Does Biofluorescence Vary with Ontogeny and Body Size in the Arizona Tiger Salamander (*Ambystoma mavortium nebulosum*)?

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Abstract

Biofluorescence occurs when absorbed higher energy light is re-emitted as a lower energy "glow." Studies of this capability in tetrapods are rapidly expanding but its function, if there is any, remains largely unknown. While most studies focus on this phenomenon in solely metamorphosed adults, this study aims to examine how biofluorescence in *A. m. nebulosum* changes with age, body size, and key developmental milestones, like metamorphosis and sexual maturity, across multiple life stages. Hatchlings, 2nd-4th year larvae, and metamorphic and paedomorphic adults were captured from multiple ponds, photographed under a blue excitation light in a portable dark room, and dorsal and ventral fluorescence was quantified. Body size was a strong positive predictor of ventral brightness while age was a stronger positive predictor for dorsal brightness. Biofluorescence was found to discretely increase during metamorphosis and sexual maturity, suggesting that these key developmental milestones are important for the functional roles of this phenomenon. Ontogenetic changes in brightness in relation to age and size suggest a number of diversified roles for biofluorescence. In larvae, it could function in predator avoidance and intraspecific foraging whereas in adults, ventral brightness could play a role in visual communication of morph and sex identification, such as for mate competition and choice. By examining ontogenetic-level biofluorescence, we can provide clearer direction into future research studying the ecological role of this phenomenon in tetrapods.

Introduction

Biofluorescence occurs when absorbed higher energy, shorter-wavelength light is subsequently re-emitted as lower-energy light from a biotic source (Lamb and Davis, 2020; Nicolaï et al., 2024). While most current studies of this phenomenon relate to aquatic animals, like cartilaginous and ray-finned fish, biofluorescence studies in tetrapods are rapidly expanding (Nicolaï et al., 2024; Park et al., 2019; Sparks et al., 2014; Lamb and Davis, 2020). In most studied organisms, emittance includes blue, green, or red fluorescence in response to excitation (Marshall and Johnson, 2017). The function of fluorescence in many of these organisms, if there is any, remains largely unclear, but is thought to play a possible role in sexual selection, visual communication, and/or predator avoidance (Nicolaï et al., 2024).

Since biofluorescence was first discovered in the Eastern Red-backed Salamander (*Plethodon cinereus*), this trait is suspected to be present throughout most of Caudata with 8 out

of 10 families exhibiting fluorescence (Muñoz, 2018; Lamb and Davis, 2020). The mechanism resulting in this "glow" is uncertain but is thought to be related to pigments in dermal chromatophores, reflective guanine-containing structures, bony elements beneath the skin, or proteins contained in glandular secretions of lymph (Taboada et al., 2017; Lamb and Davis, 2020; Anthony et al., 2023). Most salamanders emit wavelengths within the green spectrum (520-560nm) when exposed to blue light (440-460nm) and occasionally ultra-violet light (Cox and Fitzpatrick, 2023). This green fluorescence is thought to be perceived by other caudates due to the presence of green rods and blue-sensitive cones in their eyes, further aiding in low light color differentiation (Chen et al., 2008). Post-metamorphosis, terrestrial *Ambystoma tigrinum* salamanders were found to have lost these blue-sensitive cones and increased their green rod density, producing higher light sensitivity and indicating a potential difference in fluorescence perception between aquatic larvae and terrestrial adults (Chen et al., 2008).

Along with a previous study that discovered ontogenetic differences in biofluorescence in three species of mole salamander, quantitative analysis of biofluorescence in the polyphenic Arizona Tiger Salamander (*Ambystoma mavortium nebulosum*) revealed that fluorescent traits varied substantially between larvae and adults (Lamb et al., 2025; Neufell et al., in prep.). Larvae were found to exhibit low-contrast fluorescence, or greater crypsis, in comparison to adults, while terrestrial metamorphs were found to display significantly higher-contrast fluorescence than aquatic paedomorphs (Neufell et al., in prep.). With salamander larval stages having been found to differ in both ecological conditions and physiological traits, it is likely that the ecological role of biofluorescence in visually-driven behaviors could change over the span of an individual's life (Neufell et al., in prep.). For instance, it is predicted that larval fitness increases by decreasing conspicuousness in the form of lower-contrast fluorescence to avoid predation and enable camouflage, but once an individual matures, more conspicuous signaling, or brighter fluorescence, can be used in sexual selection to increase fitness (Neufell et al., in prep.; Nicolaï et al., 2024).

Although many roles have been hypothesized for fluorescence in salamanders, very few empirical efforts have been completed to investigate its functional significance. Furthermore, as most confirmed accounts of biofluorescence focus on metamorphosed adults, little is known about shifts in biofluorescence that occur in early life history stages as a salamander develops into adulthood and how those shifts vary based on life history, body size, and developmental

milestones (metamorphosis and sexual maturity). The objective of this study is to examine how and when biofluorescence changes in an ontogenetic series by sampling a variety of body sizes in multiple ponds.

Biofluorescent responses to blue excitation in Arizona Tiger Salamanders at each life stage (hatchlings, 2nd-4th year larvae, metamorphic adults, and paedomorphic adults) were photographed and quantified. Such values were then compared to evaluate differences in fluorescence between ages, body sizes, and pre-and post-developmental milestones. I hypothesized that biofluorescence would increase continuously with factors like age and body size but would increase discretely, or non-continuously, after key developmental milestones like metamorphosis and sexual maturation. By examining both ontogenetic and population-level biofluorescent shifts, this study can provide insight for future studies investigating the potential ecological roles of biofluorescence in tetrapods.

Methods

The Whiteman Lab studies a montane population of Arizona tiger salamanders (*A. m. nebulosum*) at The Nature Conservancy's Mexican Cut Nature Preserve (MCNP) in western Colorado (3,640 m asl; 39.02°N, 107.06°W). This field site contains over 24 subalpine ponds that vary in both hydroperiod and other biotic and abiotic factors (Wissinger and Whiteman 1992). The ponds are separated by several kilometers from other ponds, reducing the likelihood of dispersal to and from the watershed (Whiteman, Wissinger, & Brown, 1996). Larval development in these individuals can range from 14 months to 5 years due to short growing seasons, low temperatures, and density-dependent effects allowing the opportunity to study the possible changes in biofluorescence in a variety of life stages of the same species (Wissinger & Whiteman, 1992; Neufell et al., in prep.). Since 1990, this salamander population has been monitored via individual marking with toe clips or PIT tags along with data like snout-vent length (SVL), total length, mass, sex, capture date, and pond being collected.

In this study, 2nd-4th year larvae, metamorphic adults, and paedomorphic adults (of both sexes) were captured either by dipnet or hand in Ponds 1, 5, and 12 and hatchlings were captured from Pond 8 (Fig. 1). These specific ponds were chosen because they provide the variation across age and size that was necessary for this study. Each captured salamander was photographed in a blacked-out plexiglass box under a blue excitation light with a 500nm long-

pass filter and Sony A6400 mirrorless camera with settings of ISO 10,000, shutter speed 1/60, FSTOP 2.8, white balance daylight, and set to manual (Chairez et al., in prep) (Fig.2). Individuals were photographed both ventrally and dorsally in a portable dark room and subsequently released into their pond of origin and therefore not held for any extended period. Using ImageJ software, the fluorescence of each salamander was quantified. Two images, dorsal and ventral, of each salamander were imported and converted to grayscale to refine the brightness intensity values from a scale of 0-255 (0 being the darkest, black, and 255 being the brightest, white; Neufell et al., in prep.). The Region(s) of Interest (ROI) for both surfaces were outlined using the freehand tool in ImageJ. Dorsally, the ROI included the tip of the snout to the base of the hind legs, and ventrally, the ROI included the tip of the snout to the bottom of the cloaca. For each individual sampled, the mean dorsal and ventral brightness value was calculated and subsequently compared among age cohorts, body sizes, and pre-and post-developmental milestones.

Many of the statistical tests performed were grouped in some way by morph. Morph was defined by the group labels: “larvae” which are non-metamorphosing larvae, “paedos” which are paedomorphic adults, and “metas” which include both fully metamorphic adults and larvae in varying stages of metamorphosis. To test the strength of age, SVL, and body condition (mass/SVL) as predictor variables for ventral and dorsal brightness, multiple stepwise regressions were performed in the forward direction utilizing a p-value threshold of 0.25 as entry criterion. The first stepwise regression was performed on all morphs, the next was performed on only individuals classified as larvae and paedos, and the last was conducted on only larvae and metas. The relationship between both ventral and dorsal mean brightness and SVL (snout vent length) and age were each further analyzed via separate linear regressions. Individual linear regressions were also conducted on each morph, including larvae, paedos, and metas, separately, further testing SVL as a predictor variable for brightness. Subsequently, an ANCOVA was utilized to analyze the interaction between morph and SVL for ventral mean brightness only. To analyze the relationship between metamorph stage and sexual maturity as predictor variables for ventral and dorsal mean brightness, separate ANOVA tests were performed including all morphs. Sexual maturity stages were defined as “immature”, a sexually immature individual, “adolescent”, an individual in the process of sexually maturing, and “adult”, a sexually mature individual. Furthermore, metamorph stages were defined as “rare”, “medium”, “medium well”,

"well done", and "full meta" where "rare" individuals were those just beginning to metamorphose and "full meta" individuals were fully metamorphic adults having completed metamorphosis. Sexual maturity was then further analyzed as a predictor variable for brightness on only metas, and separately on only larvae and paedos together also using ANOVA. JMP Student Edition 19 was used to conduct all statistical analyses and an $\alpha = 0.05$ acted as a threshold for statistical significance across all tests.

Results

In the stepwise regression conducted on all morphs, SVL was the only significant positive predictor for ventral mean brightness ($F_{1,192} = 33.7$, $p < 0.0001$) with the overall regression explaining 55.4% of the variance. In a similar stepwise regression conducted on dorsal mean brightness, age was found to enter the model first as the strongest significant positive predictor ($F_{1,250} = 51.2$, $p < 0.0001$) followed by body condition ($F_{1,250} = 13.7$, $p = 0.0003$) with the overall model explaining 17% of the variance. As SVL was found to be the strongest predictor of ventral brightness, and age of dorsal brightness, subsequent linear regressions were conducted to better understand these results. Age was found to be a significant positive predictor of both ventral mean brightness ($F_{1,193} = 75.8$, $p < 0.0001$) and dorsal mean brightness ($F_{1,251} = 36.6$, $p < 0.0001$) (Fig. 3A & 3B). Age explained approximately 28.2% of variation in ventral mean brightness and 12.7% of variation in dorsal mean brightness. SVL was also found to be a significant positive predictor of both ventral mean brightness ($F_{1,193} = 239.3$, $p < 0.0001$) and dorsal mean brightness ($F_{1,251} = 7.6$, $p = 0.006$) (Fig 4A & 4B). SVL explained approximately 55.4% of the variation in ventral brightness but only 2.9% of variation in dorsal brightness. In the morph-specific linear regressions that were conducted, SVL was found to be a significant positive predictor of ventral mean brightness in larvae ($F_{1,62} = 73.8$, $p < 0.0001$; $R^2 = 0.543$), paedos ($F_{1,105} = 46.5$, $p < 0.0001$; $R^2 = 0.307$), and metas ($F_{1,22} = 20.0$, $p = 0.0002$; $R^2 = 0.476$). In further individual linear regressions that were performed, SVL was not found to be a significant predictor of dorsal mean brightness in larvae ($F_{1,74} = 1.6$, $p = 0.22$) nor paedos ($F_{1,119} = 3.4$, $p = 0.07$) but was found to be a slightly significant positive predictor for dorsal mean brightness in metas ($F_{1,54} = 5.3$, $p = 0.03$; $R^2 = 0.089$). Throughout all analyses, ventral ROIs were brighter than dorsal ROIs (Fig. 3, 4, 5)

As SVL was found to be a significant positive predictor of ventral brightness in all morphs, an ANCOVA was conducted which revealed a significant interaction between SVL and morph and their effect on ventral mean brightness ($p = 0.018$; Fig. 6). Specifically, the slope of the line of best fit for larvae was significantly different than the slope of the line of best fit for both adult morphs.

Dorsal, but not ventral mean brightness was significantly different between metamorphic stages (dorsal: $F_{4,51} = 3.9$, $p = 0.007$; ventral: $F_{4,19} = 0.11$, $p = 0.98$) (Fig. 7). A post-hoc Tukey HSD test showed that the "full meta" stage had a significantly higher dorsal brightness mean than "medium well" ($p = 0.03$) and "rare" ($p = 0.03$); no other pairwise comparisons were significant. In contrast to metamorphic stage, sexual maturity significantly altered both dorsal and ventral mean brightness (dorsal: $F_{2,250} = 21.5$, $p = <0.0001$; ventral: $F_{2,192} = 45.6$, $p = <0.0001$) (Fig. 8). A post-hoc Tukey HSD test showed that all three stages of sexual maturity (immature, adolescent, and adults) significantly differed in ventral mean brightness (all $p < 0.03$) but only adults and immatures were significantly different in dorsal mean brightness ($p < 0.0001$).

Discussion

Biofluorescence is a rapidly developing subject that has been primarily focused on many aquatic species of invertebrates and fish, but over the past decade has been expanding into the field of tetrapods (Nicolaï et al., 2024; Park et al., 2019; Sparks et al., 2014; Lamb and Davis, 2020). While most studies focus on this phenomenon in metamorphosed adults, this study takes a deeper look at how biofluorescence varies in early life history stages and how it might change with key developmental milestones and ontogenetic variables like age and body size. In this study, I found that SVL is a strong positive predictor of ventral brightness while age is a stronger positive predictor for dorsal brightness. Furthermore, biofluorescence was observed to abruptly increase during key developmental milestones like metamorphosis and sexual maturity, suggesting some level of importance for these stages in the potential functional role of this phenomenon.

Body size, as measured by SVL, was found to be the strongest positive predictor variable for ventral brightness, meaning the longer an individual was, the brighter their ventral surface was (Fig. 4A). In many amphibians, body size is often used as a proxy for fitness. In *Ambystoma*

talpoideum, for example, body size at first metamorphosis was directly associated with adult traits closely related to fitness where individuals with a larger body size at time of metamorphosis became larger adults at first reproduction in both sexes and females had an earlier age at first reproduction (Semlitsch et al., 1988). Since SVL was found to be such a strong positive predictor for ventral brightness, it is possible that ventral brightness could also be used as a fitness indicator within this population. For example, an individual that is much brighter on its ventral surface than many of its conspecifics could also be seen as a higher fitness individual. This brighter ventral fluorescence in larger adults could thus act as a sexually selected signal for both sexes, playing a role in both mate competition and mate choice. It is possible that both sexes could be utilizing ventral brightness to assess the fitness of their chosen mate.

For example, female body size has been shown to be positively correlated with female reproductive success in several salamander species. In the Eastern Red-Back Salamander, females with a larger SVL had both larger clutch sizes and offspring with larger SVLs, which helped to enhance offspring survival. Furthermore, there also existed a positive relationship between maternal SVL and subsequent egg production in preparation for the next reproductive bout. This indicates that these larger females tended to have relatively higher fitness than smaller females (Wise and Jaeger, 2021). Males, therefore, could be utilizing ventral brightness as a strategy to target large and thus higher fitness females quickly, which would in turn increase the fitness of the male that mates with her. Females could also be using ventral brightness in female choice to assess male size, and thus fitness, in a similar manner. Not only do larger males tend to signal higher survival rates and growth ability (Hernández-Pacheco et al., 2020; Doyle et al., 2010) but larger males also have been found to have greater success in mate competition via greater number of encounters with females, increased shoving of females to initiate courtship, and increased interrupting of other courting males and depositing of spermatophores (Howard et al., 1997). In this way, males might also be using ventral brightness to assess dominance in other conspecific males. With courtship behaviors similar to that of *A. m. nebulosum* (Whiteman et al., 1999), in a study on *A. t. tigrinum*, longer body length in males was found to provide many advantages in male-male competition during courtship (Howard et al. 1997). Assessment of body size by competing males was evident in the chosen tactic used by interrupting males to court chosen females. If the courting male was smaller than the incoming male, then the intruder male would shove the female away from the courting male and begin courtship, but, conversely, if the

courting male was larger, a female mimicry tactic was adopted by the intruder. This mimicry involves the intruder male both performing female behaviors to deceive the courting male, while also courting the female at the same time. Males could be using ventral brightness to assess body size and thus dominance of other males and consequently, decide on the type of tactic to use when interfering in a courtship event (Howard et al., 1997).

Next, age was found to be a stronger significant predictor variable for dorsal brightness than SVL, meaning older individuals tended to have a brighter dorsal surface (Fig. 3B). Although age was found to be the strongest predictor for dorsal brightness, it still only explained 28% of the variation. This is most likely due to the large amount of individual variation that existed within dorsal brightness across salamanders (Fig. 3B & 4B). The fact that ventral and dorsal brightness were found to have different predictor variables could mean that if biofluorescence is serving as a sort of "information source" within this population, then conspecifics could be receiving varying types of information depending on the surface of the body they are viewing, suggesting diversified roles for these different body parts. Like previously mentioned, ventral brightness could be used to assess conspecifics within the population during certain activities like when floating, swimming, or foraging in the water column leaving their ventral side to be exposed and visible (Lamb et al., 2025). Furthermore, during courtship behavior in this population, males conduct a "wheelbarrow shove" in which they push their snouts into the ventral surface slightly behind the front legs of the female (Whiteman et al., 1999). This interaction might also allow males to "get a glance" of the ventral brightness levels of females during courtship behavior suggesting an ecological role in mating for this phenomenon.

On the other hand, dorsal brightness might play a larger role in crypsis and predator avoidance, especially in more vulnerable, larval stages. Fernandez and Collins (1988) found that color in *A. t. nebulosum* was highly correlated with substrate color, water transparency, and environmental albedo in order to increase crypsis in diverse habitats. Anecdotally, it was noted during this study that color of an individual's dorsal surface seen with the naked eye was generally consistent with their dorsal brightness level under the blue excitation light. This could thus potentially indicate not only the matching of color to environmental factors like substrate color and water clarity but also simultaneous matching of biofluorescence to these factors as well to increase crypsis.

Ventral brightness was always brighter in our sampled individuals (Fig. 5). Although they have similar minimum range values on the y-axis, Fig. 3A and 4A have much higher maximum values than Fig. 3B and 4B, supporting this conclusion. Thus, this indicates a less conspicuous dorsal brightness within the population. Larval salamanders could be using this cryptic dorsal biofluorescence as a means of camouflage to avoid being detected by potential prey and/or to evade predators like many aquatic invertebrates within freshwater pond ecosystems and cannibalistic conspecifics (Lamb et al., 2025). Furthermore, many birds-of-paradise (Paradisaeidae) utilize biofluorescence, emitted as wavelengths within the green-yellow spectrum similar to many caudate populations, to enhance visual cues used during mating displays. Thus, it is possible that the wavelengths that many caudate populations fluorescence could be perceived by birds or other predators, raising the question of what role biofluorescence plays in interspecific predator-prey interactions (Martin et al., 2025). It is currently unknown though if predatory birds such as herons, mergansers, and kingfishers have these same capabilities. Overall, strong predation from cannibalistic conspecifics, visual predators outside the ponds, and aquatic invertebrates could have selected for this more cryptic dorsal fluorescence in larval individuals and paedomorphic adults over time. This less vibrant dorsal brightness could also function during foraging to avoid being detected by potential prey. Larvae and adult paedomorphs are confined to permanent ponds and thus face more intense intraspecific foraging competition than associated metamorphic adults (Whiteman et al., 1996). More conspicuous biofluorescence could give certain individuals an edge in this competition for resources.

As larval salamanders tend to be buried in the bottom of their aquatic habitats or lay on top of substrate, detritus, or aquatic vegetation in the ponds, their dorsal surface is made primarily visible (Lamb et al., 2025). Many larval salamanders have a countershading effect where they have a more vibrant ventral side with a less vibrant dorsal side which contributes to further camouflage (Lamb et al., 2025). With a darker dorsal side, background matching can occur from the top-down, like in many marine fish, and a slightly more vibrant ventral surface can help reduce an individual's shadow from below. Depending on the location of the prey or predator, this could serve as a further advantage during foraging and/or predator-avoidance activities (Sparks et al., 2014; Lamb et al., 2025).

SVL and morph were found to significantly interact (Fig. 6), such that the slope of the larvae line of fit was significantly different from the slopes of both adult morphs. In the larvae to

adult morph transition, the rate at which ventral mean brightness is increasing with SVL is changing in a non-continuous fashion, with the overall ventral brightness always being higher for metamorphic adults. This non-continuous or "discrete" change is happening during important transitional periods within a salamander's lifetime, like sexual maturity and/or metamorphosis. This indicates that these key developmental milestones could be integral to what the ecological role is of the "glow" within this population. Supporting this idea, both ventral and dorsal brightness were found to significantly increase through the defined stages of sexual maturity. Ventral brightness significantly increased across all stages of sexual maturity (Fig. 8A) while dorsal brightness is seen to significantly increase across immature individuals and adults (Fig. 8B). Because significant differences in brightness were found between the defined stages of sexual maturity, these ontogenetic differences support the role of biofluorescence as an indicator of sexual maturity within the population and, as described earlier, sexual selection (Neufell et al., in prep.).

Ventral mean brightness was unexpectedly not significantly different among any of the metamorph stages (Fig. 7A). Dorsal mean brightness, however, increased over the course of this transition, with "full metas" having being significantly brighter than the "medium well" and "rare" individuals (Fig. 7B). With a more "discrete" increase occurring in ventral brightness over the course of metamorphosis and a more incremental, continuous increase occurring in dorsal brightness, it appears that brightness on either surface is changing at different rates during this key developmental milestone. These distinctive visual changes throughout this transitional stage could indicate a potential role in morph identification.

Overall, ontogenetic changes in both ventral and dorsal brightness in biofluorescence in relation to variables like age and size suggest a diversified number of ecological roles that this "glow" could play. In larvae and smaller paedos, dorsal brightness could serve as a form of crypsis or predator avoidance whereas in adults, ventral brightness could serve as a form of visual communication in morph and sex identification, mate competition and mate choice, or play a role in assessing an individual's fitness.

Future analyses that should be conducted utilizing this data include analyzing pond effects, "spottiness", and considering sex differences. I sampled from three different permanent ponds at the MCNP and would be interested to see if the overall trends I am seeing from analyses conducted on all individuals would vary when split up by pond. Very little is still known about

what environmental factors affect biofluorescence and how they affect it. Many of these ponds vary in water clarity, substrate reflectance, vegetation makeup, and invertebrate communities which could all potentially be microenvironmental factors that change the overall trends we are seeing in salamander brightness levels. Furthermore, additional data is needed about water turbidity per pond and transmission spectra at different depths to gauge how salamanders might be perceiving this brightness differently in different ponds (Lamb et al., 2025). Secondly, many of these salamanders, especially older paedomorphic and metamorphic adults, have been found to have varying sized dark spots. These dark spots do not fluoresce under blue excitation light (Fig. 9). In my current analysis, when brightness values were quantified, this "spottiness" was not considered. In future analyses, I aim to recalculate the mean ventral and dorsal brightness values by taking the mean of the 20 brightest pixels of a salamander image, effectively removing the "spottiness" of an individual from the quantified brightness. By comparing these recalculated brightness means to the original mean values from this study, I could better see what impact these dark spots, and their abundance, might have on these trends. Finally, from previous research by Neufell et al., it is known that there are differences in biofluorescence between sexes in this population, with males having a higher brightness contrast than females. These sex differences should be considered within this data to see what role they might play in these larger trends and, thus, if the "glow" could serve as a visual cue in distinguishing between sexes (Martin et al., 2025).

Conclusion

This study takes an in-depth look at how biofluorescence changes throughout early life history stages, across key developmental milestones, and with ontogenetic variables like age and body size. SVL was a strong positive predictor of ventral brightness suggesting the potential role of ventral brightness as a fitness indicator and a sexually selected signal within the population, functioning in both mate competition and mate choice. Conversely, age was a stronger positive predictor for dorsal brightness. With different predictor variables for ventral and dorsal brightness, this could suggest diversified roles for different parts of the body. Ventral brightness could be used to assess conspecifics within the population while dorsal brightness could play a larger role in camouflage, predator avoidance, and intraspecific foraging in the more vulnerable larval stages. Furthermore, biofluorescence was seen to abruptly increase during key

developmental milestones with significant increases occurring throughout the stages of sexual maturity and metamorphosis, suggesting some level of importance of these stages in the potential functional role of this phenomenon. Altogether, these ontogenetic shifts in both ventral and dorsal biofluorescence suggest a diversified number of ecological roles that this phenomenon could play within this population from sexual selection to predator avoidance depending on the individual. Future research should focus on testing how key microhabitat factors impact this phenomenon, what role individual patterning and sex plays, and evaluating receiver perception of this "glow" to further clarify the ecological role of this phenomenon in *A. m nebulosum*, and more broadly, tetrapods.

Tables and Figures

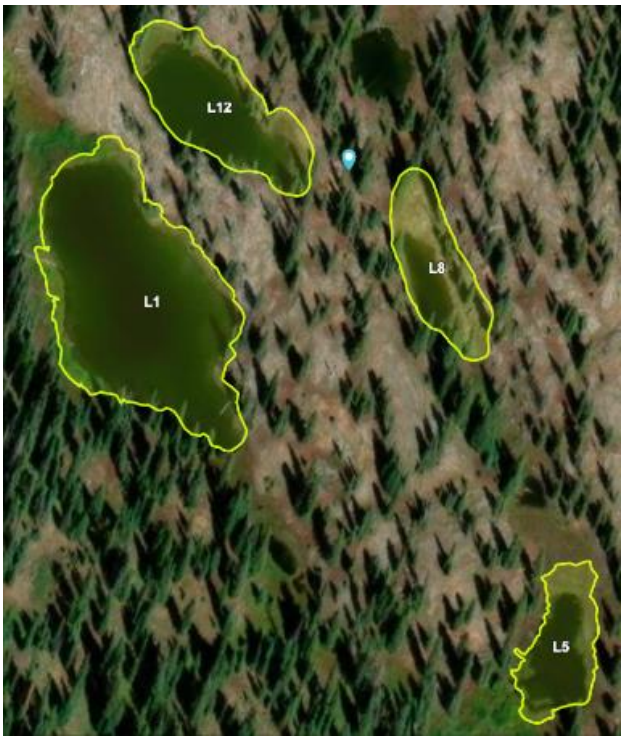
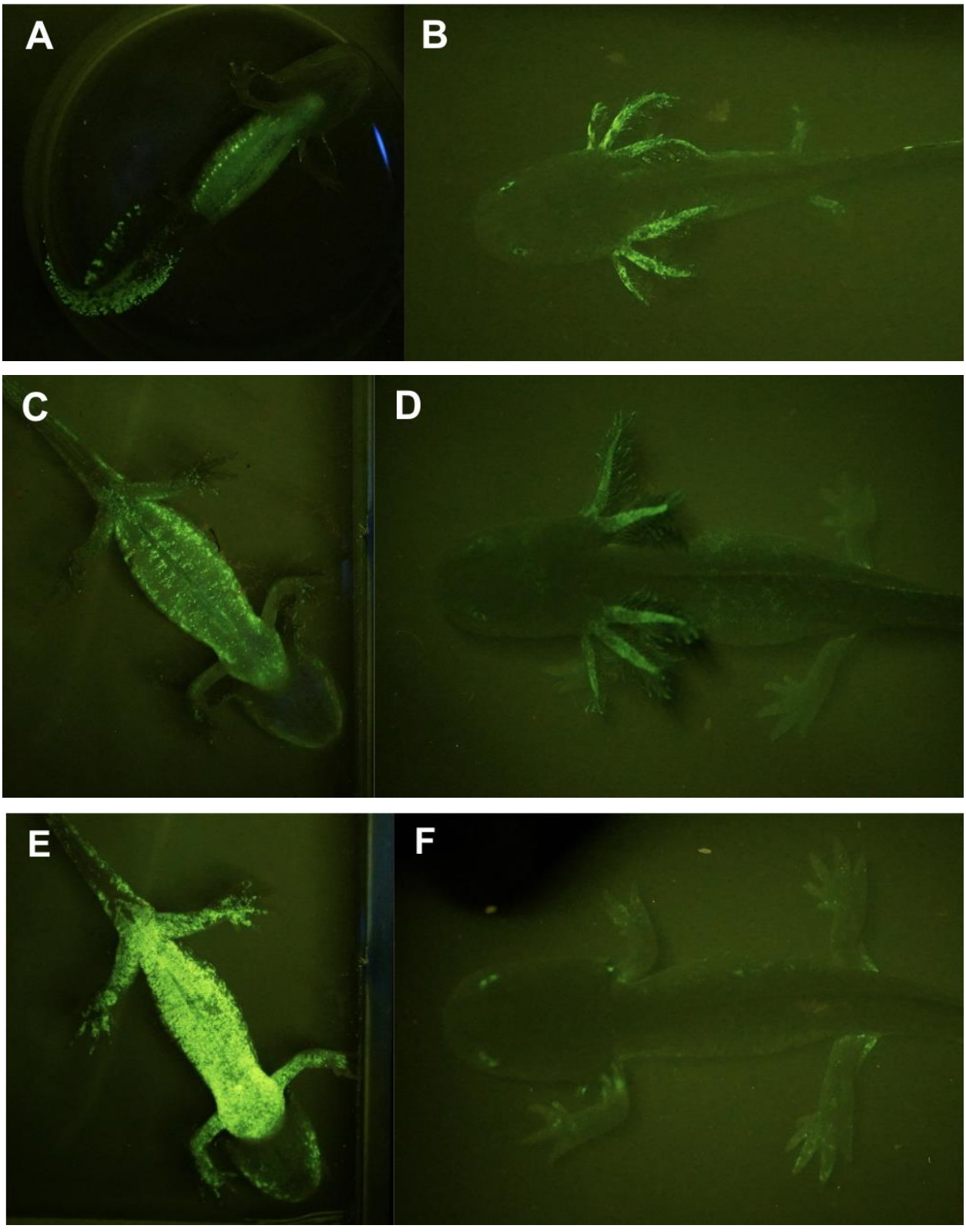


Fig 1. Map of sampled ponds, L01, L05, L08, and L12 at the Mexican Cut Nature Preserve. The blue marker represents the weatherport where animals were processed and photographs were taken.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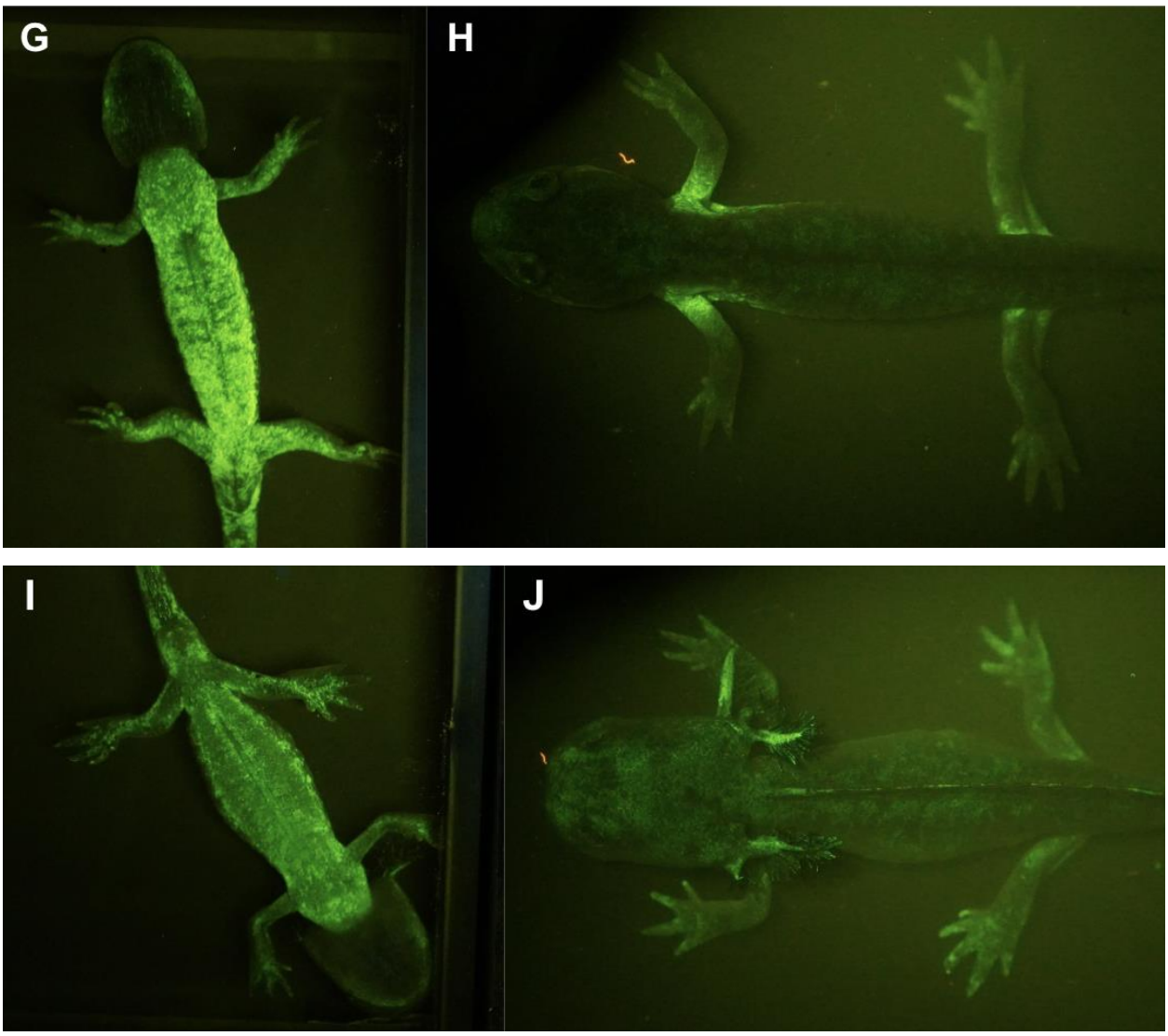

Fig. 2. Photographs of *A. m. nebulosum* biofluorescent response across life stages. Ventral and dorsal views are shown for hatchlings (A, B), 2nd -year larvae (C, D), "rare" metamorphosing larvae (E, F), young metamorphic adult (G, H), and paedomorphic adult (I, J).

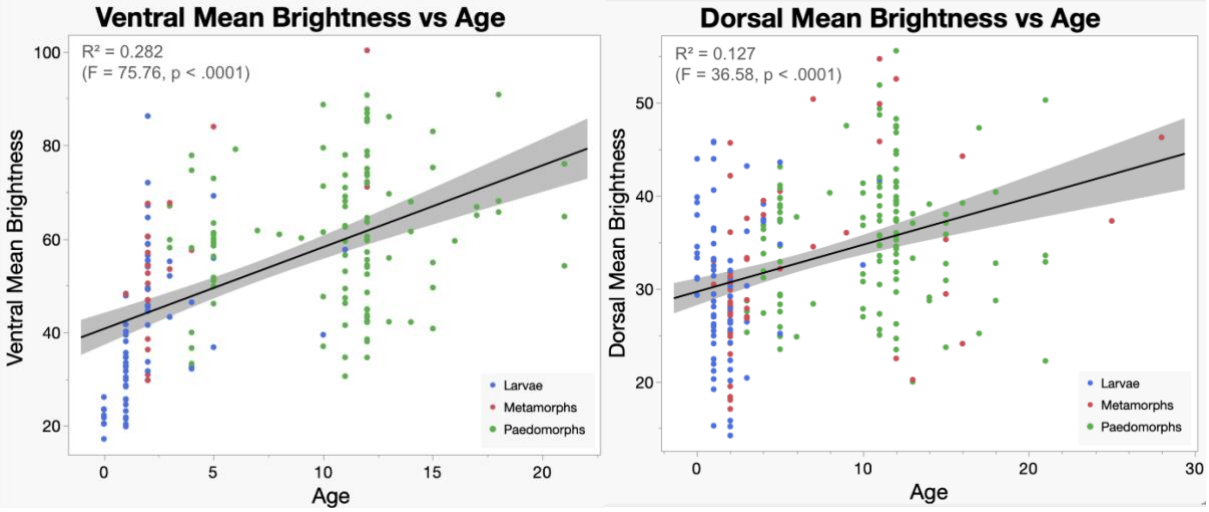

Fig 3. Linear regression analyzing the relationship between age and ventral mean brightness (left; A) and dorsal mean brightness (right; B).

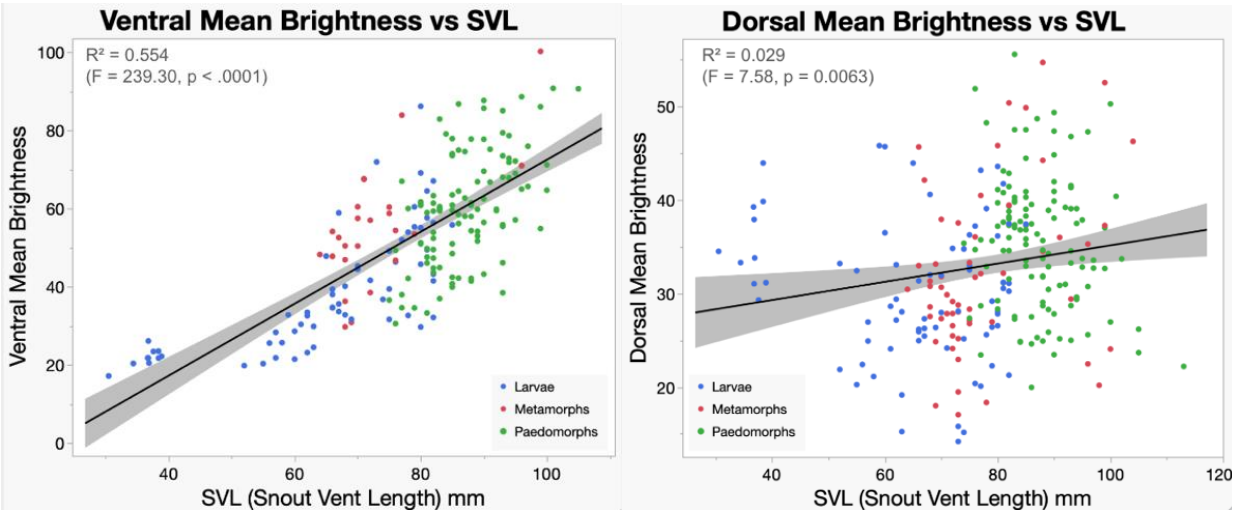

Fig 4. Linear regression analyzing the relationship between SVL (Snout Vent Length) in mm and ventral mean brightness (left; A) and dorsal mean brightness (right; B).

Fig 5. Photographs of *A. m. nebulosum* biofluorescent response. Overall brightest dorsal individual with a mean of 50.4 (left) and brightest ventral individual with a mean of 100.3 (right).

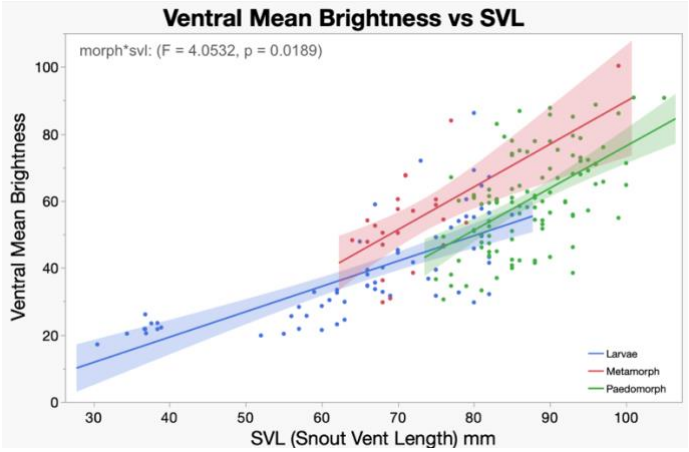

Fig 6. ANCOVA test analyzing the interaction between SVL (Snout Vent Length) in mm and morph in ventral mean brightness.

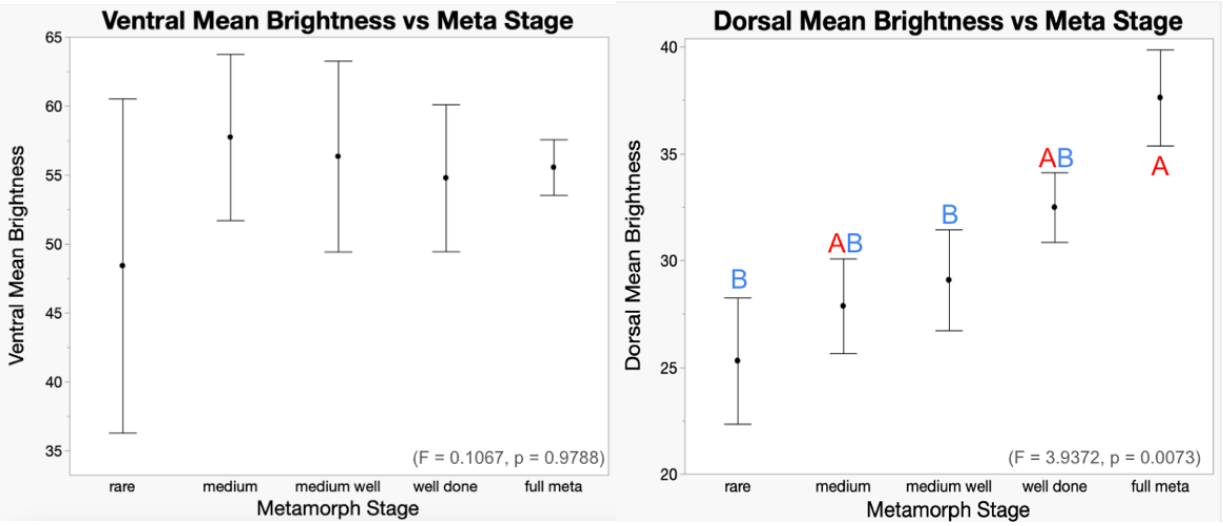

Fig 7. ANOVA test analyzing the relationship between metamorph stage and ventral mean brightness (left; A) and dorsal mean brightness (right; B).

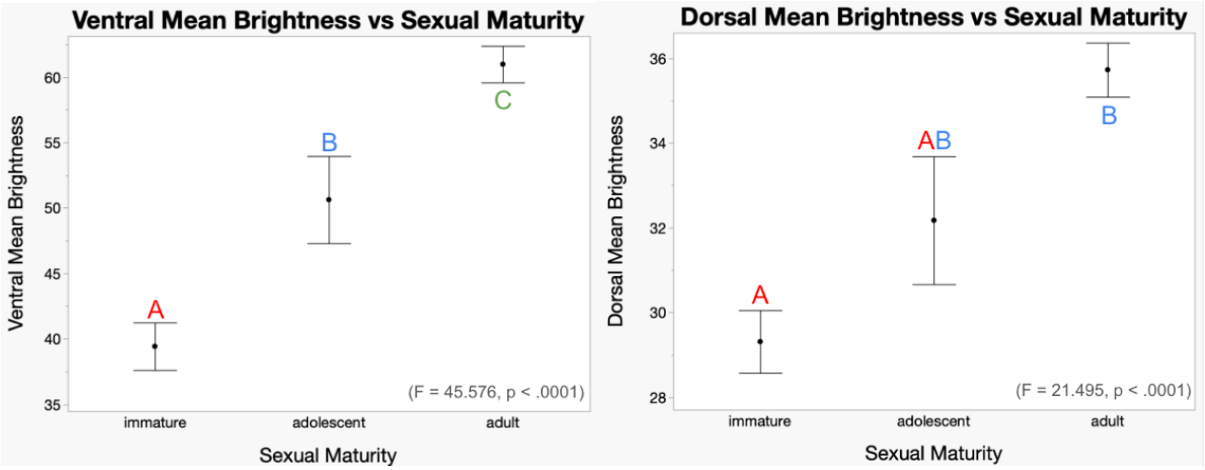

Fig 8. ANOVA test analyzing the relationship between sexual maturity and ventral mean brightness (left; A) and dorsal mean brightness (right; B).

Figure 9. Photographs of a spotty adult paedomorph under natural lighting (top) and spotty adult metamorph under blue excitation light (bottom).

References

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