The effects of UV-B on the survival of North American Amphibian species

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1. Abstract

Amphibian species are highly affected by local and global climate changes due to unique morphology which allows them to live in both aquatic and terrestrial environments. UV-B radiation has increased globally since the 1970's (Bancroft *et al.* 2008), and has been shown to cause deformities and lethality in amphibian species (Romansic *et al.* 2009; Calfee *et al.* 2006). This study examines the connection between amphibian fitness and UV-B radiation. Spotted salamander larvae (*Ambystoma maculatum*) and wood frog tadpoles (*Rana sylvatica*) were compared across three levels of UV-B exposure (0%, 80%, and 100%) for their ability to survive predation. Tadpoles and larvae survival did not correspond to UV-B levels; however, spotted salamanders were shown to be more capable of surviving predation. Despite the lack of response to UV-B, the results indicate that natural differences between species benefit survival of spotted salamanders in the short term, and further environmental factors may influence success of species survival over long term periods.

2. Introduction

Declines in biodiversity have been recorded worldwide, with amphibian populations especially affected (Blaustein and Bancroft, 2007). Sochi *et al.* (2008), using an ecological database of species, found that as many as 32% of observed amphibian species are threatened with extinction, while 43% are experiencing declines in population. Decline in amphibian biodiversity is particularly noteworthy because loss is occurring in areas undisturbed by human activity, such as remote, high altitude lakes and ponds. A singular cause for diebacks in amphibian populations has not been identified (Carey *et al.*, 2001). Most researchers believe that the cause of this loss results from a combination of both biotic and abiotic factors, with temperature, pH, bacteria, dissolved organic content (DOC), and other factors all linked to the decline of amphibian species. The global decline of amphibians has attracted much attention in the scientific community because this particular class of vertebrates has long been used as an indicator of environmental stress (Lawler *et al.* 2009).

One possible cause of the susceptibility of the Class Amphibia to environmental change is their characteristic permeable skin; these organisms do not possess scales, fur, or other protective coverings. Larval amphibians possess permeable skin that undergoes constant exchange with its aquatic environment. Similarly, amphibian eggs do not possess a hard casing or shell. These characteristics increase embryonic sensitivity to changes in the environment through increased exposure to contaminants and temperature fluctuations (Blaustein and Bancroft, 2007).

A number of recent studies have been performed to evaluate the impact of invasive species, disease, habitat loss, changes in pH, precipitation, temperature, and increased ultraviolet-B (UV-B) exposure on amphibians (Alton *et al.* 2009; Ankley *et al.* 2004; Bancroft *et al.* 2008; Romansic *et al.* 2009;). Many believe that it is a complex combination of these factors that has led to the recorded amphibian decline (Alton *et al.*, 2009). The synergistic effect of multiple stressors can combine to contribute to amphibian decline; when UV-B exposure is combined with other stressors (low pH, nitrates, pathogens, etc.) mortality rates are higher than with UV-B alone (Bancroft *et al.*, 2007). No study has provided evidence of a single factor as the cause of the current amphibian decline.

One abiotic factor that has received great scrutiny due to its relationship with amphibian mortality is UV-B. In recent decades, ozone depletion, caused in part by pollution, has elevated natural levels of UV-B (light wavelengths of 290-320 nm) (Croteau *et al.*, 2008). Amphibian species exposed to UV-B radiation during the embryonic and larval stage experience higher rates of predation and population declines (Alton *et al.*, 2009). The embryonic stage in particular can expose amphibians to potential risk from UV-B; some amphibian species lay their eggs in shallow waters to protect the developing offspring from larger predators and swifter currents (Croteau *et al.*, 2008). Shallow waters provide less protection from UV-B due to less debris and dissolved organic content, which both prevent the penetration of UV-B through water to developing embryos. UV-B exposure can also be impacted by the structure of egg masses. Eggs can be laid in large round masses, or in strings and thin sheets (Croteau *et al.*, 2008). A higher percentage of the eggs laid in clumps are protected from high levels of UV-B. Spotted salamanders (*Ambystoma maculatum)* lay their eggs in masses measuring 5-10 cm in length (Calfee *et al.* 2006) protected by a thick, jelly coating. Wood frogs (*Rana sylvatica*) have a similar egg clutch diameter; however, clutches are less spherical with more surface area (Tedder and Allen 1989). A greater surface area exposes a higher percentage of embryos to UV-B than more spherical clutches, creating a greater risk of deformity due to UV-B.

The larval stages of spotted salamanders and wood frogs also vary. Wood frogs take on average 45 days to mature from eggs to juvenile frogs (Heinrich 2000). Spotted salamander larvae have a highly variable larval lifespan; metamorphosis can occur in 2-4 months or after a period of overwintering (Phillips 1992). Exposure over a longer developmental period may lead to negative effects such as predation and deformity. Evolutionary differences between species, such as depth of egg masses, structure of egg clutches, and length of embryonic and larval development, may lead to a greater risk of predation for one species over another due to mutations that result from UV-B exposure in early development.

Increased UV-B levels also raise mortality in amphibians when coupled with changes in temperature, pH and contaminant levels (Alton *et al.*, 2009). A study conducted by Alton *et al.*

(2009) revealed that Striped Marsh frog (*Limnodynastes peronii*) tadpoles exposed to UV-B were found to be significantly smaller than tadpoles that received no UV-B treatment. Additional morphological disadvantages, such as shallower bodies and tails, in the UV-B treated tadpoles increased the risk of predation, and tadpole size and survivability experienced the greatest declines when treatments of UV-B exposure and predation were combined (Alton *et al.*, 2009). Romansic *et al.* (2009) documented the susceptibility of the Cascades frog (*Rana cascadae*) to limb malformation due to UV-B radiation. Despite the risk of UV-B exposure, Bancroft *et al.* (2008) found that the tadpoles of the Western toad (*Bufo boreas*) and the Pacific tree frog (*Pseudacris regilla)* sought warmer temperatures to stimulate their growth. The inherent behavior of amphibian larvae therefore shows an attraction to warmer temperatures; this instinct increases exposure to UV-B radiation and its negative effects. Adult amphibians exposed to UV-B radiation in embryonic and larval stages often display deformities such as hindleg reduction (Ankley *et al.*, 2004) and suppressed immune system function (Raffel *et al.*, 2006).

Despite many studies examining the effects of UV-B on amphibian survival, few studies have compared the effects of UV-B on the survival rate of two or more species. This study examined the survival rate of one anuran species and one caudate species in the presence of a common predator after exposure to high, low, and control levels of UV-B radiation during larval development. My first hypothesis is that individual species will be more susceptible to predation after exposure to high levels of UV-B. As many studies indicate (Alton *et al.* 2009; Croteau *et al.* 2008; Romansic *et al.* 2009), increased levels of UV-B during development influence the success of amphibians. My second hypothesis is that amphibian species with shorter larval stages will withstand UV-B radiation better than other species. This hypothesis was developed based on previous research that shows that tadpoles with shorter larval generations expose themselves to direct sunlight to increase body temperature and thus increase development (Bancoft *et al.* 2008). Species that expose themselves to higher rates of UV-B as a part of their life cycle will be more likely to tolerate exposure. My hypothesis will compare the survival rates of two species to determine which is the least susceptible to predation after exposure to UV-B radiation.

3. Materials and Methods

Collection and Maintenance of Amphibians

Two amphibian species, *Rana sylvatica* (wood frogs), and *Ambystoma maculatum* (spotted salamanders), were collected for observation during the larval stage of development. Collection of larvae took place between May 16 and July 21 2001 at several vernal ponds within the University of Notre Dame Environmental Research Center (UNDERC) (Figure 1). Ninety larvae were collected for both species and housed separately in plastic tubs measuring 0.49 meters in diameter. Wood frog tadpoles were fed TetraFin Goldfish Flakes (Tetra Werke, Melle, Germany). Salamander larvae were fed with zooplankton from Tenderfoot Lake captured using vertical tows. All larvae were fed once daily during UV-B treatment and predation trials. Tanks were stored outdoors for the duration of the experiment. Temperature and light cycles were held constant to match natural levels of all factors in local aquatic habitats.

UV-B Treatment

Immediately after capture, larvae were separated into three groups of ten for treatment with UV-B radiation. To create replicate trials, each treatment group contained three separate populations of ten larvae each, totaling 30 larvae per treatment and 90 larvae per species. All trials were conducted simultaneously to eliminate bias from larvae age. Larvae were exposed to UV-B during daylight hours from approximately 7am to 8pm for 7 days. Three levels of UV-B exposure were used during the experiment: high (100% UV-B), reduced (80% UV-B), and control (0% UV-B). The tubs containing larvae in the control treatment were covered by Mylar filters cut into 0.6 x 0.6 meter squares purchased from U.S. Plastics Corp. (Lima, OH). The low treatment tubs were covered with the same dimensions of window screening obtained from UNDERC storage. The high treatment received no filter to allow 100% UV-B exposure.

Predation

The predation trial began on the day following the end of the UV-B exposure period. Diving water beetle larvae (*Dytiscus verticalis*) were used as a natural predator for *Rana sylvatica* and *Ambystoma maculatum*. Predator larvae were collected from the same habitats that were used as a source for amphibian larvae (Vernal Pond U and Wood Duck Pond). One randomly selected predator was introduced into each treatment. Each of the treatments was observed once daily at 10 am after the introduction of the predator. At each time interval the number surviving predation was recorded for 5 days following the introduction of the predator.

Statistical Analysis

All data were run with Systat 13(Systat Software Inc.) were analyzed for normality using the Shapiro-Wilkes normality test. Outliers were removed from the data set to establish normality before ANOVA's were performed. P-values will be considered significant if values are less that $\alpha = 0.05$. Each species was examined separately using a repeated measure ANOVA to determine the influence of the 3 levels of UV-B on the success of larvae in the face of a predator over time. The results of the ANOVA indicate if a difference exists between the survival of a species at the different levels of radiation. The two species were further compared across the three treatments (high, low, control) for number surviving predation at each daily interval using a two-way repeated measure ANOVA. This analysis provided a statistical analysis to determine whether there is a significant difference between the survival of a single species within the three

UV-B treatments. The ANOVA also compared the difference between the overall survival of one species against the other species. Finally, the interaction between UV-B exposure and species was analyzed using the interaction effect.

4. Results

Rana sylvatica

There were no significant differences between the survival rates of wood frog tadpoles within the three UV-B treatments ($F_{2,2}$= 0.15 p= 0.86 Figure 2). All three treatments lost tadpoles at an approximate rate of 2 tadpoles per day. This pattern was consistent for each UV-B treatment for all three days. The most tadpoles consistently survived in the 0% UV-B treatment.

Ambystoma maculatum

As with *R. sylvatica*, there were no significant differences between the three treatments on the survival of larvae ($F_{2,2}$= 0.029 p= 0.97 Figure 3). The individual treatments did not decrease at a steady rate and no individual treatment had the highest number of surviving larvae across the three days of the trial.

Comparison of Species

The survival of spotted salamanders differed significantly from the survival of wood frogs ($F_{1,\,2}$= 11.34 p= 0.006), however, the difference was not caused by a response to UV-B ($F_{2,\,2}$= 0.22 p= 0.67). At each day of the trial, an average of 3 more spotted salamander larvae survived predation than wood frog tadpoles. Over the three day predation trial, the survival of the two species differed (Figure 4), but this response was largely due to predation over time ($F_{2,\,2}$= 49.21 p= 0.000002).

5. Discussion

Global climate change and ozone depletion have led to increased levels of UV-B in natural habitats (Croteau *et al.* 2008). This shift has greatly influenced declining amphibian populations, through interactions with temperature, pH, precipitation, habitat loss, and other factors (Alton *et al.*, 2009). High levels of UV-B exposure has been shown to cause deformity and mortality among frog and salamander populations (Romansic *et al.* 2009; Calfee *et al.* 2006). The results of this study suggest that over a short interval of exposure, tolerance of UV-B does not determine the success of individual species. Higher levels of radiation did not significantly impact the survival of individuals within a single species, and likewise, UV-B did not determine the difference in survival between species.

Wood frog tadpoles were expected to survive predation better due to a shorter larval stage indicating a potential tolerance of UV-B (Bancoft *et al.* 2008); less exposure to UV-B should lead to fewer deformities and other problems that would make tadpoles of this species susceptible to predators. However, UV-B did not significantly impact survival over the course of this experiment in either species. In contrast, species identity was the only factor that affected survival, with three more salamander larvae surviving predation than wood frog tadpoles on each day of the trial. This suggests that other adaptations must have determined survival in the presence of a predator. Larval salamanders are known to be cannibalistic (Phillips 1992) and are likely to have evolved defenses to ward off predators. In contrast, wood frogs develop rapidly, metamorphosing into a frog an average of 45 days after emerging from eggs (Heinrich 2000). Much energy must be invested into this process, whereas larval salamanders may take up to a year to metamorphose (Phillips 1992). Allocation of energy to growth rather than defense might serve as a detriment to wood frog survival under predation.

Short term exposure to UV-B did not act as a determining factor of fitness between species. Natural differences between species, including adaptations to survive immediate predation, were likely causes of the overall success of spotted salamander larvae. Despite the lack of influence caused by UV-B, long term effects of heightened levels of UV-B should not be discounted. This study examined only the effect of short-term UV-B exposure on amphibian species. The interaction of UV-B and temperature, as well as other abiotic and biotic factors, should be explored in future studies. Changing environmental factors influence habitats at a local scale. These changes may benefit species better equipped to tolerate fluctuations of UV-B and other factors. Small-scale shifts may cause changes in the structure of dominant species within a habitat. The long term effects of UV-B and related environmental factors should continue to be monitored to understand the dynamic influence of UV-B on amphibian species.

6. Acknowledgements

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8. Figures

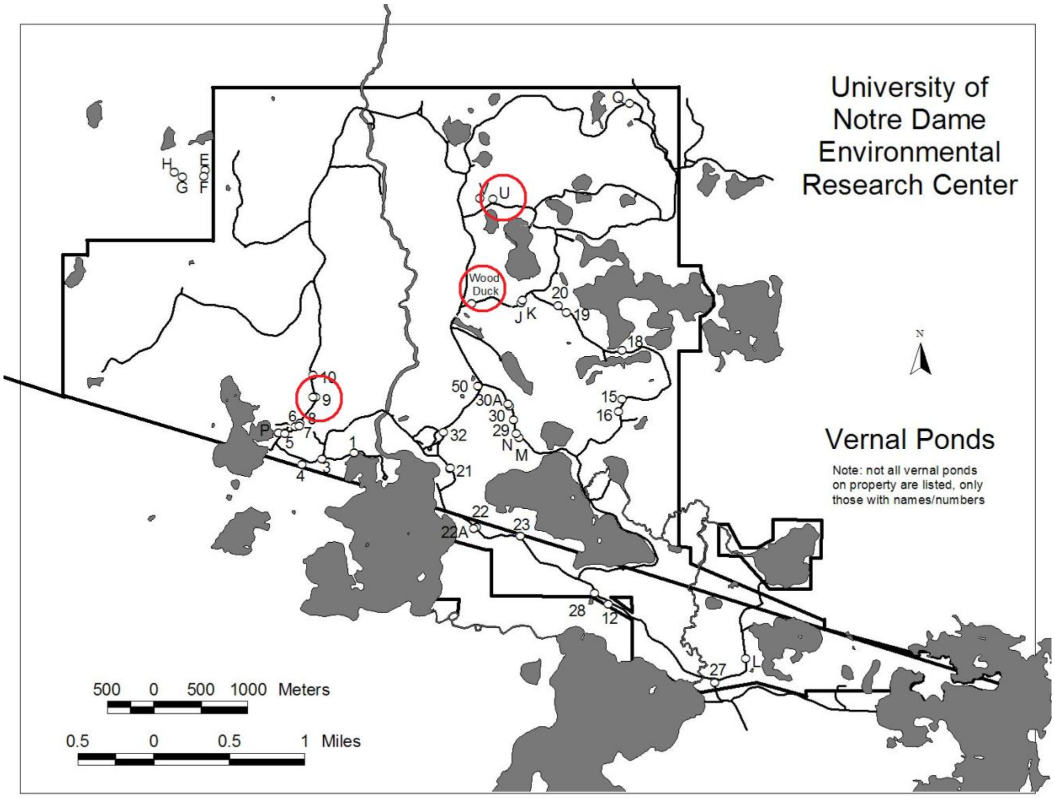

Figure 1. Map of vernal ponds on University of Notre Dame Environmental Research Center’s property. Circled ponds indicate sites of amphibian larvae and predator capture.

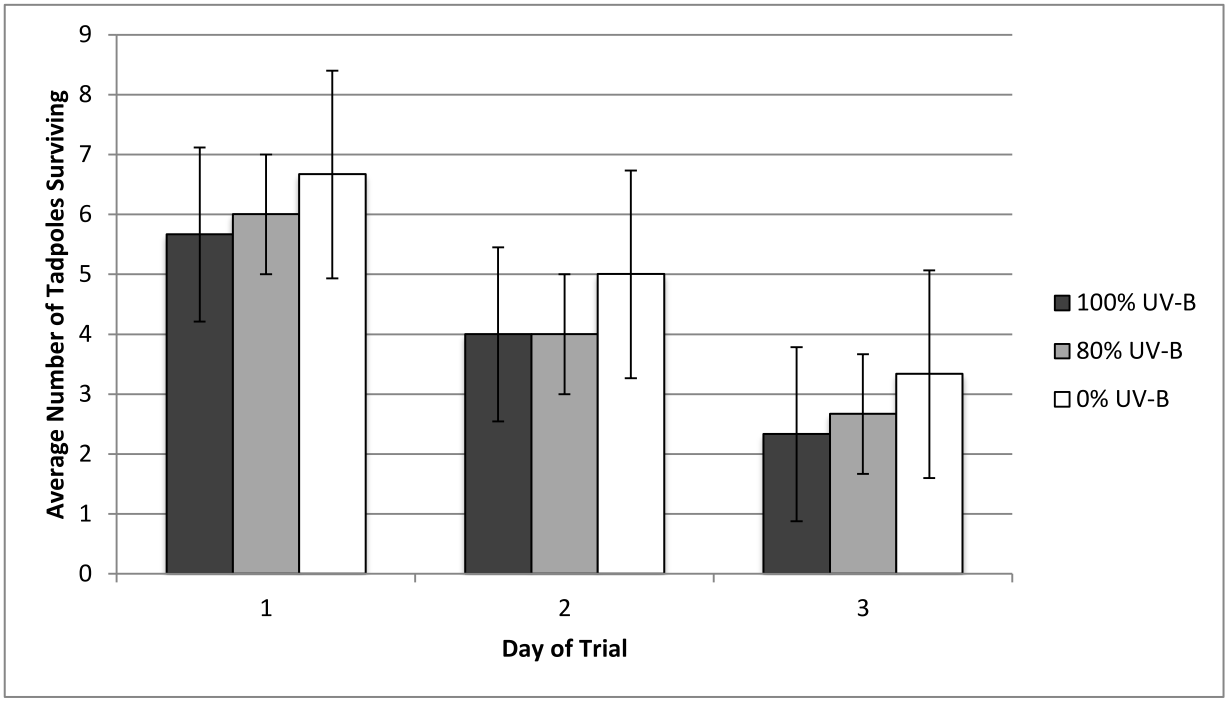

Figure 2. Average number of *R. sylvatica* tadpoles surviving predation under 3 levels of UV-B for each of 3 days of the predation trial with ± standard error bars.

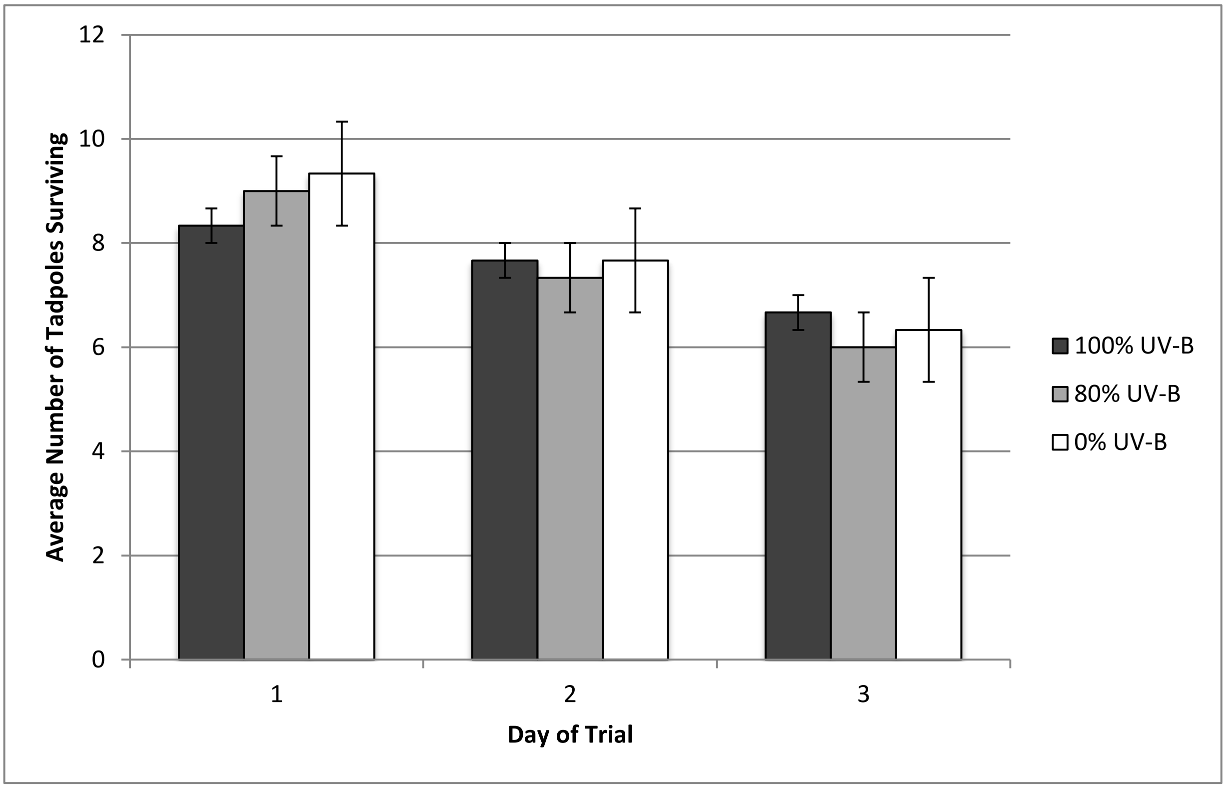

Figure 3. Average number of *A. maculatum* larvae surviving predation under 3 levels of UV-B for each of 3 days of the predation trial with ± standard error bars.

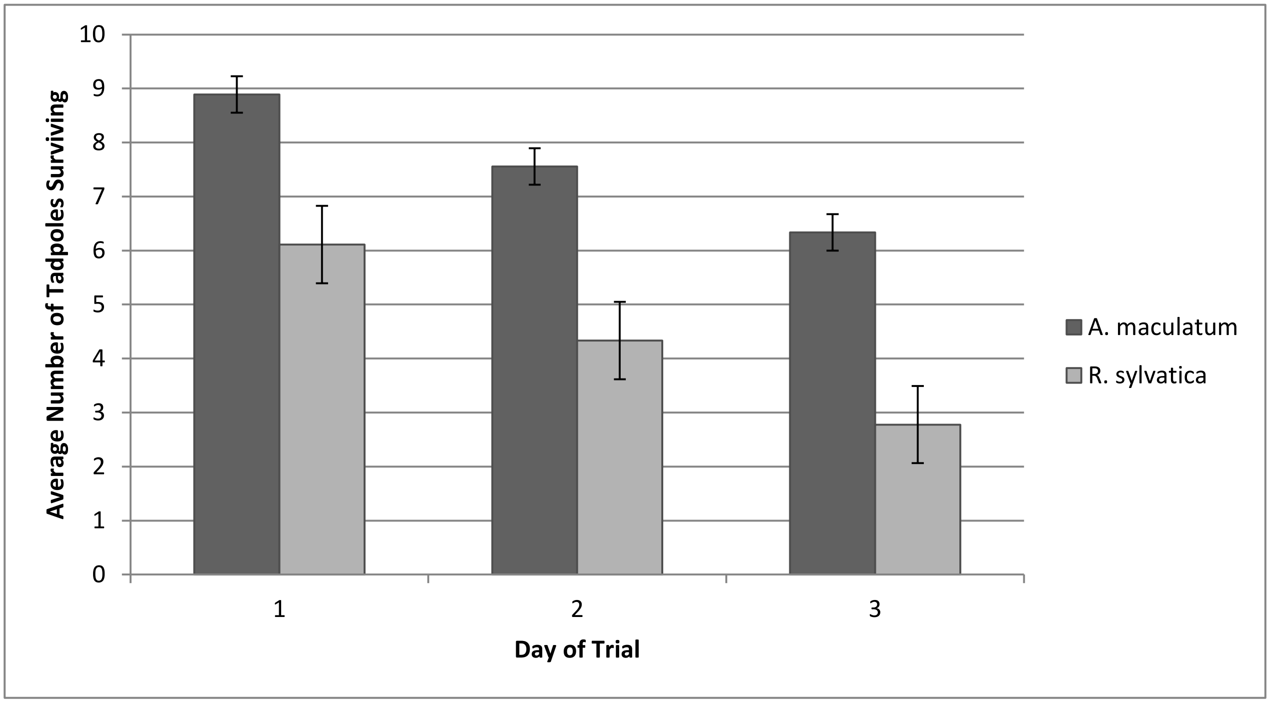

Figure 4. Average number of *A. maculatum* larvae and *R. sylvatica* tadpoles surviving predation for all 3 levels of UV-B on each of 3 days of the predation trial with ± standard error bars.