Relationships Between Vernal Pool Reptile and Amphibian Species Composition and Spotted Salamander (*Ambystoma maculatum)* Egg Mass Density in the North Carolina Piedmont

by

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Executive Summary

Spotted salamanders (*Ambystoma maculatum*) are one of many amphibian species that rely on vernal pools as breeding habitat within the northeastern United States (Egan & Paton, 2004; Karraker & Gibbs, 2009). Spotted salamander larvae are common prey for reptile and amphibian species, particularly those with aquatic life stages (Anderson et al., 2017; Hopey & Petranka, 1994; Petranka et al., 1998; Trauth & McAllister, 1995; Urban, 2007; Walls & Williams, 2001). Interspecific and intraspecific competition in crowded habitat can also result in significant mortality of spotted salamander larvae (Walls & Williams, 2001). To increase the odds of larval survivorship, spotted salamanders engage in oviposition site selection based on environmental characteristics (Davenport et al., 2017; Egan & Paton, 2004; Kern et al., 2013; Petranka & Holbrook, 2006) and prefer to breed in isolated vernal pools, areas with lower road densities (Veysey et al., 2011), and pools without fish populations (Hopey & Petranka, 1994). Due to their status as an indicator of species of vernal pool health, it is essential to consistently monitor spotted salamander populations. Spotted salamander egg mass density is a widely accepted metric of population size (Egan & Paton, 2004); therefore, continuous observation of breeding pools provides a valuable inventory of spotted salamander populations in an area.

Although many studies have evaluated the effects of environmental characteristics on spotted salamander egg mass density (Davenport et al., 2017; Egan & Paton, 2004; Kern et al., 2013), few studies have identified the relationships between reptile and amphibian presence and spotted salamander egg mass density. In this study, I evaluated the potential effects of reptile and amphibian vernal pool species composition and environmental characteristics on spotted salamander egg mass density through an observational study in the North Carolina Piedmont. Results from this study will assist in future spotted salamander population surveys, contributing to wetland and amphibian management.

I selected 21 vernal pools within Durham and Orange County to serve as my study sites. I partnered with the Duke Forest and Triangle Land Conservancy to conduct research in their managed forest habitats. During the spotted salamander breeding season (March 2022 – May 2022), I collected data on spotted salamander egg mass density, marbled salamander larval density, reptile and amphibian presence or absence, and environmental characteristics. I used linear regression models to analyze my data and identify variables correlated with spotted salamander egg mass density.

My analysis identified 5 variables significantly correlated with spotted salamander egg mass density:

1. Egg mass density has the strongest correlation with Viperid presence. Higher number of Viperid species at a site is correlated with higher average egg mass density.
2. Higher number of Scincid species at a site is correlated with higher average egg mass density.
3. Salamandrid presence has the weakest correlation with egg mass density; however, higher number of Salamandrid species at a site is correlated with higher average egg mass density.
4. More acidic pH values are correlated with higher average egg mass density.
5. The presence of early breeding spotted salamanders is correlated with higher average egg mass density.

These results suggest that spotted salamanders do not engage in reptile and amphibian predator avoidance, despite their tendency to avoid predatory fish (Hopey & Petranka, 1994). The benefits of finding suitable habitat likely outweigh the costs of predation and resource competition. Due to proximity of the study sites to urban spaces, roads, and agricultural land, optimal vernal pool habitat may be scarce within Durham and Orange County. This would lead to congregation of species with similar habitat requirements, restricting predator avoidance through habitat choice. Predators are also likely to congregate in habitat with high prey availability, explaining the correlation between predatory Viperid and Salamandrid species presence and high spotted salamander egg mass density. The correlation between Scincid presence and high egg mass density is likely due to congregation of these species in resource rich habitat. Additionally, Scincids may provide burrows recruited by spotted salamanders, due to their known use of small mammal burrows as terrestrial habitat (Faccio, 2003; Madison, 1997; Regosin et al., 2003, 2004).

The correlation between acidic pH levels and high egg mass density is likely due to the relatively acidic soil pH in the area (Xi et al., 2008), resulting in spotted salamander tolerance for acidic conditions. Similar studies have found that spotted salamander tolerance for pH levels can vary by region (Portnoy, 1990; Pough, 1976). Furthermore, early breeders will likely select quality breeding habitat, which is often recruited by other salamanders during the breeding season, explaining the correlation between the presence of early breeders and high egg mass density.

Since this study was conducted over a single breeding season, results may vary if the project were continued over several years, resulting in a larger sample size. Additionally, the environmental history of the study area is expected to play a large role in spotted salamander oviposition and breeding site selection. Because of this, spotted salamander breeding site selection is expected to vary across regions, due to differing environmental characteristics across habitats. Future research on effects of predation on larval spotted salamander density will provide further results on interspecies interactions and cascading effects of predation on spotted salamander populations. Additionally, research on the efficiency of the egg mass coating in preventing predation by different species could provide context on direct pressures of predation on egg mass density.

This data contributes to spotted salamander management within the North Carolina Piedmont. Monitoring breeding pool use can assist in evaluation of spotted salamander population size, allowing for implementation of management plans as necessary. Context on interspecies and environmental interactions between spotted salamanders is a necessary component to management techniques. Inventories of breeding pools within the Duke Forest will contribute to community science amphibian breeding monitoring initiatives.

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Abstract

Spotted salamanders (*Ambystoma maculatum*) are essential indicators of vernal pool health, yet little research has identified the effects of interspecies interactions on spotted salamander oviposition. In this study, I evaluated the relationships between vernal pool reptile and amphibian species composition and spotted salamander egg mass density within Durham and Orange County. I observed significantly higher egg mass densities in pools with more Salamandrid, Viperid, and Scincid species despite threats of predation. High egg mass densities were also correlated with the presence of early breeding spotted salamanders and more acidic pH levels. Results suggest that spotted salamanders select breeding sites based on environmental conditions, rather than in avoidance of predation or resource competition. These findings are likely influenced by scarcity of optimal breeding habitat within the study area due to history of urbanization and agricultural land use.

Introduction

Many studies have evaluated the effects of vernal pool environmental characteristics on spotted salamander (*Ambystoma maculatum*) egg mass density (Davenport et al., 2017; Egan & Paton, 2004; Kern et al., 2013), yet few have evaluated effects of species co-occurrence on this variable. Spotted salamander larvae serve as prey for many aquatic species (Anderson et al., 2017; Hopey & Petranka, 1994; Petranka et al., 1998; Trauth & McAllister, 1995; Urban, 2007; Walls & Williams, 2001); therefore, presence of predatory species can reduce spotted salamander breeding success. Egg mass density serves as a metric of spotted salamander population size (Egan & Paton, 2004) and can indicate breeding female population (Veysey et al., 2011). This study will quantify relationships between vernal pool reptile and amphibian composition and spotted salamander egg mass density, which will assist in accuracy of future population surveys.

Results can evaluate potential mutualistic, predatory, or commensal relationships between spotted salamanders and other reptile and amphibian species within the North Carolina Piedmont.

Spotted Salamander Breeding

Spotted salamanders breed almost exclusively in vernal pools, which are essential habitats for early life stages of many amphibian species (Egan & Paton, 2004; Karraker & Gibbs, 2009). Vernal pools are seasonal wetlands with a short hydroperiod that prevents the settlement of common spotted salamander predators such as fish (Hopey & Petranka, 1994). Vernal pools retain water during the winter and spring before drying out during the summer.

During the wet season, spotted salamanders migrate from terrestrial burrows to breed within these seasonal wetlands (Faccio, 2003). Female spotted salamanders deposit egg masses during the breeding season, which often attach to vegetation or sit at the bottom of the pool (Egan & Paton, 2004). Egg masses typically hatch within 4-7 weeks (Veysey et al., 2011), producing aquatic larvae.

Interspecific Interactions

Amphibian species with aquatic life stages, such as the marbled salamander (*Ambystoma opacum*), red-spotted newt (*Notophthalmus viridescens*), and wood frog (*Lithobates sylvaticus*) commonly prey on spotted salamander larvae, decreasing spotted salamander survivorship (Petranka et al., 1998; Urban, 2007; Walls & Williams, 2001). Snakes and fish also prey on spotted salamander larvae and adults, contributing to reduction of spotted salamander biomass (Davenport et al., 2017; Egan & Paton, 2004; Hopey & Petranka, 1994; Trauth & McAllister, 1995). Spotted salamanders engage in predator avoidance through site selection; however, this has only been observed in response to fish presence (Davenport et al., 2017; Egan & Paton, 2004; Petranka et al., 2004; Petranka & Holbrook, 2006). Inter and intra specific competition indirectly reduces survivorship of spotted salamander larvae due to resource competition (Walls & Williams, 2001), indicating further pressures of shared habitat.

Despite pressures of predation and resource competition, spotted salamanders adapt behavior, morphology, and habitat use to facilitate coexistence with other species (Anderson & Whiteman, 2015; Brodman & Jaskula, 2002; Semlitsch, 1988; Urban, 2010; Ward & Sexton, 1981). To reduce predation, spotted salamanders exhibit defensive morphology of egg masses; the protective jelly coating on egg masses successfully prevents penetration by predatory species when intact (Semlitsch, 1988; Ward & Sexton, 1981). Egg masses also vary in color, with opaque egg masses experiencing significantly decreased predation by tadpoles (Petranka et al., 1998). Research has found that larval spotted salamanders display differing morphology across populations to prevent predation, exhibiting rapid evolution as a prey response (Urban, 2010). Despite this, larval spotted salamanders often succumb to predation to a greater degree than embryonic spotted salamanders (Davenport et al., 2017) and rely heavily on underwater vegetation to avoid predators (Brodman & Jaskula, 2002).

Environmental Characteristics

Spotted salamanders engage in breeding pool site selection based on varying environmental characteristics (Davenport et al., 2017; Egan & Paton, 2004; Kern et al., 2013; Petranka & Holbrook, 2006). Studies have found that spotted salamanders have a preference for breeding pools with high vegetation complexity, high canopy cover, (Egan & Paton, 2004), deeper water, and more submerged vegetation (Kern et al., 2013). Spotted salamanders also select for pools in areas with lower road density and prefer isolated vernal pools (Veysey et al., 2011).

Implications

Wetlands are highly susceptible to environmental consequences of human development, which alters habitats of pool breeding amphibians (Veysey et al., 2011). As a result, amphibians face threats such as urbanization and habitat fragmentation, which reduces water quality of vernal pools and limits spotted salamander migration during the breeding season (Veysey et al., 2011). Consequently, spotted salamanders experience significant road mortality while traveling to breeding pools, raising concern for populations in areas with high road densities (Gibbs & Shriver, 2005). Due to the importance of amphibian species as indicators for environmental wellbeing, it is essential to properly monitor amphibian species and prepare comprehensive management plans as necessary.

In this study, I evaluated the relationships between vernal pool reptile and amphibian species composition and spotted salamander egg mass density within the North Carolina Piedmont. I also evaluated relationships between vernal pool environmental characteristics and spotted salamander egg mass density in the study area to account for habitat-influenced oviposition. Presence of predatory species within vernal pools is expected to reduce egg mass density since spotted salamanders frequently engage in site selection and predator avoidance (Davenport et al., 2017; Egan & Paton, 2004; Kern et al., 2013; Petranka & Holbrook, 2006). Pressures of resource competition in pools recruited by amphibians with similar habitat requirements as spotted salamanders may also reduce oviposition in vernal pools. Results are likely to vary across regions, particularly in landscapes with differing environmental histories and habitat structure.

Materials & Methods

Study Organism

Spotted salamanders (*Ambystoma maculatum*) are a species of mole salamander native to eastern North America. Spotted salamanders inhabit terrestrial forest habitats (Homan et al., 2004), residing in underground burrows or beneath forest debris for the majority of the year (Faccio, 2003; Homan et al., 2004). Adult spotted salamanders utilize small mammal burrows as terrestrial refuge (Faccio, 2003; Madison, 1997; Regosin et al., 2003, 2004). In the Spring, this species migrates up to 219 m to vernal pools to breed (Faccio, 2003), where females will lay 1-4 egg masses (Veysey et al., 2011). Although spotted salamanders remain terrestrial during their adult and juvenile life stages, this species breeds in an aquatic environment and remains aquatic during its embryonic and larval life stages. Other pool breeding amphibian species utilize vernal pools during the wet season, resulting in pressure on spotted salamander populations from predation and resource competition. To account for these pressures, spotted salamanders exhibit morphological adaptations that prevent predation of egg masses, such as a protective jelly coating (Semlitsch, 1988; Ward & Sexton, 1981), or opaque color of the egg mass (Petranka et al., 1998).

Study site

This study was conducted at vernal pools within the North Carolina Piedmont (n=21) in Durham and Orange County. 18 pools were located at sites managed by the Duke Forest; 1 pool was located in the Brumley Nature Preserve, managed by the Triangle Land Conservancy; 2 pools were located in unmanaged, public land (Table 1). The forest types in this area predominantly consist of pine, pine-hardwood, upland hardwood, and bottomland hardwood. Due to past agricultural use of the study area, the forests are currently undergoing a transition back to a hardwood dominant state (Taverna et al., 2005; Xi et al., 2008). This area receives a mean annual precipitation of 1.1 m (Taverna et al., 2005) and is home to both seasonal and permanent wetlands. The soils in this area are generally acidic, with a mean topsoil pH of 4.76 throughout the Duke Forest (Taverna et al., 2005).

Table 1: Study Sites			
Site	Location	Latitude	Longitude
1	Duke Forest	36.01013	-78.97243
2	Duke Forest	36.01135	-78.97776
3	Duke Forest	36.01262	-78.97950
4	Duke Forest	36.01439	-78.97747
5	Duke Forest	36.01657	-78.98501
6	Duke Forest	36.02123	-78.98389
7	Duke Forest	36.02066	-78.98391
8	Duke Forest	36.02798	-78.99294
9	Brumley Nature Preserve	36.02897	-79.05150
10	Duke Forest	35.99993	-79.02505
11	Duke Forest	35.99820	-79.03215
12	Public Land	36.05674	-78.96598
13	Public Land	35.96960	-79.07610
14	Duke Forest	36.02090	-78.98146
15	Duke Forest	36.02652	-78.97897
16	Duke Forest	35.98959	-79.03494
17	Duke Forest	35.99996	-79.04105
18	Duke Forest	36.00846	-79.04156
19	Duke Forest	35.99227	-79.04134
20	Duke Forest	35.98010	-79.01700
21	Duke Forest	36.02440	-78.98234

I identified spotted salamander breeding pools using community sourced data from iNaturalist, HerpMapper and a breeding pool list provided by the Duke Forest. Individual pools were identified as those 50 m or farther away from a site. Pools within 50 m of an identified site were considered a single complex site.

Data Collection

Environmental Characteristics

I collected data on pool characteristics that are known to have significant effects on egg mass density from previous research: pH, canopy cover, surface area, and temperature (Egan & Paton, 2004; Horne & Dunson, 1994; Veysey et al., 2011). I approximated pool surface area (m^2) by measuring pool diameter across two transects and calculating the area enclosed using the area of an ellipse formula. Canopy cover was recorded as categorical data describing tree cover in the study site as low, moderate, or high. Temperature and pH of each pool were measured using an Oakton PCSTestr 35 device to control for pond parameters that may affect oviposition. Amphibian breeding and development is impacted by pH levels; therefore, pH measurements will account for some variation in spotted salamander breeding in the study sites (Horne & Dunson, 1994; Portnoy, 1990; Pough, 1976).

Egg Mass Surveys

Spotted salamander breeding began later than typically recorded in the area, as the first identified egg masses of the season appeared in March 2022. Data were collected from March 2022 until May 2022, when egg masses had fully disappeared from the breeding pools. I used a quadrat measuring 1m by 1m to sample egg mass density at each site. Pools with a width less than 1m were sampled using a 0.5 m by 0.5 m quadrat, ensuring that the full area of the quadrat was within the pool and did not extend onto dry land. I placed the quadrat in a random location within the breeding pool and sampled different sections of each breeding pool four times, ensuring no overlap into areas that were previously sampled. Pools that were too small to be sampled 4 times were sampled as many times as possible without overlapping quadrat boundaries. For each quadrat sample, I measured the depth of the pool at the sampled area. I counted all egg masses within the quadrat boundary from all depths. Quadrat volume (m^3) was calculated with the dimensions of the quadrat and depth of the pool, using the area of a rectangular prism formula. Egg mass density (egg masses/m^3) was calculated using the quadrat volume (m^3) and egg mass counts (Rothenberger et al., 2019). Several pools were sampled more than once for egg mass density; however, the breeding season ended before each pool was sampled multiple times.

Species Co-Occurrence Surveys

Minimally invasive methods were used to collect species co-occurrence data to avoid potential harm of herpetofauna in this study. I used visual and auditory surveys, iNaturalist data, and museum data to identify co-occurring reptile and amphibian species at the study sites. Frog species were identified using auditory surveys and were recorded as present or absent at each site. I conducted visual surveys to assess the presence or absence of herpetofauna. I performed visual searches within a 10 m radius of the pool for 5 minutes at each study site. These surveys included a visual scan of the area around the pool and scans underneath forest debris. Species identified and number of each species were recorded for each site. Pools were sampled for co-occurring species several times during the breeding period. Some pools were only sampled once due to time constraints at the end of the breeding season.

Marbled salamander larval density was calculated at each site using dip net sampling. I began sampling marbled salamander larvae density once spotted salamander egg masses appeared in vernal pools and ended sampling once egg masses were no longer in breeding pools or until pools were dry. Using a dip net, I swept each pool 4 times at different areas of the pool, ensuring that the sweeps did not overlap in area. I standardized each dip net sweep to approximately 1 m. I then measured the depth of the net frame during the sweep (m) and the width of the net frame (m) for each sweep. I used the depth of the net frame, width of the net frame, and length of the sweep to calculate the volume of the sampled area (m^3) using the area of a rectangular prism formula.

Organisms captured in the net were transferred to a container filled with water from the sampled pool. I counted the number of marbled salamander larvae captured in the sweep and noted the presence of other amphibian species. Sampled organisms, water, and pool litter were then returned to the pool of origin. I wore nitrile gloves throughout the duration of the sampling process to ensure safe handling of amphibians. Water volume (m^3) sampled by the dip net and the number of marbled salamander larvae collected in each sweep were used to estimate marbled salamander density within each pool (larvae/m^3). The protocol (A188-22-11) for this field work was reviewed and approved by the Duke IACUC committee.

Statistical Analysis

Overview

To answer my research questions, I used linear regression models to fit my data. I created a multiple linear regression model to identify potential effects of breeding pool characteristics and species co-occurrence on egg mass density. To identify potential effects of breeding pool characteristics and species co-occurrence on egg mass presence, I created a binomial regression model. I identified interactions between presence of early breeders and egg mass density using a simple linear regression model.

Breeding Pool Characteristics, Species Co-Occurrence, & Egg Mass Density

I created a multiple linear regression model to evaluate potential effects of breeding pool characteristics and species co-occurrence on spotted salamander egg mass density.

Independent variables included the number of species of reptile and amphibian families at each site and the breeding pool characteristics: pH, canopy cover, surface area, and temperature. The dependent variable is egg mass density (m^3). I met the model's assumptions using residual and AV plots.

To observe the potential effects of breeding pool characteristics and species co-occurrence on breeding pool site selection, I created a binomial regression model. Independent variables included the number of species within reptile and amphibian families at each site and the breeding pool characteristics: pH, canopy cover, surface area, and temperature. The dependent variable is presence or absence of egg masses, indicating breeding pool site selection.

The multiple linear regression model and binomial generalized linear model incorporated all sample sites in the data set, including those with egg mass densities of 0. This ensured that I could test for co-occurring species that drastically reduce egg mass density or deter oviposition within a pool. Both models were fit using backward stepwise regression.

Early Breeding Spotted Salamanders & Egg Mass Density

To test the correlation between the presence of early breeding spotted salamanders and egg mass density in vernal pools, I analyzed my data using a simple linear regression model.

The independent variable of this model is the presence or absence of spotted salamander larvae. The presence of spotted salamander larvae indicates the presence of early breeding spotted salamanders and absence of spotted salamander larvae indicates the absence of early breeding spotted salamanders. The dependent variable of the model is egg mass density (m^3).

Assumptions of the model were tested using residual and AV plots and the model met the assumptions of simple linear regression. Many of the sampled breeding pools did not contain egg masses; therefore, pools with egg mass densities of 0 were removed from the data set for this model. Since this analysis only accounted for currently active breeding pools, the inclusion of pools with an egg mass density of 0 would reduce the accuracy of these test results.

Results

Breeding Pool Characteristics, Species Co-Occurrence, & Egg Mass Density

Egg mass density was significantly correlated with 4 variables from the model ($R^2 = .9511$): number of Salamandrid species ($P = .006971$), number of Viperid species ($P = 1.29e\text{-}12$), number of Scincid species ($P = 0.000441$), and pH ($P = 0.005145$).

Viperid presence had the strongest correlation with egg mass density. Sites with more Viperid species had higher average egg mass densities than sites with fewer Viperid species present (Figure 1). Higher egg mass densities were also observed at sites with more Scincid species (Figure 2). The number of Salamandrid species at a site had the weakest correlation with egg mass density; however, higher egg mass densities were observed at sites with more Salamandrid species (Figure 3). pH was the only environmental factor significantly correlated with egg mass density. Higher egg mass densities were observed in pools with more acidic pH values. pH values at study sites did not drop below 4.36, which is close to the mean topsoil pH of the study area (Figure 4).

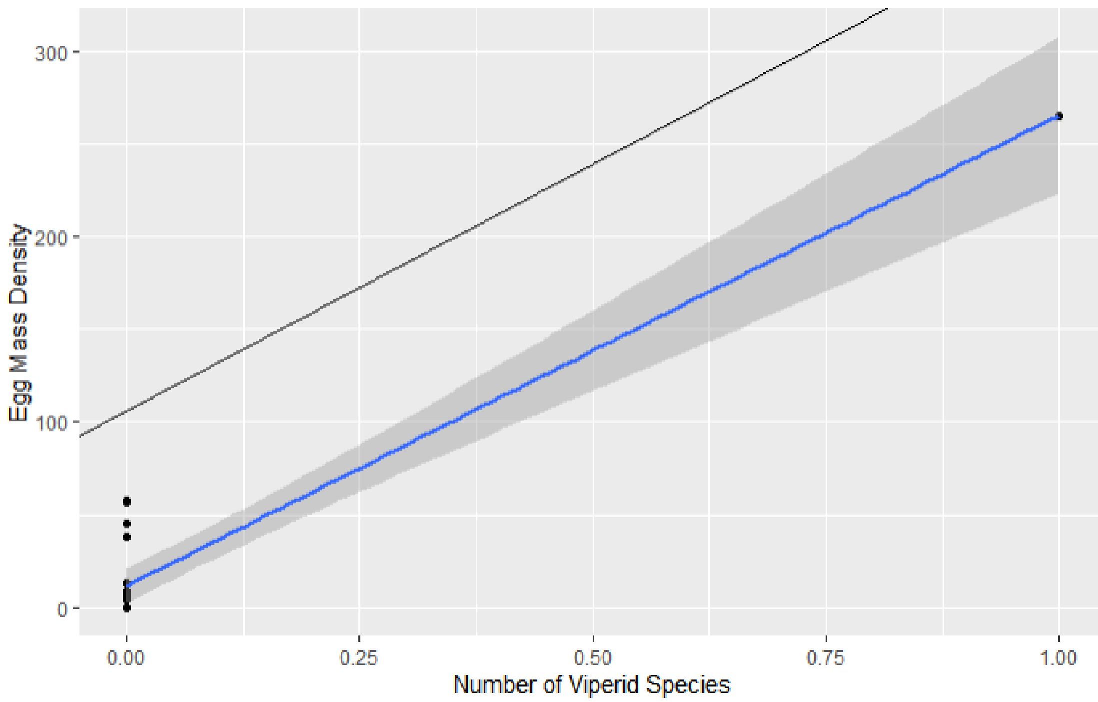

Figure 1: Correlation between number of Viperid species and spotted salamander egg mass density (egg masses/m^3).

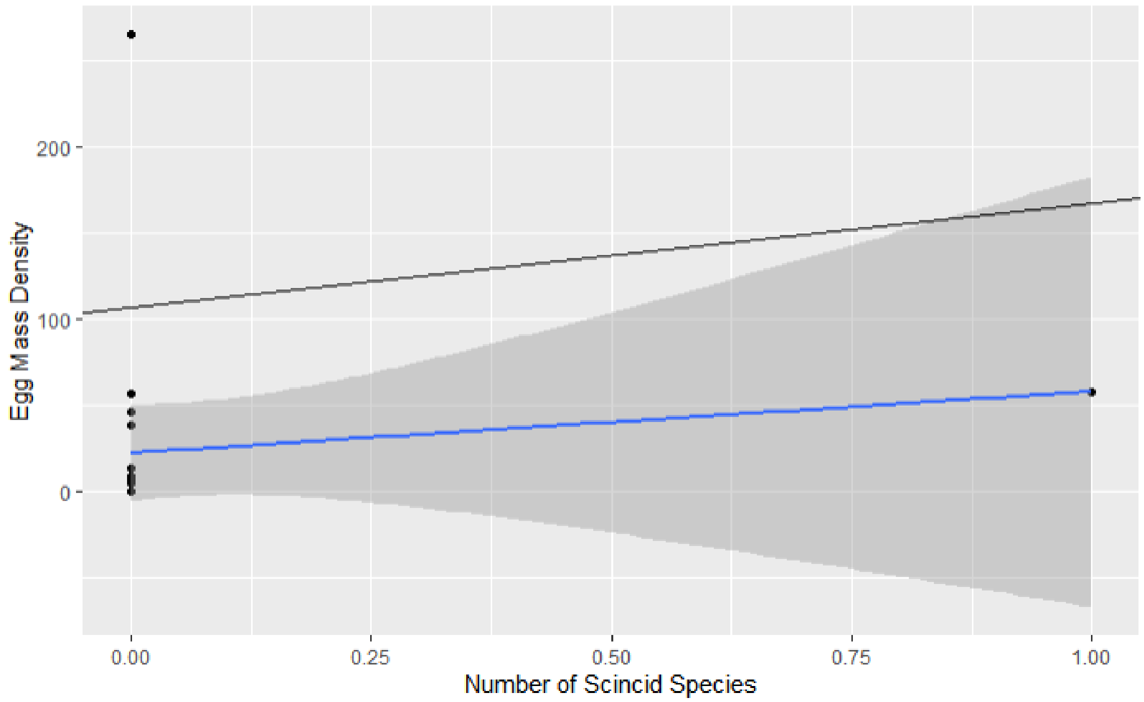

Figure 2: Correlation between number of Scincid species and spotted salamander egg mass density (egg masses/m^3).

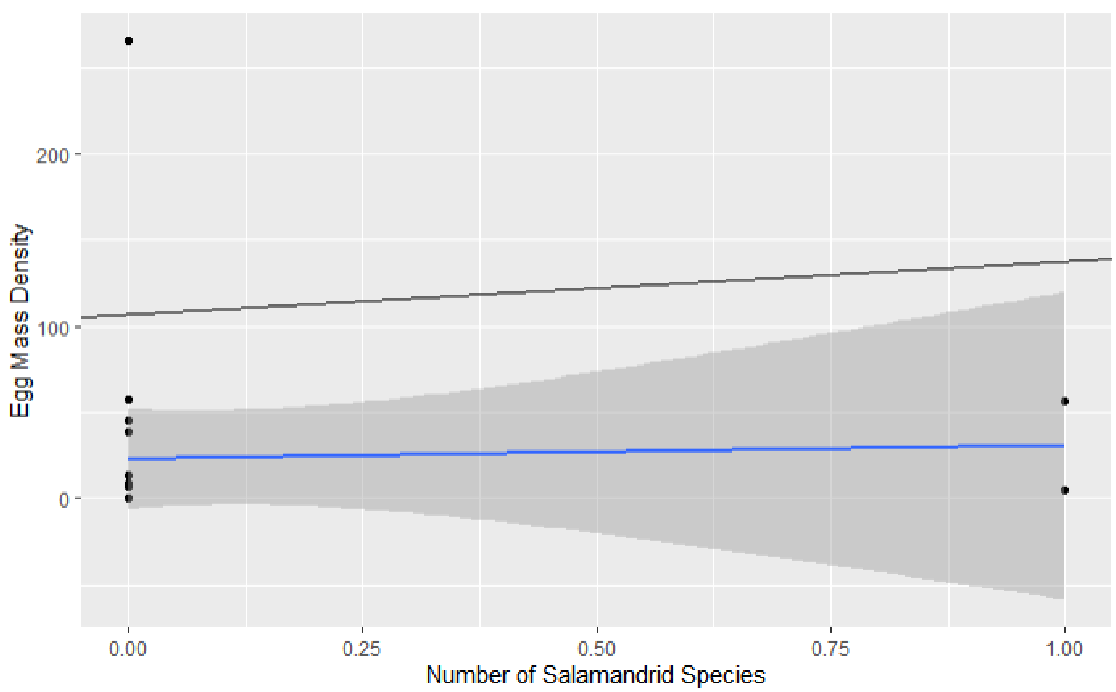

Figure 3: Correlation between number of Salamandrid species and spotted salamander egg mass density (egg masses/m^3).

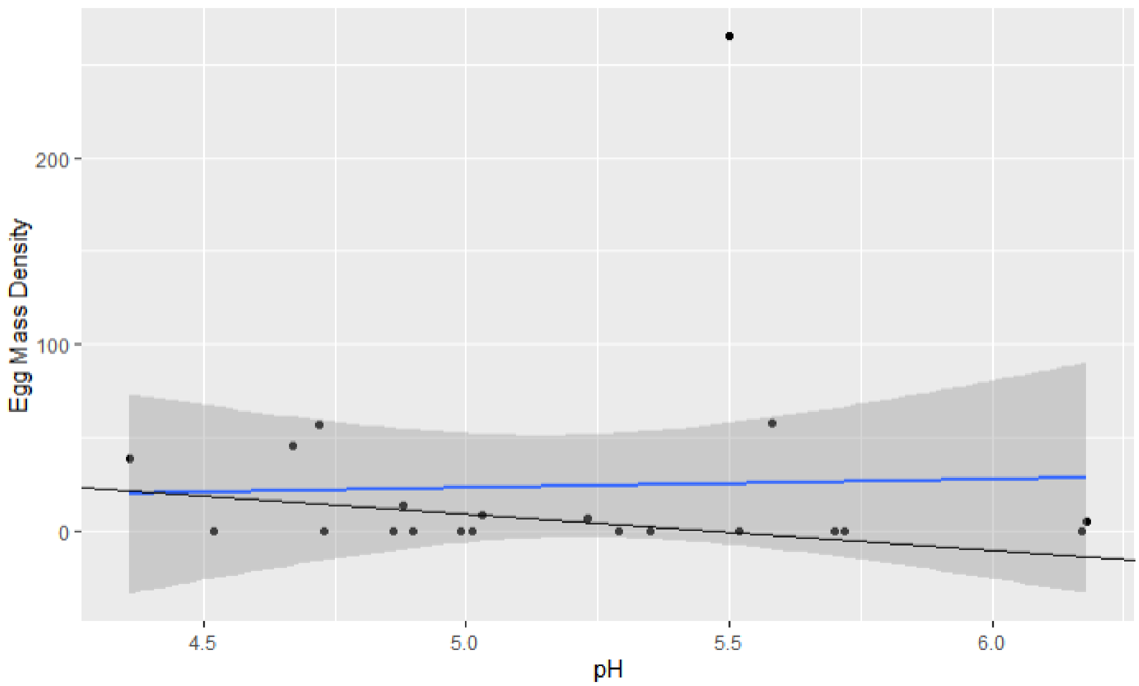

Figure 4: Correlation between pH and spotted salamander egg mass density (egg masses/m^3).

Species co-occurrence and breeding pool characteristics had no significant correlation with the presence or absence of egg masses. This suggests that site selection is not influenced by species co-occurrence. Alternatively, lack of significance from these tests may indicate that the sample size is too small to observe significant effects.

Early Breeding Spotted Salamanders & Egg Mass Density

I observed a significant correlation between spotted salamander larvae presence and spotted salamander egg mass density (P = 0.0461, R^2 = .2162). Pools with spotted salamander larvae were found to have higher egg mass densities than those without spotted salamander larvae (Figure 5). These findings suggest that the presence of early breeding spotted salamanders indicates significantly greater use of a breeding pool.

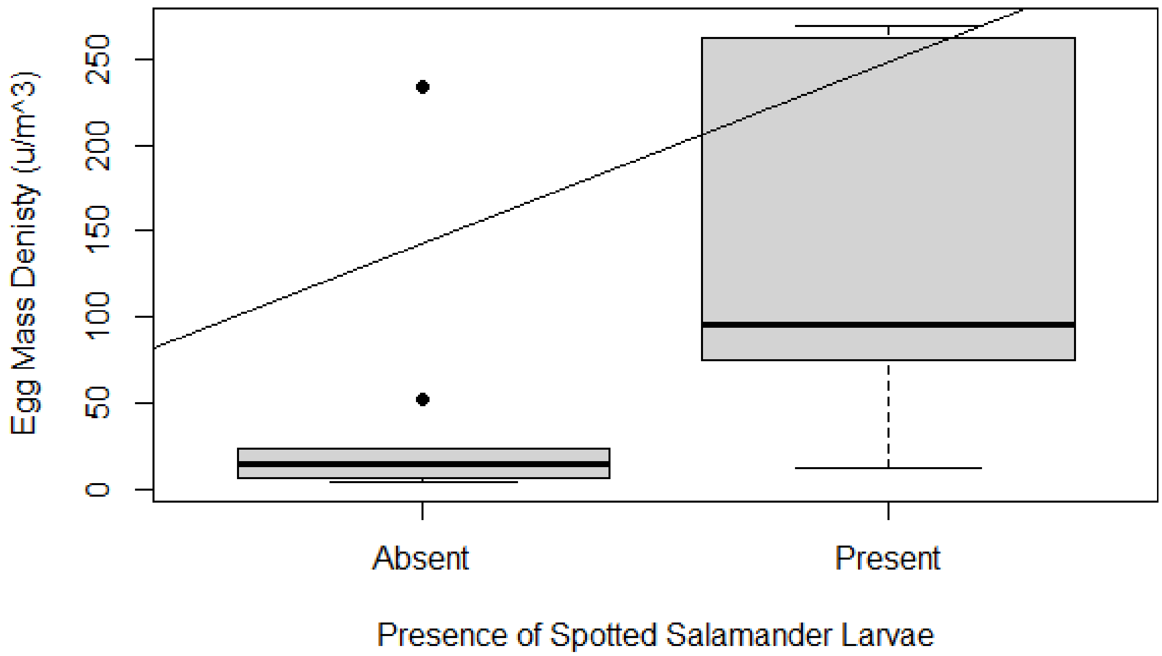

*Figure 5: Relationship between early breeding spotted salamanders and egg mass density***.**

Discussion

Species Co-Occurrence & Egg Mass Density

Although presence of Salamandrid species can decrease the survivorship of spotted salamander larvae (Urban, 2007), I observed higher spotted salamander egg mass densities in vernal pools with more Salamandrid species. Salamandrids share similar ecological requirements as spotted salamanders while in their aquatic life stages; therefore, congregation in similar habitat is expected. Due to efficiency of the egg mass coating in preventing predation by amphibian and reptile species (Semlitsch, 1988; Ward & Sexton, 1981), Salamandrids pose minimal threat to spotted salamanders during the embryonic life stage. Due to adaptations employed by spotted salamanders to protect egg masses from predation, it is more common for predatory species to reduce density of spotted salamander larvae rather than spotted salamander egg mass density.

Although presence of Salamandrids in spotted salamander breeding pools may increase pressures of predation and limit resource availability during the larval stage, these pressures do not seem to influence spotted salamander oviposition.

Additionally, high amphibian populations are likely to attract predators. Salamandrids and Viperids are likely to congregate in pools with greater densities of spotted salamander egg masses due to availability of a consistent food source upon larval development. My results suggest that the benefits of suitable habitat outweigh the costs of predation, as I did not observe decreased egg mass density in pools with more potentially predatory reptile, amphibian, and fish species. Despite evidence that spotted salamanders engage in site selection to avoid fish predation (Davenport et al., 2017; Egan & Paton, 2004), I did not observe predator avoidance through site selection. This variance in results is possibly due to the small sample size of ponds with fish in the study and may reflect different results if more fish populated ponds were included.

Scincids are not known to prey on spotted salamanders during their egg or larval stages due to their terrestrial life history; rather, Scincids feed on a diet of insects or plant material. The positive correlation between Scincids and spotted salamander egg mass density is likely due to desirable habitat qualities and high resource availability. Correlations between high egg mass density and greater number of Scincid species may be due to burrowing capabilities of skinks. Spotted salamanders may utilize skink burrows as terrestrial refuge (Wu et al., 2015), similar to spotted salamander use of small mammal burrows for habitat (Faccio, 2003; Madison, 1997; Regosin et al., 2003, 2004).

Environmental Characteristics & Egg Mass Density

Environmental history of the study area is expected to influence amphibian habitat selection. Due to high levels of urbanization in Durham and Orange County, access to breeding pools with optimal conditions within the study area may be limited. Spotted salamanders are known to deposit fewer egg masses in areas with higher road density and select for breeding pools that are more isolated (Veysey et al., 2011). This may lead salamanders to migrate to more isolated vernal pools with optimal environmental conditions to increase the odds of larval survivorship. Findings suggest that spotted salamanders within this area are not able to engage in frequent predator avoidance due to limited habitat choice, explaining the positive correlation between egg mass density and co-occurrence of Salamandrids and Viperids.

Spotted salamander tolerance for acidic pH levels can vary across study areas (Portnoy, 1990; Pough, 1976). Pough (1976) found significant embryonic mortality in pools with a pH < 6 in the Ithica, New York region. In contrast, Portnoy (1990) observed high rates of embryonic mortality in pools with a pH < 4.5 within Cape Cod, Massachusetts. Similar to Portnoy's (1990) findings, I observed higher spotted salamander egg mass density in pools with more acidic pH levels, with pool pH reaching a minimum of 4.36. Due to the acidic pH of soils in the study area (Taverna et al., 2005), spotted salamanders within Durham and Orange County likely have a tolerance for acidic pH values and therefore prefer slightly acidic pool conditions. These results indicate geographic adaptations of spotted salamanders to accommodate relative pH of an area. Furthermore, other factors affecting water quality can affect amphibian sensitivity to pH levels, as acidic pH paired with high tannin-lignin concentrations can reduce amphibian embryonic survival (Portnoy, 1990). Because of this, a combination of pH levels and other water quality components contribute to amphibian survival and breeding success.

Early Breeding Spotted Salamanders & Egg Mass Density

I found that vernal pools visited by early breeding spotted salamanders have significantly higher egg mass densities than pools visited later in the season. High egg mass densities indicate greater use of these pools by the breeding population or lower rates of egg mass predation in pools chosen by early breeders. Research on egg mass predation shows that intact egg masses are largely immune to predation by most predators (Semlitsch, 1988; Ward & Sexton, 1981); therefore, decreased egg mass density in response to predation is unlikely. The presence of early breeding spotted salamanders potentially indicates high pool quality, thus leading to high egg mass density throughout the breeding period. Since spotted salamanders engage in breeding site selection (Davenport et al., 2017; Egan & Paton, 2004; Kern et al., 2013; Montieth & Paton, 2006; Petranka et al., 2004; Petranka & Holbrook, 2006), early breeding spotted salamanders likely select pools that possess desirable environmental qualities. Despite drawbacks of resource competition between conspecifics (Anderson & Whiteman, 2015), such pools are frequently selected by spotted salamanders throughout the breeding season, leading to higher overall egg mass densities.

Limitations

Due to time limitations on this project, data collection occurred over a single breeding season. Continuation of this project over multiple breeding seasons could potentially provide further results on oviposition in the study area.

Availability of breeding pools likely plays a large role in spotted salamander breeding site selection; therefore, results are expected to differ regionally. Since Durham and Orange country are characterized by high levels of agriculture and urbanization, prioritization of habitat choice may be different than other regions with different environmental histories.

The small sample size of fish populated pools possibly contributed to lack of significant effects of fish co-occurrence on egg mass density. Finding a large sample size of fish populated pools used as spotted salamander breeding habitat may be challenging, as spotted salamanders generally avoid fish populated pools. Larger pool sample size could also contribute to more significant results on egg mass presence or absence. A larger team collecting field data would contribute to greater sample sizes and could potentially identify further trends.

Future Research Directions

Due to spotted salamander preference for isolated habitat, road distance from each of the sampled vernal pools can contribute to identification of preferential breeding pool characteristics. Evaluation of environmental characteristics such as vegetation complexity, level of submerged vegetation, water depth, and hydroperiod could identify if the breeding pools in the study are considered desirable breeding habitats for spotted salamanders.

Overall efficiency of spotted salamander egg mass coatings in protecting embryos from significant mortality due to predation is disputed (Rowe et al., 1994; Stout et al., 1992). Additionally, efficiency of predator consumption of spotted salamander embryos vary (Rowe et al., 1994; Stout et al., 1992; Ward & Sexton, 1981), with most predators posing minimal threat to intact egg masses (Ward & Sexton, 1981). Future research on the impact of co-occurring insect, crustacean, and gastropod species on egg mass density would further evaluate the efficiency of certain species as egg mass predators and determine if predator density can significantly decrease egg mass density in breeding pools. Predation has a greater effect on spotted salamander abundance during the larval stage (Davenport et al., 2017), therefore effects of predatory co-occurring species can be better observed by collecting data on larval density.

Implications

This study will assist in evaluating the stability of spotted salamander populations in the North Carolina Piedmont, which can be used to evaluate overall health of vernal pool ecosystems. Contribution to data on vernal pool interspecies interactions can assist in creating more comprehensive management plans in the future that account for conservation of multiple species.

Due to yearly changes in breeding pool use of spotted salamanders, this research provides updated information on active breeding sites within Durham and Orange County. Study sites within the Duke Forest contribute to amphibian population monitoring and habitat use. Additionally, results can assist in monitoring stability of breeding site availability within Durham and Orange County.

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