Can Social Dynamics and Conspecific Density Influence the Intensity and Distribution of Tiger Salamander Biofluorescence?

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Abstract

Salamanders have been used in a variety of experiments to determine the health of various ecosystems. Biofluorescence was recently discovered in amphibians, but little is known about its possible 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 climate change and other human impacts can affect salamanders and their ecosystem health. This project aims to focus on tiger salamander biofluorescence, and how its distribution and intensity is affected by conspecific density and social dynamics. During 2025, we found that different light exposures affected tiger salamander larval biofluorescence. Building off of this result, we now aim to understand if conspecific density and the social dynamics that occur among larvae might also influence this phenomenon. To conduct this research we captured Arizona tiger salamander larvae from a single pond to control for any differences among the ponds. They were manipulated in an experimental design with four different density treatments. We took ventral and dorsal biofluorescence photos of them before and after the duration of the experiment, using a blue excitation light and a 500 nm long-pass filter to accurately capture their biofluorescence. The brightness of each salamander was quantified using ImageJ software. I hypothesized that the salamanders exposed to increased density in small bins would lead to a brighter glow because of the potential social role of biofluorescence or the increased stress

from being in a small space with another larva competing for food. Both ventral and dorsal biofluorescence photos did not show significant interactions with the treatments. A small trend in the dorsal data could be seen so this suggests that density may not be plastic as sun exposure was for them last year. This potential trend is worth revisiting in future studies. Future analysis of the data collected during this study is still underway, due to the unexpected occurrence of metamorphosis in some of the larvae.

Introduction

Salamanders have sensitive skin that is very susceptible to environmental changes and stressors. Due to this sensitivity, their presence in an environment has been utilized as a highly effective bioindicator of ecosystem health (Siddig et al., 2019), including the effects of climate change (Sumanasekara et al., 2015). The information their presence provides can help us learn more about the ecosystems in which they reside and how they are being affected by anthropogenic changes (Southerland et al., 2004).

It has recently been discovered that salamanders, as well as other amphibians, can glow in striking green, blue, and red colors (Figure 1)(Lamb & Davis, 2020). Biofluorescence occurs in salamanders when they absorb high wavelengths of light, like ultraviolet or blue light. This absorption of light results in their brilliant fluorescent glow, which is light reemitted at a longer wavelength(Cox & Fitzpatrick, 2023).

Figure 1. Biofluorescing salamander, dorsal shot.

Since biofluorescence is a very recent discovery in amphibians, there is very little known about how it works.

A particularly important question about this phenomenon 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 (Lamb & Davis, 2020).

These uncertainties show the importance of continued research into biofluorescence in amphibians. In 2025, I conducted an experiment that showed that biofluorescence is environmentally-dependent, such that salamander larvae that were exposed to different amounts of light biofluoresced differently. In that

experiment, larvae were reared individually, and I was unable to test the potential density or social context of biofluorescence. Building upon this research, the objective of this study is to experimentally determine how the potential interaction of larval density and social cues affects the intensity and distribution (on the body) of salamander biofluorescence.

I hypothesized that increased density would lead to an overall brighter glow because of the potential social role of biofluorescence. Specifically, I predicted an interaction between increased conspecific density and glow intensity, such that salamanders will glow brightest in environments with higher salamander densities and smaller bins because of the potential social role of biofluorescence.

Methods

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, to control for any differences among the ponds (Figure 2). 2nd-year larvae were chosen due to the

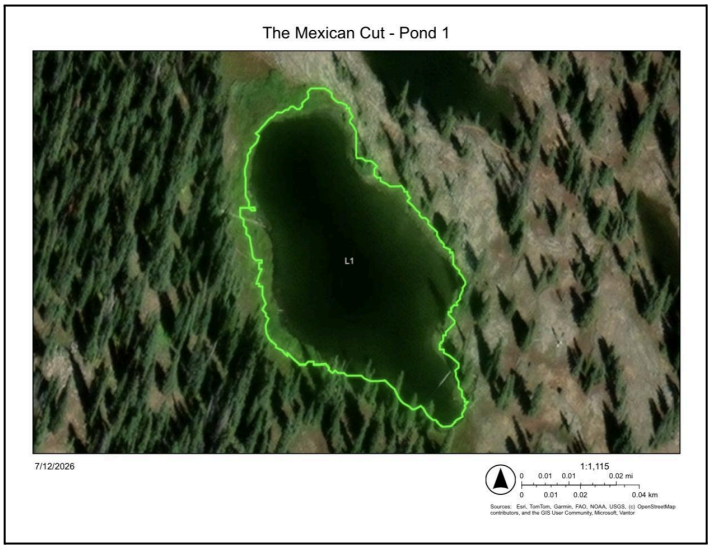

Figure 2. Map of pond 1 at the Mexican Cut.

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 as well as our previous one (Neufeld et al., 2025). This specific pond was chosen to control for differences among the ponds and because it is the largest pond at MCNP, it also has the largest density of salamanders.

This project was a 2x2 experimental design with four density treatments (1 larva in a small tank, 2 larvae in a small tank, 1 larva in a large tank, and 2 larvae in a large tank) which allowed us to explore the effects of density and potential social dynamics. There were 6 replicates of each treatment, which required 36 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 12.4 liter plastic storage boxes for the small tank treatments and 16 liter plastic storage boxes for the large tank treatments. They were positioned on the floor of a Weatherport (Figure 3). An empty tank was used to

Figure 3. Experiment setup in weatherport.

monitor daily temperature, UV, and light exposure using a multispectrum light meter. After 14 days, larvae were photographed, measured, and carefully released back into Pond 1. Larvae were photographed (dorsal and ventral) in a portable dark room. They were photographed under a blue excitation light using a 500 nm longpass filter to accurately detect their biofluorescence using a Sony A6400 mirrorless camera (ISO 10,000, shutter speed 1/60, FSTOP 2.8, manual mode) to accurately capture biofluorescence, as in 2025.

The brightness of each larva was quantified utilizing ImageJ, using previous methods (Neufeld et al., 2025). 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. After outlining, the increased image was deleted and the ROI was added to the grayscale unedited tiff and quantified. The ROI was made of the full body for both ventral and dorsal sides to quantify brightness of the entire animal. The brightness means and maximums were calculated for this ROI. (Figure 4)

Figure 4. Dorsal shot of salamander ROI outlined, in grayscale, on ImajeJ.

Results

The large bins had significantly more light entering them than the small bins (paired t = 4.02, p < 0.001, d.f. = 20). Although this was the case, the salamanders

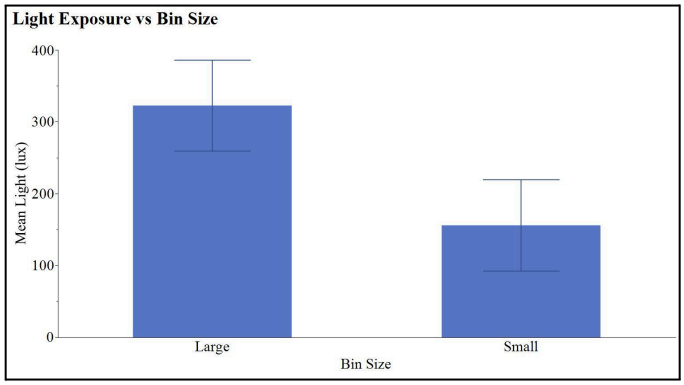

Figure 5. Graph showing results from the paired t-test to check for lux light variation within the different bins.

did not vary significantly in biofluorescence in terms of the different bin sizes and the different light intensities coming in (t= -0.81, p =0.4297, d.f. = 20).

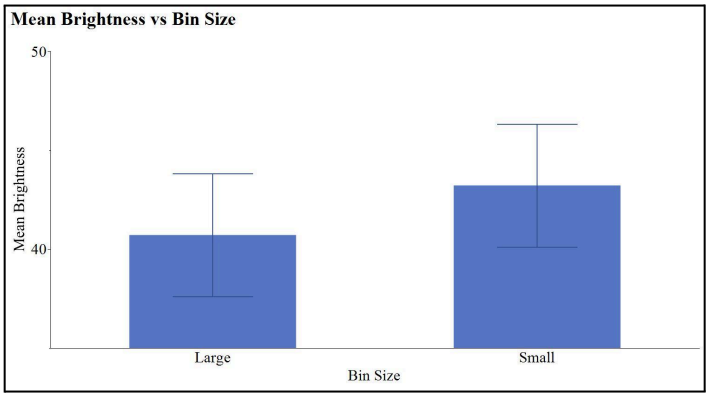

Figure 6. Graph of all mean salamander biofluorescence within the different bins.

Overall, ventral salamander biofluorescence brightness did not significantly change over time and over all the experiments. Dorsal brightness did significantly change over time, over all the experiments (paired $t = 2.89$, $p < 0.003$).

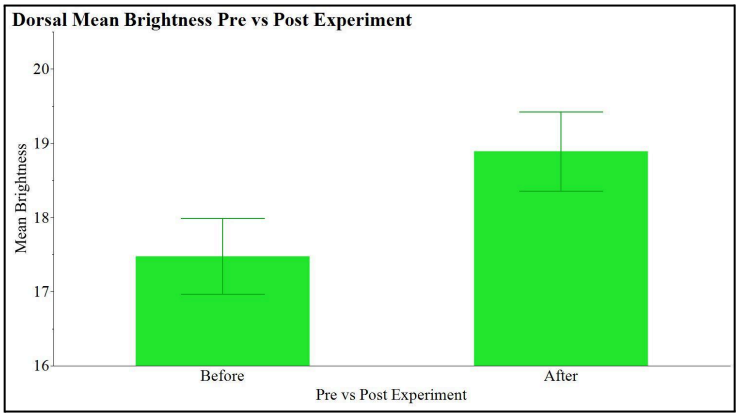

Figure 7. Overall mean dorsal brightness across all treatments, before and after experiment.

There was no significant interaction between dorsal salamander biofluorescence and the different density treatments ($F = 1.78$, $p = 0.0904$).

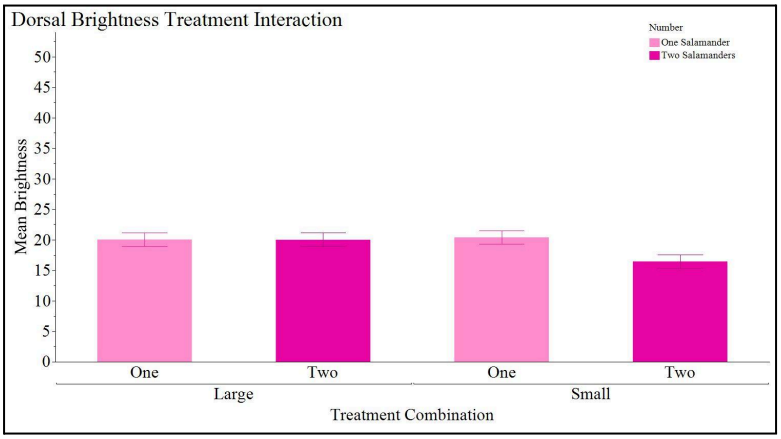

Figure 8. Dorsal brightness interaction with treatment combinations.

There was also no significant interaction between ventral salamander biofluorescence and the different density treatments (F = 0.01, p = 0.9906,).

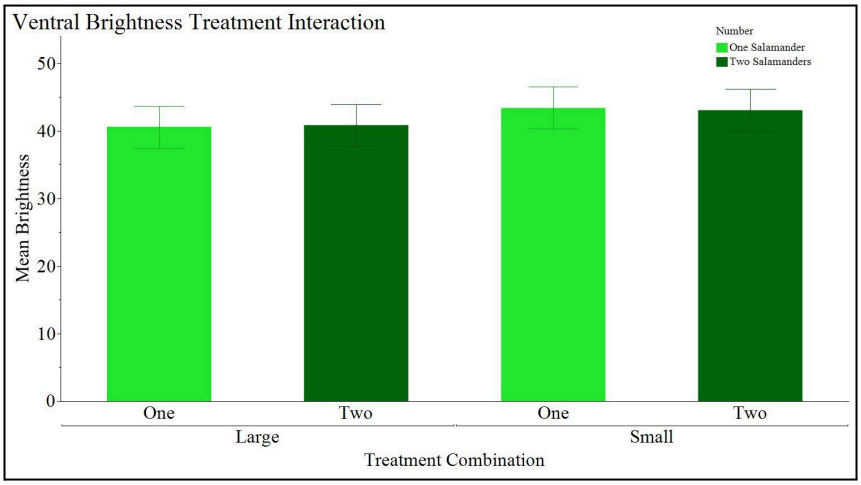

Figure 8. Dorsal brightness interaction with treatment combinations.

Analysis is still in the process for this dataset.

Discussion

The results of this study have potentially profound implications. When ponds dry quickly, salamanders experience high stress due to increased competition, water quality changes, and increased risk of disease (Petranka, 1989), all of which could influence their biofluorescent glow. These stress factors can cause high salamander mortality rates, so a potential change of glow could serve as an early indicator of

salamander stress or health (Figure 9).

Figure 9. Deceased salamander.

Evaluating the results, the sunlight conditions varied among the different sized bins, but this did not significantly impact how individual salamanders biofluoresced over time. This could have been due to a different wavelength of light that was not measured, such as blue light; the unexpected metamorphosis of some individuals may have also played a role in some of the data received. Although the ventral biofluorescence images did not appear to change over time, the dorsal images showed noticeable changes. Specifically, all salamanders exhibited increased dorsal biofluorescence over time, further supporting the results from last year's sunlight exposure experiment. The ventral data collected this year showed that the treatments did not impact the biofluorescence of the salamanders. Additionally, there was also no significant interaction between dorsal biofluorescence intensity and density treatments. There was a small trend in the dorsal biofluorescence data, this suggests that social cues might not be as plastic of an effect as sunlight exposure was for them last year. Finally, it may also mean that two salamanders

together might have not been enough or they might just need more time to exhibit a clearer trend.

There were a few limitations and unexpected occurrences in this experiment. Some of the larvae within the experiment began to metamorphose, which does not typically happen at this age. They could have been cued for this before the experiment was set up, although none of them seemed to be undergoing metamorphosis when initially collected. For this reason, it is especially important to revisit the data, taking into account which individuals started metamorphosis. The ventral data might have also been affected by the scraped plexiglass barrier that was used to take the ventral photos. This caused some images to blur out the darker parts of the salamanders which might have impacted many of the before shots. Ultimately, while some things were harder to control than others, there are a few things that could be controlled in future experiments to avoid affecting some of the results.

For future experiments or directions, it would be great to set up more replicates in longer term studies with more density treatments to see if a clearer result could be revealed. Different ventral shot equipment would help avoid any minor photo analysis errors that could have occurred. Additionally, it would be insightful to see if diseased salamanders glow differently from healthy salamanders. Maybe there is a certain level of stress they need to go through to change in biofluorescence. Further analysis of the data we already have, could help us see if there were any hidden trends due to some of the individuals that started metamorphosis.

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