

Bio-fluorescence in Cannibalism Interactions in MCNP *Ambystoma mavortium nebulosum*

Population

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Abstract:

Many animals bio-fluoresce. Various amphibians have recently been identified as bio-fluorescent. The adaptive value of bio-fluorescence is, as of yet, unknown. Hypothesis abound from camouflage to mate selection, but little research has been completed to confirm them. We studied whether a cannibalistic species *Ambystoma mavortium nebulosum* found at the Mexican Cut Nature Preserve (MCNP), recognizes fluorescence in conspecific hatchlings and whether it uses fluorescence as a cue in it's cannibalistic interactions. We did not find support for this hypothesis.

Intro:

Bio-fluorescence is common in the animal kingdom with species of insects, mammals, reptiles, birds, and fish all exhibiting fluorescence. The recent findings that many amphibians are bio-fluorescent has sparked much public and scientific interest (Lamb & Davis, 2020). Bio-fluorescence occurs when an organism is exposed to light and re-emits light at a lower wavelength. The adaptive value of bio-fluorescence in amphibians is unknown, but several hypotheses exist.

At least one salamander species, *Plethodon metcalfi* has been identified as bio-fluorescently sexually dimorphic (Cox & Fitzpatrick, 2023). The presence of dimorphism in the fluorescent patterns of some amphibian species suggests that fluorescence may be used in mate selection. Other hypotheses are that bio-fluorescence serves in communication, con-specific recognition, or as a camouflage (Lamb & Davis, 2020). For it to be camouflage, either the pond substrate itself or enough of the plant and insect community would also have to fluoresce. Another possibility that isn't given much consideration in the literature is whether fluorescence may be important for prey detection. As of yet, little research has been completed regarding the significance of bio-fluorescence in salamanders. The population of *Ambystoma mavortium nebulosum* in the Mexican Cut Nature Preserve has been under study at the

Rocky Mountain Biological Laboratory since 1982. It offers an excellent opportunity to explore whether bio-fluorescence can be detected by conspecific amphibians and the role that fluorescence may play in predator-prey interactions (Whiteman and Wissinger, 2005).

A. m. nebulosum exhibits two adult morphs: paedomorphs and metamorphs. Paedomorphic adults are sexually mature but remain predominantly aquatic and retain larval characteristics such as gills. Metamorphic adults lose their gill frills and leave their natal ponds. The two morphs breed successfully with one another (Whiteman et al, 1999), and there are various reasons why an individual might undergo paedomorphosis rather than metamorphosis, although the exact purposes are not fully understood. *A. m. nebulosum* is the apex predator of the pond ecosystems studied at MCNP and is a gape-limited predator that cannibalizes small larvae, especially first year hatchlings of their own species. Cannibalism is a fairly common occurrence and a powerful force in these populations and has been shown to drive population fluctuations in the MCNP ponds (Wissinger, 2010).

Lucy Crayton (unpublished data, 2023) confirmed that both morphs of *A. m. nebulosum* fluoresce, and that fluorescence develops over time. Hatchlings exhibit very little fluorescence, ventrally, near the legs with expanding fluorescent area as they grow until they reach sexual maturity with both paedomorphs and metamorphs exhibiting extensive fluorescence on much of their bodies including the dorsal surface (Crayton, unpublished data, 2023). The retinal structures of typical salamanders would indicate that they likely can detect fluorescence. As *A. m. nebulosum* fluoresces in green wavelengths and salamanders typically have red rods which are most sensitive to green light (Lamb & Davis 2020; Chen et. al, 2008). It is unknown whether fluorescence impacts their behavior in any way. The prominence of cannibalism in these populations raises the question: Does the

fluorescence of hatchling larvae attract adult conspecifics? Though the fluorescence of hatchlings is fairly minimal, adults may be able to detect it, and, potentially use it as a cue for locating potential prey. Currently, salamander bio-fluorescence is a new discovery, and there is plenty that the scientific community doesn't yet know. We performed trials with adults reacting to fluorescing hatchling larvae to test whether there is a *A. m. nebulosum* is attracted to bio-fluorescence.

Methods:

To address whether bio-fluorescence plays a role in the cannibalistic interactions of *A. m. nebulosum*, we performed a microcosm experiments adapted from experiments that were piloted at Allegheny College by Dr. Lee M. Demi (Personal Communication 2024). These experiments occurred overnight at the MCNP. Adult paedomorphic salamanders caught from Pond 12 for the long term research project at MCNP were used in these experiments before they were returned to their ponds. In each of three 4 foot artificial pools, which were filled with water from Pond 12, we placed 6 adult salamanders (3 females and 3 males) and 4 minnow traps baited in different ways. Trap 1 was baited with 2 larval hatchling salamanders under blue light made by Bobopai to excite their fluorescence and held in a floating minnow box to prevent them from being cannibalized during the experiment. Trap 2 was baited with two more larval hatchling salamander that were not under excitation light, but were also protected from cannibalism in the same manner. Trap 3 was an unlit and unbaited trap, and Trap 4 was an unbaited trap that was lit by a blue light of the same model as Trap 1. All larval bait salamanders were sourced from Pond 8. The adult salamanders were given a brief acclimation period before the traps were placed and baited. After the traps were set the experiment was left overnight to

allow the 6 adult individuals to choose a trap. The next morning the abundance in each trap was recorded and

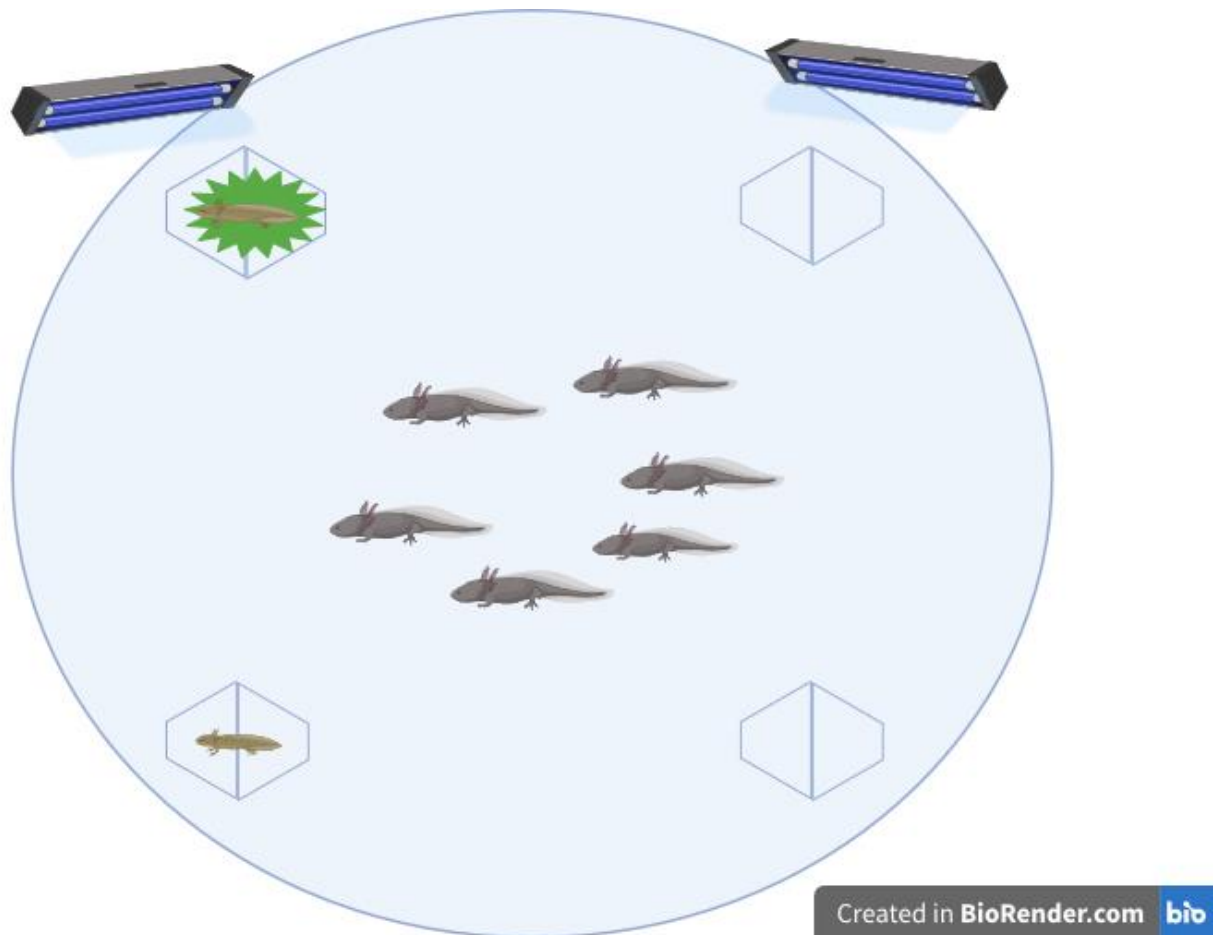
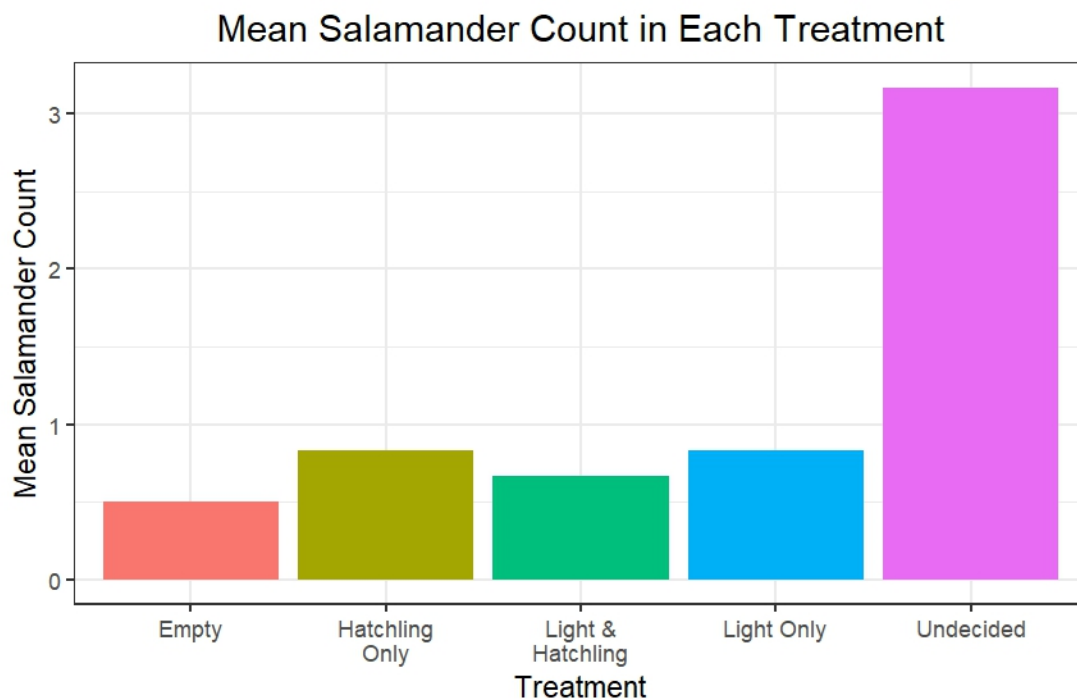


Figure 1: A visualization of the experimental pools with adult paedomorphic salamanders as choosers and larvae in traps as bait.

salamanders were returned to the ponds from which they were caught. This experiment was repeated across 2 nights, August 8th and 9th of 2024. The choices made by the adult salamanders reveal whether they exhibit a preference for fluorescing conspecifics. It was expected that adults would generally demonstrate a preference for traps containing larval salamanders, but a more pronounced preference for fluorescing larvae.

Results:

In fact, *A. m. nebulosum* showed no preference for either lit or unlit larvae. We analyzed the data using Chi-Squared tests. Significantly more salamanders made no choice at all than entered any of the 4 treatment traps (Figure 2, $p = 6.18 \times 10^{-5}$). It should be mentioned that one male was able to escape the experimental pools during the experiments on the night of August 8th, he was counted as undecided for the analysis. When undecided salamanders were removed from the analysis no treatment was significantly more attractive than any of the others ($p = .8856$). An analysis of sex effects showed no significant difference in trap choice between the sexes (Figure 3, $p = .3482$).



Average number of salamanders observed in each treatment

Figure 2:

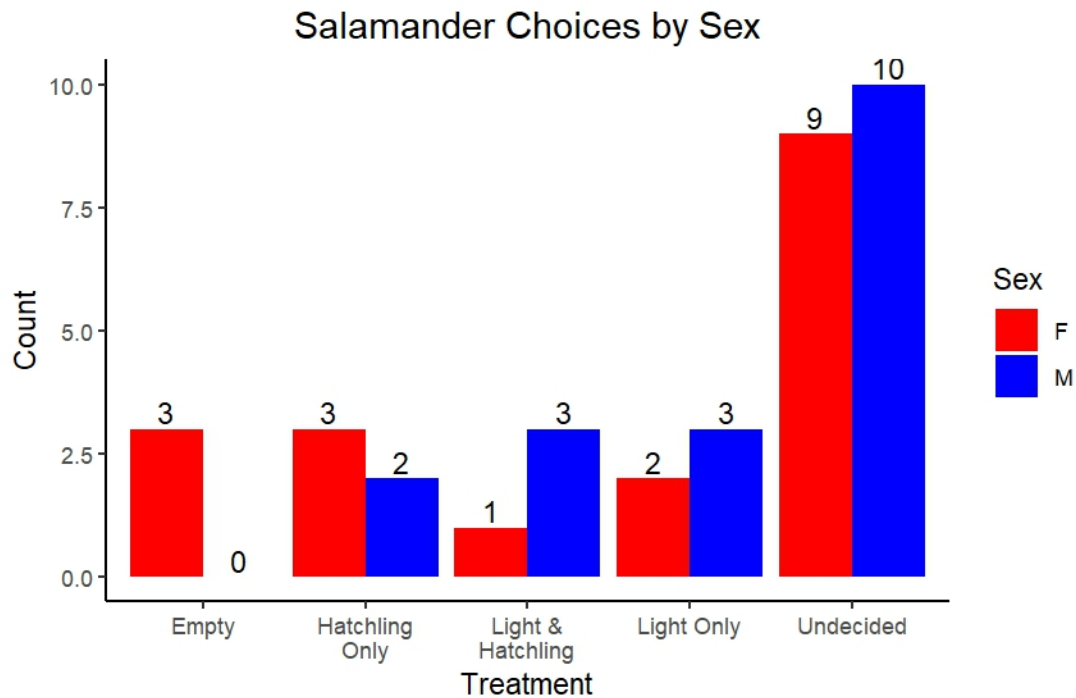


Figure 3: Total

salamander counts of each sex found in each trap across all experimental pools

Discussion:

The results of this experiment do not offer support for the hypothesis that bio-fluorescence of larval salamanders increases the attraction of potentially cannibalistic adult conspecifics. None of our treatments were preferred over remaining outside of traps. And, among the animals that did choose none of our treatments were more popular than the others so it's impossible to conclude that any of them have any influence on the behavior of *A. m. nebulosum*. It is possible that this is due to faulty experimental design rather than an actual lack of attraction.

Salamanders were only considered to have chosen a given treatment if they were actually found within the corresponding trap, however it's possible that the traps were too difficult to enter so that only the most intrepid individuals were recorded as having made a choice. Often, salamanders were found underneath of traps, but were counted as undecided because they weren't inside. It's possible that they in fact did prefer or were pursuing the bait of the trap they were found beneath, but failed to enter, with the current experimental design I can't say. The depth of the water may have contributed to the potential difficulty of entering traps as we weren't able to fully submerge the entrances of the traps.

It may also be that minnow traps are simply ineffective means of capturing *A. m. nebulosum*. We attempted to capture salamanders using the 8 minnow traps in Pond 1 and only caught 16 total animals (Wilborn, unpublished data, 2024). Pond 1 is the most densely populated pond at MCNP and contains hundreds of individuals. Our capture rates compared with the population suggest that minnow traps may not be effective for *A. m. nebulosum*. Though, this was only done once, and it would be irresponsible to draw any firm conclusions from this activity, it is a possibility that should not be ignored. Perhaps more testing on the effectiveness of minnow traps on this species should be conducted.

It was rather surprising, given the cannibalistic tendencies of the population, (Wissinger, 2010) that there was no preference for either of the traps baited with hatchlings. There was free exchange of water between the minnow boxes that held them and the rest of the pool so chemical cues would have been present. It's possible that the visibility was obscured by the lattice of the traps or slight discoloration of the minnow boxes thereby reducing attraction. The most likely reason for the lack of preference for larvae was the lack of a starvation period. Typically, cannibalism experiments hold animals for around 24 hours before they are used in experiments to ensure that they are hungry (Wissinger, 2010; Thomas, Personal Communication, 2024). This wasn't done for these experiments due to a lack of time and the expectation that it would likely not be necessary for the MCNP salamanders thanks to the relative lack of resources available at MCNP due in part to the high elevation and associated short growing season.

The broader questions of whether conspecifics recognize bio-fluorescence in *A. m. nebulosum* and the potential purposes of bio-fluorescence in this organism remain unresolved. From this experiment we cannot conclude that bio-fluorescence plays any role in *A. m. nebulosum*'s cannibalistic interactions, although this data doesn't deny the possibility and future research may still find support for this hypothesis.

References

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