University of North Carolina Asheville
Journal of Undergraduate Research
Asheville, North Carolina
May 2018

Predation rates on *Ambystoma maculatum* (spotted salamander) polymorphic egg masses

Laura Fullmer
Biology Department
University of North Carolina Asheville
Asheville, NC 28804 USA

Faculty Advisors: Dr. Rebecca Hale, Professor Caroline Kennedy

Abstract

A polymorphism results when a gene has multiple alleles, and polymorphic species can have many different forms. *Ambystoma maculatum* (spotted salamander) lays polymorphic egg masses that appear either clear or white. This study examined the effects of egg mass morph on predation rates in the natural environment to determine possible advantages of the polymorphism. It was hypothesized that clear egg masses would be predated upon more heavily because white masses have a survivorship advantage under predation. This study examined the relationship between egg mass volume and embryo number for masses of both morphs, surveyed natural predators in the field, and used volume as an indicator of predations rates. Twelve different taxa were surveyed as possible predators in the field, with *Ambystoma opacum* being most prevalent. Change in egg mass volume was not significant for either morph, but white masses experienced a relatively smaller change in volume than clear masses. The lack of significant difference in the change in volume suggests that morph does not significantly influence predation rates. It is expected that some other fitness advantage maintains the white morph rather than greater survivorship under predation.

Keywords: Polymorphism, *Ambystoma maculatum, Ambystoma opacum, predation*

1. Introduction

Some organisms naturally occur in many forms, as genetic variation within a species leads to different phenotypes. It can be difficult to explain why a polymorphism might persist within a population: if one phenotype contributes to slightly higher fitness, the other phenotypes should disappear over time. Co-occurring polymorphisms might be due to phenotypic advantages as specific phenotypes experience greater success in certain stages of life or when subjected to varied selective pressures. For example, Noonan and Comeault discovered that the color and pattern variations of *Dendrobates tinctorius* (strawberry poison dart frog) are subject to both natural and sexual selective forces. Specifically, predation and mate choice affect phenotypic frequency of *D. tinctorius*.[1] It was found that novel phenotypes were attacked significantly more by avian predators than cryptic or local phenotypes.[1]

Another well documented polymorphism occurs within *Ambystoma maculatum*, the spotted salamander. *Ambystoma maculatum* are native to North Carolina and breed in vernal ponds during late winter and early spring. Females oviposit approximately 2-4 egg masses per season with each containing around 50-80 eggs.[2] A protective jelly matrix surrounds the eggs, constituting a mass, which is often attached to branches and debris below the surface. The egg masses are heritably polymorphic, appearing either white/opaque or clear/translucent.[3] White masses appear milky and tend to be firmer than clear masses, especially as they age and absorb water, due to a crystalline protein that is not present in clear masses.[2,4] However, both morphs become inhabited by a symbiotic green algae *Oophilia amblystomatis*, which helps supply oxygen to the developing embryos under photosynthetic conditions.[4] The embryos, in turn, supply the algae with carbon dioxide and a home in the gelatinous matrix. Some common predators of *A. maculatum* eggs and larvae include *Rana sylvatica* (wood frog) tadpoles, *Notophthalmus viridescens* (eastern newts), caddisfly larvae, and other *Ambystoma* species.[2]

Many studies have examined the polymorphism in *Ambystoma maculatum* (spotted salamander). The biological and chemical components that produce the different phenotypes are well understood, yet the advantages behind each remain unclear. Research conducted by Elsea Brown at the University of North Carolina at Asheville focused on the relationship between *Oophilia amblystomatis* and *Ambystoma maculatum* in the hopes of understanding the advantages of the clear mass morph.[5] Brown hypothesized that clear morphs in high light conditions would better facilitate algal productivity.[5] She discovered that under some conditions, embryos from clear eggs masses are more likely to survive to hatching when protected from predation and that survivorship does differ significantly with egg mass morph.[5] However, it is still unknown what maintains the white morph.

It is possible that the white morph is advantageous under predation, as white masses tend to be firmer than clear masses. This might make embryos in white masses less accessible to some species because predators are unable to physically break through the gelatinous matrix. Although Stenhouse found that larval survivorship is affected by the presence of predators in the environment, such as *Ambystoma opacum* (marbled salamander),[6] whether predation rates differ between morphs is still unsure. Thus, this study focused on answering the following questions: (1) How does morph affect rates of predation on *A. maculatum* egg masses in the natural environment? And (2) What species of predators are feeding on *A. maculatum* in the natural environment? It was hypothesized that clear masses would be more heavily predated upon than white masses under natural conditions and that primary predators of the masses would include *Notophthalmus viridescens* (eastern newt) and *Rana sylvatica* (wood frog).

2. Methods

2.1. Determining Volume/Embryo Relationship

Both clear and white masses were randomly collected from Sandy Bottom Wetland Preserve in Buncombe, NC on three separate days. The masses were used to establish the relationship between egg mass volume (ml) and embryo number. Egg mass volume was measured by displacement with a 2000 ml graduated cylinder. The graduated cylinder was filled to an established, designated initial volume (typically 200 ml) with water, and then an egg mass was added to the liquid. Excess liquid was drained off the masses and debris was removed before the masses were placed into the cylinder for measurement. The final volume of the egg mass and water was recorded, and the egg mass volume was determined by subtracting the initial water volume from the final volume.

The number of embryos in an egg mass was determined after measuring mass volume. Two different counting methods were used on masses. The first counting method consisted of counting visible embryos by placing masses between two pieces of Plexiglas and lighting them from behind via a white-light transilluminator. Grid transparencies were placed on top of the glass, and egg masses were viewed from above. The second method entailed physically breaking apart the gelatinous matrix of an egg mass in a shallow plastic bin and then transferring each individual embryo to a separate container via pipettes. Embryos were counted using clickers to maintain precision and accuracy.

2.2. Field Study

White and clear egg masses were randomly collected and retrieved from Sandy Bottom Wetland Preserve. Eighty egg masses were collected on different dates in three groups: the first group consisted of 20 masses, the second group consisted of 32 masses, and the final group consisted of 28 masses. Eighty cages were constructed out of green hexagonal mesh wire and black plastic zip-ties to hold egg masses in the field. Wire was cut using standard scissors and assembled into cages by hand. Cages varied in size, but most cages were constructed by cutting a T-shape out of a 14 x 16 in. rectangle. Other cages were put together from the remaining pieces and fragments of wire to eliminate waste. Variation in cage size was beneficial, as some experimental masses were smaller than others. Each egg mass was placed in a cage, and the cages were sealed with zip-ties. Small circular tags with 3-digit numerals were attached to the outside of the cages for easy identification. Tags were either green or red: red indicated a clear mass, and green indicated a white mass. Twenty white PVC pipe poles were set up in the field. Ten poles were placed in a line down a shady channel, whereas the other 10 poles were spaced out circularly in an open, sunny, and grassy area. Four cages

were attached to each pole in a random fashion using zip-ties: 2 white masses and 2 clear masses per pole. During the field experiment, water temperature (°C), dissolved O_2 content (mg/L), and total depth (cm) were measured at each pole on a weekly basis. After several weeks in the field, masses were retrieved according to group. The first group of 20 masses was collected from the field for initial measurement on March 6 except for a single white mass that was collected on March 8 to make the number of clear and white masses equivalent: 10 clear and 10 white. Cages were set out on March 8, and the masses were then retrieved for final measurement on April 15. The second group of 32 masses was collected for initial measurement on March 17. Sixteen masses were clear, and 16 masses were white. Cages were set out on March 19 and retrieved for final measurements on April 20. The third group of 28 masses was collected for initial measurements on March 26. Fourteen masses were clear, and 14 masses were white. Cages were set out on March 27 and were retrieved for final measurement on April 21.

2.3. Surveying the Natural Community

The aquatic fauna of Sandy Bottom Wetland Preserve was sampled on four different days during the months of March and April. Cylindrical metal trash cans were used as throw traps. The open grassy area and shallow channel of the wetland were walked as traps were randomly thrown every few steps by walking in zigzag patterns. Small dip nets were used to sweep the water inside of the trash can and collect animals. Animals were identified visually without a key. The taxa and number of animals found with each throw were recorded. Three samples were taken on March 8. Throw one was at a total depth of 15.5 cm, and throw three was at a total depth of 12.0 cm. No depth measurement was taken for throw two. On March 17, fourteen samples were taken with seven of the throws in the shallow channel and seven throws in the open grassy area. Thirteen samples were taken on March 22, and seventeen samples were taken on April 13.

2.4. Statistical Analyses

The relationship between egg mass volume and embryo number was examined with Pearson correlation analyses for each morph and both morphs combined. A t-test was used to determine if egg mass volume and embryo number differed significantly between morphs. Change in egg mass volume for both morphs was analyzed using ANOVA and a Mixed-effect model took into account pole number. Three masses were left out of the Mixed-effect model because pole numbers were unable to be determined. Correlation analyses determined the relationships between the dissolved oxygen (mg/L), temperature (C), and total depth (cm) of the water at each pole number. All statistical analyses were performed in RStudio.

3. Results

3.1. Volume/Embryo Relationship

The relationship between egg mass volume (ml) and embryo number for both morphs was positive. The relationship between volume and embryo number was not significant for white masses but was for clear masses (Fig. 1; White: $y = 38.50 + 0.41x$, $t= 1.69$, $R^2= 0.240$, $p= 0.126$; Clear: $y = 31.83 + 0.53x$, $t= 6.41$, $R^2= 0.622$, $p < 0.0001$). When clear and white masses were combined for analysis, there was a significant increase in volume with embryo number ($y = 32.38 + 0.51x$, $R^2= 0.513$, p-value < 0.0001).

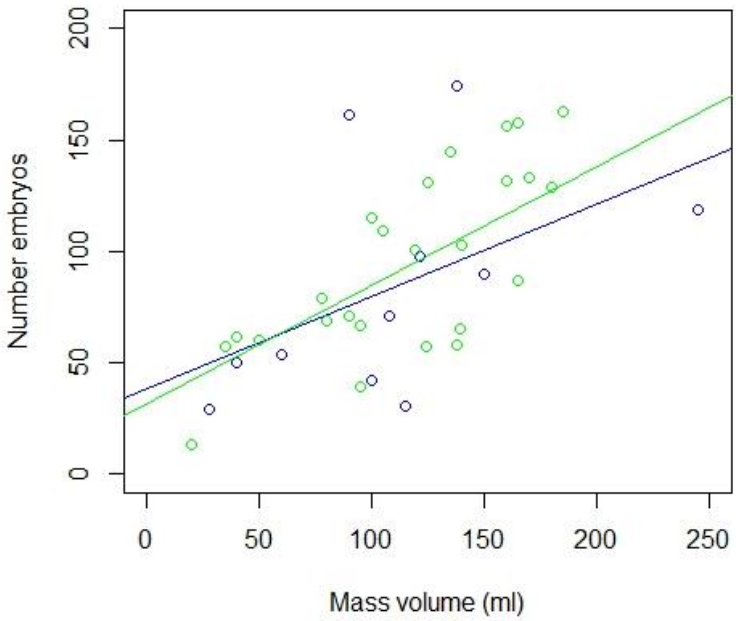

Figure 1. Egg mass volume and embryo number relationships.

Figure 1. Egg mass volume (ml) and embryo number for white masses (blue dots) and clear masses (green dots) of *Ambystoma maculatum* (spotted salamander) (White: $y = 38.50 + 0.41x$, $t = 1.69$, $R^2 = 0.240$, p-value= 0.126; Clear: $y = 31.83 + 0.53x$, $t = 6.41$, $R^2 = 0.622$, p-value < 0.0001).

3.2. Field Study

Site conditions varied with dates and with pole number. Total water depth at the site increased from March 23 to April 20 by an average of 11.69 cm. Poles located in the open, grassy area tended to have higher water dissolved oxygen content than poles located in the shaded channel (Fig. 2). Temperature was fairly consistent across poles, while the total water depth at poles in the shaded channel tended to be greater (Fig. 2). Qualitatively, temperature increased slightly over time (Fig. 3), while dissolved oxygen decreased slightly over time (Fig. 4).

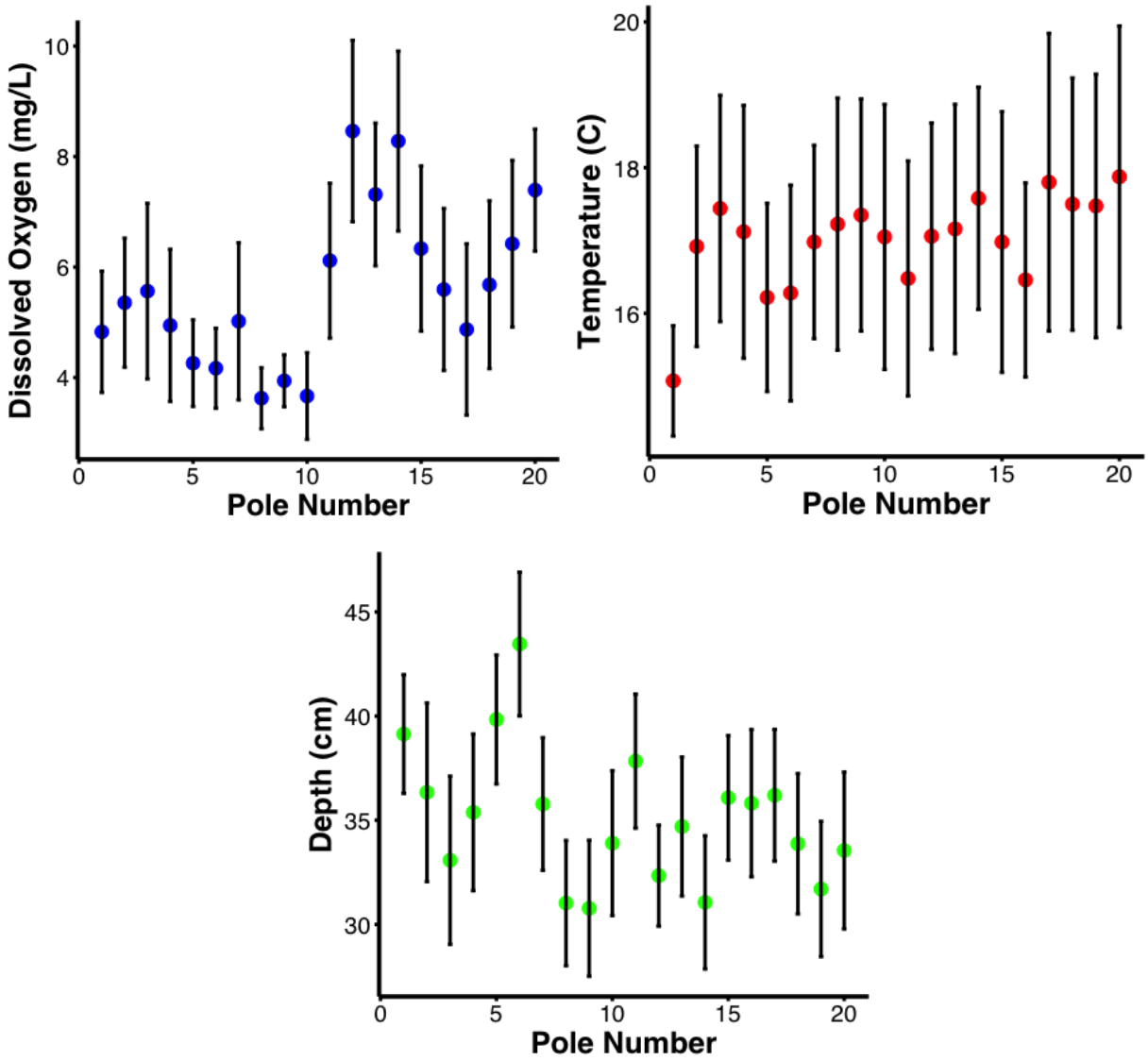

Figure 2. Mean (±SE) dissolved oxygen (mg/L), temperature (C), and total depth (cm) for water at Sandy Bottom Wetland Preserve.

Figure 2. Mean (±SE) dissolved oxygen (mg/L), temperature (C), and depth (cm) for water at Sandy Bottom Wetland Preserve at each site location (pole number) over the months of March and April. Variation in depth at a single pole was due to increased rainfall.

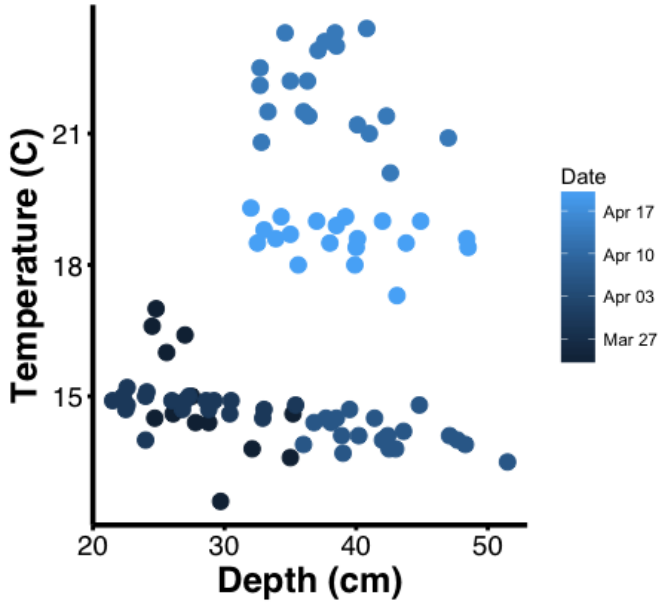

Figure 3. Relationship between temperature (C) and total depth (cm) of water.

Figure 3. Relationship between temperature (C) and depth (cm) of water at Sandy Bottom Wetland Preserve over the months of March and April.

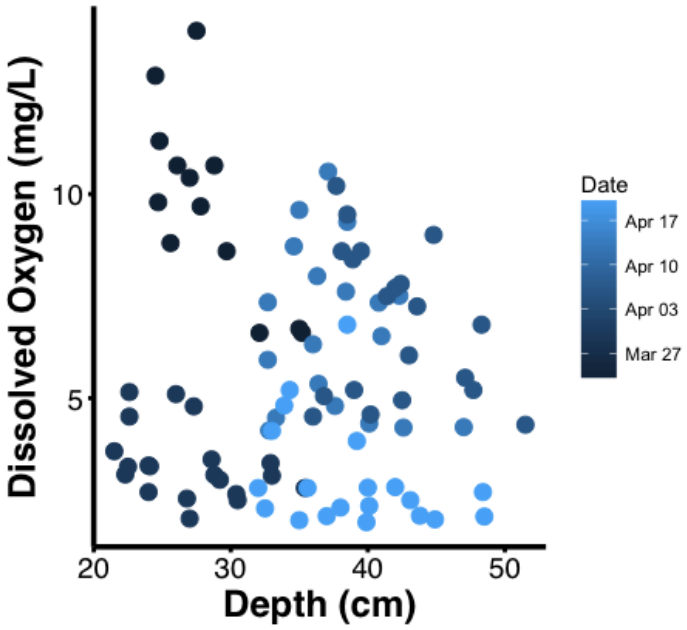

Figure 4. Relationship between dissolved oxygen (mg/L) and total depth (cm) of water.

Figure 4. Relationship between dissolved oxygen (mg/L) and depth (cm) of water at Sandy Bottom Wetland Preserve over the months of March and April.

Most egg masses increased in volume over the course of the experiment (mean change in volume= 232). However, change in egg mass volume was not significant for either morph ($F_{1,74}$= 0.0549, p= 0.815). White masses experienced a change in volume 3.495 ml less than clear masses (Fig. 5). Egg mass volume did not differ significantly with pole number ($F_{1,53}$= 0.141, p= 0.708).

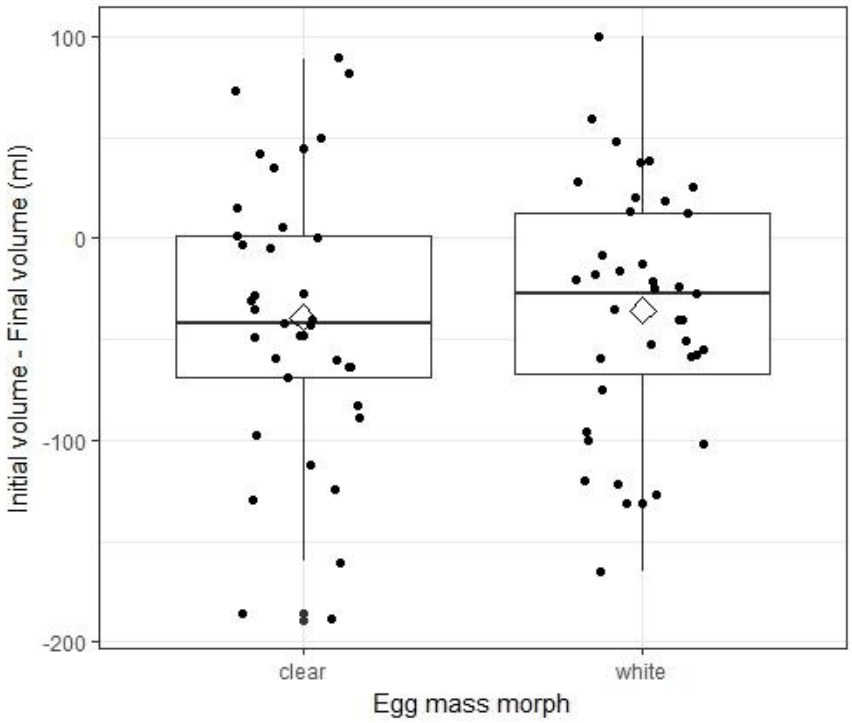

Figure 5. Change in egg mass volume (ml).

Figure 5. Change in egg mass volume (ml) for white and clear masses of *Ambystoma maculatum* (spotted salamander). Diamonds represent mean volumes (mean change in volume= 232, F= 0.0549, p= 0.815).

3.3. Survey of the Natural Community

The natural community surveyed at Sandy Bottom was fairly diverse. Twelve different taxa were identified: *A. maculatum*, *A. opacum*, *Anax*, Zygoptera, Trichoptera, Notonectidae, *N. viridescens*, *L. sylvaticus*, Amphipoda, Isopoda, Annelida, and Chironomidae (Table 1). A total of 66 individuals were sampled. *A. opacum* was most prevalent with 16 individuals. Notonectidae was least prevalent with only one individual. The *A. maculatum* specimen sampled was a clear egg mass.

Table 1. Aquatic fauna observed as possible natural predators of *Ambystoma maculatum* (spotted salamander) at Sandy Bottom Wetland Preserve during 4 days of randomized trash can sampling (N=47 throws, N=66 animals).

	Taxa											
Date	*A. maculatum*	*A. opacum*	*Anax*	Annelida	Trichoptera	Zygoptera	Notonectidae	*N. viridescens*	*L. sylvaticus*	Amphipoda	Chironomidae	Isopoda
3/8/17	0	0	0	0	0	0	0	3	3	0	0	0
3/17/17	0	8	2	0	0	0	1	0	0	3	3	5
3/22/17	1	6	0	2	0	0	0	2	0	0	0	0
4/13/17	1	2	0	5	3	4	0	2	0	1	0	1

4. Discussion

Other studies have found lower predations rates among white masses.[2] However, unlike this study, such studies have been under unnatural, microcosmic conditions where the type of predator is controlled, and predators have less choice and freedom. Petranka et al. examined predation only by *Rana sylvatica* (wood frog) tadpoles, and the experiment was done in kiddie pools.[2] More recently Jacobsen did a mesocosm study at field site in which *Rana sylvatica* (wood frog) tadpoles were confined with clear and white masses and found that they nibbled more on clear.[7] This study examined predation rates under natural conditions in the field, so predators where not limited in options or movement. Therefore, it might be reasoned that although white masses appear to be less favorable to predators under unnatural predatory conditions, they do not have a natural advantage over clear. Also, the survey of the natural community in this study suggests that *Rana sylvatica* (wood frog) might not be a primary predator of *A. maculatum* at Sandy Bottom Wetland Preserve, as no wood frog tadpoles were found in the trash can samples.

Surprisingly, final egg mass volumes tended to be greater than initial volumes for both white and clear morphs. This is likely explained by the swelling of the gelatinous matrices of egg masses as masses absorbed water overtime in the field. This swelling confounds the accurate determination of predation rates on white and clear masses, since many of the masses were swollen to volumes greater than what they were at the start of the field experiment. Using volume as an indicator of predation rates, then, might not be the best method for future experiments. The embryo number of egg masses, however, did increase with volume for both white and clear morphs with volume explaining about 50.0% of variation in embryo number. Since embryos from the masses of this study were all early in development and younger than Harrison's stage 10, the regression lines of the Pearson correlation analyses could be used in the future to predict embryo numbers of *A. maculatum* egg masses. Nonetheless, the lack of significant difference in the change in volume of white masses and the similar slopes of the linear regressions for the change in volume of each morph indicate that white and clear masses of *A. maculatum* have common significant relationships and conflicts with the hypothesis that white masses have a survivorship advantage under predation. This suggests, then, that morph does not significantly influence predation rates.

The increase in water temperature with total water depth at Sandy Bottom likely occurred as a result of overall temperature and rainfall increasing as spring progressed from March to April. Variation in depth correlated more with time and rainfall patterns making the site deeper throughout the course of the study. Cold water tends to hold more oxygen, so this might explain why dissolved oxygen tended to decrease as the water at the site got deeper and warmer over the season. Temperature of the water might influence the breeding habits of *A. maculatum* and the development and success of *A. maculatum* embryos. Namely, Baldauf found that temperature and humidity seem to influence spring migration and breeding in *A. maculatum* the most, as increased temperatures and snow water run-off trigger migration.[8] Temperature might impact the relative survivorship of clear and white egg masses depending on algal symbionts, as higher temperature would increase embryo metabolism and facilitate the mutualistic relationship between *A. maculatum* embryos and *Oophilia amblystomatis*. Since Brown found that clear masses tend to have higher survival and greater success than white masses when inhabited by algae under certain conditions,[5] clear masses

with algae would likely have higher survivorship in the field relative to white when the mutualism is enhanced by warmer temperatures.

Moreover, predation on *A. maculatum* egg masses might be quantified more precisely by observing predator behavior and feeding rates in the field via direct observation and videotaping, and some other future studies might examine morph advantages of *A. maculatum* after hatching to see if there are survivorship advantages for hatchlings and juveniles of a particular morph when under predation.

5. Acknowledgements

The author wishes to express her appreciation to Dr. Rebecca Hale and Professor Caroline Kennedy for their mentorship, help in the laboratory and field, and assistance in drafting this paper. The work of Mischa Derrico and Natriefia Miller is appreciated, as is Dr. Timothy Forrest's guidance in data analyses. Access to Sandy Bottom Wetland Preserve, laboratory facilities, and equipment were provided by the University of North Carolina at Asheville, and transportation was graciously provided by Mischa Derrico and Dr. Rebecca Hale.

6. References

1. B.P. Noonan & A. Comeault, "The Role of Predator Selection on Polymorphic Aposematic Poison Frogs", *Biology Letters* 5 (2009): 51-54.
2. J.W. Petranka, A.W. Rushlow, & M.E. Hopey, "Predation by Tadpoles of *Rana sylvatica* on Embryos of *Ambystoma maculatum:* Implications of Ecological Role Reversals by *Rana* (Predator) and *Ambystoma* (Prey)", *Herpetologica* 54 (1998): 1-13.
3. B.C. Ruth, W.A. Dunson, C.L. Rowe, & S.B. Hedges, "A Molecular and Functional Evaluation of the Egg Mass Color Polymorphism of the Spotted Salamander, *Ambystoma Maculatum*", *Journal of Herpetology* 27 (1993): 306-314.
4. L.M. Hardy & M.C. Lucas, "A Crystalline Protein Is Responsible for Dimorphic Egg Jellies in the Spotted Salamander, *Ambystoma Maculatum* (Shaw) (Caudata: Ambystomatidae)." *Comparative Biochemistry and Physiology Part A: Physiology* 100A (1991): 653-660.
5. C. Brown, "Differential Fitness Maintains Polymorphism in *Ambystoma maculatum*," University of North Carolina at Asheville (2016).
6. S. Stenhouse, N. Hairston, & A. Cobey, "Predation and Competition in *Ambystoma maculatum* Larvae and Laboratory Experiments", *Journal of Herpetology* 17 (1983): 210-220.
7. C. Jacobsen, "Predation and Mutualism: Conflicting Selection Pressures Maintain Spotted Salamander (*Ambystoma maculatum*) egg mass dimorphism", Appalachian State University (2015).
8. R.J. Baldauf, "Climactic Factors Influencing the Breeding Migration of Spotted Salamander, *Ambystoma maculatum* (Shaw), *Copeia* 1952 (1952): 178-181.