

Katie Killian

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Biofluorescence in Arizona Tiger Salamanders (*Ambystoma mavortium nebulosum*) as an  
indicator of sexual readiness

Keywords: sexual selection, biofluorescence, salamanders, cloacal fluorescence, tail fluorescence

Abstract

Biofluorescence, the emission of visible light by organisms following the absorption of shorter wavelengths of light, has been identified recently in various amphibians but remains poorly understood in salamanders. This study investigates the potential relationship between biofluorescence and sexual readiness in *Ambystoma mavortium nebulosum*, the Arizona tiger salamander. Specifically, we analyzed maximum gray values of the tail, cloaca, and full body through creating regions of interest from fluorescence images of the underbelly of animals in relation to morph (paedomorph and metamorph), sex, and degree of cloacal swelling, which serves as an external indicator of sexual readiness. Fluorescence was quantified using ImageJ from standardized photographs of 146 individuals. ANOVA tests revealed significant differences in tail brightness between morphs ( $p > 0.05$ ), confirmed by Tukey's HSD post hoc tests (adjusted  $p < 0.05$ ). Although sex differences in tail brightness were initially detected, post hoc comparisons did not reveal significant pairwise differences. Cloacal brightness appeared to vary with swelling level ( $p < 0.05$ ), but this was not confirmed by adjusted post hoc tests. Full body brightness level showed no significant variation across morphs, sexes, or level of cloacal swelling. These findings suggest a morph-specific difference in tail biofluorescence, with other potential sexual or reproductive signals warranting farther study. Future analyses will incorporate

a larger dataset, individual level comparisons over time, and additional body regions to better understand the biological role of fluorescence in salamander courtship and communication.

## Introduction

**Biofluorescence** is a natural phenomenon that has been studied in a number of different organisms and is characterized by the absorption of shorter wavelengths by an organism, which are then reemitted at longer wavelengths. Biofluorescence may have a variety of different biological roles, including signaling and visual cues between organisms, predator avoidance, and courtship (Nicolai et al., 2024).

Biofluorescence in amphibians remains understudied and is not yet well understood. In frogs, fluorescence is produced primarily by the lymph glands (Taboada et al., 2017). Biofluorescence in this context has been shown to significantly increase both the brightness and night vision of the animal, suggesting that fluorescence may be important to visual perception. Tree frogs fluoresced in the bright blue to green range (390nm – 430nm), which was relatively similar to the range recorded for some species of salamanders: green to yellow in response to blue and UV excitation light. Most importantly, peak emissions from the study organisms fell into the green range of light, setting the animal apart from its surroundings, which fluoresce in the red to yellow range under blue excitation light, further suggesting that fluorescence may play an important role in perception (Lamb & Davis, 2020).

In some study organisms, biofluorescence has been identified as a possible mechanism of sexual selection. The bony tubercles of chameleons, a sexually dimorphic trait, have been shown to fluoresce under UV light (Prötzel et al., 2018). The plumage of the blue winged parrotlet also

**Commented [HW1]:** For your final paper (or maybe now, you can just get it started ahead of time), you should broaden this in terms of sexual selection. It would be good for you to read up on some of that—the ideas surrounding brightness in all kinds of species for example, and then focusing on those species that might do something similar to what we are thinking, like how baboons can show sexual readiness with bright colors on their butts. Stuff like that. Probably start with one or at most two extra paragraphs to build a general framework for the study beyond the glow stuff.

Your abstract would be better if you started out with a general sentence as well...

Think about papers (and proposals) as an hourglass, that thing you see villains in old movies turning over, like you have one minute. They are broad at the top (beginning, intro), then narrow down (end of intro, methods, results) then broaden back again (discussion, end of discussion more general than beginning, just like start of intro is more general than the end). Keeping that in mind will help you a lot in science no matter what you end up doing.

exhibited differences in wavelength and intensity of fluorescence between sexes under UV light (Barreira et al., 2012).

Arizona tiger salamanders (*Ambystoma mavortium nebulosum*) have been shown to fluoresce, with each distinct life history stage and morph showing a distinct pattern of biofluorescence (Neufell et al., in review.) Slight brightness differences between sexes were identified, with females fluorescing more conspicuously than males. However, whether these differences in brightness can be attributed to sexual dimorphism or to serve a role in courtship is still unclear.

Some salamanders fluoresce in the cloacal region (Lamb & Davis, 2020), which is important for breeding behavior, suggesting that the Arizona tiger salamander may very well use biofluorescence in courtship. Confirmed courtship behaviors of tiger salamanders heavily involve use of the tail, and include both mate choice by the female, and mate competition between males. Behaviors include tail tapping of the female by the male, as well as shoving of the female by the male away from other males. Reproductive success has been positively correlated to the tail length and tail height of male tiger salamanders (Howard et al., 1996). Fluorescence readings in the tail of tiger salamanders may also give indication of whether biofluorescence and mating status are correlated. We aim to discover whether salamanders fluoresce brighter if they are more sexually ready, exhibiting more cloacal swelling, similar to the way wild baboon females exhibit sexual swelling in their hind region as an indicator of their sexual reproductive value (Domb & Pagel, 2001).

In this study, we use cloacal fluorescence readings paired with tail fluorescence readings taken over the course of the breeding season to determine whether biofluorescence correlates with sexual readiness, categorized by the state of swelling of the cloaca, as well as whether

biofluorescence in the tail and cloacal region is significantly different between sexes. More specifically, biofluorescence will be quantified for all animals for the head, belly, cloaca, tail, and full body. These values will be compared with sexual readiness recorded on the same day of capture; cloacal swelling observations can range from not swollen to very swollen.

We hypothesize to see that animals that are experiencing more swelling in their cloacal areas will glow brighter for both paedomorphs and metamorphs. Additionally, we hypothesize to see that males will glow brighter in their cloacal and tail areas during active breeding times, as the female chooses in the mate choice of tiger salamanders.

## Methods

### Capture and Initial Processing

Arizona Tiger Salamanders were caught opportunistically out of ponds in the Mexican Cut Nature Preserve owned by the Nature Conservancy (Figure 1). All morphs, sexes, and life stages were captured out of sixteen subalpine ponds in the period from late June to late July. Salamanders were caught either by hand or using dipnets. Snout-vent-length (SVL), total length, mass, sex, morph, date and pond of capture, and cloacal state were recorded for each salamander into a database containing the life history of all previously caught animals.

Figure 1.

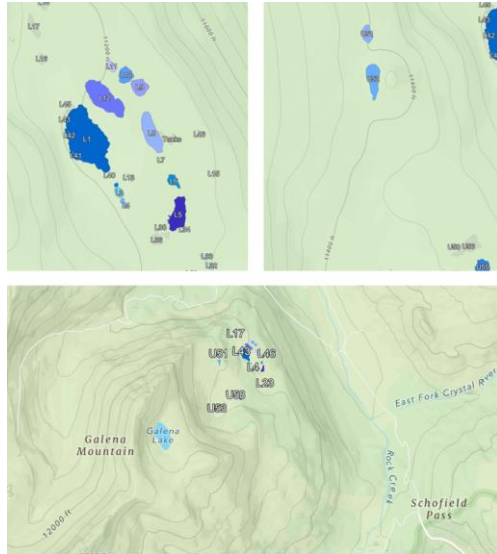


Figure 1. Mexican Cut Nature Preserve Ponds. Upper Left: Lower Cut ponds including ponds 1, 2, 3, 4, 5, 6, 8, 9, 10, 11, 12, and 18. Upper Right: Upper Cut ponds including ponds 51, 52, and 55. Bottom: Broader view of research site.

#### Photography of Fluorescence

The fluorescent responses of salamanders were photographed using a 500nm long-pass lens from Nightsea, paired with a Sony a6400 mirrorless camera under blue light excitation (Nightsea Xite Flashlight) in a portable darkroom. The photos were taken in Manual mode using ISO 10,000, an aperture of f/2.8, and a shutter speed of 1/60 second. Photographs were taken of the underside of 504 distinct mature animals from underneath a fiberglass sheet to capture both the cloacal and tail regions. 117 salamanders were opportunistically recaptured over the course of the season, and were photographed a second time.

During photographing, salamanders were held in an 8x10x20 cm clear plastic box sitting atop the fiberglass sheet containing 200mL of water taken from the pond of capture of that specific animal. A sheet of black plastic was placed over the box to reduce glare, followed by two layers of darkroom cloth. The blue excitation light was clamped 25 cm underneath the fiberglass sheet; photographs were taken level with the excitation light to keep the distance between animal and camera lens constant.

Following photography, animals were returned to the same ponds they were initially captured from.

#### Fluorescence Analysis

Brightness of fluorescence was quantified using ImageJ. One image per salamander was imported into ImageJ and then converted to grayscale, simplifying the brightness intensity measurement from a scale ranging from 0 (black) to 255 (white). This grayscale image was then saved as a tiff. To more clearly outline the regions of interest (ROIs), the brightness of the grayscale image was increased. ROIs were outlined in ImageJ using the freehand tool, and included a snapshot of the tail to the dark stripe situated right behind the cloaca ("tail"), an ROI of the cloaca beginning by drawing a straight line connecting the front end of the two back legs to the line already drawn for the tail ("cloaca"), an ROI of the head to the throat ("head"), and then an ROI representing the space between the head and cloaca ROI ("belly") (Figure 2). An ROI was also made of the full body, "full body" to quantify brightness over the entire animal. The legs themselves were not included in any of the ROIs due to variation in position as well as difficulty in tracing.

Figure 2.

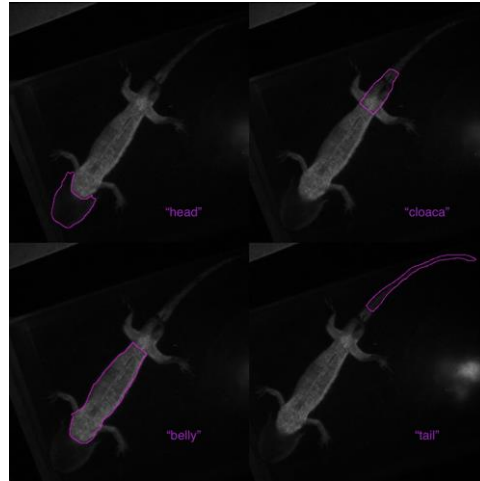


Figure 2. Visual representation of regions of interest (ROIs) in ImageJ in fluorescence image analysis.

Once drawn, these ROIs were then applied to the original grayscale image and the mean, maximum, and minimum were calculated for each ROI.

#### Statistical Analysis

A series of ANOVAs were performed on a subset of animals to examine the effects of sex, morph, and cloacal swelling on the maximum gray values of the full body, cloaca, and tail, as measured in ImageJ. When significant effects were detected, post hoc pair wise comparisons were conducted using Tukey's HSD test to adjust for multiple comparisons.

Results

Areas of the body compared in this study included the cloaca, the tail, and the full body brightness for both morphs, paedomorphs and metamorphs. ANOVA results for full body brightness revealed no significant differences between morphs, sexes, or cloacal swelling levels ( $p > 0.05$ ; Figures 3 and 4).

Figure 3.

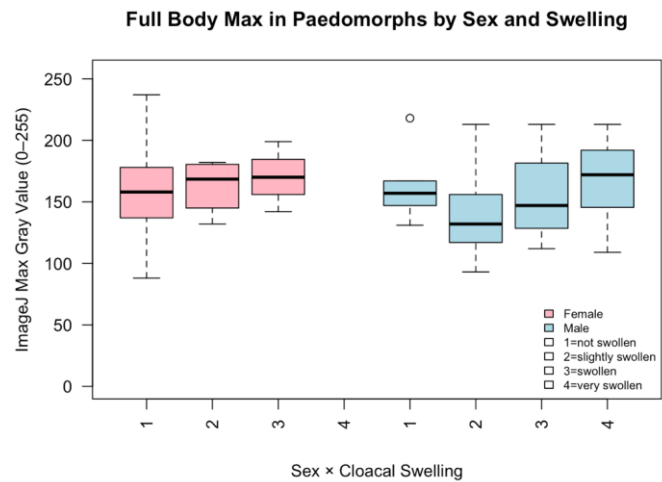


Figure 3. Sex and Cloacal Swelling of Paedomorphs vs. Full Body Max Gray Value (0-255)

Figure 4.

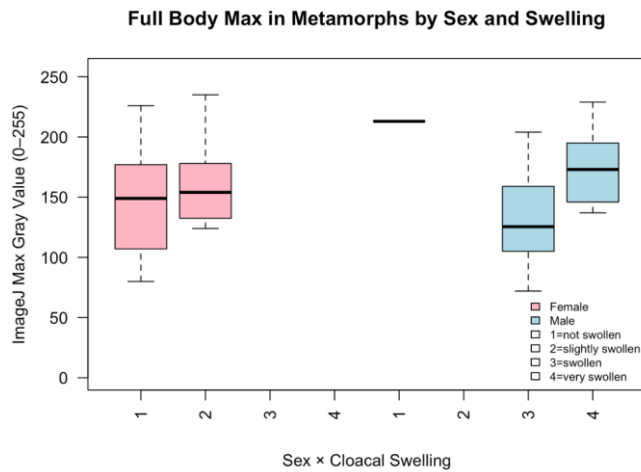


Figure 4. Sex and Cloacal Swelling of Metamorphs vs. Full Body Max Gray Value (0-255).

Differences between sexes for maximum tail brightness were statistically significant (ANOVA,  $p < 0.05$ ), however, post hoc tests with Tukey adjustment did not find any pairwise differences significant at the 0.05 level (adjusted  $p > 0.05$ ). ANOVA additionally identified statistically significant differences between morphs and tail brightness, ( $p < 0.05$ ), which was confirmed by post hoc Tukey's HSD tests (adjusted  $p < 0.05$ ) (Figures 5 and 6). ANOVA comparisons for the effect of sex and morph on tail brightness, morph and level of cloacal swelling on tail brightness, and sex and level of cloacal swelling on tail brightness were not significant ( $p > 0.05$ ).

Figure 5.

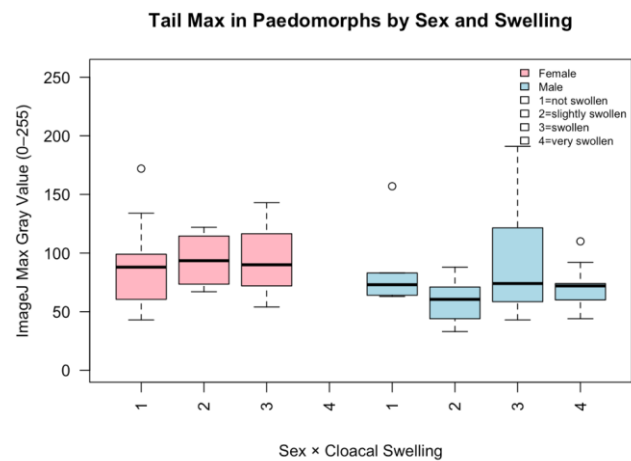


Figure 5. Sex and Cloacal Swelling of Paedomorphs vs. Tail Max Gray Value (0-255).

Figure 6.

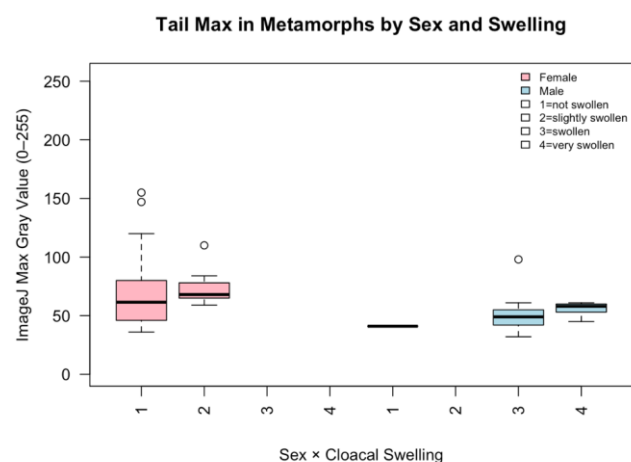


Figure 6. Sex and Cloacal Swelling of Metamorphs vs. Tail Max Gray Value (0-255).

The cloacal region comparisons between sex, morph, and cloacal swelling using ANOVA suggested that brightness level varied with level of cloacal swelling ( $p < 0.05$ ). Post hoc analysis using Tukey's HSD, however, did not confirm significant pairwise differences, (adjusted  $p > 0.05$ ). Other comparisons, including those between cloacal brightness and sex, morph, and their interaction with cloacal swelling, did not yield statistically significant results ( $p > 0.05$ ) (Figures 7 and 8).

Figure 7.

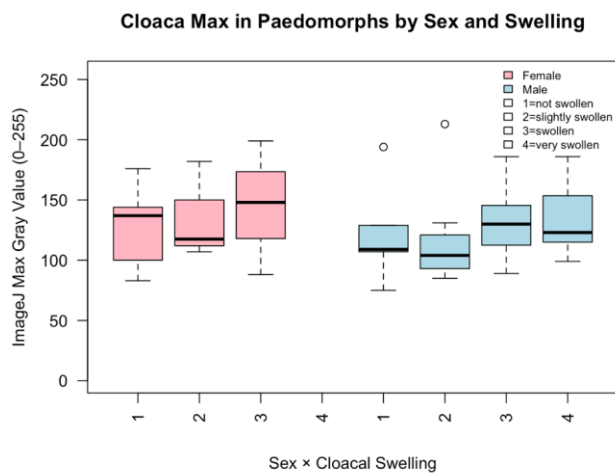


Figure 7. Sex and Cloacal Swelling of Paedomorphs vs. Cloaca Max Gray Value (0-255).

Figure 8.

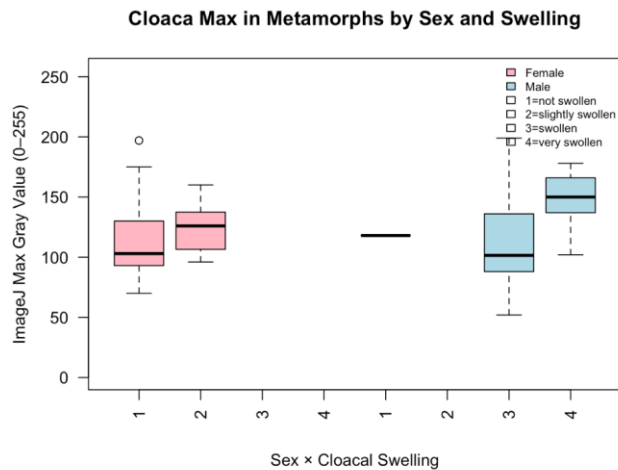


Figure 8. Sex and Cloacal Swelling of Metamorphs vs. Cloaca Max Gray Value (0-255).

## Discussion

Analysis of full body brightness showed no significant differences between morph, sex, or cloacal swelling level. This could be due to the amount of variation that was present between the animals that were photographed, both within sex, and within morph. Tiger salamanders have been shown to be incredible morphologically variable, with the two morphs that were studied in this study, as well as a cannibal morph. Amount of phenotypic variation in tiger salamanders has been linked to a number of environmental stressors, including habitat, genetics, predation, competition, body size, and kinship (Collins et al., 1993). As such, it is very possible that the biofluorescence of tiger salamanders is also a trait that has a broad range of phenotypic variance.

Tail brightness was found to be statistically different between morphs. A difference in overall brightness is corroborated by previous work, where life history morphs were shown to

have both distinct brightness levels and patterns (Neufell et al., in review), however a difference in tail brightness adds a level of intricacy to this detail.

While a significant difference in tail brightness between sexes was found initially using ANOVA, post hoc testing found no significant pairwise differences between tail brightness and sex. It is possible that this could be due to a small sample size, as only a subset of the full dataset was analyzed. For the purposes of this paper and discussion, only 146 salamanders of the 504 distinct animals photographed were analyzed within ImageJ and included in the dataset. Tests examining the differences between the sexes and tail brightness will have to be repeated following the inclusion of all salamanders photographed.

The same applies to results found regarding cloacal brightness, namely that cloacal brightness varied with level of cloacal swelling. Again, Tukey's HSD did not reveal any significant pairwise comparisons between cloacal brightness and level of cloacal swelling. It is important to note, however, that for this analysis and this specific result, salamanders were compared to other salamanders and not to themselves. There are many variables that could potentially play into biofluorescence in addition to cloacal swelling not specifically tested here, including age, pond, and size. In the future, it would be important to test cloacal brightness against cloacal swelling again, this time in the context of the same salamanders. 117 salamanders were photographed multiple times over the course of the season. Testing brightness of individuals against themselves allows for the removal of some of these extra variables that were not controlled for in this initial analysis, such as age, size, and pond of capture.

In the future, more analysis will be done with the data that was collected for this experiment. The biofluorescence of all animals will be quantified, and then statistics will be run again with a larger dataset. Due to the amount of data collected for each animal, there are a

multitude of traits that can be tested against biofluorescence levels. As such, other variables will be brought into the analysis, including pond of capture, age of animal, and size of animal. Recaptured salamanders will be analyzed for differences in cloacal swelling over the duration of the breeding season, and how this might have affected the overall brightness, tail brightness, and cloacal brightness of the animal. Additionally, head brightness data was collected, and this will be analyzed as well to see if head brightness varies morph, sex, or level of cloacal swelling.

Ultimately, much research is still to be done on the topic of biofluorescence in Arizona tiger salamanders in relation to sexual selection and sexual readiness. No strict conclusions can be drawn from this study yet, but the results do suggest that tail brightness does vary significantly between morphs. Certain other traits may still vary with biofluorescent responses, but more study needs to be conducted. Focus should especially be placed on the relationship between cloacal swelling and cloacal brightness, as well as the potential sexual dimorphism in tail brightness.

## References

- Barreira, A. S., Lagorio, M. G., Lijtmaer, D. A., Loughheed, S. C., & Tubaro, P. L. (2012). Fluorescent and ultraviolet sexual dichromatism in the blue-winged parrotlet. *Journal of Zoology*, 288(2), 135–142. <https://doi.org/10.1111/j.1469-7998.2012.00931.x>
- Collins, J. P., Zerba, K. E., & Sredl, M. J. (1993). Shaping intraspecific variation: Development, ecology and the evolution of morphology and life history variation in tiger salamanders. *Genetica*, 89, 167–183. <https://doi.org/10.1007/BF02424512>
- Domb, L. G., & Pagel, M. (2001). Sexual swellings advertise female quality in wild baboons. *Nature*, 410, 204–206.
- Howard, R. D., Moorman, R. S., & Whiteman, H. H. (1997). Differential effects of mate competition and mate choice on eastern tiger salamanders. *Animal Behaviour*, 53(6), 1345–1356. <https://doi.org/10.1006/anbe.1996.0359>
- Lamb, J. Y., & Davis, M. P. (2020). Salamanders and other amphibians are aglow with biofluorescence. *Scientific Reports*, 10(1), 2821. <https://doi.org/10.1038/s41598-020-59528-9>
- Neufell, T., Owen, D. A. S., Crayton, L., & Whiteman, H. H. (in review). *Biofluorescence Varies Ontogenetically in a Polyphenic Amphibian*.
- Nicolaï, M. P. J., Bok, M. J., Abalos, J., D'Alba, L., Shawkey, M. D., & Goldenberg, J. (2024). The function and consequences of fluorescence in tetrapods. *Proceedings of the National Academy of Sciences of the United States of America*, 121(24), e2318189121. <https://doi.org/10.1073/pnas.2318189121>

Prötzel, D., Heß, M., Scherz, M. D., Schwager, M., Padje, A. van't, & Glaw, F. (2018).

Widespread bone-based fluorescence in chameleons. *Scientific Reports*, 8(1), 698.

<https://doi.org/10.1038/s41598-017-19070-7>

Taboada, C., Brunetti, A. E., Pedron, F. N., Carnevale Neto, F., Estrin, D. A., Bari, S. E.,

Chemes, L. B., Peporine Lopes, N., Lagorio, M. G., & Faivovich, J. (2017). Naturally occurring fluorescence in frogs. *Proceedings of the National Academy of Sciences*,

114(14), 3672–3677. <https://doi.org/10.1073/pnas.1701053114>