Influence of water chemistry and predator communities on the egg mass polymorphism of *Ambystoma maculatum*

by

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Dedication

I would like to dedicate this thesis to my parents, Dr. Howard Neufeld and Dr. Claudia Cartaya-Marin and to my wonderful grandmother, Mana. I owe them thanks for their endless support and encouragement and for helping me be the best version of myself.

Abstract

Spotted salamanders (*Ambystoma maculatum*) migrate each spring to temporary ponds to mate. The females lay their eggs in two morphs: clear and opaque. The presence or absence of hydrophobic protein crystals in the egg mass jelly influence whether the mass will be clear or opaque. Previous studies have hypothesized that water chemistry and predator communities may influence which color polymorphism is more prominent in a site by creating the ideal environment for one of the egg mass morphs. For this study, I examined associations between water chemistry, predator communities and egg mass morphology. I measured water chemistry, the abundance of clear and opaque egg masses at three sites in Watauga County, North Carolina each with multiple breeding ponds (n=16). Additionally, in each pond, I used aquatic light traps to sample vertebrates and invertebrate communities during both the spring and late summer. The proportion of clear egg masses ranged from 0-58.5%. However, I found no significant relationship between water chemistry or predator communities and the proportion of clear egg masses. These results suggest that the frequency of egg mass polymorphisms in Watauga County breeding ponds appears unrelated to water chemistry or predator communities. Future investigations into how environmental factors influence the frequency of Spotted Salamander egg mass polymorphisms should examine larger numbers of breeding ponds across a broader range of environmental conditions to better elucidate the factors that influence the prevalence of clear or opaque egg masses.

Introduction

Spotted salamanders (*Ambystoma maculatum*) are common in the eastern North America and range from Georgia north to Canada (Urban et al. 2015). In late winter or early spring, large numbers of *A. maculatum* migrate to breeding ponds during rainy, warm nights to mate and deposit egg masses (Urban et al. 2015). Like many amphibians, *A. maculatum* is sensitive to aquatic pollutants and generally choose temporary ponds that are in woodland areas rather than roadside or urban habitats where pollutant levels may frequently be higher (Turtle 2000). Egg clutches are attached to submerged vegetation and are surrounded by a thick, protective jelly coating that occurs in two morphs: clear and opaque (Ruth et al. 1993, Urban et al. 2015). These two egg morphs are genetically encoded and caused by the presence or absence of a hydrophobic protein crystal in the egg layer; opaque egg masses contain these proteins but clear masses do not (Ruth et al. 1993). The thick jelly coating on the eggs acts as a protective layer from predators as well as environmental elements, such as adverse water chemistry (Altig and McDiarmid 2007). Many predators of amphibian eggs have a difficult time reaching the eggs because of the thick jelly layer (Walters 1975).

Despite decades of study, it is still not clear why *A. maculatum* produces two egg mass color morphs or what environmental conditions favor production of one morph over the other. Both color morphs are commonly found together in the same breeding populations. Whether a female will lay an opaque versus clear egg mass does seem to be under genetic control (Ruth et al. 1993). Females in captivity will lay only an opaque mass or a clear egg mass, but never both. Females with white ovisacs lay opaque egg masses and females with black ovisacs lay clear egg masses (Hardy and Lucas 1991).

Ambystoma maculatum egg masses have a symbiotic relationship with the green algae (*Oophila amblystomatis*), which may help facilitate the exchange of gases through the thick jelly layer (Pinder and Freit 1994). Jacobsen (2015) found that algal densities were higher in clear egg masses suggesting that the clear egg masses may facilitate higher rates of algal photosynthesis, which presumably contributes to increased hatching success (Jacobsen 2015).

There is evidence that the frequency of the egg mass polymorphism may be influenced by predator communities (Shu et al. 2015). Ponds with predatory fish typically have low numbers of breeding amphibian species, however some amphibians with large clutches, including the spotted salamander, may co-occur with predatory fish (Hecnar and M'Closkey 1997). Although *A. maculatum* larvae are top predators in many ponds, their eggs are consumed by Wood frog (*Rana sylvatica*) tadpoles (Petranka et al. 1998). Jacobsen (2015) found that clear egg morphs were preferred by *R. sylvatica* tadpoles over opaque egg morphs. Additionally, larvae of the caddisfly, *Banksiola dossuaria* are also reported to consume unhatched embryos as well as newly-hatched *A. maculatum* larvae (Stout et al. 1992, Rowe et al. 1994). Petranka et al. (1998) found that opaque egg masses were preyed upon less frequently than clear egg masses, however Rowe et al. (1994) found that caddisfly larvae had no preference for clear or opaque eggs.

Water chemistry may also play a role in the frequency of opaque egg masses. King (2016) examined water chemistry and predator communities from mountain and Piedmont breeding ponds in North Carolina. He found that water chemistry was correlated with the frequency of egg mass polymorphism in mountain ponds but also that opaque egg masses were only found in mountain ponds. Larvae hatching from opaque egg masses may have higher survivorship in waters with lower nutrients compared to the survivorship than those hatching from clear egg masses in waters with lower nutrients (Pintar and Resetarits 2017). King (2016) suggested that *A. maculatum* may breed in ponds within their ideal pH range, though some temporary ponds have high rates of acidification (Rowe et al. 1994). Pough and Wilson (1977) found that *A. maculatum* bred in ponds with a pH range of 6-10, however ponds with pH ranging from 7-9 had the greatest hatching success rates. Amphibians are vulnerable to acid deposition in water (Pierce 1985) and likely choose oviposition sites that fall within their ideal pH ranges. King (2016) found that high-elevation ponds typically had lower pH and lower proportions of clear egg masses compared to lower-elevation ponds with higher pH and a higher proportion of clear egg masses. Some *A. maculatum* egg predators including Wood frogs and the caddisfly (*Ptilostomis postica*) are tolerant of low pH levels and may influence egg mass survivorship (Rowe et al. 1994). Thus, water chemistry may influence both egg mass polymorphisms directly by affecting larval development and survival and indirectly by influencing predator communities.

Here I investigate associations between water chemistry, predator communities, and egg mass polymorphism in high-elevation (~1000m) ponds in northwestern North Carolina. I predict that sites with a lower pH and lower DO will have high proportions of opaque egg masses. Additionally, I predict that predator communities will influence the frequency of clear egg masses but only in high-DO ponds because survival of larvae from opaque egg masses in low-DO/low-pH ponds is likely to be low.

Methods

I measured water chemistry and predator communities in spotted salamander breeding ponds during February-April 2018 and again in September 2018. I sampled three sites, each with several breeding ponds (n=16 ponds total) in Watauga County, North Carolina. I used a YSI Pro Series Multimeter Water Quality Meter (Yellow Springs, OH) to measure water temperature, pH, NO_3 mg/l, DO concentration (mg/l), Specific conductance (μ s/cm), and salinity (ppt). Additionally, I counted the egg masses and recorded the number of clear and opaque masses in each pond. To measure predator community structure, I placed 3-4 cayalume light stick-baited mesh funnel traps in each pond during April and September (Hillsenhoff and Tracy 1982, Bennett et al. 2012, Madden and Jehle 2012). Traps were deployed at dusk and retrieved the following morning. I field identified, counted, and released all vertebrates and preserved and retained all invertebrates for identification in the lab.

I computed the abundance and diversity of both invertebrate and vertebrate predators as well as the total number of predators in each pond and used SPSS software and SigmaPlot v.14 to conduct all statistical analyses. I used Spearman correlations to examine associations between the proportion of clear egg masses and both spring and late summer water quality and predator data. Since this is a broad ecological survey where there could be many confounding factors affecting the variables being measured and because my sample size was relatively small I set my statistical significance level (alpha) to 0.10. (Toft and Shea 1983).

Results

I found that the proportion of clear egg masses was significantly related to spring water temperature-warmer ponds had higher proportions of clear egg masses relative to colder ponds (r=-0.678, p=0.004). Additionally, egg mass color was significantly related to specific conductance-ponds with higher conductance levels had higher proportions of opaque egg masses (r=0.601, p=0.066).

In contrast, I found no relationship between the proportion of clear egg masses and spring DO (r=-0.265, p=0.314, n=16) and pH (r=-0.363, p=0.163, n=16). However, pond DO and pH were positively correlated (r=0.662, p=0.005, n=16), likely because DO concentrations both directly and indirectly influence pH (Boto and Bunt 1981). Interestingly, there was a marginally significant negative correlation between late summer DO and % clear egg masses (r=-0.535, p=0.098, n=10) suggesting that the frequency of opaque egg is lower in ponds with lower year-round DO levels.

I found few relationships between vertebrate and invertebrate communities and water chemistry or the frequency of opaque egg masses. There was no relationship between spring DO, pH, and the total numbers of invertebrate and vertebrate taxa. One pond at the Brookshire park site was a statistical outlier because of a high number of vertebrates (313) were trapped at the site, the majority of which were newly-hatched larval spotted salamanders and thus not likely to be egg mass predators. Once I removed the outlier from Brookshire Park barbed wire right pond the correlation improved to (r=-0.753, p=0.016, n=9), which shows a negative correlation between DO and total vertebrates. There was no relationship between late summer pH and % clear egg masses ($r=-0.231$, $p=0.490$, $n=10$) and no relationship between late summer DO and the total number of invertebrate and vertebrate taxa.

Discussion

Environmental variables including water chemistry, habitat permanence, and predator communities impart different selection pressures on pond breeding amphibian egg mass size, hatch rate, larval survival and emergence timing (Moore and Townsend Jr. 1998). Temporary ponds vary more in their physical and chemical environment compared to more permanent ponds (Williams 1996). Dissolved oxygen concentrations vary both temporally and spatially in breeding ponds and have been shown to induce behavioral changes including egg mass placement (Pechmann et al. 1989). Temperature changes in temporary breeding ponds can affect life cycle stages of insects (Williams 1996). Amphibians are very sensitive to changes in water chemistry and may avoid areas that are not conductive to survival (Karraker et al. 2008). Roadside ponds in areas that may use salt to deice the roads have higher rates of local extinction (Karraker et al. 2008). At Meat Camp, there was a roadside pond that didn't have any egg masses or other amphibian vertebrates. This could be due to runoff from the road creating an environment that was harmful to amphibians. Even though I did not observe a statistically significant relationship between spring DO and the proportion of clear egg masses, I found the lowest proportion of clear egg masses in ponds with very low and very high DO concentrations and the highest proportions in ponds with intermediate DO levels.

Late summer water chemistry data provided information relevant to developing *A. maculatum* larva as well as for the predator communities. Although I did not find any relationships between late summer DO and invertebrate and vertebrate communities, they provide, along with pond permanence data, insights into conditions that may limit the types of predators that can persist in these ponds. In the spring, I caught more vertebrates whereas in late summer more invertebrates, including predatory beetles and dragon fly larvae were encountered.

It is still unclear to what degree opaque egg masses restrict the transfer of oxygen to developing larvae. *Ambystoma maculatum* egg mass jelly has low rates of water diffusion (Pinder and Freit 1994), suggesting that during later developmental stages (i.e., when larvae and presumably eggs are larger) oxygen would be less likely to reach the center of the egg masses. Although studies on whether the diffusion of oxygen into the center of egg masses varies between clear and opaque morphs have not be done, their study suggests that egg masses with a higher concentration of the symbiotic algae are able to sustain the egg masses at later developmental stages by providing supplemental oxygen to centrally located eggs. Jacobsen (2015) found that egg masses with more algae have increased developmental and hatching rates relative to masses with less algae.

Although *A. maculatum* typically breed in temporary ponds that lack predatory fishes, (Urban et al. 2015) many other predators including frog tadpoles, dragon flies, beetles and caddisfly larvae occupy these habitats (Caldwell et al. 1980). Traps detected fish in several of the ponds sampled at the Brookshire Park and Meat Camp sites. These ponds are more permanent than many of the other ponds sampled and retain water even during dry years (M. Gangloff, pers. Obs). Other studies have found in more permanent ponds, predation may be more important to the survival of salamander eggs and larvae whereas in temporary ponds, competition is more important (Sredl and Collins 1992). It is possible that fish predation in these more permanent sites may have influenced the observed ratios of egg mass morphs, however, egg mass counts were made soon after oviposition and fishes were generally uncommon and limited to an apparently introduced population of Golden shiners (*Notemigonus crysoleucas*) in one of the deeper Meat Camp ponds.

Salamander larvae are often the top predators in temporary ponds and consume a range of prey including other amphibian larvae, macroinvertebrates and zooplankton (Wissinger et al. 1999). However, because of hatching time differences, *A. maculatum* tend to be preyed upon more heavily than other *Ambystoma* larvae because they are often the last species to hatch in ponds with multiple *Ambystoma* taxa or *R. sylvatica* (Walls and Williams 2001). Although Wood frogs egg masses and tadpoles were found in many of these ponds, there are no other *Ambystoma* species known to breed in Watauga County.

DO can impact the breeding behavior of amphibians and larval development (Atlas 1938). The level of DO can also impact the hatching success, with eggs in low-DO ponds showing lower hatching success rates (Sacerdote and King 2009). Sacerdote and King (2009) found that laboratory-raised *A. maculatum* larvae had optimal hatch rates at DO concentrations > 4 mg/l, whereas larvae in field enclosures had optimal hatch rates at DO concentrations of 5-6 mg/l. Spring DO levels in Watauga County breeding ponds averaged 6.53 mg/l, which is consistent with the results found in Sacerdote and King's (2009) study. In contrast, the mean DO concentration measured during late summer was 3.05 mg/l. It is unclear whether DO concentrations influence larval survival in this region but DO strongly influences the survival of predators including fishes. I did not observe any meaningful relationships between DO and invertebrates or vertebrate predators but studies targeting larger numbers of ponds across broader environmental gradients are needed to better understand how much water quality data are needed to get a more complete understanding of how conditions in breeding ponds affect egg mass polymorphisms.

King (2016) found that sites at higher elevations tended to have more opaque egg masses, but that the frequency of color morphs was more variable compared to lower elevations where egg masses were predominately clear. Although I also found that opaque egg masses were more common than clear egg masses in my high-elevation sites (many of which were also sampled by King), ponds at Brookshire Park (a site that was not sampled by King) had the highest proportion of percent clear egg masses (38.9% and 58.5%). It is presently unclear why there are more opaque egg masses as well as more egg mass variability at higher elevations. Future studies targeting breeding ponds at intermediate elevations (300-1000m) may provide some insights into these questions and help to separate the effects of water chemistry and the predator communities on the proportion of egg masses.

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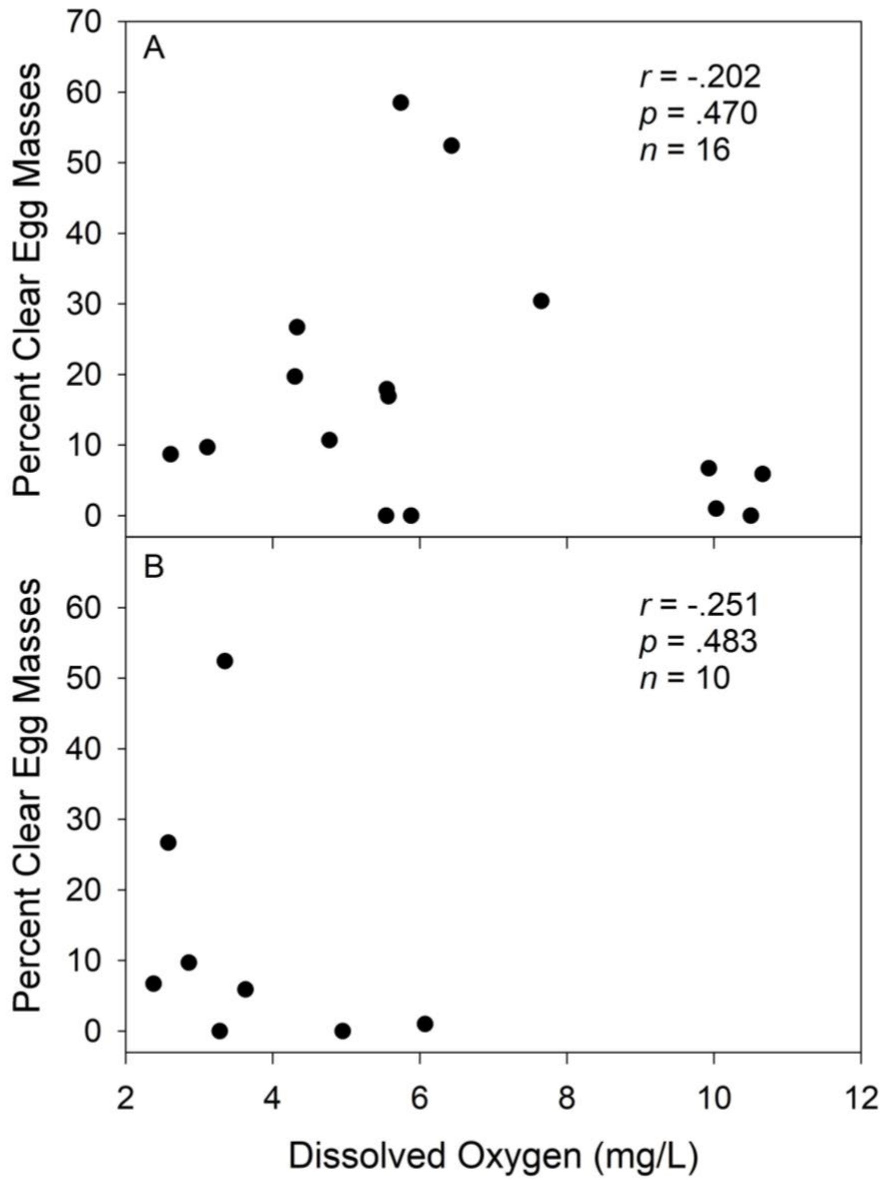

Figure 1. Relationship between Dissolved Oxygen (DO) concentration and the percentage of clear spotted salamander egg masses observed in (A) spring and (B) late summer in Watauga County, NC. Although there is no statistically significant relationship between variables, low-to moderate DO ponds had proportionally higher numbers of clear egg masses whereas high-DO ponds had primarily opaque egg masses.

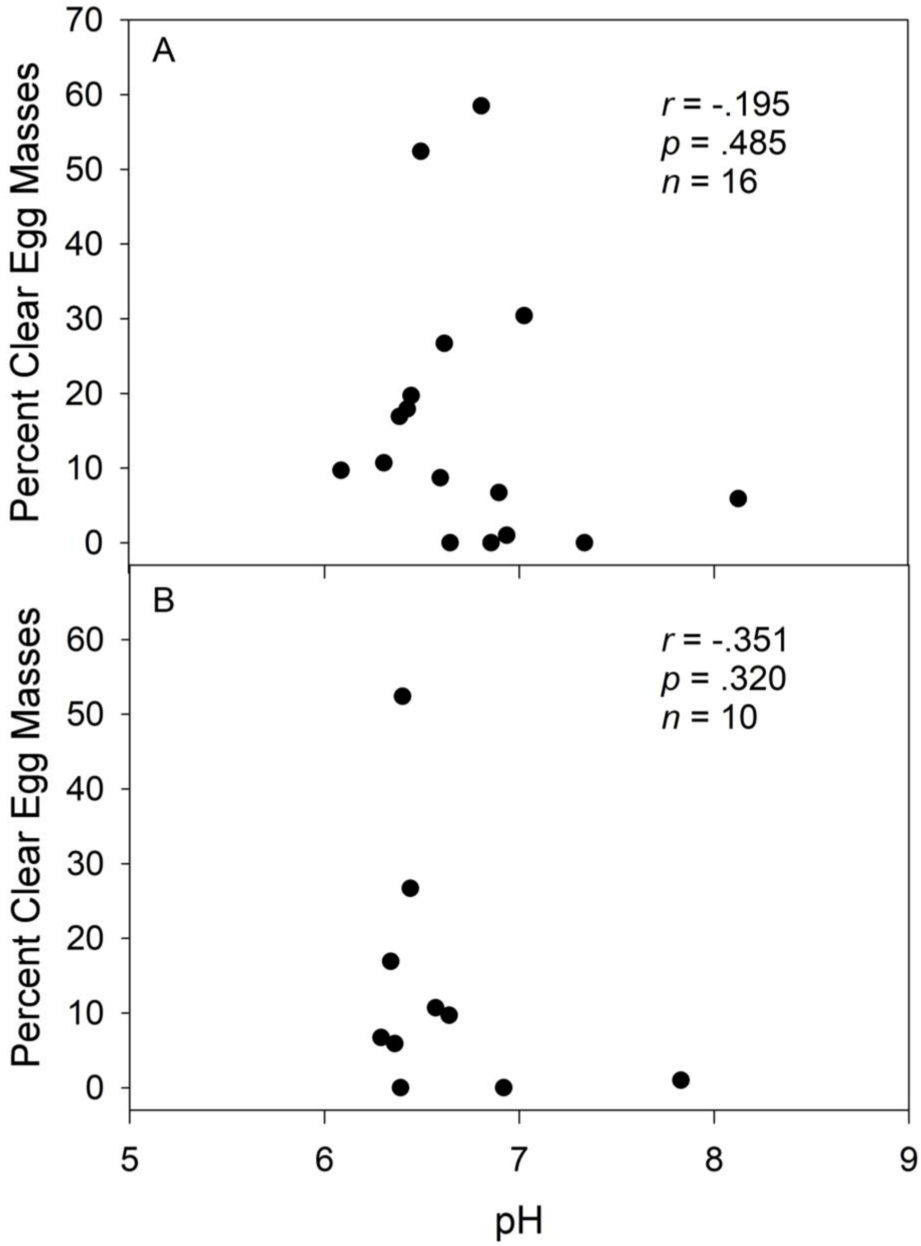

Figure 2. Relationship between pH and the percent clear Spotted salamander egg masses observed in (A) spring and (B) late summer in breeding ponds in Watauga County, NC. There is no statistically significant relationship between the two variables.

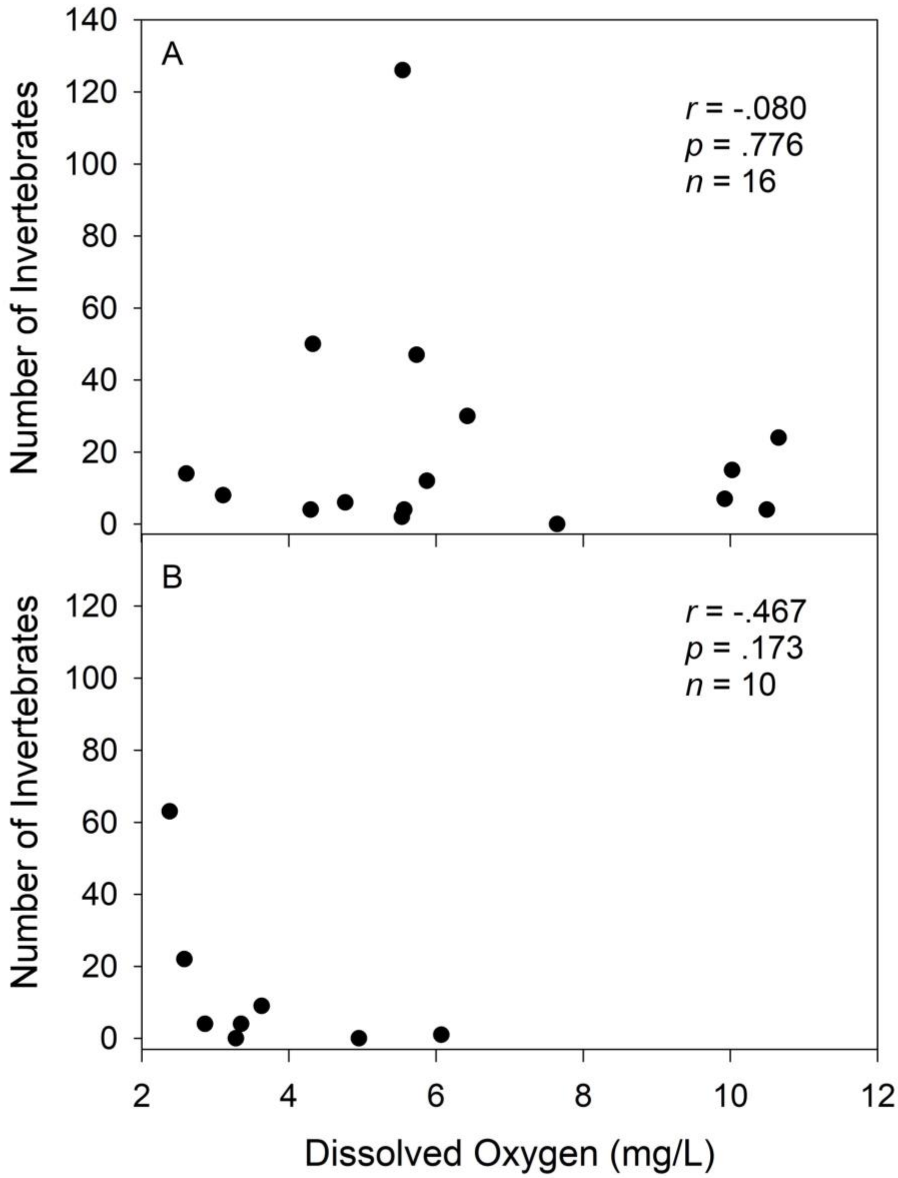

Figure 3. Relationship between Dissolved Oxygen concentration and the total invertebrates trapped at each breeding pond in Watauga County, NC during (A) spring and (B) late summer. There is no statistically significant relationship between the two variables.

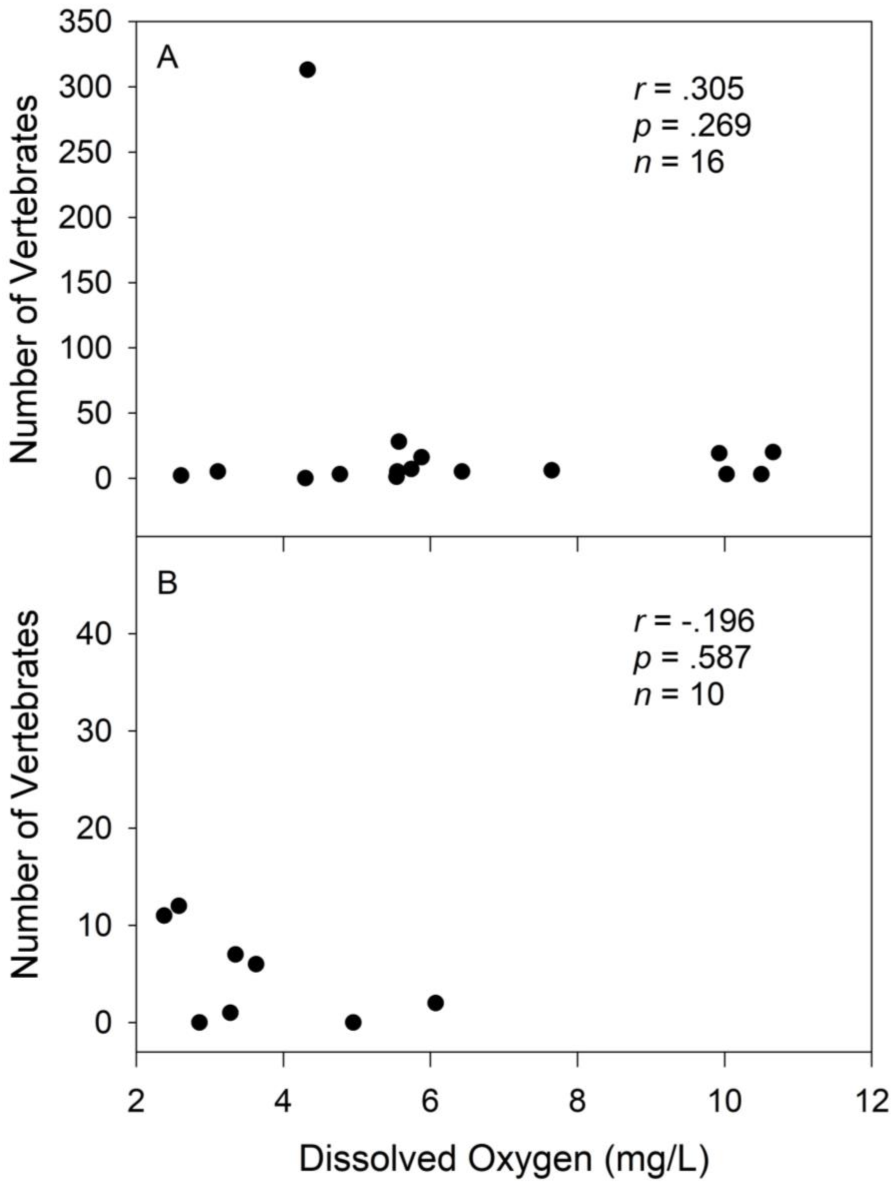

Figure 4. Relationship between Dissolved Oxygen concentration and the total vertebrates counted at each breeding pond in Watauga County, NC during (A) spring and (B) late summer. There is no statistically significant relationship between the two variables.

Table 1. Latitude and Longitude coordinates, elevation, and the total number of *Ambystoma maculatum* egg masses and the proportion of clear egg masses measured at 17 breeding ponds in Watauga county, North Carolina in April 2018.

Site	Pond	Latitude	Longitude	Elevation (m)	Number of Egg Masses	Percent Clear Egg Masses
Twin Rivers	Pond 1	36.16527	-81.75166	899	102	1.0
	Pond 2	36.16528	-81.75555	897	5	0.0
	Pond 3	36.16500	-81.75527	898	68	5.9
	Manmade	36.16333	-81.75806	903	45	6.7
	Natural Pond	36.16333	-81.75806	903	18	0.0
Brookshire	Barbed Wire	36.22917	-81.63750	937	221	38.9
	Forked Pond	36.22805	-81.64306	937	147	58.5
	Septic Pond	36.22806	-81.64361	937	0	0
	Ted Mackeral Pond	36.22916	-81.64166	937	23	30.4
Meat Camp	Pond 1	36.26361	-81.62833	943	23	8.7
	Pond 2	36.26389	-81.62833	944	93	9.7
	Pond 3	36.26389	-81.62917	944	7	0.0
	Boardwalk	36.26389	-81.62889	944	28	10.7
	Flooded path	32.26389	-81.62833	944	0	0.0
	Picnic table	32.26444	-81.63000	945	65	16.9
	Cattails	36.26389	-81.62917	944	66	19.7
	Flag 20	36.26417	-81.62944	945	39	17.9

Table 2. Water chemistry parameters and the total number of vertebrates and invertebrates (taxa richness and abundance) sampled at 15 ponds in Watauga County, North Carolina in April 2018.

Spring						
Site	Pond	DO* (mg/L)	pH	Clear Egg Masses (%)	Number of Vertebrates	Number of Invertebrates
Twin Rivers	Pond 1	10.03	6.94	1.0	3	15
	Pond 2	5.54	7.34	0	1	2
	Pond 3	10.66	8.13	5.9	20	24
	Manmade	9.93	6.90	6.7	19	7
	Natural Pond	10.50	6.86	0	3	4
Brookshire	Barbed wire left	4.33	6.62	26.7	313	50
	Barbed wire right	6.43	6.50	52.4	5	30
	Soccer Field	7.65	7.03	30.4	6	0
	Forked Pond	5.74	6.81	58.5	7	47
Meat Camp	Pond 1	2.61	6.60	8.7	2	14
	Pond 2	3.11	6.09	9.7	5	8
	Pond 3	5.88	6.65	0	16	12
	Boardwalk	4.77	6.31	10.7	3	6
	Picnic table	5.57	6.39	16.9	28	4
	Cattails	4.30	6.45	19.7	0	4
	Flag 20	5.55	6.43	17.9	5	126

*Dissolved Oxygen

Table 3. Water chemistry parameters and the total number of vertebrates and invertebrates (taxa richness and abundance) sampled at 9 ponds in Watauga County, North Carolina in September 2018.

		Late Summer			
Site	**Pond**	**DO* (mg/L)**	**pH**	**Number of Vertebrates**	**Number of Invertebrates**
Twin Rivers	Pond 1	6.07	7.83	2	1
	Pond 2	2.86	6.92	1	0
	Pond 3	3.63	6.36	6	9
	Manmade	2.38	6.29	11	63
	Natural Pond	4.95	6.39	0	0
Brookshire	Barbed wire left	2.58	6.44	12	22
	Barbed wire right	3.35	6.40	7	4
Meat Camp	Pond 2	2.86	6.64	0	4
	Boardwalk	0.57	6.57	5	0
	Picnic table	0.80	6.34	0	55

*Dissolved Oxygen

Appendix 1. Invertebrate taxa encountered in light trap sampling of 9 salamander breeding ponds at the Twin Rivers Development and Brookshire Park in Watauga County, North Carolina in April (S) and September (LS) 2018.

	Twin Rivers										Brookshire Park							
	Pond 1		Pond 2		Pond 3		Manmade		Natural Pond		Barbed Wire		Soccer Field		Forked Pond		Septic Pond	
Invertebrates	**S**	**LS**	**S**	**LS**	**S**	**LS**	**S**	**LS**	**S**	**LS**	**S**	**LS**	**S**	**LS**	**S**	**LS**	**S**	**LS**
Chaoboridae	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Chironomidae	0	0	0	0	0	0	0	93	0	0	0	0	0	0	0	0	0	0
Clams	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Coleoptera	0	1	0	0	6	0	6	2	2	0	0	0	0	0	0	0	0	0
Corixidae	0	0	0	0	0	0	0	7	0	0	0	0	0	0	0	0	0	0
Culicidae	0	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0
Diptera	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Ephemeroptera	8	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Gerridae	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0
Hemiptera	0	0	0	0	16	0	0	0	0	0	0	0	0	0	0	0	0	0
Hirudinea	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Homoptera	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Isopod	0	0	0	0	0	0	1	0	0	0	0	5	0	0	0	0	0	0
Megaloptera	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0
Nepidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Notonectidae	0	1	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0
Odonata	1	3	0	0	0	0	0	29	0	0	0	1	0	0	1	0	0	0
Physidae	0	0	0	0	2	0	0	3	0	0	0	59	0	0	0	0	0	0
Planorbidae	0	0	0	0	0	28	0	0	0	0	80	0	0	0	6	0	0	0
Plecoptera	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Trichoptera	6	0	2	0	0	0	0	0	0	0	0	0	0	0	40	0	0	0
Unknown Arthropod	0	0	0	0	0	0	0	42	0	0	0	6	0	0	0	0	0	0

Appendix 2. Vertebrate taxa encountered in light trap sampling of 9 salamander breeding ponds at the Twin Rivers Development and Brookshire Park in Watauga County, North Carolina in April (S) and September (LS) 2018.

	Twin Rivers										Brookshire Park							
	Pond 1		Pond 2		Pond 3		Manmade		Natural Pond		Barbed Wire		Socer Field		Forked Pond		Septic Pond	
Vertebrates	**S**	**LS**	**S**	**LS**	**S**	**LS**	**S**	**LS**	**S**	**LS**	**S**	**LS**	**S**	**LS**	**S**	**LS**	**S**	**LS**
Anuran larvae	1	4	0	0	1	12	1	26	1	0	218	55	6	0	6	0	0	0
Anuran adult	0	1	0	0	0	0	0	0	2	0	3	1	0	0	0	0	0	0
Blacknose Dace	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Blue Gill	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Crayfish	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Larval newt	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0
Adult newt	0	0	1	0	19	5	16	6	0	0	0	0	0	0	0	0	0	0
Royside dace	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Adult salamander	2	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0
Spotted salamander larvae	0	1	0	0	0	0	0	0	0	0	97	0	0	0	0	0	0	0
Shiners	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Snapping turtle	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0

Appendix 3. Invertebrate taxa encountered in light trap sampling of 8 salamander breeding ponds at the Meat Camp Environmental Studies Area in Watauga County, North Carolina in April (S) and September (LS) 2018.

	Meat Camp															
	Pond 1		Pond 2		Pond 3		Boardwalk		Flooded Path		Cattails		Picnic Table		Flag 20	
Inverts	**S**	**LS**	**S**	**LS**	**S**	**LS**	**S**	**LS**	**S**	**LS**	**S**	**LS**	**S**	**LS**	**S**	**LS**
Sphaeriidae	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Coleoptera	0	0	4	3	1	0	0	0	0	3	0	0	2	1	0	0
Corixidae	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0
Culicidae	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Diptera	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ephemeroptera	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Hirudinea	0	0	0	1	0	0	0	0	0	0	0	0	0	0	3	0
Homoptera	1	0	0	0	0	0	0	0	0	0	0	0	0	0	123	0
Isopod	0	0	0	0	0	0	0	0	0	0	3	0	1	2	0	0
Megaloptera	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Nepidae	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Odonata	0	0	0	1	0	0	0	0	0	3	0	0	0	1	0	0
Physidae	1	0	0	2	1	0	0	0	0	0	0	0	0	47	0	0
Plecoptera	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0
Trichoptera	0	0	4	0	10	0	4	0	0	0	1	0	1	0	0	0
Unknown Arthropod	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0

Appendix 4. Vertebrate taxa encountered in light trap sampling of 8 salamander breeding ponds at the Meat camp Environmental Studies Area in Watauga County, North Carolina in April (S) and September (LS) 2018.

	Meat Camp															
	Pond 1		**Pond 2**		**Pond 3**		**Boardwalk**		**Flooded Path**		**Cattails**		**Picnic Table**		**Flag 20**	
Vertebrates	**S**	**LS**	**S**	**LS**	**S**	**LS**	**S**	**LS**	**S**	**LS**	**S**	**LS**	**S**	**LS**	**S**	**LS**
Anuran larvae	0	0	0	0	1	0	0	0	0	2	0	0	25	0	0	0
Adult newt	1	0	4	0	19	0	0	4	0	0	0	0	3	0	5	0
Rosyside dace	1	0	1	0	0	0	3	0	0	0	0	0	0	0	0	0
Shiners	0	0	0	1	0	0	0	5	0	1	0	0	0	0	0	0
Snapping turtle	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0

Appendix 5. Spearman correlations between water chemistry, percent clear egg masses, and the number of invertebrates sampled at 15 ponds in Watauga County, NC in April 2018. Bold text indicates statistically significant correlations at the alpha level 0.1.

	DO	pH	SPC	Temp	NO_3	% Clear	Inverts
pH	**r= 0.658 p= 0.008 n= 15**						
SPC	**r= -0.732 p= 0.002 n= 15**	**r= -0.586 p= 0.022 n= 15**					
Temp	r= -0.163 p= 0.562 n= 15	r= -0.396 p= 0.144 n=15	r= 0.162 p= 0.564 n=15				
NO_3	r= -0.262 p= 0.345 n= 15	r= -0.341 p= 0.214 n= 15	**r= 0.498 p= 0.059 n=15**	r= 0.119 p= 0.672 n= 15			
% Clear	r= -0.202 p= 0.470 n= 15	r= -0.195 p= 0.485 n= 15	r= 0.199 p= 0.478 n= 15	r= -0.431 p= 0.108 n= 15	r= 0.029 p= 0.917 n= 15		
Inverts	r= -0.080 p= 0.776 n= 15	r= -0.108 p= 0.702 n= 15	r= -0.313 p= 0.256 n= 15	r= -0.068 p= 0.809 n= 15	r= 0.025 p= 0.930 n= 15	r= 0.292 p= 0.291 n= 15	
Verts	r= 0.305 p= 0.269 n= 15	r= 0.225 p= 0.420 n=15	r= 0.068 p= 0.811 n= 15	r= 0.006 p= 0.984 n= 15	**r= 0.633 p= 0.011 n= 15**	r= -0.090 p= 0.750 n= 15	r= -0.084 p= 0.766 n=15

% Clear-Percentage of clear egg masses, SPC- Specific conductance, inverts- invertebrates, verts- vertebrates, temp- temperature

Appendix 6. Spearman correlations between water chemistry, percent clear egg masses, and the number of invertebrates sampled at 11 ponds in Watauga county, NC in September 2018. Bold text indicates statistically significant correlations at the alpha level 0.1.

	DO	pH	SPC	Temp	NO_3	% Clear	Inverts
pH	**r= 0.590 p= 0.073 n= 10**						
SPC	**r= -0.724 p= 0.018 n= 10**	r= -0.109 p= 0.764 n= 10					
Temp	r= 0.275 p= 0.441 n= 10	r= -0.116 p= 0.749 n= 10	**r= -0.675 p= 0.032 n= 10**				
NO_3	r= -0.413 p= 0.236 n= 10	r= -0.213 p=0.555 n= 10	**r= 0.649 p= 0.042 n=10**	**r= -0.570 p= 0.085 n= 10**			
% Clear	r= -0.251 p= 0.483 n= 10	r= -0.351 p= 0.320 n= 10	r= 0.032 p= 0.930 n=10	**r= 0.676 p= 0.032 n= 10**	r= -0.106 p= 0.771 n= 10		
Inverts	r= -0.467 p= 0.173 n= 10	r= -0.419 p= 0.228 n= 10	r= -0.116 p= 0.750 n= 10	r= 0.206 p= 0.569 n=10	r= 0.036 p= 0.922 n= 10	r= 0.054 p= 0.882 n= 10	
Verts	r= -0.196 p= 0.587 n= 10	r= -0.333 p= 0.348 n= 10	r= -0.289 p= 0.418 n= 10	r= 0.542 p= 0.106 n=10	r= -0.214 p= 0.552 n= 10	r= 0.423 p = 0.223 n= 10	r= 0.355 p= 0.314 n= 10

% Clear-Percentage of clear egg masses, SPC- Specific conductance, inverts- invertebrates, verts- vertebrates, temp- temperature