

**Effects of ultraviolet radiation  
on salamander larvae**

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## **Abstract**

Populations of many amphibian species appear to be declining. Though there is no known single cause for this decline, their widespread distribution seems to suggest involvement of global agents--increased ultraviolet radiation, for example. The objective of my study was to determine the effects of UV radiation on salamander larvae. My hypothesis was that UV radiation would have an adverse effect on the survivorship, morphology, length, and weight of these larvae. My field experiment suggested otherwise, however, in the case of survivorship, length, and weight. The larvae that had been grown for 29 days under normal conditions were longer, heavier, and had increased survivorship than the ones that were UV-protected. My experiment did show, though, that the UV-protected larvae exhibited a decreased level of morphological damage than the control. All of my results are statistically insignificant, however.

## Introduction

One of today's major environmental concerns is the depletion of the ozone layer. Estimates indicate the possibility of a 3% to 7% reduction in the total ozone content of the atmosphere--a reduction that would have serious ecological effects--as a result of the continued industrial use of these gases at about the present level. Because of the slow diffusion of gases into the stratosphere, the expected time to reach such ozone reductions would ordinarily take 100 years. A return of the atmosphere to natural (preindustrial) conditions after a complete halt in the atmospheric release of these gases could also be expected to take more than a century (Crutzer, 1995).

This is cause for great concern for amphibians, as increased levels of ultraviolet (UV) radiation as a consequence of the diminished ozone layer is now being postulated as a potential factor in reported declines of amphibian eggs (Grant, 1995). Amphibians are sensitive indicators of environmental change--most have thin, permeable skin and their gelatinous eggs lack a shell to shield developing embryos (Black, 1995).

Though these worldwide declines and even extinctions of some frogs, toads and salamanders have appeared since the mid-1980's not much research has been done in the area of UV damage on amphibian eggs. Any research done in this area has been primarily directed towards isolated cells and tissue lines or on embryonic stages using UV-C. Ultraviolet-C, however, is not environmentally relevant because most of the energy contained in this wave band is filtered out by the atmosphere (Grant, 1995). Blaustein (1994) was the first to examine the effect of ultraviolet radiation on frog and toad eggs in the field. Blaustein's (1994) results seem to provide evidence that the natural levels of UV-B were killing these amphibian eggs, especially those out in the

open.

It seems that amphibian eggs have evolutionarily adapted to minimal amounts of UV radiation. Those eggs exposed to sunlight tend to have melanin deposits over the animal hemisphere, whereas most eggs deposited in places not exposed to sunlight lack the pigment. It is hypothesized that these melanin deposits may function to protect the embryo from ultraviolet radiation or to increase the temperature of the egg through greater heat absorption. While experimental exposure of eggs to intense UV light caused mortality and abnormal development, darkly pigmented eggs are more resistant to radiation than paler eggs (Duellman, 1986).

The objectives of this study were to determine the effects of UV radiation on salamander larvae. These salamander eggs were collected from a vernal pond and half were exposed to UV light while the other half were not. My hypothesis was that a smaller percentage of the larvae exposed to the UV radiation would actually survive, and of those that did survive would exhibit an increased amount of morphological deformities and a decrease in size and weight compared to those larvae not exposed to UV radiation.

## **Materials and Methods**

### *Study Site*

The field experiment was conducted in the Upper Peninsula of Michigan at the University of Notre Dame Environmental Research Center. The study site was located on Vernal Pond 24 in Vilas Co., WI. The experiment was conducted in a reasonably accessible, shallow area, with the depth of water varying from 3 to 4 ½ feet and the

pond becoming shallower as the summer progressed. The water temperature was on average 21 degrees Celsius, and the oxygen level was measured to be 1.05 ml/g. Care was taken to pick an area not shaded by trees, and each experimental unit was located approximately 3 meters from the water's edge.

### *Experimental Design*

On June 8, 1996, 24 salamander egg masses were collected in ziplock bags from Vernal Pond 5. The eggs were found in the form of jelly masses attached to vegetation. The masses were usually on or near the surface of the water with approximately 30-100 eggs per mass. The eggs were kept cold via ice packs as they were transported back to the research facility. There, fifteen eggs were carefully cut off of each jelly mass and put in separate ziplock bags to be taken to the study site. The remaining eggs were kept in labeled rubber tubs with respirators to be observed in lab.

Fifteen eggs were put in each of 24 plastic baskets. These baskets had been covered on all sides with wire mesh (which had been carefully secured with duct tape) so as to allow water flow, yet impede the eggs/larvae from escaping and predators from entering the baskets. Baskets were divided between two experimental units which were situated side-by-side in the water. Each unit contained 12 baskets which were arranged in two rows of six. Two sunlight treatments were employed on the baskets: unfiltered sunlight, (+)UV, and sunlight filtered to remove ultraviolet wavelengths, (-)UV. The (+)UV baskets were covered with a sheet of regular plexiglas (secured with duct tape on only one end to allow lifting of the plexiglas), while the (-)UV baskets were covered with UV-absorbing plexiglas. The 24 baskets were randomly assigned as

(+)UV and (-)UV. Baskets 1, 5, 6, 9, 10, 12, 13, 15, 16, 20, 21, and 23 were under the (-)UV category.

Each set of 12 baskets was attached to a floating wooden apparatus. The wooden frame, 1.27m x 1.09m, had a stabilizing crossbar across the center, with five plastic rods U-bolted at the ends and at the crossbars. Using micro filament, each basket was tied in four places to the plastic rods to keep them securely attached. Long pieces of styrofoam were later tied to the frame to ensure that the basket tops were barely grazing the top of the water. The racks themselves were anchored down with bricks attached to opposing corners.

The site was visited daily and observations of the weather and water temperature inside and outside the baskets were made. Each basket was also examined for the number of eggs/larvae present and this data was recorded, as were necessary observations. At this time the plexiglass covers were also dusted and cleaned.

The salamander larvae were removed from the experimental units and transported to lab in ziplock bags on July 6, 1996. Once in lab, they were measured, weighed, and examined under a dissecting scope for morphological abnormalities.

## **Results**

Of the 360 salamander eggs (180 in each treatment) used in the experiment, a total of 93 survived until the end of the 29 day experiment. The surviving larvae included 51 (+)UV and 42(-)UV larvae with the number of larvae surviving in each basket varying from 0 to 15. Due to this high variation, there was no statistically

significant effect of UV radiation on survivorship ( $n=362$ ,  $p=0.309$ ) (Fig.1).

The average length of the (+)UV salamanders, at 13.10 mm, was found to be longer than that of the (-)UV salamanders, which was 12.75mm (Fig.2). The high variation in the data shows that the length did not change with treatment ( $n=93$ ,  $p=.239$ ).

The data from the weight analysis show similar results. The average weight of the (+)UV larvae was calculated to be 17.11 mg, while the average weight of the (-)UV was lower at 16.68 mg (Fig.3). Again, the high variation concludes that there is no significant effect of UV radiation on larval weight ( $n=93$ ,  $p=.599$ ).

In terms of morphological damage, such as disfigurement or absence of forearms or gills, the (+)UV larvae exhibited 13 cases of such effects in comparison to the eight observed in the (-)UV larvae (Fig. 4). These results, due again to high variation, show no difference between treatments ( $n=93$ ,  $p=.950$ ).\_

## **Discussion**

The results of my experiment, though not significant, show that salamander eggs exposed to UV light actually have a greater rate of survivorship and increased length and weight than those not exposed to UV light. Limitations in my experimental design may have caused these unexpected results. First of all, the eggs gathered were not, in some cases, newly hatched, as it was very difficult to not only bag extremely young eggs but also to keep them alive during the transition period from one vernal pond to another. Therefore, some of the eggs may have been older than others, resulting in an obvious source of error. The older eggs would necessarily be longer and heavier than

the younger ones, due to no experimental controls, but rather sheer age difference. This is seen in comparisons of the average larval length of each basket ( $n=93$ ,  $p=.010$ ) and the average weight ( $n=93$ ,  $p=.05$ ).

The low percentage of survivorship may have been largely due to predation and/or the larvae escaping. I am fairly certain a predation problem existed since no carcasses of the missing larvae were found, but they were definitely disappearing. During my observations of the baskets, I would find fewer larvae in some of them as the days progressed. In the baskets, themselves, I would find various insects I removed if possible. In addition, I failed to remove frog larvae I saw in baskets 1, 2, 10, and 20, as I thought they would not be harmful to my salamander eggs/larvae. After ending the experiment I realized all of those baskets ended with only 0-1 larvae, leading me to believe the frog larvae was the cause of the low survivor rate. Though I do not believe it was possible for the larvae to escape through the tiny holes of the wire mesh, after taking the baskets out of the water found that a little of the mesh from basket 8 had come out of the duct tape. This could have led to the escape of those salamanders.

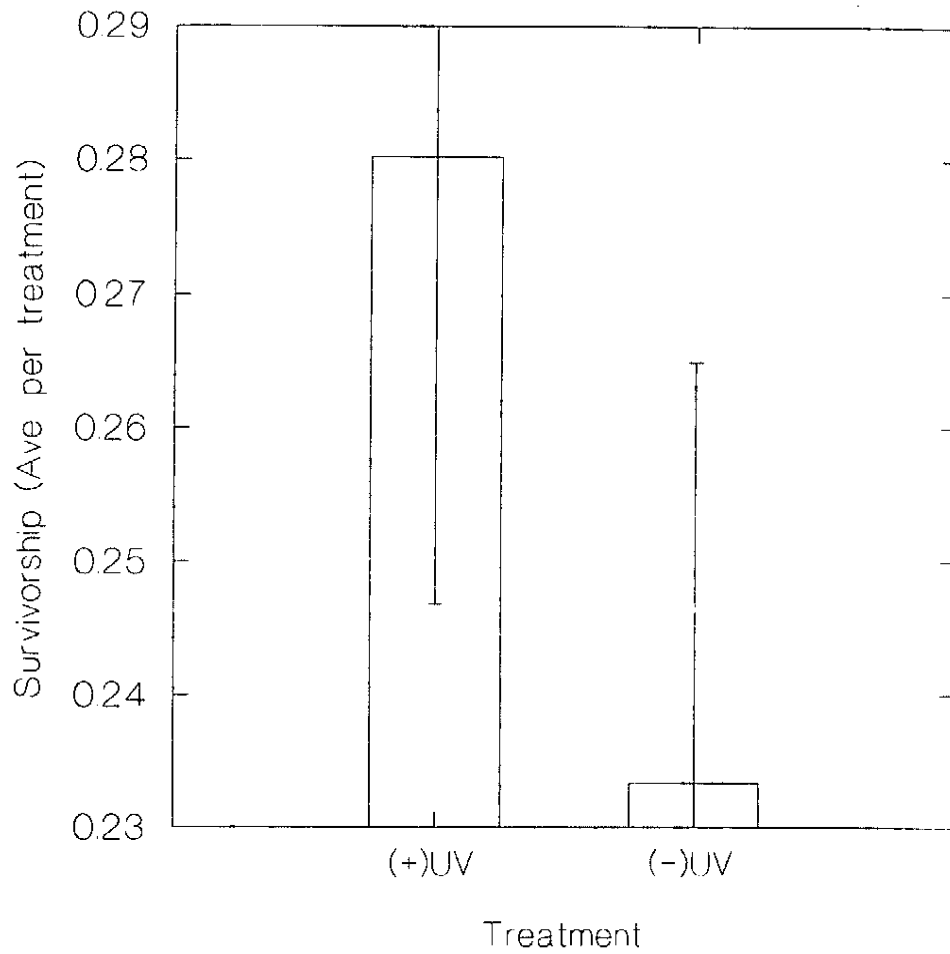
The duration of the experiment may have been a shortcoming, as well. 29 days may not have been sufficient time to get valid results. Though, during the majority of this time, the days were bright and sunny, we did have seven overcast, rainy days. Also, during my observations, I noticed the larvae tended to sit in the corner or on the edges of the basket, so they may not have been getting as much sun as they would have had they been in their natural habitat. Furthermore, the plexiglas, though cleaned regularly, tended to get condensation and dust accumulation on it, which would impede sunlight penetration.

The temperature inside each basket was fairly constant with that outside the basket, at about 24 degrees Celsius. The masses, however, felt very warm to the touch--not as they did when removed from the vernal pond in the beginning. I have not come up with an adequate explanation for this observation.

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## SURVIVORSHIP



**Fig. 1. Survivorship among (+)UV and (-)UV at the end of the 29 day experiment**

## LENGTH

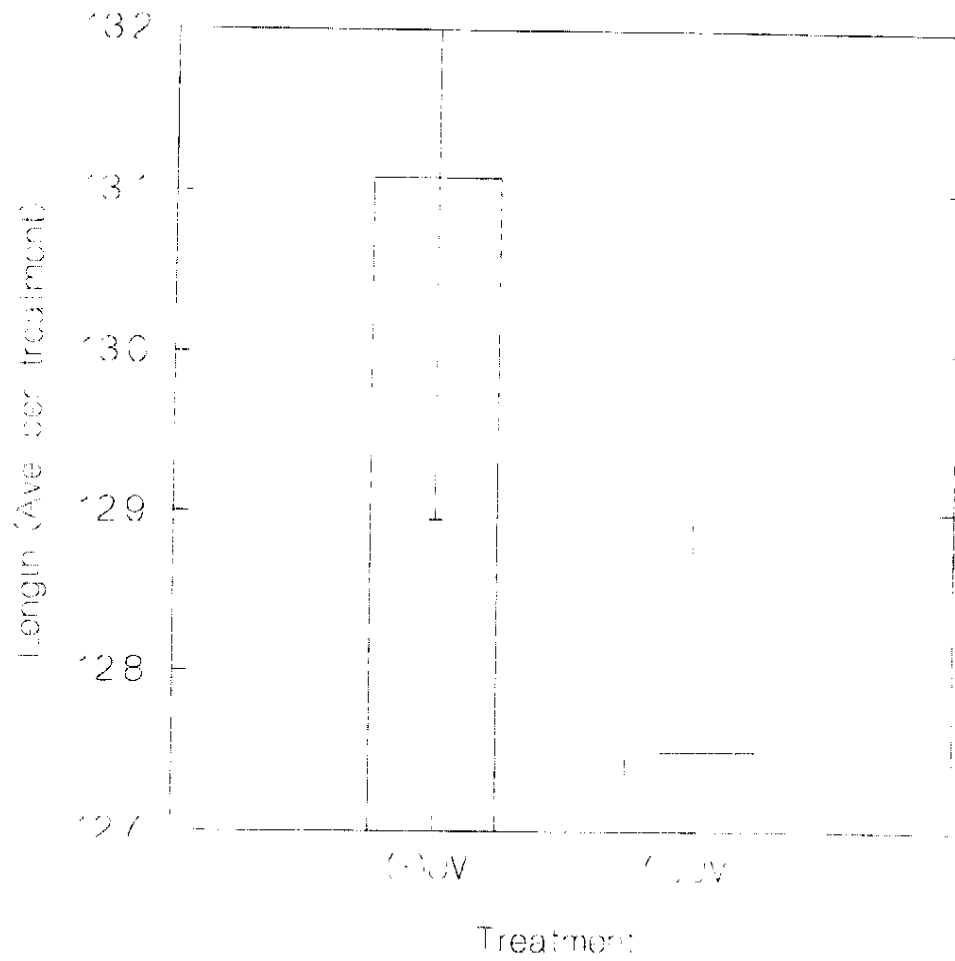


Fig. 2. Average length of (+)UV and (-)UV at the end of the 29 day experiment

# WEIGHT

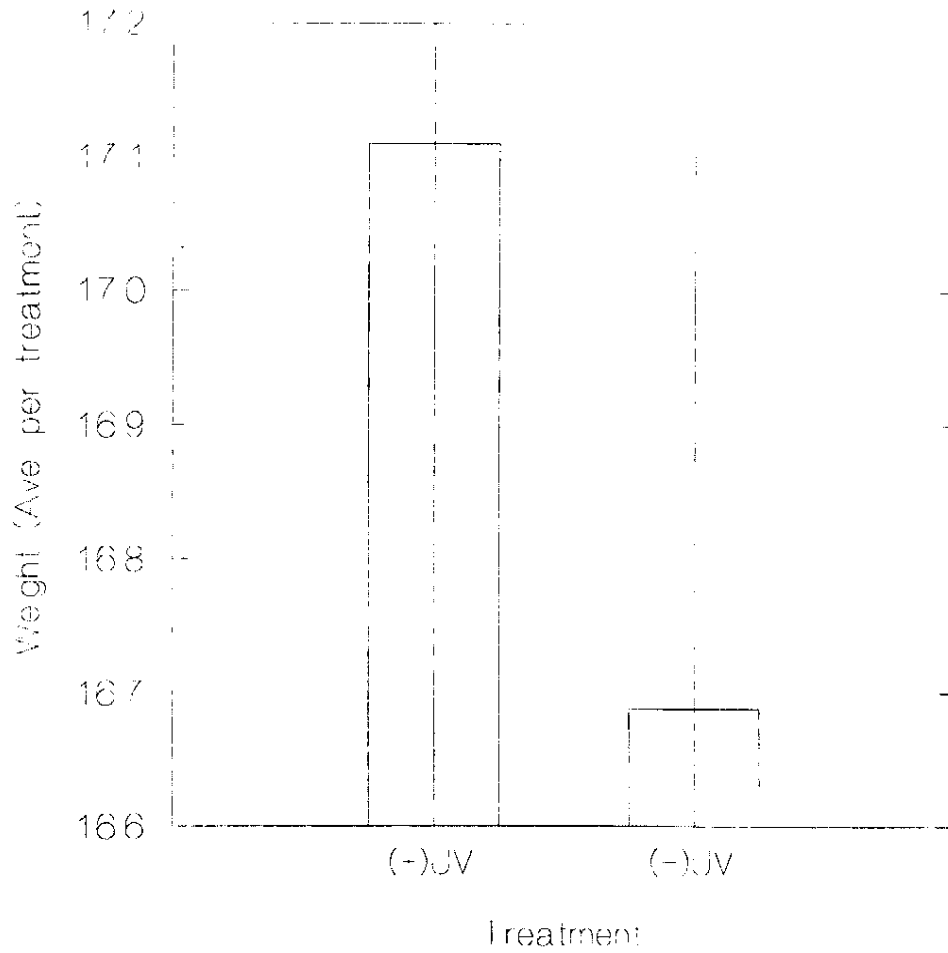


Fig. 3. Average weight of (+)UV and (-)UV at the end of the 29 day experiment

## MORPHOLOGICAL ABNORMALITIES

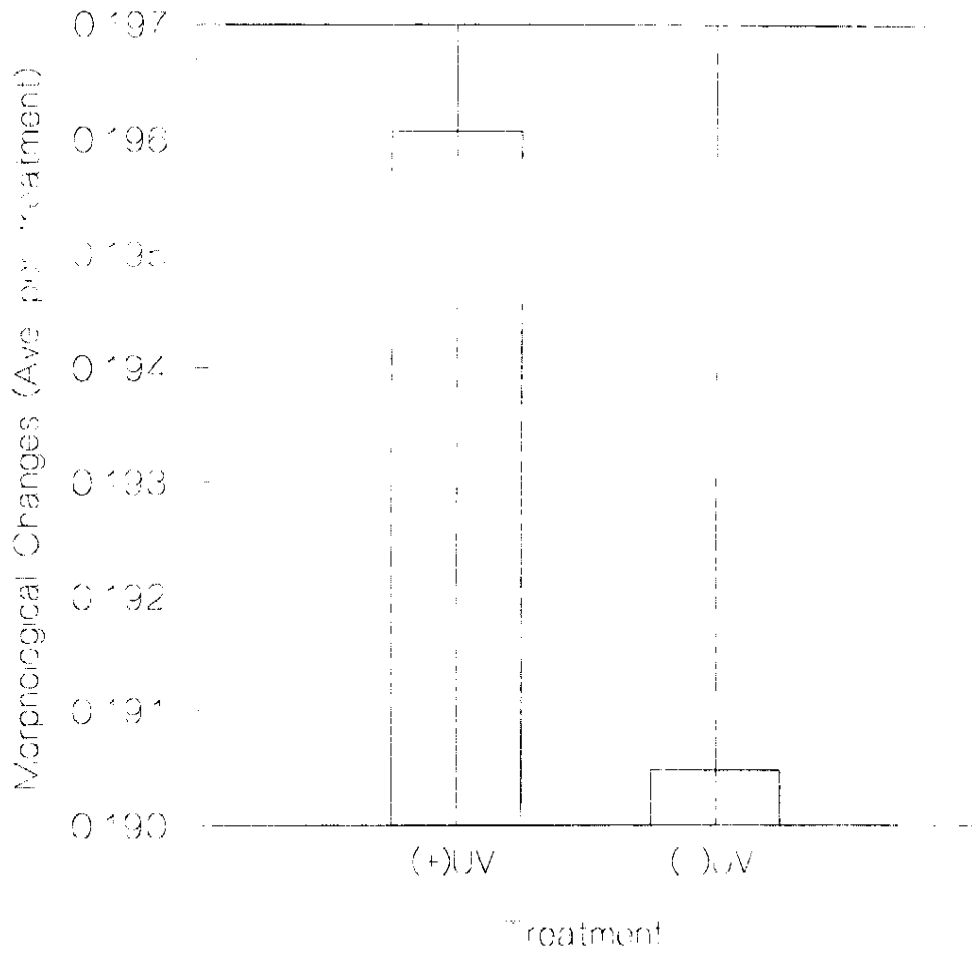


Fig. 4. Average morphological abnormalities of (+)UV and (-)UV at the end of the 29 day experiment

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