

Lucille Crayton

Mentor: Howard Whiteman

Title: Changes in the biofluorescence of *Ambystoma mavortium nebulosum* with age

Introduction

From being able to understand members of the same species to understanding cues given by predators, communication between individuals is what drives the continued existence of any given species. There are many mechanisms used by individuals to convey different cues to others, including but not limited to color, vocal cues, body language, and more (Green & Marler 1979). Many of these mechanisms have been well studied and are widely accepted by the scientific community. However, as time goes on, additional methods of communication are being defined.

Take, for example, biofluorescence. Fluorescence more generally occurs when a higher wavelength of light, usually within the visual spectrum, is absorbed by an object and then reemitted as a lower wavelength of light within the visible spectrum (Lamb & Davis 2020). Biofluorescence specifically refers to the process in which a living organism performs this re-emittance of light, instead of an abiotic object. Biofluorescence has been studied since the mid-1900s, but has been relatively limited to marine species and systems (Lippincott-Schwartz 2006, Sparks et al. 2014). In the last several years, researchers have begun exploring fluorescence in a wider range of species, ranging from mammals to amphibians. Amphibians stand out when looking at these species, as there is evidence for them being able to perceive fluorescence in others. Multiple salamander species, including several which exhibit fluorescence in some way, have green rods in their eyes; many of these species gain additional rods when they metamorphose (Chen et al. 2008). However, very little is understood about the purpose of this fluorescence in their systems.

An opportunity to explore the purpose and mechanisms of fluorescence in salamanders comes in Colorado. Recent field research at the Mexican Cut Nature Preserve, owned by the Nature Conservancy and managed by the Rocky Mountain Biological Laboratory (RMBL), has shown that Arizona tiger salamanders, *Ambystoma mavortium nebulosum*, exhibit biofluorescence. The salamanders at the Cut have been studied since the 1950s and have been individually marked since 1990 (Wissinger & Whiteman 1992). The salamanders have a unique life history. They exhibit facultative paedomorphosis, wherein some individuals retain their larval characteristics into adulthood while others metamorphose and leave the ponds (Whiteman et al. 1996). When observed for fluorescence, many individuals appeared to have similar but unique patterns depending upon their morph.

However, in order for adult individuals to utilize fluorescence for the purposes of communication, they must first either have or develop fluorescence. At the Cut, salamanders are often broken into cohorts based on the year they hatched. For larval salamanders, knowing the

cohort for individuals allows us to keep track of growth and sexual development as they age. In this case, it also provides the unique opportunity to gain an understanding of how biofluorescence may change over the course of larval development. This summer, I focused on examining the biofluorescence of larval salamanders in their first three years to determine commonalities between both individuals and cohorts, as well as explore how biofluorescence may change as the salamanders age.

Methods

The observational methods used at Mexican Cut have been established and consistently used since 1990 (Wissinger and Whiteman 1992, Whiteman et al. 2012, Lackey et al 2019). The process for photographing the salamanders was developed and implemented this summer but were based on previous work done on fluorescence in salamanders (Lamb & Davis 2020, Cox & Fitzpatrick 2023).

Salamanders were scooped out of a given pond using a long handled net and then brought back to a Weatherport for processing. To begin, the salamanders were checked to determine if they were recaptures or not; this was done by checking for a passive integrated transponder (PIT) tag or for a unique toe clip (Brown et al 2022). If an animal does not have a PIT tag or clips, and is able to receive a tag at the time, they were given one once measurements were completed. The salamanders were weighed and measured for snout-vent length (SVL) and total length with a measuring board. We also recorded gill health (if applicable), body condition, morph, sex, and whether the salamander was reproducing (determined via cloaca condition). All measurements were recorded into an Access database, which has been maintained and updated since 1990.

Once processing was complete, individuals were randomly selected to be photographed. The salamanders were taken to a dark room. Each individual was placed in a five-by-five black tupperware to obtain pictures of their dorsal side and then placed in a glass-bottomed aquarium to get pictures of their ventral side. Photos were taken from around 15 centimeters above or below the animal. Each individual was photographed under both regular and blue excitation light, using a standard flashlight and Nightsea Xite Flashlight respectively. Once all individuals had been photographed, they were returned to their pond of origin and released. A note was made in the database of which individuals were photographed.

Once field work had been completed, the photographs were sorted by individual and placed into a Dropbox folder. A spreadsheet with each individual's information was created, and the folder with an individual's photographs was linked to the spreadsheet. For the purposes of this project, only larval salamanders with a known cohort were analyzed. Photos were further sorted by quality and then imported into two different programs. The first was ImageJ for analysis of both intensity and extent of fluorescence, while the second was blank to compare pattern similarity across individuals.

Results

At the moment, the photo analyses discussed in the methods section have not been performed. They will be completed over the next several months. The results for the purposes of RMBL's paper requirements will be descriptions of some of the common trends we noticed while photographing and beginning analysis.

For hatchlings, salamanders born within this calendar year, there was relatively little fluorescence. The dorsal side of each hatchling was almost entirely dark, with no dots or specks of fluorescence along them. The ventral side was similarly dark, although the space behind the front legs was beginning to fluoresce slightly. For most of the hatchlings observed, there were some speckles along the top and bottom of the tail. The smallest hatchling had limited speckles and the tail remained mostly dark, while the biggest hatchling had significantly more fluorescence along the tail.

For second-years, salamanders born in the previous year, there was more fluorescence and individuals had more variation between one another than hatchlings did. The dorsal side of the second years remained relatively dark, with limited fluorescence if they had any at all. However, the ventral side began to show fluorescence across most of the torso, with the legs, cloaca, and head remaining dark. Similarly, the tail was almost completely covered in patches of both fluorescent and non-fluorescent areas. Some second-years also had a line of dots along their sides, approximately following the separation between the dorsal and ventral sides. Interestingly, there also seemed to be some connection to size and fluorescence within the same cohort, particularly when comparing second-years from different ponds to one another. In particular, larger second-years did not have the line of dots along their side that smaller second-years had.

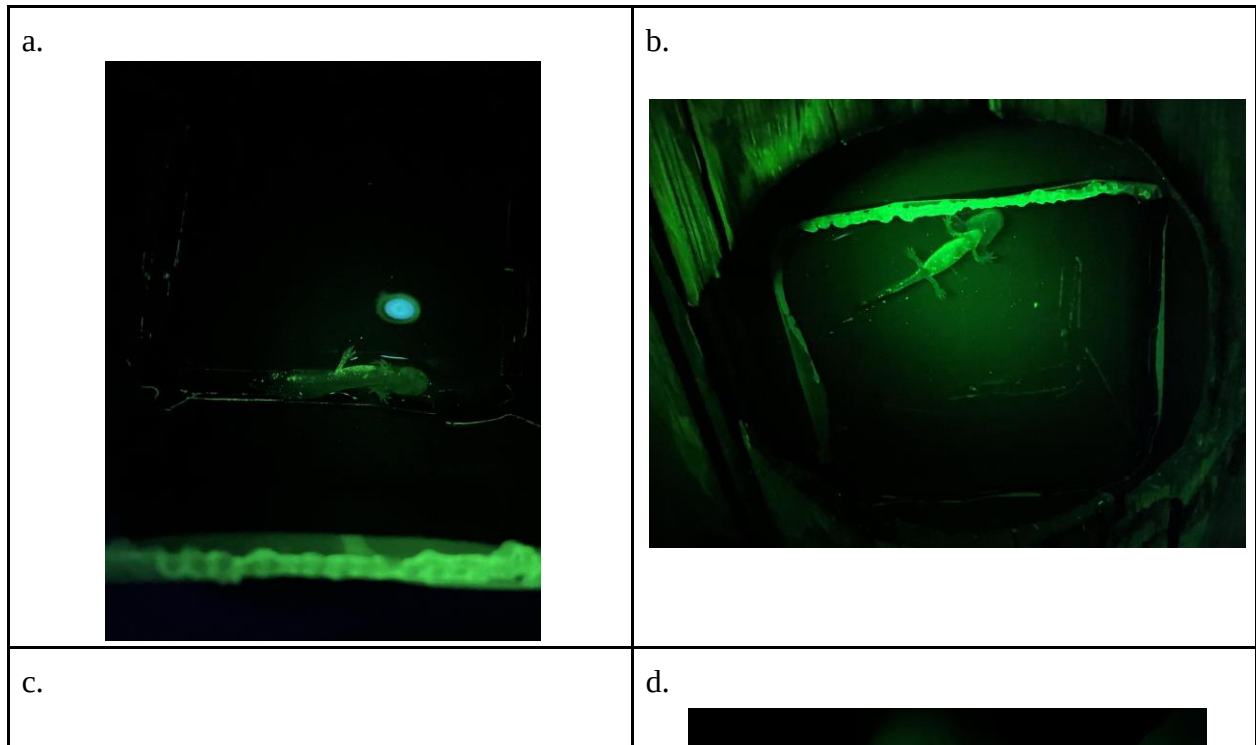


Figure 1. Section a shows the dorsal side of a second-year larva of a *Desmognathus fuscescens* of a second-years with a bright blue circular spot on the head and a glowing green line along the body. Section b shows the ventral side of a second-years with a bright blue circular spot on the head and a glowing green line along the body. Section c shows the dorsal side of a second-years with a bright blue circular spot on the head and a glowing green line along the body. Section d shows the ventral side of a second-years with a bright blue circular spot on the head and a glowing green line along the body. The areas around the legs and cloaca begin to fill in with more fluorescence, gradually losing the dark areas that were previously present (Fig. 2, sec. b). It was around this cohort where individuals began to look more similar to mature salamanders, particularly paedomorphic adults.

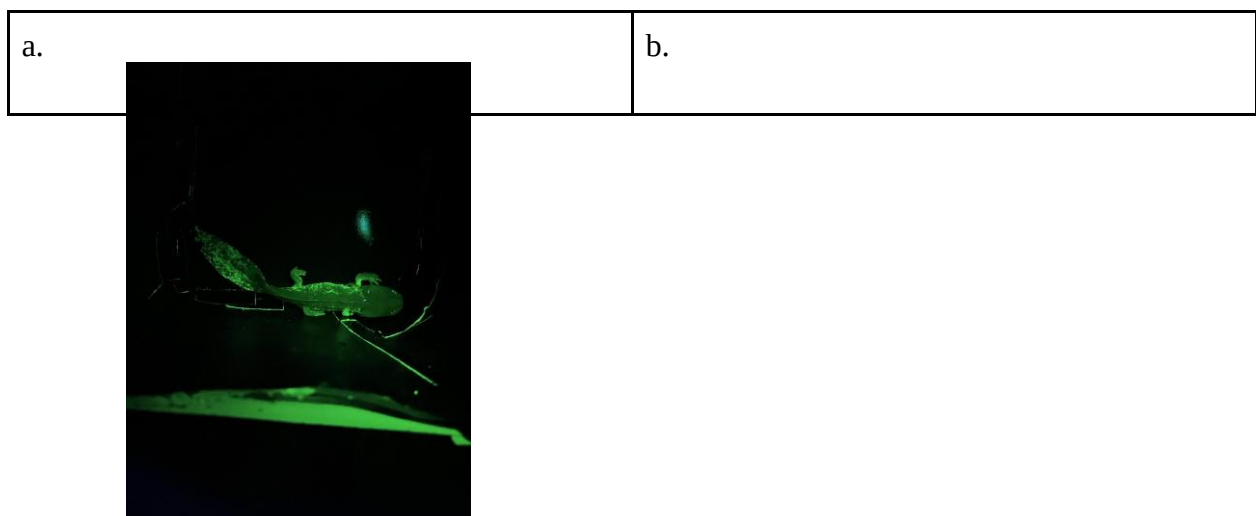
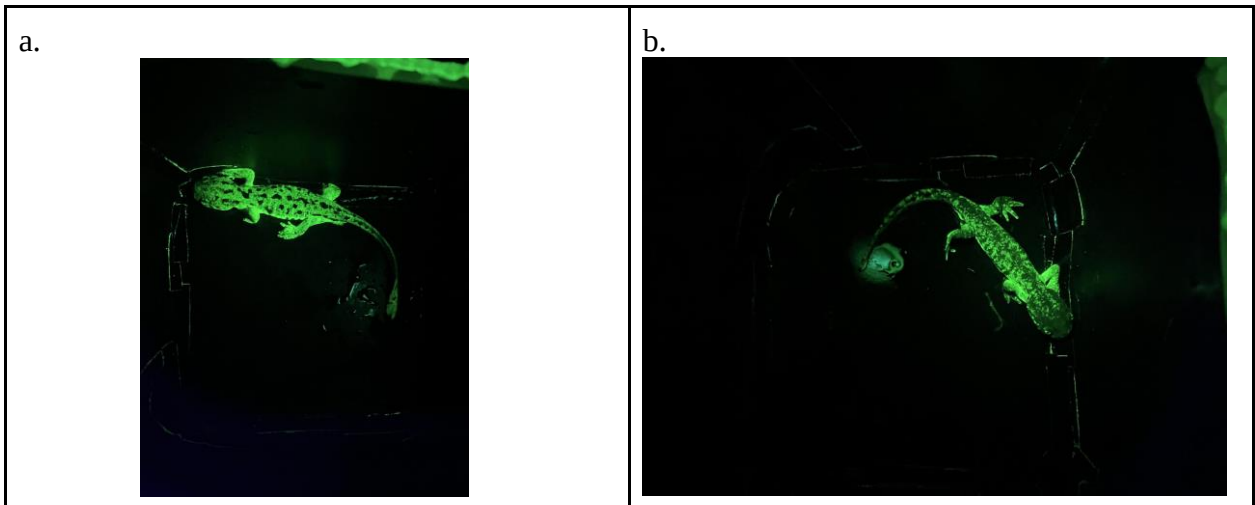




Figure 2. Section a shows the dorsal side of a third-year. Section b shows the ventral side of a third-year.

Unfortunately, fewer fourth-years were able to be photographed or observed. Of those that were, two had metamorphosed and one was larval. One was sexually mature while the other two were immature. The metamorphic individuals looked quite different from one another, with one looking more similar to adult metamorphs, which exhibit both brighter and more dorsal fluorescence, and the other looking somewhat like a paedomorphic adult, exhibiting dimmer and less dorsal fluorescence (Fig. 3, sec. a & b). Interestingly, their ventral sides followed this pattern, although they were more comparable to one another than the dorsal side (Fig. 3, sec. c & d). As for the larval third-year, the fluorescence looked very similar to many paedomorphs photographed with few differences between itself and a sexually mature individual (Fig. 3, sec. e & f).



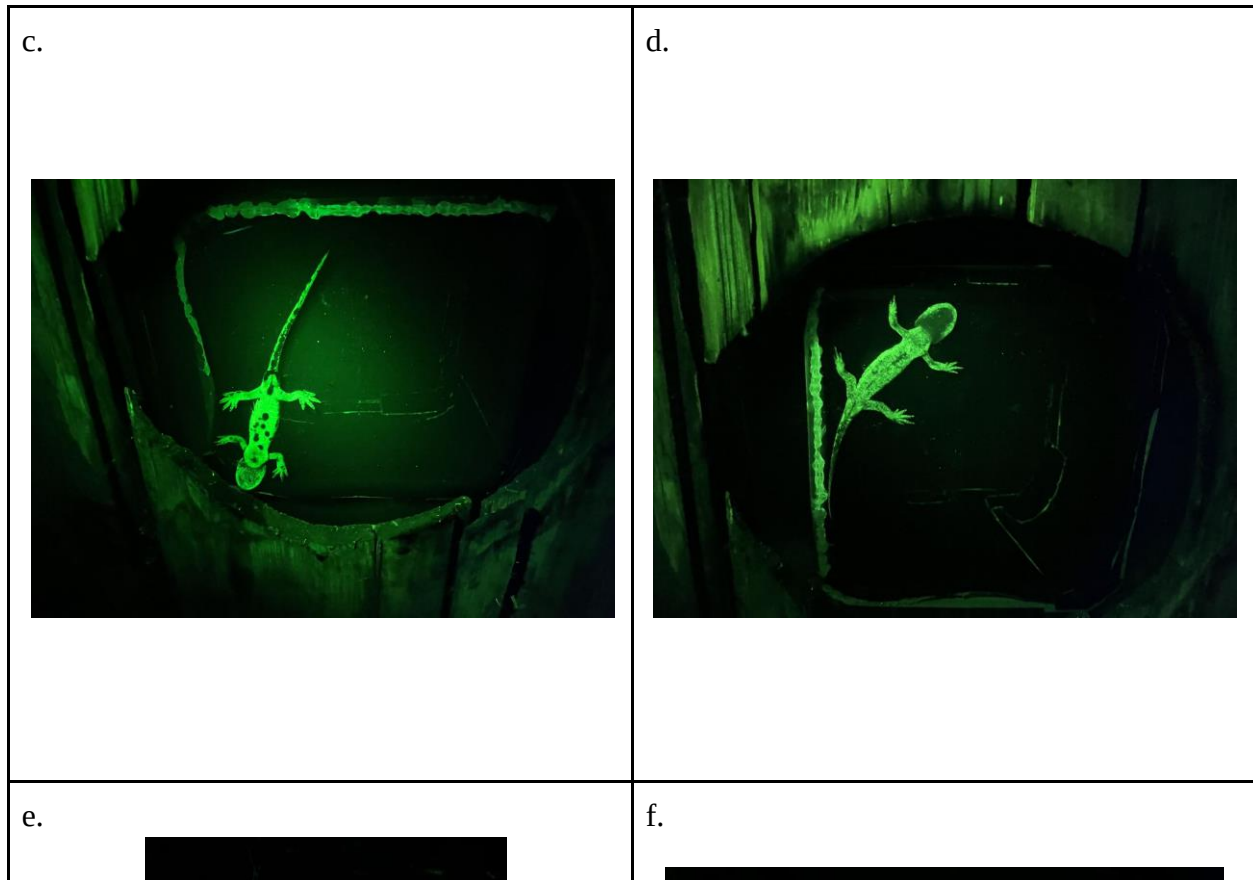


Figure 3. Salamanders. Panels c and d show the dorsal side of the larval and metamorphosed individuals, respectively. Panels e and f show the ventral side of the larval and metamorphosed individuals, respectively.

All in all, the fluorescent development of the salamanders over the two years of the study shows that the fluorescent development of the salamanders is a result of the fluorescent development of the salamanders.

Discussion

In order to understand the role of fluorescent development plays in the life history of individuals, it is important to acknowledge that relatively little is known about the role of biofluorescence in salamanders generally. It has been hypothesized that fluorescence may be a form of communication, camouflage, or simply a left-over from other processes that serves no function for the salamanders themselves (Lamb & Davis 2020). Many of these theories rely on the idea that salamanders can perceive fluorescence, as was suggested by Chen et al. in 2008. Based on the observations of larval salamanders over the course of this summer, there is some support for both the camouflage and communication hypotheses, as well as the theory that tiger salamanders can perceive fluorescence.

This population of salamanders has been shown to cannibalize larval individuals, both as larvae

and as adults (Whiteman et al. 1996, Denoël et al. 2006, Wissinger et al. 2010). Hatchlings, as well as some smaller second-years, face strong predation from larger larval and paedomorphic salamanders, in large part due to their small size; these salamanders are gape-limited, so once larval salamanders reach a certain size, even the largest paedomorphs can no longer prey on them (Wissinger et al. 2010). Additionally, smaller salamanders, particularly hatchlings, may face predation from some of the invertebrates found in the ponds; little is known about either invertebrate fluorescence or an invertebrate ability to perceive fluorescence. However, the idea of using fluorescence, or a lack thereof, as a way to camouflage themselves makes a certain amount of sense. Assuming that the salamanders can see fluorescence, the lack of fluorescence found in hatchlings and smaller first-years may be a strategy used to avoid predation until they are large enough to no longer be an option for cannibalistic individuals or any invertebrate predators. Similarly, there could be some level of mimicry occurring once salamanders reach a certain size. In a preliminary set of observations, one of the larger invertebrate predators, *Dytiscus dauricus*, also showed fluorescence along its sides in a row of dots that visually appear very similar to those found on the second-years. No solid analysis was completed on the invertebrate fluorescence and, given time constraints this summer, any further exploration of the topic will need to be done at a later time.

Communication is incredibly important in the natural world, particularly when considering interactions between individuals of the same species. Given the potential for salamanders to perceive fluorescence in other individuals, fluorescence could be a powerful tool for intraspecific communication. There was a clear lack of fluorescence around the cloacal region of larval salamanders into their third-year, even as they begin to look more and more similar to a mature adult salamander. This lack of fluorescence may be used by other salamanders as an additional indication of immaturity during the breeding season, although, given that we are unsure of the potential function of fluorescence in breeding, this should be taken with a grain of salt.

There remain many questions about fluorescence in *A. m. nebulosum*, as well as other salamander species. While this project has increased our understanding of both the existence of fluorescence in larval salamanders and changes in fluorescence across larval development, the questions of how these salamanders are fluorescent and why they may be fluorescing are unanswered. Potential for future projects exploring physiology of salamander eyes and skin, fluorescence across different invertebrate prey species, and sexual selection abound.

Literature Cited

- Cox, J. L., & B. M. Fitzpatrick. 2023. Biofluorescent sexual dimorphism revealed in a southern Appalachian endemic salamander, *Plethodon metcalfei*. *Scientific Reports* 13(3588): <https://doi.org/10.1038/s41598-023-29051-8>.
- Chen, Y., S. Znoiko, W. J. DeGrip, R. K. Crouch, & J. Ma. 2008. Salamander Blue-sensitive Cones Lost During Metamorphosis. *Photochemistry and Photobiology* 84(4): 855-862.
- Denoël, M., H. H. Whiteman, & S. A. Wissinger. 2006. Temporal shift of diet in alternative cannibalistic morphs of the tiger salamander. *Biological Journal of the Linnean Society* 89:373-383.
- Green, S. & P. Marler. 1979. The Analysis of Animal Communication. In: *Social Behavior and Communication: Handbook of Behavioral Neurobiology*, edited by P. Marker & J. G. Vandenbergh. Plenum Press New York London 3: 73-158.
- Lippincott-Schwartz, J. 2006. Biofluorescence: the making of a new technology. *Nature Cell Biology* 8(1212): <https://doi.org/10.1038/ncb1106-1212>.
- Lamb, J. Y., & M. P. Davis. 2020. Salamanders and other amphibians are aglow with biofluorescence. *Scientific Reports* 10(2821): <https://doi.org/10.1038/s41598-020-59528-9>.
- Sparks, J. S., R. C. Schelly, W. L. Smith, M. P. Davis, D. Tchernov, V. A. Pieribone, & D. F. Gruber. 2014. The Covert World of Fish Biofluorescence: A Phylogenetically Widespread and Phenotypically Variable Phenomenon. *PLoS One* 9(1):e83259 <https://doi.org/10.1371/journal.pone.0083259>.
- Whiteman, H. H., S. A. Wissinger, and W. S. Brown. 1996. Growth and foraging consequences of facultative paedomorphosis in the tiger salamander, *Ambystoma tigrinum nebulosum*. *Evolutionary Ecology* 10: 429– 422.