

The Effects of Natural Sun Exposure on the Intensity and Distribution of
Salamander Biofluorescence

Julianne Chairez

Whiteman Lab

8/5/25

Abstract

Salamanders have been used in a variety of different experiments to determine the health of various ecosystems. Biofluorescence was recently studied in amphibians in a study done in 2020. Not much is known about all its functions, more studies are needed to fully understand how it works and how it can be affected by environmental factors. Learning more about the functions of salamander biofluorescence could lead to a greater understanding of how pollutants and human impacts can affect salamanders and the health of their ecosystems. This project aimed to focus on tiger salamander biofluorescence and how its distribution and intensity is affected by the exposure of natural sunlight. Unmarked, 2nd year larval salamanders were taken from a single diverse pond to control for any differences among the ponds. The larvae were exposed to three different light treatments being dark, dimmed, and light. Within these light treatments there were also two time treatments that were six and twelve days. Their biofluorescence was photographed before and after the time treatments with a blue excitation light and a 500 nm long-pass filter. All images were quantified using ImageJ. The results from this experiment support the idea that salamander biofluorescence may be partly environmentally dependent and this could provide useful information for future studies to further understand the functions of amphibian biofluorescence. Furthermore, more extensive research into this topic could provide a new indicator of ecosystem health for use in conservation.

Introduction

Salamanders are effective bioindicators of ecosystem health.¹ Due to their sensitive skin, they're very susceptible to environmental changes and stressors, and may help us understand the effects of climate change.² With this information we can learn more about the ecosystems in which they reside and how they're being affected by anthropogenic changes.³

It has recently been discovered that salamanders as well as other amphibians can glow in striking green, blue, and red colors.⁴ Biofluorescence occurs in salamanders when they absorb high wavelengths of light, like ultra-violet or blue light. This absorption of light results in their brilliant fluorescent glow, which is light reemitted at a longer wavelength. Salamanders have been specifically seen to be glowing at different shades and variations of green. Studies have been conducted to determine more information about this new phenomenon.⁵

Since biofluorescence is a very recent discovery in amphibians, there is very little known about how it works. A particularly important question is understanding whether biofluorescence is environmentally sensitive, as this might allow researchers to use biofluorescence as a bioindicator of environmental change. It has been hypothesized that biofluorescence may have many functions such as mate signaling, other types of communication, or predator avoidance. It is also possible that biofluorescence is simply an inherited trait that has no function and is

not environmentally sensitive. These uncertainties show the importance of continued research into biofluorescence in amphibians.

The objective of this study was to experimentally determine how natural sunlight exposure affects the intensity and distribution (on the body) of salamander biofluorescence. This research will specifically help us to understand how one environmental parameter, sunlight, affects biofluorescence. I hypothesized that salamanders would glow brighter if exposed to sunlight, when compared to those kept in full darkness, due to the increased absorption of higher UV levels, and that the longer a salamander is kept in darkness, the less it will glow relative to those exposed to sunlight.

Methods



Figure 1. The subjects were collected from Pond 1, labeled L1 in the figure.

This study was conducted at a site that is already established by the Whiteman Lab, at the Mexican Cut Nature Preserve (MCNP) which is owned by The Nature Conservancy and is located near Gothic, Colorado. Unmarked, 2nd-year

larval salamanders were captured from Pond 1 at MCNP (**Figure 1**), to control for any differences among the ponds. Larvae were chosen due to the potential plasticity of their biofluorescence. At this stage of life, they are still growing and their biofluorescence is changing which makes them the best subjects for this experiment.⁶

This specific pond was chosen because it is the largest pond at MCNP, and it has the largest number of salamanders. This project was a 3x2 factorial design, with three light treatments and two time treatments. The three time treatments were ambient light with no cover, dimmed light with a white cover, and dark with a black cover. Within these three treatments there were also two time treatments of six and twelve days of exposure.

There were ten replicates of each treatment, which required 30 larval salamanders. All larvae were measured, matched for size across treatments, and photographed before entering the experiment. Salamanders were kept, monitored, and fed *ad libitum* in separate 5.7-liter Sterilite boxes that were randomly positioned on the floor of a Weather Port. (**Figure 2**)

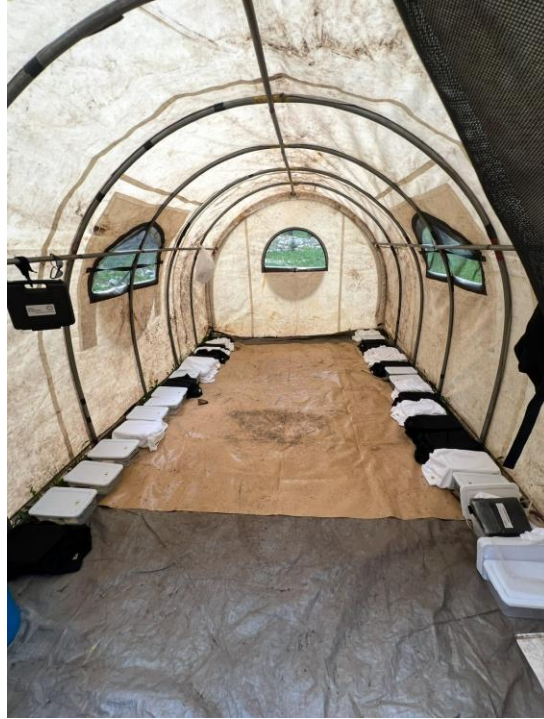


Figure 2. Subjects were arranged along the sides and randomized to try and control for light differences entering the Weather Port.

Three empty tanks, one for each treatment, were used to monitor daily UV light exposure using a SPER Scientific UV light meter. The temperature of each container was also taken to check for all factors that could have affected their biofluorescence.

After their time treatments, larvae were photographed, measured, and carefully released back into Pond 1 after the full 12 days. Larvae were photographed in a portable dark room using top and bottom shots. They were photographed under a blue excitation light using a 500 nm long-pass filter to accurately detect their biofluorescence. The camera used was a Sony A6400 mirrorless camera. It had specific settings in place such as; ISO 10,000, shutter

speed 1/60, FSTOP 2.8, and was on manual to accurately capture and show the biofluorescence.

The brightness of each larvae was quantified using ImageJ using previously determined methods⁶. These methods consisted of importing two images per larval salamander of their dorsal and ventral sides into ImageJ and then converting them to grayscale. They were converted to grayscale to simplify the brightness intensity measurement from a scale of 0 (black) to 255 (white). This grayscale image was saved as a tiff and to clearly outline the regions of interest (ROIs), the brightness of the grayscale image was increased. The ROIs were outlined in ImageJ using the freehand tool. The ROI was made of the full body for both ventral and dorsal sides to quantify brightness of the entire animal. The mean and maximum brightness were calculated for this ROI.



Figure 3. An example photo of the images analyzed, specifically the ventral side of the larvae.

Results

There was a significant relationship between UV and treatment (kw, $X^2=29.19$, $df=2$, $p<0.001$). Specifically, the ambient treatment was higher than the dark treatment (Pairwise Willcox, $p<0.001$). Ambient was higher than dimmed (Pairwise Willcox, $p<0.001$). Dimmed and dark treatments were not different.

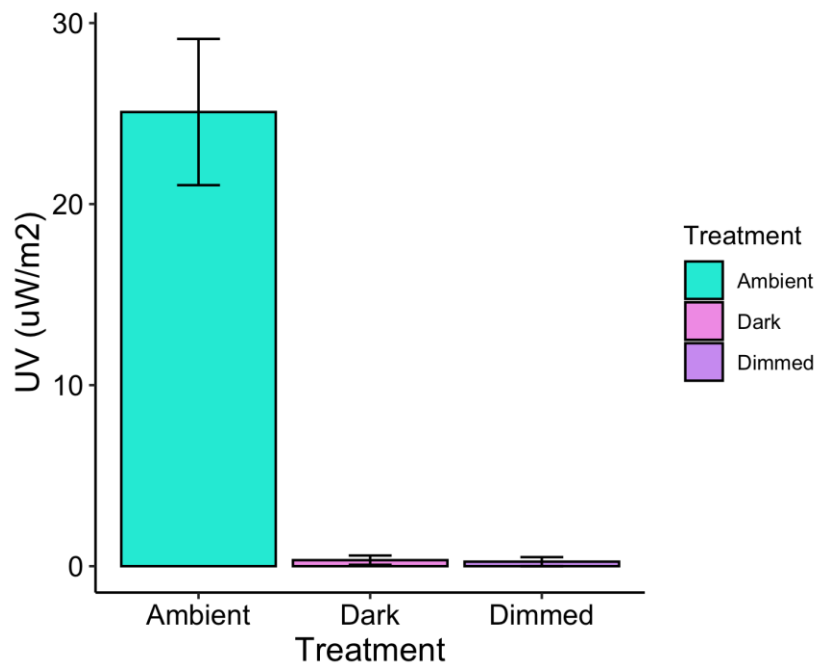


Figure 4. This shows the drastic difference of UV exposure between ambient and the other treatments. Dark and dimmed were not different.

There was no significance between the temperature differences among the three treatments ($F=0.18$, $p=0.83$).

For the dorsal side of the salamander, brightness was affected by treatment (ANOVA, $F=7.029$, $p=0.002$), by the amount of time exposed to the light treatment,

(ANOVA $F=150.7$, $p<0.001$), and the interaction between treatment and time (ANOVA, $F=3.177$, $p=0.018$).

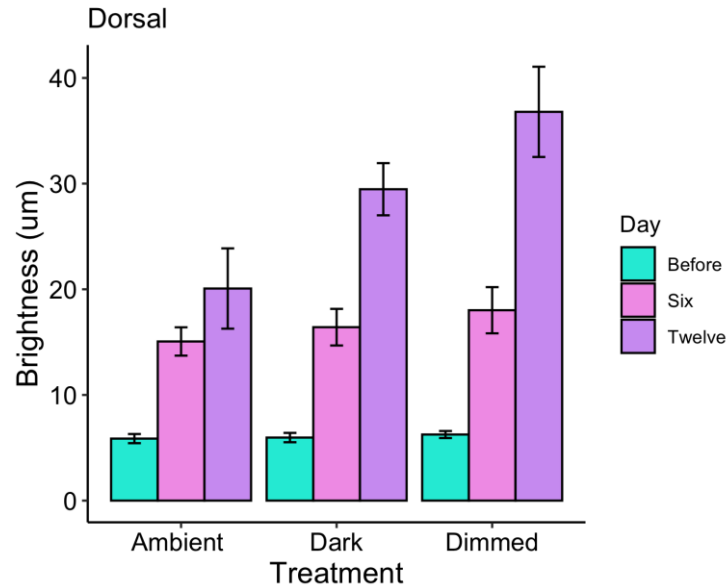


Figure 5. This shows the brightness growth differences between the light and time treatments on the dorsal side.

For the ventral side of the salamander, brightness was also affected by treatment (ANOVA, $F=5.398$, $p=0.006$) and by the amount of time exposed to the given treatment (ANOVA, $F=64.177$, $p<0.001$). There was no interaction between treatment and day on the ventral side.

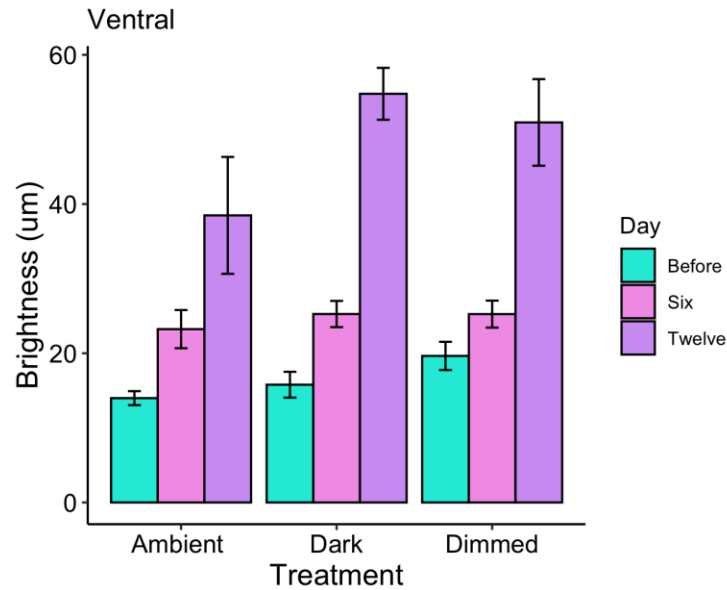


Figure 6. **This shows the brightness growth differences between the light and time treatments on the ventral side.**

Discussion

The results from both figures 5 and 6 support the hypothesis that amphibian biofluorescence is partly environmentally dependent. There was a difference in the way the larval salamanders changed throughout the different treatments, and they changed in all the different treatments as time passed. This behavior could be utilized in different ways. There are many species that use light or glow to catch and hunt prey⁷. This could be a potential use for their biofluorescence getting brighter in darker conditions. They could also use their biofluorescence to communicate with each other and see others. Knowing this data could help with future studies and to further understand the uses of salamander biofluorescence. The results gave a deeper understanding into how it all works and opens many doors for future research.

Throughout this experiment there were gaps throughout the process where error could have been introduced. For example, during photo capturing there may have been some errors in the accuracy of the photos. The container that kept the larval salamanders for photographing was too big, which allowed them to always move around. The photo taking skills could have also been a contributing factor to error for the before pictures specifically. Overall, there are different steps that should be taken in future research studies to control for these possible errors in the data.

For future studies, there are some things that could be improved for another project like this one. A smaller photo container could be useful to keep the larvae from moving too much. A more controlled lab setting could also help regulate how much light is reaching them. It would also be useful to have a longer-term study to see if anything really changes within a longer period in controlled conditions. Photo capturing techniques should be proficient from the start to take good and accurate biofluorescence photos for all salamanders before the treatments. It would also be interesting to test different salamander ages and morphs to see how much they can change as well.

Overall, this experiment and data has the potential to lead us to more advancements in a variety of topics. This data not only gives more insight into how salamander biofluorescence works but it also opens the doors for further research into this behavior. More research can potentially help with conservation efforts for certain ecosystems that contain salamanders and even different amphibian species.

Their biofluorescence could be utilized as a bioindicator of an ecosystem's health, which could be incredibly valuable and useful for conservation efforts.

References

1. Siddig, A. A., Ochs, A. & Ellison, A. M. Do terrestrial salamanders indicate ecosystem changes in New England forests? *Forests* 10, 154 (2019).

2. Sumanasekara, V. D. W., Dissanayake, D. & Seneviratne, H. T. J. Review on use of amphibian taxa as a bio-indicator for watershed health and stresses. in *NBRO Symposium Proceedings* vol. 1 (2015).
3. Southerland, M. T. *et al.* Stream salamanders as indicators of stream quality in Maryland, USA. (2004).
4. Lamb, J. Y. & Davis, M. P. Salamanders and other amphibians are aglow with biofluorescence. *Sci. Rep.* 10, 2821 (2020).
5. Cox, J. L. & Fitzpatrick, B. M. Biofluorescent sexual dimorphism revealed in a southern Appalachian endemic salamander, *Plethodon metcalfi*. *Sci. Rep.* 13, 3588 (2023).
6. Neufeld, T. M., Owen, D. A. S., Crayton, L. & Whiteman, H. H. Biofluorescence varies ontogenetically in a polyphenic amphibian. *Nat. Commun.*
7. Chuang, C.-Y., Yang, E.-C. & Tso, I.-M. Deceptive color signaling in the night: A nocturnal predator attracts prey with visual lures. *OUP Academic* (2007). Available at: <https://journals.aai.org/beheco/article-abstract/19/2/237/212321?redirectedFrom=fulltext>. (Accessed: 9th August 2025)

