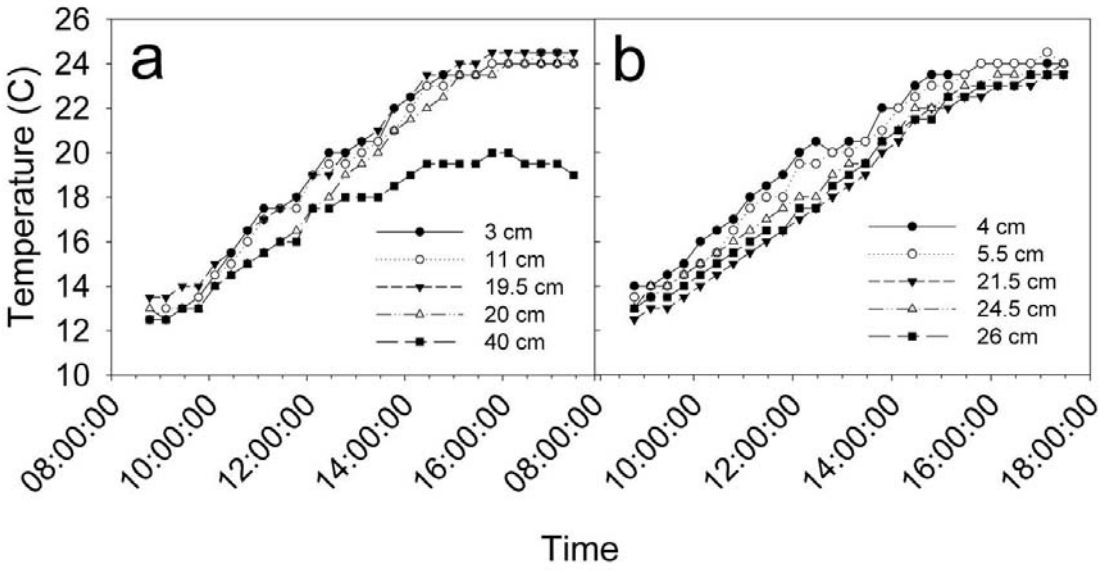
Appendix E. Depth profiles for all ponds at the Potholes.

APPENDIX F



Appendix F. Thermal contour plots for Susan's Pond on 20 July 2006. Dark line indicates the bottom of the pond. Data are morning (A), mid-day (B), and evening (C) temperatures in degrees Celsius.

APPENDIX G



Appendix G. Thermal profiles in Pond B (panel A) and Pond C (panel B) at the Potholes. Each line represents temperatures measured at the depth indicated.