BIOTIC AND ABIOTIC FACTORS INFLUENCING HEADWATER STREAM ORGANISMS IN THE SOUTHERN APPALACHIANS

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by
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BIOTIC AND ABIOTIC FACTORS INFLUENCING HEADWATER STREAM ORGANISMS IN THE SOUTHERN APPALACHIANS

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ABSTRACT

Patterns in species distributions and the formation of community assemblages are driven by combinations of factors. While, the roles of biotic and abiotic factors in ecological and evolutionary phenomena are well-understood, few investigations have focused on these interactions involving morphologically dissimilar taxa. My research aimed to determine whether interactions were strong among morphologically dissimilar species with widely overlapping assemblages in southern Appalachian streams and if environmental conditions contributed to the strength of these interactions. First, I conducted field surveys across 8 stream sites in the summer of 2018 to estimate the occupancy probabilities and body condition of salamanders in response to the presence of heterospecifics and habitat variables. I predicted that salamander occupancy estimates and body condition would be lower in streams where fish and/or crayfish were present and higher in streams with greater canopy cover, slope, and proportions of larger substrates. My results suggested that stream salamander occupancy was driven by habitat variables, not species interactions, and their body condition was negatively associated with the presence of fish.

Second, I used a two-pronged approach (*in situ* and *ex situ* methods) to identify the potential abiotic and biotic factors that influence the spatial patterns associated with body condition and refuge use for both stream salamanders and crayfish. My artificial stream experiment involved three species identity treatments (salamander-salamander, crayfish-crayfish, and salamander-crayfish) and two refuge density treatments (low and high) to determine if the presence of heterospecifics and lower refuge densities negatively affected body condition, refuge use, cohabitation, and survival. Field surveys were performed across four streams to examine the influence of macro- and micro- habitat characteristics on interspecific cohabitation between salamanders and crayfish. The results from my experiment suggested that the cohabitation and refuge use rates, growth, and mortality of salamanders was not affected by the presence of crayfish, nor by refuge density. Consistent with the results from my experiment, species identity and habitat variables were not associated with cohabitation patterns between salamanders and crayfish. Overall, conclusions from my thesis imply that stream salamanders and crayfish may simply coexist, but that fish can negatively affect the body condition of salamanders, thereby potentially driving salamander assemblages and distributions.

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Animal Care and Use Committee (IACUC) protocol permit 19-11, and all study animals were collected under the North Carolina Wildlife Resources Commission permits 18-SC01281 and 19-SC01312, Tennessee Wildlife Resources Scientific Collection permits 1296 and 1816, and the USDA Cherokee National Forest permit 2610.

DEDICATION

To my family, fiancé, and pets, I could not have done this without your unconditional support and love.

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FOREWORD

Chapters 1 and 2 of this thesis cover the spatial and temporal scales of species assemblages in streams and the small-scale, short-term mechanistic experiment based on larger patterns. Both chapters within this thesis will be submitted to the peer-reviewed journal, *Freshwater Science*. The content of this thesis has been prepared within the formatting and style guidelines for publication for this journal

CHAPTER 1: OCCUPANCY AND INTERSPECIFIC INTERACTIONS OF SALAMANDERS, FISH, AND CRAYFISH IN SOUTHERN APPALACHIAN STREAM COMMUNITIES

ABSTRACT

Understanding the factors and mechanisms involved in species distributions and community assemblages is important for explaining ecological and evolutionary phenomena. The interaction between abiotic and biotic factors can be especially important for organisms that have specific life history requirements or are experiencing declines. Many amphibian species are threatened or near-threatened and stream-breeding salamanders in the southern Appalachians are faced with numerous stressors that may alter their diversity and abundance in stream ecosystems. Streams often undergo high variations in the quality and quantity of resources, thereby, potentially inducing changes in the strength of interactions associated with predation and competition. Predation, competition, and the interaction between these factors and abiotic conditions have been recognized as important factors regulating abundance and distributions among semi-aquatic salamanders. In these stream communities, predatory interactions have been suggested to be most important between individuals of different life stages or sizes, whereas, competition is more common between similar-sized individuals. Although stream salamander responses to the combined effects of abiotic and biotic factors have been documented in the southern Appalachians, existing field-based studies do not involve interactions with distantly related taxa that are native to this region. The focus of my study was to determine whether salamander populations exhibited patterns in occupancy and body condition as a response to the presence of heterospecifics and habitat variables, either directly, or as a function of those variables driving distributions of these taxa.

Salamanders, fish, crayfish, and aquatic habitat data were collected from eight streams in two counties (Monroe, TN and Macon, NC) during the summer of 2018. The body condition of salamanders was compared across sub-reaches where fish were present or absent. Both single-species and two-species occupancy models were used to determine if species occurrence was dependent on abiotic and biotic factors. My results suggested that salamander occupancy was not influenced by the presence of heterospecifics but that their co-occurrence was driven by several abiotic factors. However, fish presence negatively influenced the body condition of salamanders, indicating potential negative implications for salamander growth or survival. Overall, the lack of an effect on salamander occupancy in my study indicates that this is a result of coevolution with other salamanders and taxa (crayfish and fish), thus facilitating the potential for local adaptation to reduce the costs associated with competition. Furthermore, the strength of predatory interactions may not be strong enough in my study sites to influence salamander distributions.

INTRODUCTION

The extent to which an organism responds to environmental conditions depends on their physiological and ecological limitations, resulting in individuals selecting habitats within these constraints that maximize their survival, growth, and reproduction (Werner and Anholt 1983, Davis 2005, Gunnarsson et al. 2005, Ellegren and Sheldon 2008, Rittenhouse et al. 2009, Isaksson 2015). The concept of "environmental filtering" describes a nested hierarchy of abiotic factors acting as "filters" that only allow species with optimal traits for each "filter" to occupy a location (Bazzaz 1991, Woodward and Diament 1991). Furthermore, species become more specialized as the abiotic conditions become more particular to a location, thus, the availability of optimal conditions dictates the direction and strength of biotic interactions (i.e., predation, competition, mutualism, parasitism, and commensalism) at these local scales (Sugihara 1980, Kolasa and Biesiadka 1984, Kolasa and Strayer 1988, Dunson and Travis 1991). The interaction between abiotic and biotic factors may be especially influential for species and populations that are specialists, sensitive to changes, or are already in decline.

Amphibians are the most threatened vertebrates in the world (Stuart et al. 2004). These declines are largely attributed to disease outbreaks, habitat destruction, prevalence of contaminants, and factors associated with global climate change (Stuart et al. 2004). Amphibian biodiversity is high in the southern Appalachians, although, noticeable declines in the abundance of salamanders in this region have been reported (Walls 2009, Milanovich et al. 2010, Caruso and Lips 2013). Stream-breeding salamander populations in the southern Appalachians are faced with numerous stressors that may alter their diversity and abundance in stream ecosystems (Crawford and Semlitsch 2008, Peterman and Semlitsch 2009, Vazquez et al. 2009, Milanovich et al. 2010, Peterman et al. 2011, Price et al. 2011). Streams often undergo high variations in the quality and quantity of resources, thereby, potentially inducing changes in the strength of interactions associated with predation and competition (Vannote et al. 1980, Wipfli et al. 2007, Peterman et al. 2008). Stream amphibian diversity and abundance has been negatively associated with reductions in canopy cover because higher canopy cover density is important for regulating temperature, promoting moisture retention, and hindering UVB radiation exposure (Blaustein 1998, Caissie 2006, Peterman et al. 2011). Furthermore, amphibian movement is dependent on these characteristics due to their susceptibility to desiccation, growth inhibition, and reduced survival (Spotila 1972, Sugalski and Claussen 1997, Blaustein 1998, Placyk and Graves 2001). Many stream salamanders also rely entirely on cutaneous respiration and, as a result, their occurrence is positively associated with the high prevalence of riffles and water flow that oxygenate streams (Hairston 1949, Organ 1961, Davic and Orr 1987, Lowe et al. 2004). Additionally, salamander microhabitat selection is likely dependent on the availability of large substrates because they often use them as refuge during high flow events to avoid being flushed downstream (Lowe et al. 2004). Because of these constraints in microhabitat conditions, biotic factors can further limit salamanders.

Predation, competition, and the interaction between these factors and abiotic conditions have been recognized as important factors regulating abundance and distributions among semi-aquatic salamanders (Krzysik 1979, Keen 1982, Southerland 1986). The outcome of both competition and variations in environmental conditions in Desmognathine communities likely allows for the coexistence of several congeners through spatial niche partitioning (Hairston 1949, Organ 1961, Tilley 1968, Peterman et al. 2008). Stream-containing habitats are dynamic and offer a gradient of microhabitats ranging from fully aquatic to terrestrial, thereby promoting niche partitioning and coexistence. Stream salamanders often occur in high densities and are likely integral as both prey and predators in riparian-aquatic food webs (Davic and Welsh 2004, Peterman et al. 2008, Keitzer and Goforth 2013). In these communities, predatory interactions usually occur between individuals of different life stages or sizes, whereas, competition is more common between similar-sized individuals (Tilley 1968, Hairston 1980, Colley et al. 1989). With regards to competition, a dominant species is considered to have greater fitness than the other species due to their asymmetrical ability in acquiring a shared limited resource or tolerating the presence of a common enemy (Volterra 1926, Lotka 1932). Therefore, it would be logical that intraspecific competition would be strongest between individuals of the same size because they would require the same habitat and prey size. This has been demonstrated in early studies, where smaller salamander species are excluded from the more aquatic and forced to inhabit the more terrestrial areas of a stream by larger *Desmognathus* species (Hairston 1949, Organ 1961, Hairston 1987, Crawford and Semlitsch 2007). For example, seal salamander (*Desmognathus monticola*) individuals of the same life stage will compete over habitats containing different particle sizes (Roudebush and Taylor 1987). Moreover, if juvenile densities are too high in these habitats, then interactions with adults will further limit their habitat selection to avoid potential predation (Roudebush and Taylor 1987). Thus, with intraspecific predation, differences in sizes may be the most influential in the outcomes of interactions. Additionally, it has been suggested that the most plausible interaction between heterospecifics among Desmognathine communities is predation (Hairston 1980).

Competitive and predator-prey interactions have also been exhibited between salamanders and either fish or predatory arthropods in experimental studies (Resetarits 1991, 1995, Ennen et al. 2016). These taxa often have similar diets and microhabitat preferences; therefore, competitive asymmetry is likely strong because of their morphological differences and niche requirements. This interaction has been observed between spring salamander (*Gyrinophilus porphyriticus*) larvae and small-sized adult brook trout (*Salvelinus fontinalis*). In which, the latter was the larger predator and negatively influenced spring salamander growth, survival, and habitat use (Resetarits 1995). However, the strength of competition between these species is expected to decrease as their sizes become more similar, thus, acting as a potential driver of coexistence (Werner and Gilliam 1984). A different experiment concluded that Appalachian brook crayfish (*Cambarus bartonii*) did not demonstrate competitive nor predatory interactions with Desmognathine salamanders (Resetarits 1991).

Ecological and evolutionary phenomena can be explained by identifying the underlying forces causing patterns of species distributions and community assemblages (McPeek and Holt 1992, Hastings 1993). Although stream salamander responses to the combined effects of abiotic and biotic factors have been documented in the southern Appalachians, existing field-based studies do not involve interactions with distantly related taxa that are native to this region. My study aimed to assess whether salamander populations exhibited patterns in occupancy and body condition as a response to the presence of heterospecifics and habitat variables, either directly, or as a function of those variables driving distributions of these taxa. I predicted that salamanders captured in stream reaches where fish and crayfish were present would demonstrate lower occupancy probabilities and body condition relative to salamanders in reaches where fish and crayfish were absent. While, fish and crayfish occupancy probabilities would remain unassociated with salamander presence or absence. Additionally, I sought to identify if the largest salamander species influenced the occupancy of smaller salamanders as a function of either competition or predation.

METHODS

Study Sites

The southern Appalachian Mountains are recognized as encompassing unique soil, geology, landform, and climatic dynamics (Stein et al. 2000). Moreover, these features promote salamander biodiversity due to the abundance of streams that are high-gradient, low-order, low temperature, and high canopy cover (Petranka 1998, Stein et al. 2000, Kozak and Wiens 2010). My study was conducted across two high-gradient and two low-gradient headwater streams in western North Carolina (Macon County), in addition to, one high-gradient and two low-gradient streams in eastern Tennessee (Monroe County). For these seven sites, a 105-m reach was established immediately upstream of road intersections and were divided into three 25-m sub-reaches, separated by 15-m gaps.

Focal Species

My study involved some of the most numerically dominant species endemic to southern Appalachian headwater streams. In headwater streams consume similar prey items, such as invertebrates and amphibian larvae and eggs (Usio and Townsend 2004, Cruz and Rebelo 2007, Lodge et al. 2011, Barrett et al. 2012, Sepulveda et al. 2012) and also occupy refuges (e.g. rocks and logs; Benvenuto et al. 2008, Keitzer et al. 2013). Our focal species included three species of salamanders (*Desmognathus monticola* [seal salamander], *D. quadramaculatus* [black-bellied salamander], and *Eurycea wilderae* [Blue Ridge two-lined salamander]), two species of fish (*Semotilus atromaculatus* [common creek chub] and *Rhinichthys atratulus* [blacknose dace]) and one species of crayfish (*Cambarus bartonii*). Species belonging to the *Desmognathus* genus are lungless and rely entirely on cutaneous respiration as both larvae and adults (Hairston 1949, Organ 1961, Davic and Orr 1987). *D. quadramaculatus* inhabit densely forested stream channels and banks and is the largest salamander species in my study region where adult snout-to-vent lengths (SVL) are often between 57 and 94 mm (Bruce 1993, Petranka 1998, Peterman 2008). *D. monticola* are also stream-associated but occupy stream edge microhabitats for frequently and their SVL can range from 46-80 mm (Bruce and Hairston 1990, Titus and Larson 1996). Like Desmognathines, *E. wilderae* are lungless and adults exhibit cutaneous respiration, however, their gills are highly vascularized during their larval period (Hairston 1949, Organ 1961, Davic and Orr 1987). *E. wilderae* are usually smaller than these Desmognathines with SVL between 30 and 49 mm (Sever 1999). Compared to *D. quadramaculatus* and *D. monticola*, they use microhabitats differently where reproductively mature individuals will leave stream areas in mid-summer after they deposit their eggs and then return in the winter (Bruce 1982, Petranka 1998).

As for my focal fish species, *S. atromaculatus* is a small minnow that reaches total lengths between 120 and 300 mm in low order streams, whereas, the length of *R. atratulus* can range from 5 to 100 mm (Reed and Moulton 1973, Copes 1978). A distinguishing feature between these species is the relatively larger gape of *S. atromaculatus*, compared to that of *R. atratulus* (Ward and Coburn 2008). *C. bartonii* are a medium sized (carapace length = 10-40 mm) crayfish and represent most of the invertebrate biomass in Appalachian headwater streams (Woodall and Wallace 1972, Huryn and Wallace 1987, Griffith et al. 1994).

Field surveys

Surveys were conducted between May and August 2018 and all sites were visited once every two weeks during this sampling period for a total of three visits. Stream salamanders were collected by performing time-constrained visual encounter surveys (VES) by two people for a total of 20 minutes per sub-reach. During these searches, cobble and other objects were overturned and an aquarium net was used to collect salamanders and crayfish. After VES, a haul seine was used to collect salamanders, fish and crayfish by placing it 2 m downstream of the person flipping rocks and was repeated as we moved upstream for a total of eight times for each 25-m stretch. SVL and mass were determined for each salamander, which were used to calculate body condition. All salamanders, fish, and crayfish were identified to species. Salamanders were marked with fluorescent dye using visible implant elastomers (VIE); marks were placed just above their forelimbs or hindlimbs after the surveys concluded and then they were released. These marks were unique to each sub-reach for a given surveying period within one stream.

Substrate composition, canopy cover, and the slope of each 25-m reach were also measured *in situ* once per stream sub-reach for the field season after sampling was completed. These variables were selected because previous studies found significant associations between these variables and occupancy (Lowe et al. 2004, Kiffney and Roni 2007, Ward et al. 2008, Cecala et al. 2018). Riparian canopy cover was calculated with a densitometer from the center of each sub-reach. Substrate composition was categorized into six groups based on the Wentworth scale (sand, silt, gravel, cobble, boulder, bedrock, and organic material; Peoples et al. 2011) and were assessed within the wetted boundaries of the entire sub-reach.

Statistical analyses

Estimates of species occupancy in streams can be important for evaluating the abiotic and biotic influences on the distributions of stream species. Recently, occupancy modeling has been implemented to evaluate co-occurrence patterns among species and has incorporated site-level covariates to identify potential drivers of ecological patterns (Peoples et al. 2011, Peoples and

Frimpong 2016, Arnhold et al. 2018, Baecher and Richter 2018, Chen et al. 2019). For my study, one out of eight streams were removed from the analyses because it was dry for two of the survey dates. To identify the potential abiotic factors involved in species occurrence and co-occurrence for the remaining sites, I first log-transformed substrate proportions due to autocorrelation. I then identified all abiotic variables that had relationships with raw species abundance values using a canonical correspondence analysis (CCA; Ter Braak 1986) in RStudio v3.3.1 (R Core Team 2016). To determine if the occurrence of all focal species was influenced by abiotic characteristics, I estimated their occupancy probabilities using single-species, single-season occupancy models in Program "PRESENCE" v2.12.17 (MacKenzie et al. 2006, Hines 2006). I used three basic models for all species, including one where detection is held constant across sites but the occupancy probability can vary (Ψ, $\rho(.)$), one where both occupancy and detection are held constant across sites and surveys ($\Psi(.)$, $\rho(.)$), and the third model where occupancy is held constant across sites but the probability of detection can vary among surveys ($\Psi(.)$, $\rho(t)$. Each site covariate that was significant from the CCA was incorporated into the first model (Ψ, $\rho(.)$) and all candidate models within 10% of the most robust models were selected for model-averaging based on the Akaike Information Criteria (Richmond et al. 2010).

I used two-species occupancy models to quantify the effect of fish and crayfish presence on salamander occupancy and to estimate occupancy of *D. monticola* and *E. wilderae* when *D. quadramaculatus*, the larger species, was present (Mackenzie et al. 2004). These models are important for estimating the strength and direction of species interactions and determining if habitat covariates facilitate these interactions. All parameterization was implemented as in Richmond et al. (2010), which estimates the probability of occupancy of species A (Ψ^{A}); probability of occupancy of species B when species A is present (Ψ^{BA}); probability of occupancy of species B when species A is absent (Ψ^{Ba}); probability of detection of species A when species B is absent (ρ^{A}); probability of detection of species B when species A is absent (ρ^{B}); probability of detection of species A when both species are present (r^{A}); probability of detection of species B when both species are present and species A is detected (r^{BA}); and the probability of detection of species B when both species are present and species A is not detected (r^{Ba}). Using established methods, I compared models where the occupancy and detection of species B were independent of species A ($\Psi^{BA} = \Psi^{Ba}$; $r^{BA} = r^{Ba}$) with models where species A influenced the occurrence ($\Psi^{BA} \neq \Psi^{Ba}$; $r^{BA} = r^{Ba}$), detection ($\Psi^{BA} = \Psi^{Ba}$; $r^{BA} \neq r^{Ba}$), or both parameters ($\Psi^{BA} \neq \Psi^{Ba}$; $r^{BA} \neq r^{Ba}$) of species B (Peoples and Frimpong 2016, Arnhold et al. 2018). To identify the potential abiotic factors that are involved in species co-occurrence, site covariates from the most robust single-species occupancy models were also incorporated into the two-species models.

Body condition, represented by the scaled mass index (SMI), is a condition index that is representative of an individual's energy reserves by relating mass to length and has been used in previous field surveys and experiments involving stream organisms (Davenport and Lowe 2016, Ennen et al. 2016, Liles et al. 2017, Hoffacker et al. 2018). This was calculated for salamanders (*D. quadramaculatus*; Peig and Green 2009, 2010) to assess the strength of their interactions with fish across sub-reaches where fish were or were not detected during any survey. Salamander body condition was compared using analysis of variance (ANOVA) models with the "aov" function in RStudio v3.5.2 (R Core Team 2018). I did not evaluate the effects of fish presence on the body condition of other focal salamander species (*D. monticola* and *E. wilderae*) because their body condition data was too limited.

RESULTS

Overall, one salamander (*Desmognathus quadramaculatus*; *n* = 212) and one crayfish (*Cambarus bartonii*; *n* = 1377) species were captured in all sub-reaches (*n* = 21; Table 1-1). *Rhinichthys atratulus* (n = 117) was captured in more sub-reaches (n = 9 and 7, respectively; Table 1-1) than *Semotilus atromaculatus* (n = 35). One salamander species (*Eurycea wilderae*; n = 23) was detected in 10 sub-reaches, while *D. monticola* (n = 16) was found in seven sub-reaches (Table 1-1). As for my raw species co-occurrence data, both *D. quadramaculatus* and *C. bartonii* were captured in all sub-reaches where fish and other salamander species were detected (Table 1-1). *D. monticola* was found in less sub-reaches (n = 4; Table 1-1) where *E. wilderae* was present than in sub-reaches (n = 5 and 5; Table 1-1) where *S. atromaculatus* and *R. atratulus* were captured. Lastly, E. wilderae was captured in fewer sub-reaches (n = 2; Table 1-1) where *S. atromaculatus* was detected, compared to the number of sub-reaches (n = 8; Table 1-1) where *R. atratulus* was captured.

Single-species occupancy

The probability of occupancy for *D. quadramaculatus* (Table 1-2; Figure 1-1; w_i = 0.493), *D. monticola* (Table 1-1; Fig. 1-1; w_i = 0.518), and crayfish (Table 1-2; Fig. 1-1; w_i = 0.337) was not influenced by site-specific nor survey-specific variables. Single-season, single-species models indicated that the probability of occupancy for only one species (*R. atratulus*) was driven by a site-specific predictor variable (slope; Table 1-2; Fig. 1-2; w_i = 0.407). Stream slope negatively influenced the probability of occupancy (Table 1-2; Fig. 1-2). The best models for *E. wilderae* (Table 1-2; Fig. 1-3a; w_i = 0.475) and *S. atromaculatus* (Table 1-2; Fig. 1-3b; w_i = 0.709) specified that occupancy probabilities were dependent on the surveying period. The occupancy probabilities for *E. wilderae* (Fig. 1-3a) decreased throughout the field season, whereas, *S. atromaculatus* (Fig. 1-3b) occupancy was greater during the beginning and ending of the field season.

Species-interaction occupancy

My most robust two-species models did not support my hypotheses regarding interspecific species interactions. The results from these models suggest that fish (*R. atratulus* and *S. atromaculatus*) presence did not negatively affect the occupancy of any salamanders (Tables 1-3 and 1-4). The best-fit models involving *S. atromaculatus* and salamander co-occurrence indicated that its presence did not affect the occupancy probability of *D. quadramaculatus* (Tables 1-3 and 1-4; w_i = 0.473), *D. monticola* (Tables 1-3 and 1-4; w_i = 0.726), or *E. wilderae* (Tables 1-3 and 1-4; w_i = 0.523). Similarly, *R. atratulus* presence did not influence the occupancy of *D. quadramaculatus* (Tables 1-3 and 1-4; w_i = 0.478), *D. monticola* (Tables 1-3 and 1-4; w_i = 0.595), or *E. wilderae* (Tables 1-3 and 1-4; w_i = 0.488). Salamander occupancy [*D. quadramaculatus* (Tables 1-3 and 1-4; w_i = 0.731), *D. monticola* (Tables 1-3 and 1-4; w_i = 0.731), and *E. wilderae* (Tables 1-3 and 1-4; w_i = 0.731)] was not significantly associated with the presence of crayfish. As for interactions between *D. quadramaculatus* and other salamander species, the most robust models indicated that occupancy probabilities of both *E. wilderae* (Tables 1-3 and 1-4; w_i = 0.692) and *D. monticola* (Tables 1-3 and 1-4; w_i = 0.555) were not dependent on the presence of *D. quadramaculatus*. Although there was no apparent interaction between my focal species, some species pairs exhibited co-occurrence that was influenced by abiotic factors. As expected from the single-species results, the occurrence of *R. atratulus* with *E. wilderae* and *D. monticola* was influenced by stream slope. The only other abiotic factors that showed significance with co-occurrence were the proportions of sand and cobble for the *S. atromaculatus-D. quadramaculatus* species pair (Tables 1-3 and 1-4; w_i = 0.473).

Body condition

The mean body condition of *D. quadramaculatus* was lower in sites where *Semotilus atromaculatus* was present (Fig. 1-4; $F_{1,186}$ = 18.59, P < 0.01). However, there was no difference between *D. quadramaculatus* body condition in sites where *R. atratulus* were present or absent (Fig. 1-4; $F_{1,186}$ = 1.697, P = 0.194), although, mean *D. quadramaculatus* body condition was 0.10 g lower in sub-reaches where *R. atratulus* was absent relative to when they were present (Fig. 1-4).

DISCUSSION

My study is the first to provide a broader understanding of the interactions, or lack thereof, that occur among salamanders, between salamanders and distantly related species, and the involvement of abiotic conditions throughout southern Appalachian headwater stream communities. I found that salamander occupancy was unrelated to interspecific interactions, however, co-occurrence patterns of some species pairs were dependent on abiotic factors. This suggests that negative interactions are not strong enough between these species to influence salamander occupancy but that some abiotic factors are important in driving their coexistence. However, my results also suggested that competitive or predatory interactions with fish may show implications of influencing the growth and survival of salamanders.

Salamander occupancy was independent of the presence of fish or crayfish, indicating that these species are exhibiting coexistence or that the presence of another species does not affect salamander distributions. The only indication of competitive or predatory interactions was apparent in the significantly lower salamander (*Desmognathus quadramaculatus*) body condition in sub-reaches where fish (*Semotilus atromaculatus*) were present relative to where they were absent. I also found that the mean body condition of *S. atromaculatus* was significantly greater than the body condition of *R. atratulus* (Cragg, unpublished data). Furthermore, *S. atromaculatus* have larger gapes than *R. atratulus*, thus allowing them to consume a broader range of prey size classes (Ward and Coburn 2008). This suggests that predation by *S. atromaculatus* may be the most influential on salamander growth and survival because salamanders are allocating energy for predator avoidance, rather than obtaining prey. Another possible explanation for salamander body condition being dependent on *S. atromaculatus*, rather than *R. atratulus*, presence would be through either exploitative or interference competition. The larger gape of *S. atromaculatus* relative to *R. atratulus* may allow them to obtain larger prey that salamanders also consume, thus reducing the abundance of, or using aggressive behavior to prevent salamanders from obtaining, that resource. Therefore, the strength of interactions between salamanders and fish are likely dependent on the size differences between these taxa. Neither *Desmognathus monticola* or *Eurycea wilderae* occupancy was dependent on the presence of *D. quadramaculatus*. This was likely a result of the smaller salamander species (*E. wilderae* and *D. monticola*) partitioning habitat to avoid encountering *D. quadramaculatus*. This result may demonstrate spatial niche partitioning, resulting in the coexistence of these species.

The only abiotic factor used in my study that was influential for single-species occupancy was the negative relationship between slope and *R. atratulus* occupancy. This is likely an indication that *R. atratulus* is not tolerant of the rapid stream flow in these high gradient reaches or that upstream travel is limited by barriers (i.e., large boulders) when stream depth is low (Burton and Odum 1945). In addition to affecting *R. atratulus* occupancy, the co-occurrence of this fish species and salamanders (*D. monticola* and *E. wilderae*) was also driven by slope. Substrate composition was also important in the co-occurrence of other species pairs. Proportions of cobble influenced *D. monticola* and *E. wilderae* co-occurrence, whereas, both sand and cobble were involved in the co-occurrence of *D. quadramaculatus* with *E. wilderae* and *S. atromaculatus*. However, the most robust single-species models did not specify that occupancy was dependent on these substrate characteristics.

The results from my species-interaction models involving salamanders and crayfish were surprising because a previous study found that their interactions can have impacts on salamander health and habitat use (Gamradt et al. 1997). However, a different experiment concluded that the presence of crayfish (*C. bartonii*) did not influence the growth or survival of two salamander species (*E. bislineata* and *Gyrinophilus porphyriticus*; Resetarits 1991). The inconsistencies with the first study is likely because the focal crayfish species in my study and in Resetarits (1991) are native to our study systems, whereas, the crayfish species in Gamradt et al. (1997) was a much larger, introduced species. As for the occupancy models with both salamanders and fish, those results were also unexpected because other studies have observed negative effects on salamander distributions (Barr and Babbitt 2002, Lowe et al. 2004). For example, larval salamander (*Eurycea bislineata*) abundance was significantly decreased in streams where fish (*Salvelinus fontinalis*) were present (Barr and Babbitt 2002). However, *S. fontinalis* and *E. bislineata* are not common in southern Appalachians and were not detected in my sites, which may explain the dissimilarity in our results. Fish (*S. atromaculatus*) negatively affected salamander body condition which is consistent with other studies (Resetarits 1991, Barr and Babbitt 2002, Lowe et al. 2004, Ennen et al. 2016). Competitive interactions have been demonstrated to occur between *S. atromaculatus* and *D. quadramaculatus* in an experiment, where salamanders experienced a reduction in body condition as a response to interactions (Ennen et al. 2016). Stream salamanders did not exhibit negative interactions, suggesting that these stream species are co-existing. However, the capture methods in my study were primarily performed in the most aquatic areas of streams and there were substantially more *D. quadramaculatus* individuals captured compared to that of *E. wilderae* and *D. monticola*. Because *D. quadramaculatus* is the largest of these species, they usually dominate stream channels, so it is possible that the other smaller species were mostly occupying the more terrestrial areas.

Abiotic factors including, slope and substrate composition (sand and cobble) were influential in species occupancy. The negative relationship between *R. atratulus* occupancy and slope is consistent with previous studies that have concluded that this species is either uncommon or absent in streams with greater slope (Burton and Odum 1945, Hitt and Roberts 2012). Stream depth was not measured in my study, but I did observe substantial decreases in water depth over the span of my surveys. This decrease in water depth may have further contributed to the negative relationship between slope and *R. atratulus* occupancy because that could limit their movement upstream. Substrate characteristics were only influential on species co-occurrence and not on single-species occupancy. This highlights the importance of studying biotic interactions and abiotic factors together because the coexistence of these species may be largely dependent on environmental conditions. One study determined that substrate sizes were influential on species detection, but not their occupancy (Cecala et al. 2018). While another study found that larger substrates were positively associated with salamander habitat selection and survival (Martin et al. 2012). Thus, if optimal substrate sizes are not limited, then it would be expected that this would also facilitate species coexistence. Salamander occupancy was also independent of canopy cover density, whereas, another study concluded that they demonstrated a significant positive relationship with riparian canopy cover (Peterman et al. 2011). However, most of the sites in my study were densely forested, therefore, canopy cover was high and homogenous and may not have been a limiting factor for my focal species.

Stream salamander occurrence was not associated with fish and crayfish presence in my study, therefore additional research could be implemented using introduced crayfish and fish to determine if salamander occupancy would be affected by species that they haven't adapted to. Such results may reveal whether local adaptation is the major mechanism preventing negative species interactions within these communities. Future experimental work should focus on species-specific responses of stream salamander body condition to the presence of focal fish species in my study. Other future studies should consider evaluating the strength of other abiotic variables (i.e., stream velocity, depth, width, and temperature) on species co-occurrence throughout time to determine if occupancy is temporally mediated. Additionally, further exploration into slope's effect on *R. atratulus* occurrence is needed to eliminate other potential abiotic factors involved and provide support of steeper slopes influencing the distribution of this species.

Few studies have evaluated interactions between stream salamanders, fish, and crayfish, especially in abiotic contexts. My results add to the growing knowledge regarding stream species co-occurrence within an occupancy framework. With regards to species occupancy, the lack of interaction among salamanders and between other taxa (crayfish and fish) in my study may be a result of coevolution, thus facilitating the potential for local adpatation to reduce the costs associated with competition. Furthermore, the strength of predatory interactions may not be strong enough in my study sites to influence salamander distributions. However, the presence of larger fish species was shown to have potential negative implications for salamander growth or survival. Identifying both the abiotic and biotic factors that mediate stream organism occurrence is important for predicting and explaining stream community dynamics. With increases in the rate of climate change and anthropogenic development, associated abiotic factors may induce changes in strength of species interactions and the availability of optimal habitat for stream organisms. This may be especially important for southern Appalachian stream amphibians, as their populations are faced with numerous stressors that may alter their diversity and abundance (Crawford and Semlitsch 2008, Walls 2009, Milanovich et al. 2010, Peterman et al. 2011, Price et al. 2011, Caruso and Lips 2013).

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Table 1-1. Raw counts of sub-reaches (*n* = 21) where six focal species were detected and their co-occurrence patterns among headwater streams in Monroe County, Tennessee and Macon County, North Carolina.

	D. quadramaculatus	***D. monticola***	***E. wilderae***	***R. atratulus***	***S. atromaculatus***	***C. bartonii***
D. quadramaculatus	21	7	10	9	6	21
D. monticola	7	7	4	5	5	7
E. wilderae	10	4	10	8	2	10
R. atratulus	9	5	8	9	6	9
S. atromaculatus	6	5	2	6	6	6
C. bartonii	21	7	10	9	6	21

Table 1-2. Single-species occupancy analyses including predictor variables for candidate models that were strongly correlated with site-specific covariates. Ψ, $p_{(.)}$: probability of occupancy when survey days are held constant; $\Psi_{(.)}$, $p_{(.)}$: probability of occupancy when site-specific covariates and survey days are held constant; Ψ, $p_{(T)}$: probability of occupancy when survey days are included. Sa: sand; c: cobble; g: gravel; sl: slope.

Model	**AIC**	**ΔAIC**	**w_i**	**k**
D. quadramaculatus				
Ψ, $p_{(.)}$	47.95	0.00	0.493	2
$\Psi_{(sa)}$, $p_{(.)}$	49.95	2.00	0.181	3
$\Psi_{(.)}$, $p_{(.)}$	49.95	2.00	0.181	3
Ψ, $p_{(T)}$	51.64	3.69	0.078	4
$\Psi_{(sa,c)}$, $p_{(.)}$	51.95	4.00	0.067	4
D. monticola				
Ψ, $p_{(.)}$	54.92	0.00	0.518	2
Ψ, $p_{(T)}$	55.06	0.14	0.483	4
E. wilderae				
Ψ, $p_{(T)}$	65.46	0.00	0.475	4
$\Psi_{(.)}$, $p_{(.)}$	65.87	0.41	0.387	3
Ψ, $p_{(.)}$	67.93	2.47	0.138	2
R. atratulus				
$\Psi_{(sl)}$, $p_{(.)}$	65.44	0.00	0.407	3
Ψ, $p_{(T)}$	67.10	1.66	0.178	4
Ψ, $p_{(.)}$	67.17	1.73	0.172	2
$\Psi_{(sl,c)}$, $p_{(.)}$	67.42	1.98	0.151	4
$\Psi_{(.)}$, $p_{(.)}$	68.41	2.97	0.092	3
S. atromaculatus				
Ψ, $p_{(T)}$	49.02	0.00	0.709	4
$\Psi_{(g)}$, $p_{(.)}$	52.07	3.05	0.154	3
Ψ, $p_{(.)}$	52.31	3.29	0.137	2
C. bartonii				
Ψ, $p_{(.)}$	34.66	0.00	0.337	2
Ψ, $p_{(T)}$	36.43	1.77	0.139	3

Table 1-3. Two-species occupancy analyses including predictor variables for models that were strongly correlated with site-specific covariates. Ψ^{A}: probability of occupancy by species A; Ψ^{BA}: probability of occupancy by species B, given that species A is present; Ψ^{Ba}: probability of occupancy by species B, given that species A is absent; ρ^{A}: probability of detection of species A, given that species B is absent; ρ^{B}: probability of detection of species B; r^{A}: probability of detection of species B, given that both are present; r^{BA}: probability of detection of species B, given that both are present and species A was detected; r^{Ba}: probability of detection of species B, given that both are present and species A was not detected. Sa: sand; c: cobble; sl: slope.

Model	AIC	ΔAIC	w_i	k
D. quadramaculatus - D. monticola				
$\Psi^{A}, \Psi^{BA} = \Psi^{Ba}, \rho^{A}, \rho^{B}, r^{A}, r^{BA}, r^{Ba}$	91.07	0.00	0.555	7
$\Psi^{A}, \Psi^{BA}, \Psi^{Ba}, \rho^{A}, \rho^{B}, r^{A}, r^{BA}, r^{Ba}$	93.07	2.00	0.204	8
D. quadramaculatus - E. wilderae				
$\Psi^{A}_{(sa,c)}, \Psi^{BA}_{(sa,c)} = \Psi^{Ba}_{(sa,c)}, \rho^{A}, \rho^{B}, r^{A}, r^{BA}, r^{Ba}$	113.19	0.00	0.692	9
$\Psi^{A}_{(sa,c)}, \Psi^{BA}_{(sa,c)}, \Psi^{Ba}_{(sa,c)}, \rho^{A}, \rho^{B}, r^{A}, r^{BA}, r^{Ba}$	115.19	2.00	0.254	10
D. monticola - E. wilderae				
$\Psi^{A}_{(c)}, \Psi^{BA}_{(c)} = \Psi^{Ba}_{(c)}, \rho^{A}, \rho^{B}, r^{A}, r^{BA}, r^{Ba}$	125.34	0.00	0.543	8
$\Psi^{A}_{(c)}, \Psi^{BA}_{(c)}, \Psi^{Ba}_{(c)}, \rho^{A}, \rho^{B}, r^{A}, r^{BA}, r^{Ba}$	127.34	2.00	0.200	9
R. atratulus - D. quadramaculatus				
$\Psi^{A}, \Psi^{BA} = \Psi^{Ba}, \rho^{A}, \rho^{B}, r^{A}, r^{BA}, r^{Ba}$	116.86	0.00	0.478	7
$\Psi^{A}_{(sl)}, \Psi^{BA}_{(sl)} = \Psi^{Ba}_{(sl)}, \rho^{A}, \rho^{B}, r^{A}, r^{BA}, r^{Ba}$	118.72	1.86	0.189	8
$\Psi^{A}, \Psi^{BA}, \Psi^{Ba}, \rho^{A}, \rho^{B}, r^{A}, r^{BA}, r^{Ba}$	118.86	2.00	0.176	8
R. atratulus - D. monticola				
$\Psi^{A}_{(sl)}, \Psi^{BA}_{(sl)} = \Psi^{Ba}_{(sl)}, \rho^{A}, \rho^{B}, r^{A}, r^{BA}, r^{Ba}$	108.91	0.00	0.595	8
$\Psi^{A}_{(sl)}, \Psi^{BA}_{(sl)}, \Psi^{Ba}_{(sl)}, \rho^{A}, \rho^{B}, r^{A}, r^{BA}, r^{Ba}$	110.89	1.98	0.221	9
R. atratulus - E. wilderae				
$\Psi^{A}_{(sl)}, \Psi^{BA}_{(sl)} = \Psi^{Ba}_{(sl)}, \rho^{A}, \rho^{B}, r^{A}, r^{BA}, r^{Ba}$	125.70	0.00	0.488	8
S. atromaculatus - D. quadramaculatus				
$\Psi^{A}_{(sa,c)}, \Psi^{BA}_{(sa,c)} = \Psi^{Ba}_{(sa,c)}, \rho^{A}, \rho^{B}, r^{A}, r^{BA}, r^{Ba}$	98.00	0.00	0.473	9
$\Psi^{A}, \Psi^{BA} = \Psi^{Ba}, \rho^{A}, \rho^{B}, r^{A}, r^{BA}, r^{Ba}$	99.21	1.21	0.258	7
$\Psi^{A}_{(sa,c)}, \Psi^{BA}_{(sa,c)}, \Psi^{Ba}_{(sa,c)}, \rho^{A}, \rho^{B}, r^{A}, r^{BA}, r^{Ba}$	100.00	2.00	0.174	10
S. atromaculatus - D. monticola				
$\Psi^{A}, \Psi^{BA} = \Psi^{Ba}, \rho^{A}, \rho^{B}, r^{A}, r^{BA}, r^{Ba}$	99.55	0.00	0.726	7
$\Psi^{A}, \Psi^{BA}, \Psi^{Ba}, \rho^{A}, \rho^{B}, r^{A}, r^{BA}, r^{Ba}$	101.50	1.95	0.274	8
S. atromaculatus - E. wilderae				
$\Psi^{A}, \Psi^{BA} = \Psi^{Ba}, \rho^{A}, \rho^{B}, r^{A}, r^{BA}, r^{Ba}$	122.36	0.00	0.523	7
$\Psi^{A}, \Psi^{BA}, \Psi^{Ba}, \rho^{A}, \rho^{B}, r^{A}, r^{BA}, r^{Ba}$	124.33	1.97	0.195	8
$\Psi^{A}_{(c)}, \Psi^{BA}_{(c)} = \Psi^{Ba}_{(zc)}, \rho^{A}, \rho^{B}, r^{A}, r^{BA}, r^{Ba}$	124.34	1.98	0.194	8
C. bartonii – D. quadramaculatus				
$\Psi^{A}, \Psi^{BA} = \Psi^{Ba}, \rho^{A}, \rho^{B}, r^{A}, r^{BA}, r^{Ba}$	72.41	0.00	0.731	7
$\Psi^{A}, \Psi^{BA}, \Psi^{Ba}, \rho^{A}, \rho^{B}, r^{A}, r^{BA}, r^{Ba}$	74.41	2.00	0.269	8
C. bartonii – D. monticola				
$\Psi^{A}, \Psi^{BA} = \Psi^{Ba}, \rho^{A}, \rho^{B}, r^{A}, r^{BA}, r^{Ba}$	77.08	0.00	0.731	7
$\Psi^{A}, \Psi^{BA}, \Psi^{Ba}, \rho^{A}, \rho^{B}, r^{A}, r^{BA}, r^{Ba}$	79.08	2.00	0.269	8
C. bartonii – E. wilderae				
$\Psi^{A}, \Psi^{BA} = \Psi^{Ba}, \rho^{A}, \rho^{B}, r^{A}, r^{BA}, r^{Ba}$	73.97	0.00	0.731	7
$\Psi^{A}, \Psi^{BA}, \Psi^{Ba}, \rho^{A}, \rho^{B}, r^{A}, r^{BA}, r^{Ba}$	75.97	2.00	0.269	8

Table 1-4. Model-averaged parameter estimates of detection and occupancy probabilities. Ψ^{A}: probability of occupancy by species A; Ψ^{BA}: probability of occupancy by species B, given that species A is present; Ψ^{Ba}: probability of occupancy by species B, given that species A is absent; ρ^{A}: probability of detection of species A, given that species B is absent; ρ^{B}: probability of detection of species B; r^{A}: probability of detection of species B, given that both species are present; r^{BA}: probability of detection of species B, given that both species are present and species A was detected; r^{Ba}: probability of detection of species B, given that both species are present and species A was not detected.

	Parameters							
Species	$\boldsymbol{\Psi}^{\mathbf{A}}$	$\boldsymbol{\Psi}^{\mathbf{BA}}$	$\boldsymbol{\Psi}^{\mathbf{Ba}}$	$\boldsymbol{\rho}^{\mathbf{A}}$	$\boldsymbol{\rho}^{\mathbf{B}}$	$\boldsymbol{r}^{\mathbf{A}}$	$\boldsymbol{r}^{\mathbf{BA}}$	$\boldsymbol{r}^{\mathbf{Ba}}$
***D. quadramaculatus* -**								
D. monticola	1.000 ± 0.000	0.755 ± 0.002		0.368 ± 0.185	0.500 ± 2.500	1.000 ± 0.000	0.123 ± 0.146	
E. wilderae	1.000 ± 0.000	0.658 ± 0.090		0.757 ± 0.119	0.500 ± 2.500	0.934 ± 0.037	0.298 ± 0.125	
***D. monticola* -**								
E. wilderae	0.922 ± 0.034	0.245 ± 0.068		0.060 ± 0.079	0.068 ± 0.124	0.212 ± 0.094	0.302 ± 0.108	
***R. atratulus* -**								
D. quadramaculatus	0.387 ± 0.061	1.000 ± 0.000		0.500 ± 2.500	0.950 ± 0.067	0.534 ± 0.113	0.757 ± 0.157	
D. monticola	0.555 ± 0.041	0.720 ± 0.052		1.000 ± 0.000	0.051 ± 0.091	0.313 ± 0.144	0.212 ± 0.128	
E. wilderae	0.497 ± 0.032	0.750 ± 0.048		0.087 ± 0.237	0.086 ± 0.102	0.623 ± 0.111	0.465 ± 0.147	
S. atromaculatus	0.434 ± 0.028	0.605 ± 0.042		1.000 ± 0.000	0.000 ± 0.000	0.439 ± 0.119	0.550 ± 0.151	
***S. atromaculatus* -**								
D. quadramaculatus	0.402 ± 0.036	1.000 ± 0.000		0.500 ± 2.500	1.000 ± 0.000	0.435 ± 0.000	0.619 ± 0.000	
D. monticola	0.381 ± 0.079	0.847 ± 0.114		0.074 ± 0.269	0.057 ± 0.079	0.597 ± 0.154	0.259 ± 0.196	
E. wilderae	0.092 ± 0.141	0.231 ± 0.156		0.000 ± 0.000	0.000 ± 0.000	0.145 ± 0.664	0.000 ± 0.000	
***C. bartonii* -**								
D. quadramaculatus	1.000 ± 0.000	0.835 ± 0.169		1.000 ± 0.000	0.500 ± 2.500	0.876 ± 0.052	0.950 ± 0.053	
E. wilderae	1.000 ± 0.000	0.500 ± 0.184		0.762 ± 0.135	0.500 ± 2.500	1.000 ± 0.000	0.174 ± 0.156	
D. monticola	1.000 ± 0.000	0.763 ± 0.194		1.000 ± 0.000	0.500 ± 0.000	0.858 ± 0.079	0.071 ± 0.080	
R. atratulus	1.000 ± 0.000	0.571 ± 0.097		0.891 ± 0.069	0.500 ± 2.500	0.868 ± 0.086	0.571 ± 0.257	
S. atromaculatus	1.000 ± 0.000	0.394 ± 0.180		0.932 ± 0.069	0.500 ± 2.500	0.786 ± 0.124	0.105 ± 0.176	

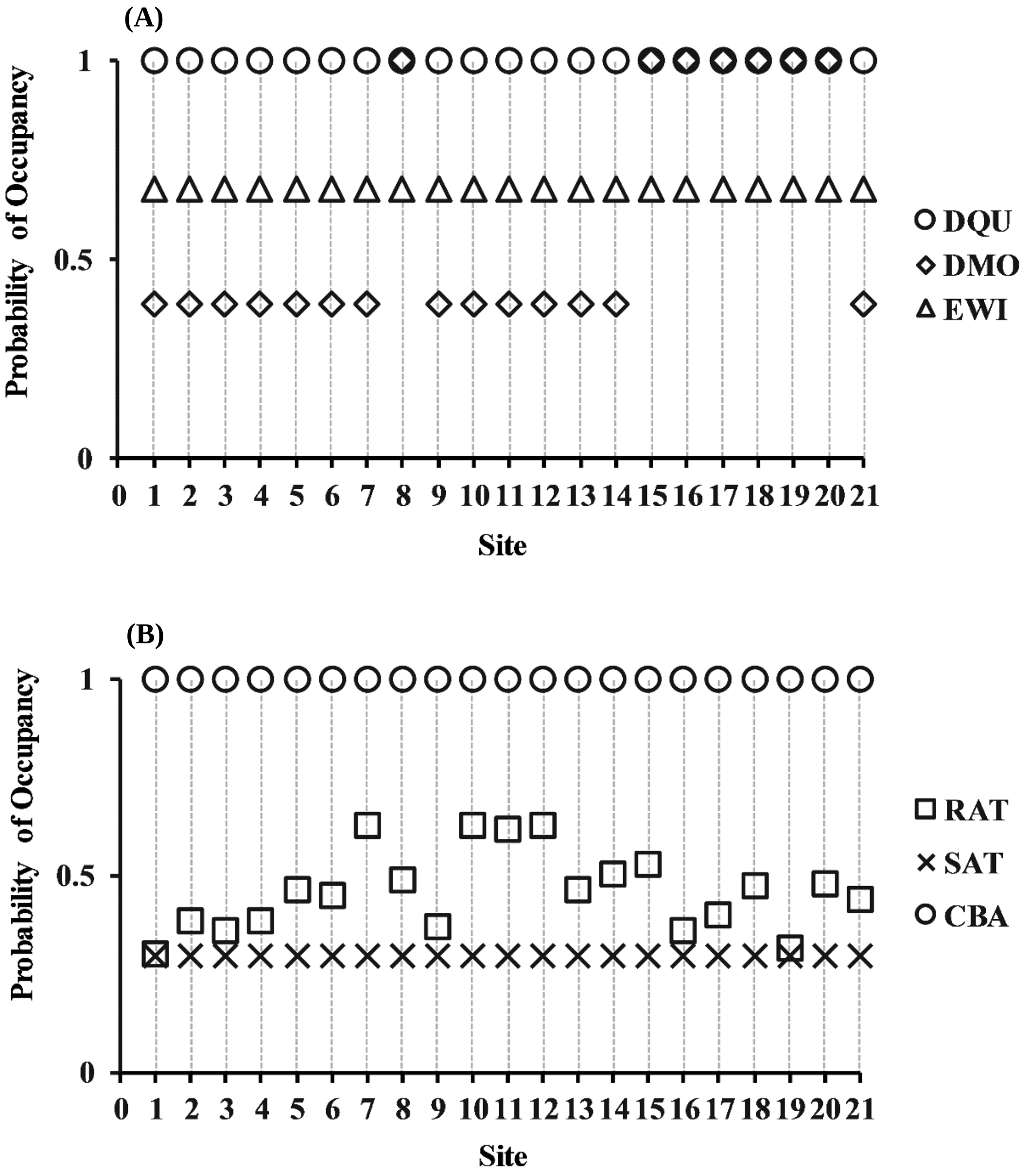

Figure 1-1. Single-species occupancy probabilities by site for A) *D. quadramaculatus* (DQU), *D. monticola* (DMO), and *E. wilderae* (EWI); B) *R. atratulus* (RAT), *S. atromaculatus* (SAT), and *C. bartonii* (CBA) at each site.

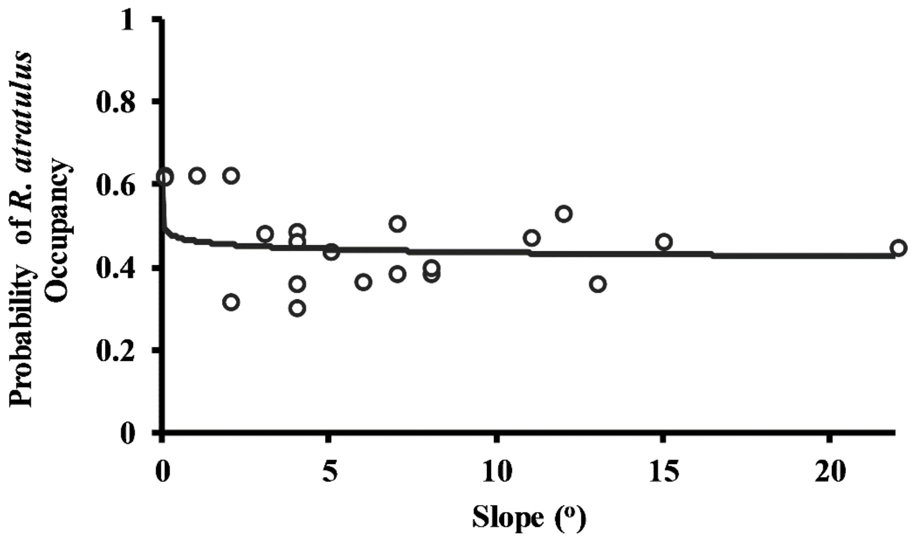

Figure 1-2. *R. atratulus* single-species occupancy probabilities and the slope across all sites located in Monroe County, Tennessee and Macon County, North Carolina. Surveys were conducted between May and July 2018.

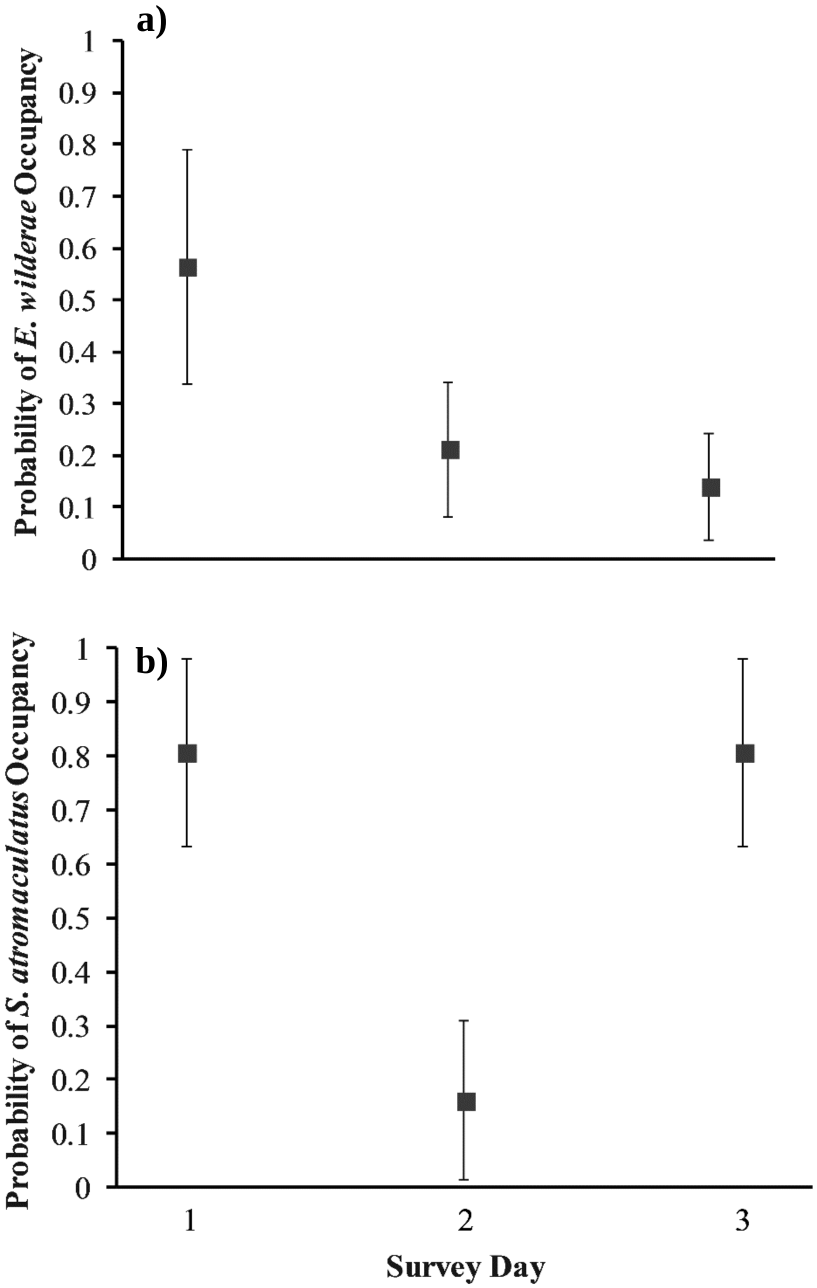

Figure 1-3. Occupancy probabilities of *E. wilderae* (a) and *S. atromaculatus* (b) as a function of survey day.

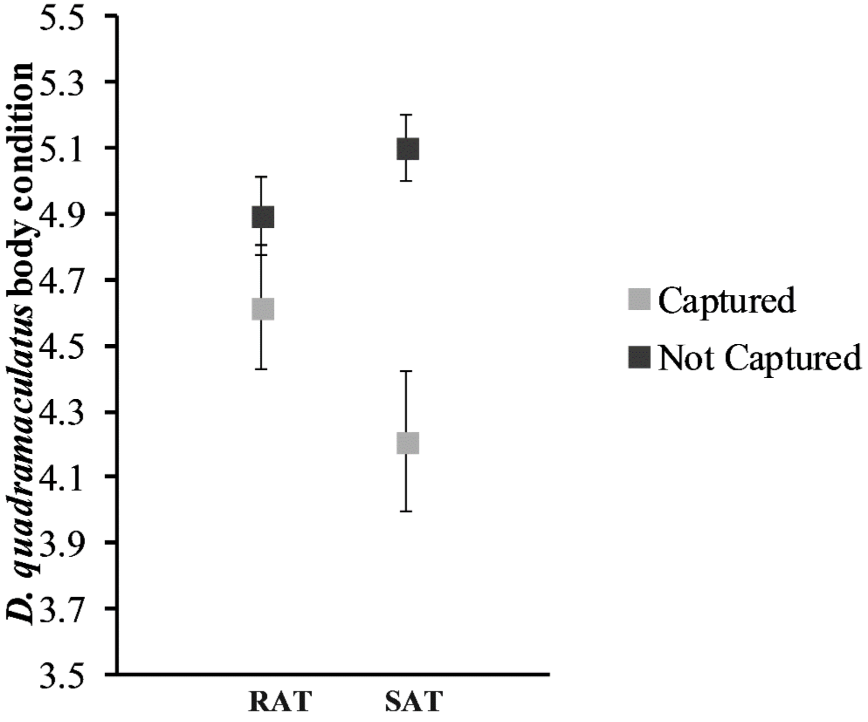

Figure 1-4. Mean body condition of *D. quadramaculatus* compared across sites where *R. atratulus* (RAT) and *S. atromaculatus* (SAT) were or were not captured at the same sites in Monroe County, Tennessee and Macon County, North Carolina. Surveys were conducted between May and July 2018.

CHAPTER 2: ROLE OF ABIOTIC FACTORS IN THE INTERACTIONS BETWEEN STREAM SALAMANDERS AND CRAYFISH IN THE SOUTHERN APPALACHIANS

ABSTRACT

The role of species interactions (i.e., predation and competition) in determining species distribution and abundance within communities is well-documented in ecological literature. Less attention has been on these interactions between morphologically dissimilar taxa in southern Appalachian streams, therefore, I investigated this by selecting a ubiquitous and widely overlapping assemblage of stream species. I used a two-pronged approach (*in situ* and *ex situ* methods) to determine the potential abiotic and biotic factors that explain the spatial patterns associated with refuge use and body size for salamanders and crayfish. My artificial stream experiment compared intra- and inter-specific success and use of refuges at two different refuge densities. Field surveys documented factors potentially associated with refuge cohabitation between C. bartonii and salamanders (*D. quadramaculatus* and *D. marmoratus*) within natural streams, in addition to, measuring abiotic factors (i.e., micro- and macro-level habitat). In my experiment, the frequency of cohabitation and refuge use, growth, and mortality of *D. quadramaculatus* was not influenced by refuge density, nor by species identity. *C. bartonii* body condition did not differ between low or high refuge densities, nor between the presence of hetero- or con-specifics. However, *C. bartonii* did occupy refuges significantly more frequently when refuge densities were higher, compared to when densities were lower. In my field surveys, neither abiotic or biotic factors were associated with interspecific cohabitation among salamanders and *C. bartonii*. Although my results failed to detect any competitive or predatory interactions between stream salamanders and *C. bartonii* over refuge, it appeared that refuge availability determined how frequently *C. bartonii* occupied refuges. Overall, these results

support existing concepts, with respect to, distantly related species being less likely to compete due to their morphological dissimilarities. However, it should be considered that there are some niche breadths between salamanders and crayfish such as diet (i.e., crayfish are often more omnivorous) which may explain why interactions between them were not strong. Although interactions between salamanders and crayfish in my study were not apparent, other biotic (i.e., presence of another potential predator or competitor such as fish) and abiotic factors (i.e., involving a treatment group with only one refuge available) that were not tested may be more influential. Understanding the underlying mechanisms involved in driving ecological responses is important for predicting how future changes in abiotic and biotic composition may impact these communities.

INTRODUCTION

Species interactions are important for determining the biological organization of natural communities (Connell 1983, Schoener 1983, Creed 2006, Rudolf 2008, Bylak 2018). One interaction, competition, can be exhibited indirectly, through the exploitation of a limited resource or by a species directly interfering with another species' ability to access a resource (Case and Gilpin 1974, Amarasekare 2002, Case et al. 2005). Therefore, competitive interactions can control the habitat use, distribution, and composition of species within communities (Connell 1983, Schoener 1983, Ennen et al. 2016, Bastianelli et al. 2017, Chen et al. 2019). In theory, competition is often strongest between closely related species due to their possession of similar phenotypes (Aarssen 1983, Abrams 1983). However, there has been evidence of these interactions occurring between distantly related taxa, despite their morphological differences (Gatz 1979, Schoener and Spiller 1987, Morin et al. 1988, Resetarits 1991, Davenport and Riley 2017). For example, some lizard species compete for prey with taxa such as spiders and birds (Wright 1981, Schoener and Spiller 1987). Larval anurans (*Anaxyrus fowleri*) have experienced significant reductions in their mass in response to competing with insects for pond periphyton (Morin et al. 1988). Other distantly related pond species, such as greater bladderwort (*Utricularia vulgaris*), a carnivorous plant species, and bluegill (*Lepomis macrochirus*) can compete for zooplankton (Davenport and Riley 2017). Which interspecific competition between distantly related taxa has been documented in terrestrial and pond communities, it has received less attention in stream systems.

Streams are unique due to their complex community structures and diversity of life stages and species, yet, there has been little focus on the interactions between distantly related species in these systems. Stream species have the potential to fill ecological niches due to their similar life history requirements and adaptations to dynamic habitats (Connell 1980, Davic and Welsh 2004, Sepulveda et al. 2012). Of the few studies that have considered competitive interactions between salamanders and other distantly related species, there are conflicting results for interactions between stream salamanders and crustaceans. One experimental study concluded that the presence of Appalachian brook crayfish (*Cambarus bartonii*) did not influence the survival nor the growth of spring salamanders (Resetarits 1991). Yet, others have found that spatial competition was apparent between recently introduced red swamp crayfish (*Procambarus clarkii*) and California newts (*Taricha torosa*) (Gamradt and Kats 2006). Considering that some of the species from the previous examples are either not common or existent in my study region, further investigations need to involve more numerically dominant species in the southern Appalachians. Furthermore, competitive interactions between brook crayfish and Desmognathine species in this region have not yet been explored.

Desmognathine salamanders and Appalachian brook crayfish are both ubiquitous throughout southern Appalachian headwater streams and with high biomass. Salamanders and crayfish in headwater streams do consume similar prey, such as aquatic invertebrates and amphibian larvae and eggs, suggesting the potential for resource competition (Usio and Townsend 2004, Richmond and Lasenby 2006, Cruz and Rebelo 2007). Both taxa also rely on cover objects (e.g. rocks and logs) for refuge from predators, ambushing prey, and anchoring during high stream flow events (Lowe et al. 2004, Benvenuto et al. 2008, Keitzer et al. 2013). Therefore, individuals may compete for food resources and interstitial spaces within streams. While it’s apparent that they coexist at larger spatial scales, it’s not well-understood how they interact at the micro-habitat scale, especially with regards to refuge occupancy. It’s possible that they exhibit interference competition for food resources or space through territorial defense thus, influencing their refuge-selection. Or, predation may be the most influential interaction between these taxa therefore, either preventing the prey taxa from inhabiting a refuge if it’s already occupied by the predator taxa or causing prey to occupy it more frequently to avoid the predator taxa.

These interactions can also be dependent on abiotic processes, operating at multiple spatial scales; however, evaluations of such dependent relationships are limited (Chesson 2000, Fields et al. 2017). If the availability of optimal habitat is limited, then competitive or predatory pressure among salamanders and crayfish may be strong. For example, if the density of refuges (i.e., large rocks) is limited in a habitat, then competition may be intensified through territorial defense, forcing the subordinate individuals or species to occupy the lower quality micro-habitat (i.e., areas with no large rocks; Jacobs and Taylor 1992, Camp and Lee 1996, Fero and Moore 2014). The risk of predation between these taxa may also be higher in this situation due to the lack of refuges available for prey to hide from predators (Camp and Lee 1996, Benvenuto et al. 2008). However, if neither interaction is important, then it’s likely that both species would be forced to occupy the same refuge thus, exhibiting their coexistence at these smaller scales. Cohabitation may indicate that neither competitive nor predatory pressure are strong enough to make them avoid refuges where the other species is present.

Understanding the mechanisms involved in the patterns of species distributions and community assemblages is important for preserving ecosystem function (Naeem et al. 1994, Tilman and Downing 1994, Paine 2002, Hooper et al. 2005). Furthermore, salamanders and crayfish represent much of the biomass present throughout southern Appalachian headwater streams and are essential components throughout these ecosystems (Woodall and Wallace 1972, Huryn and Wallace 1987, Peterman et al. 2008). Although interactions between stream salamanders and crayfish have responses have been evaluated, existing field-based studies do not lack the interplay with abiotic factors and do not involve taxa native to the southern Appalachians. Using a two-pronged approach (both field-based and experimental methods), I assessed whether these taxa exhibit predatory or competitive interactions that influence their spatial patterns and body condition and if habitat characteristics are involved in these interactions. The first objective of my research was to determine experimentally if the strength of interactions between salamanders (*Desmognathus quadramaculatus*) and crayfish (*C. bartonii*) was greater inter- or intra-specifically and if these interactions are dependent on refuge availability. First, I hypothesized that the growth, survival, and cohabitation frequency will be lower, and that refuge use frequency will be higher between heterospecifics for both taxa. Lastly, fewer spatial refuges will either intensify the exiting interactions between heterospecifics or induce interactions between individuals if species identity is not significant. The second objective of my research was to assess the spatial patterns of Desmognathine salamanders (*D. marmoratus* and *D. quadramaculatus*) and crayfish in natural streams and determine whether cohabitation patterns are associated with macro- and micro- habitat characteristics (such as rock size of capture location, substrate composition, channel characteristics, stream depth, stream width, and flow). I hypothesized that cohabitation rates between salamanders and crayfish will be positively associated with refuge and substrate size because larger rocks support more refuges for more individuals. Second, the probability of interspecific cohabitation will have a positive relationship with greater stream flow because the prevention of being displaced downstream may be more consequential than remaining near a heterospecific. Third, cohabitation between salamanders and crayfish will be more frequent in riffles because these areas are shallow and may increase their exposure to terrestrial predators. Lastly, stream size will be positively associated with interspecific cohabitation due to potentially greater risks of encountering fish.

METHODS

Experiment

For the experimental component of my research, I used artificial streams to evaluate potential competitive interactions between *Desmognathus quadramaculatus* and *Cambarus bartonii* by manipulating refuge density and species identity. I conducted the artificial stream experiment at the Tennessee Aquarium Conservation Institute (TNACI) in Chattanooga, Tennessee between December 21, 2018 and February 23, 2019. A three-by-two factorial experiment was used to manipulate the identity of competitors (two *D. quadramaculatus*, two *C bartonii*, or one *D. quadramaculatus* and one *C bartonii*) with cover object availability (two or four objects). Individuals were housed with other subjects of comparable sizes for processing before the experiment began.

A total of 24 *Desmognathus quadramaculatus* with mean snout-to-vent length (SVL) of 50 mm (range: 45-55 mm) and 24 *Cambarus bartonii* with mean carapace length (CPL) of 35 mm (range: 30-40 mm) were collected from six streams in Monroe Co., Tennessee during December 14-16, 2018. All individuals were housed individually in containers at TNACI until the experiment was initiated on December 21, 2018. All study individuals were measured for TL (total length) and mass, *D. quadramaculatus* for SVL, and *C bartonii* for CPL before and after the experiment in order to monitor any changes in body condition (Resetarits 1991, Ennen et al. 2016, Lowe et al. 2018). Change in body condition has been used to measure stream organisms' response to interspecific competition in previous experimental studies in order to assess the strength of these interactions (Resetarits 1991, Davenport and Lowe, 2016, 2018, Liles et al. 2017, Hoffacker et al. 2018). All experimental animals were marked with a unique fluorescent color combination via injection of visible implant elastomers (VIE), which allowed me to distinguish individuals during the experiment (Davenport and Lowe, 2016, 2018, Liles et al. 2017, Hoffacker et al. 2018). The location of the injection site was species-specific; *D. quadramaculatus* received a mark ventrally above their hindlimbs, while *C bartonii* were marked on their ventral abdominal tissue.

The artificial streams were twenty-four 68 L fiberglass containers with independent recirculating systems comprised of the same substrate composition (three-cm-deep layer of sand, followed by a single layer of gravel) in order to mimic natural stream conditions (Ennen et al. 2016, Liles et al. 2017, Hoffacker et al. 2018). Dechlorinated water was added to the streams with a depth of 11.5 cm between the substrate and water surface. Water remained at a constant flow rate of 40.5 L/min by using pumping systems made out of aquarium pumps, standpipes, and PVC tubing. Artificial streams were covered with window screening, tightly bound with binder clips to prevent escape. All cover object treatments were randomly assigned to artificial streams to ensure that each treatment combination. Each stream received artificial cover objects, consisting of 15 cm x 15 cm semi-transparent plexiglass squares that were elevated from the substrate by bolts inserted through the corners (McNeely et al. 1990). All species pairs were provided with equal quantities of prey items (live mayfly nymphs) every 15 days (Ennen et al. 2016, Liles et al. 2017, Hoffacker et al. 2018). Eight 12 x 12 mm leaf litter pieces were added to all artificial streams during each feeding event for *C bartonii*. The specific locations of subjects were observed, on average, every three days in the morning to evaluate rates of cohabitation and monitor their activity and refuge use.

Using the '*lme'* function in the '*nlme'* package in RStudio v3.5.2 (R Core Team 2018), mixed effects models were performed to evaluate the effect of refuge availability and subject identity on the interactions between *Desmognathus quadramaculatus* and *Cambarus bartonii* crayfish. The fixed effects included cover object density (low or high) and species identity (*D. quadramaculatus* or *C. bartonii*) and the random effects were the artificial stream number and the observation day (to account for repeated measures). The response variables for only *D. quadramaculatus* included the frequency of cohabitation occurrences, rate of mortality, and the rate of emergence from water. Response variables for both *D. quadramaculatus* and *C. bartonii* involved the change in body condition and the frequency of refuge use. The rate of mortality, emergence from water, and cohabitation for crayfish were excluded from analyses because sample sizes were too low. Body condition, represented by the scaled mass index (SMI), was calculated for all focal species (Peig and Green 2009, 2010). This condition index is representative of an individual's energy reserves by relating mass to length and has been used in previous field surveys and experiments involving stream organisms (Davenport and Lowe 2016, Ennen et al. 2016, Liles et al. 2017, Hoffacker 2018). SMI of body condition will be calculated as

$$SMI = ln(mass)\ X \left(\frac{\mu\, mass}{length}\right)^{\left(\frac{slope}{r}\right)},$$

where *ln(mass)* is the natural log of the body mass and *length* is the either the SVL (*D. quadramaculatus*) or CPL (*C. bartonii*) for an individual. μ *mass* is the sample mean mass and *slope* is *ln(mass)* over *ln(length)*, which is calculated from an ordinary least squares (OLS) regression. The slope is then divided by the Pearson correlation coefficient *r* (LaBarbera 1989). The proportional change in scaled mass was calculated as follows:

$$\Delta\ scaled\ mass = scaled\ mass_{start} - scaled\ mass_{end},$$

where *scaled* $mass_{start}$ represents the proportional measurements obtained before the experiment and *scaled* $mass_{end}$ represents those obtained at the termination of the experiment. Cohabitation frequency was calculated by denoting observations where both subjects occupied the same refuge with a '1' and a '0' for observations where cohabitation did not occur. The *C. bartonii*-only treatments were removed from the cohabitation frequency analysis because all cohabitation values were '0', which resulted in abnormally high variances among treatments. The rate of mortality was determined by denoting each deceased individual with a "1" and each survived individual with a "0". Emergence from water represents the rate of which *D. quadramaculatus* were found out of water (i.e., on top of refuges, pipes, and on artificial stream walls) and observations where this occurred were represented by a "1" and all other observations were denoted with a "0".

Field surveys

The focal species for my field-based project included two stream salamander species (*Desmognathus quadramaculatus* and *Desmognathus marmoratus*) and one species of crayfish (*Cambarus bartonii*). Field surveys were conducted between May 28 and July 7, 2019 to augment the cohabitation experiment. One 150-m stretch was established for each of my four sites located in Watauga County, NC. Each site was visited four times throughout the summer with a minimum of eight days (dependent on the weather) between visits. One rock per meter (*n* = 150) was selected to be flipped during each survey to keep effort standardized across streams (Lowe 2012, Lowe et al. 2018). Individuals that were captured were placed in separate Ziploc bags, set aside on land (until the entire site was completed), and given an identification number in the order in which they were detected. All salamanders and crayfish were identified to species and life stage, measured for length (CL for crayfish and SVL for salamanders), measured for mass (wet), and marked with elastomer. Each salamander was given a unique VIE color combination ventrally above limbs and crayfish were batch-marked (i.e., all individuals get the same color, which varied based on the week the site was visited) on their ventral abdominal tissue.

The macrohabitat and microhabitat of each flipped rock was categorized, regardless of whether an individual was present underneath. This involved recording the alpha dimension of the rock that was overturned, in addition to categorizing the surrounding substrate composition within a 50 cm radius of the center of each overturned rock. Each area was also categorized at the macro scale as being within a riffle, run, or pool within 1 m of the rock's center (Grant et al. 2009, Lowe 2012, Cecala et al. 2014, Lowe et al. 2018). After all individuals were processed and released, the stream depth, width, and flow were measured at the 0-m, 50-m,100-m, and 150-m marks along the study reach. Depth and flow were measured in intervals of 25%, 50%, and 75% across the width of the stream and the width was measured for both bankfull and wetted widths.

I compared the probability of cohabitation of stream salamanders and crayfish with the lengths of individuals, rock diameter, proportion of each substrate type (i.e. silt, sand, gravel, cobble, boulder, and bedrock), stream size (bankfull width, wetted width, and depth), and stream flow using logistic regression models implemented by the '*glm*' function in RStudio (v3.5.2). The probability of cohabitation was calculated by denoting observations where both subjects occupied the same refuge with a '1', while observations were represented by a '0' if individuals were not occupying the same refuge. The values for substrate proportions were autocorrelated, thus, values were log-transformed to avoid violating the assumptions for regression models.

Linear regression models were implemented to evaluate the factors influencing the number of individuals cohabitating together using the '*lm*' function in RStudio (v3.5.2).

RESULTS

Experiment

Desmognathus quadramaculatus mortality was not affected by species identity (*n* = 3; t = -0.536, *P* = 0.602; Tables 2-1 and 2-2; Figure 2-1b) nor refuge density treatments (*t* = 0.00, *P* = 0.715; Tables 2-1 and 2-2; Fig. 2-1b). While *D. quadramaculatus* body condition generally increased during the experiment, there was no significant difference among refuge density (Tables 2-1 and 2-2; Fig. 2-2a; *t* = 0.-0.739, *P* = 0.939) or species identity treatments (Tables 2-1 and 2-2; Fig. 2-2a; *t* = -0.857, *P* = 0.960). The mean frequency of refuge-use for *D. quadramaculatus* was not significantly different between the *D. quadramaculatus*-only and *D. quadramaculatus*-*Cambarus bartonii* treatments (*t* = -0.165, *P* = 0.869; Table 2-2; Fig. 2-2b), nor was it different between low and high refuge densities (*t* = 0.643, *P* = 0.521; Table 2-2; Fig. 2-2b). There was no significant difference between mean cohabitation frequency for *D. quadramaculatus* between species identity treatments (Tables 2-1 and 2-2; Fig. 2-1a; *t* = -0.205, *P* = 0.838) nor refuge density treatments (Tables 2-1 and 2-2; Fig. 2-1a; *t* = 1.531, *P* = 0.128). The mean frequency of emergence from the water for *D. quadramaculatus* was not influenced by species identity (Tables 2-1 and 2-2; Fig. 2-1c; *t* = 0.595, *P* = 0.553) nor refuge density treatments (Tables 2-1 and 2-2; Fig. 2-1c; *t* = -1.265, *P* = 0.208). *Cambarus bartonii* body condition was not affected by species identity (Tables 2-1 and 2-3; Fig. 2-3b; *t* = -0.857, *P* = 0.414) and refuge densities (Tables 2-1 and 2-3; Figure 2-3b; *t* = -0.739, *P* = 0.479). The mean frequency of refuge use for *C. bartonii* was greater in high refuge density (0.600 ± 0.084) treatments than in the low density (0.462 ± 0.083) treatments (Tables 2-1 and 2-3; Fig. 2-3a; $t = -2.483$, $P = 0.014$). *C. bartonii* frequency of refuge use did not differ between the *C. bartonii*-only and *D. quadramaculatus*-*C. bartonii* crayfish treatments (Tables 2-1 and 2-3; Fig. 2-3a; $t = 0.375$, $P = 0.708$).

Field surveys

The probability of interspecific cohabitation for *Desmognathus quadramaculatus* was not dependent on the following environmental factors: silt (0.068; Table 2-4; Figure 2-4), sand (0.220; Table 2-4; Figure 2-4), gravel (0.435; Table 2-4; Figure 2-4), cobble (0.400; Table 2-4; Figure 2-4), boulder (-0.081; Table 2-4; Figure 2-4), and bedrock (0.012; Table 2-4; Figure 2-4), wetted width (-0.175; Table 2-4; Figure 2-5), bankfull width (-0.199; Table 2-4; Figure 2-5), stream depth (1.469; Table 2-4; Figure 2-5), stream flow (0.658; Table 2-4; Figure 2-5), refuge diameter (-0.010; Table 2-4; Figure 2-8a), riffles (0.036; Table 2-5; Figure 2-10a), runs (0.146; Table 2-5; Figure 2-10a), and pools (0.081; Table 2-5; Figure 2-10a). Additionally, the SVL of salamanders did not determine their probability of interspecific cohabitation (-0.042; Table 2-4; Figure 2-9a). Interspecific cohabitation for crayfish was also not influenced by the following environmental factors: silt (0.009; Table 2-4; Figure 2-6), sand (0.129; Table 2-4; Figure 2-6), gravel (0.286; Table 2-4; Figure 2-6), cobble (0.619; Table 2-4; Figure 2-6), boulder (-0.050; Table 2-4; Figure 2-6), and bedrock (0.111; Table 2-4; Figure 2-6), wetted width (-0.130; Table 2-4; Figure 2-7), bankfull width (-0.148; Table 2-4; Figure 2-7), stream depth (0.016; Table 2-4; Figure 2-7), stream flow (0.812; Table 2-4; Figure 2-7), refuge diameter (-0.089; Table 2-4; Figure 2-8b), riffles (0.100; Table 2-5; Figure 2-10b), runs (0.346; Table 2-5; Figure 2-10b), and pools (0.139; Table 2-5; Figure 2-10b). The carapace length of crayfish did not influence their probability of cohabitating with salamanders (-0.011; Table 2-4; Figure 2-9b).

DISCUSSION

Experiment

The results from my experiment suggest that potential agonistic interactions between *Desmognathus quadramaculatus* and *Cambarus bartonii* are no stronger than intraspecific interactions in response to refuge availability. *D. quadramaculatus* did not differentiate in rate of occupying refuges when in the presence of conspecifics or heterospecifics, nor by the availability of refuge. Species identity did not influence the frequency of refuge use for *C. bartonii,* either. However, there was a significant positive relationship between high refuge density and the frequency of occupying a refuge. Cohabitation frequencies for *D. quadramaculatus* did not differ in the presence of either species, thus my competition hypothesis for spatial refuges was not supported. Though, cohabitation was rare among all subjects, which prevented us from having a large sample size to compare cohabitation rates between treatments. Refuge availability did not appear to limit the rates of cohabitation between *D. quadramaculatus* and *C. bartonii*, nor did it negatively impact the survival of individuals in the stream assemblages in my study. Surprisingly, *D. quadramaculatus* did not spend more time out of the water in the presence of *C. bartonii*, nor were there indications of *C. bartonii* presence affecting salamander survival. Lastly, the change in body condition for both *D. quadramaculatus* and *C. bartonii* was not influenced by refuge density nor by species identity.

Cambarus bartonii in my experiment occupied refuges significantly more frequently when there were greater densities of refuges available. Because species identity did not influence their rate of refuge use, the presence of another individual, regardless of species, may have contributed to refuge density's effect on refuge use. For example, if subjects occupied more than one refuge during the experiment (instead of establishing territory over a single refuge), then they may associate those refuges with the presence of another individual. Thus, it is possible that they avoid those refuges more frequently to reduce the potential of encountering another individual, which the lower refuge density (two refuges) treatment would not provide enough refuge options as the higher refuge density (four refuges) treatment. A previous experiment evaluated the effects of refuge availability on social behavior and habitat choice in the crayfish, *Orconectes virilis* (Fero and Moore 2014). Crayfish did not spend significantly more time occupying refuges in areas with higher refuge density than in the lower refuge density areas (Fero and Moore 2014). However, crayfish in their experiment did occupy refuges significantly more frequently during the day and in high refuge density areas (Fero and Moore 2014). If *C. bartonii* exhibit similar occupancy behavior as *O. virilis*, then this may provide a possible explanation for the higher rate of refuge use in my experiment because observations only occurred in the morning.

If agonistic behaviors towards *Desmognathus quadramaculatus* were used by *Cambarus bartonii*, I expected salamanders to escape from the water or show physical signs of injury. Previous competition experiments involving crayfish have resulted in highly aggressive actions imposed on salamander competitors, thus allowing crayfish to obtain the resource of interest (Gamradt and Kats 2006, Savvides and Louca 2015). California newts (*Taricha torosa*) spent significantly more time out of water in the presence of red swamp crayfish (*Procambarus clarkii*) than with conspecific newts (Gamradt et al. 1997). Additionally, *T. torosa* were repeatedly attacked by *P. clarkii* and exhibited wounds on their limbs and tails from such interactions (Gamradt et al. 1997). My results may differ from those for California newts likely because *P. clarkii* are a much larger, non-native crayfish species, whereas, the crayfish species used in my experiment (*C. bartonii*) are smaller and native. Because both *D. quadramaculatus* and *C. bartonii* are native to the areas where their ranges overlap, it is likely that these two species coexist through behavioral adaptations to reduce the costs associated with competition.

The lack of a change in body condition for both of my focal species is consistent with those of Resetarits (1991), which concluded that the presence of *Cambarus bartonii* did not influence the growth of two salamander species and conversely salamander presence did not affect crayfish growth. This is an indication that they co-occur and competition does not exist between these taxa. In our experiment, *D. quadramaculatus* individuals only gained in body condition, while several crayfish individuals experienced a decrease in body condition over the course of the experiment. Though not significant, *C. bartonii* appeared to exhibit an increase in body condition in the low refuge density treatment and a decrease in body condition in the high refuge density treatment.

My experiment points to several avenues for future studies. First, my experiment only had two different refuge densities, so manipulating additional refuge densities along a wider gradient (i.e., refuge quantities ranging from one to five) could explore the relationship between spatial competition among *Desmognathus quadramaculatus* and *Cambarus bartonii*. It is entirely possible that refuge availability does not influence interactions between salamanders and *C. bartonii*. Second, resource competition based on prey availability may be more influential on the strength of interactions between these species. Because *C. bartonii* and *D. quadramaculatus* often consume similar prey items, if this resource is limited or if more predatory stream organisms (i.e. fish) are present, then the effects of competition may be stronger. Although both

C. bartonii and *D. quadramaculatus* are both numerically dominant in streams, they may not frequently interact with each other.

Field surveys

I conducted field surveys to determine if habitat characteristics and the sizes of individuals influenced the co-occurrence of crayfish and salamanders. The results from the field component indicate that neither biotic nor abiotic factors are influencing the probability of cohabitation for stream salamanders and crayfish. Though substrate composition did not have a significant effect on cohabitation, there was a slight negative relationship between the probability of interspecific cohabitation and the proportion of sand and the proportion of cobble surrounding refuges for salamanders. Cohabitation for crayfish had a minor negative trend with the proportion of cobble and the proportion of boulder. I anticipated a significantly negative trend with smaller substrate particles for both salamanders and crayfish because it can reduce the amount of refuge available. However, I did not find this relationship from my survey data. If this result was significant, it would have been consistent with a previous study that discovered a negative relationship between finer substrates and some stream salamander species (Lowe et al. 2004). It is possible that the streams used in my study consisted of high enough proportions of larger rocks (i.e., cobble and boulder) that it was not a limiting factor. Future studies should consider incorporating stream sites that have greater variation in substrate composition to further evaluate whether this influences interspecific cohabitation.

Interspecific cohabitation for salamanders had a non-significant negative relationship with bankfull width and cohabitation for both salamanders, and crayfish exhibited a slight positive association with stream flow. The size of refuges did not have a significant effect on cohabitation for neither salamanders nor crayfish. But crayfish cohabitation had a slightly negative trend with the size of the refuge they were occupying. Though not significant, both salamander and crayfish cohabitation probabilities exhibited a negative trend with SVL and CPL. This suggests that larger individuals may cohabitate less frequently than smaller individuals, however, this was likely due to the small sample size of adults. Some larger and dominant crayfish species have been observed to exhibit more aggression and territoriality over refuges than the smaller, more subordinate individuals (Fero and Moore 2014). Previous work suggests that larger *D. quadramaculatus* individuals likely aggressively take over refuges occupied by smaller individuals, causing those individuals to wander more or move between refuges more frequently (Camp and Lee 1996). Furthermore, smaller salamanders likely encounter crayfish more frequently than larger adults and consequently, have a greater probability of becoming consumed due to their small size and recurrent wandering. Thus, survival of *D. quadramaculatus* larvae and juveniles may be driven by the prevalence of refuges and may be susceptible to higher ratios of *C. bartonii* and *adult D. quadramaculatus* abundance to refuge densities. Overall, the influence of abiotic factors on interspecific interactions was not apparent, but there are other avenues for future research involving other abiotic variables that were not tested in my study. Additionally, future studies should consider performing an ex-situ study to determine if individuals with greater size differences exhibit cohabitation patterns differently than similar-sized individuals and if abiotic conditions are more important in these interactions.

Cohabitation may be a potential indicator of coexistence between species but could have consequences for shared prey in a community. Both crayfish and salamanders are distantly related yet utilize similar resources (i.e., prey and refuge) while not exhibiting strong competitive interactions. However, individuals in my study may inhabit the same refuge because prey densities are higher in those specific locations. Although these two predator species did not appear to affect each other's distribution, they can affect prey (i.e., larval salamanders and macroinvertebrates) in unexpected ways (Rudolf 2006, Wooster et al. 2011). For example, when larger predators are not present, smaller conspecific predators exhibited increased densities and, thus a decrease in prey survival (Rudolf 2006). Conversely, the presence of multiple predators has been demonstrated to reduce the risk of predation for prey, which may cause substantial increases in these prey populations (Vance-Chalcraft and Soluk 2005). While I did not measure responses in prey, the lack of interference between my predatory focal species may lead to risk enhancement. The effects of the presence of multiple predators on prey should be further explored by incorporating prey (i.e., larval salamanders or macroinvertebrates) density as a response variable to treatments consisting of the presence of either only one species or two species in artifical stream experiments.

My study is one of the first to examine that relationship using both a field and experimental approach. Using this combined approach allowed me to evaluate the potential abiotic and biotic factors driving the microhabitat use and spatial distributions of salamanders in natural stream systems, while also testing for the effects of specific variables in a controlled experimental setting. My results indicate that despite having overlapping resource requirements and numerical dominance in headwater streams, competition for spatial refuges between my focal species is weak. This supports previous studies proposing the concept that distantly related species are less likely to compete because of their morphological differences as a result of local adaptation to minimize competition (Case and Gilpin 1974, Gatz 1979, Connell 1980). Due to the complex interactions existing in stream community structures, it is important for ecologists to identify the factors involved in the ecological responses exhibited by these organisms.

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Table 2-1. Effects of refuge density and species identity on salamanders and crayfish in artificial stream experiments. ‘Refuge-use’ represents the frequency of subjects using a refuge, ‘Body Condition’ represents the change in body condition, ‘Cohabitation’ indicates the frequency of subjects sharing the same refuge with another individual, ‘Emergence’ represents the frequency of salamanders leaving the water, and ‘Mortality’ represents the mean frequency of salamander survival. ‘Subject Identity’ indicates whether subjects in a treatment were of the same species, ‘Intraspecific’, or of different species, ‘Interspecific’. All values represent means ±1 SE.

	Refuge Density		**Species Identity**	
	High	Low	Intraspecific	Interspecific
Salamanders				
Refuge-use	0.302 ± 0.076	0.324 ± 0.072	0.277 ± 0.066	0.35 ± 0.081
Body Condition	0.578 ± 0.126	0.592 ± 0.121	0.59 ± 0.100	0.581 ± 0.143
Cohabitation	0.014 ± 0.021	0.046 ± 0.020	0.024 ± 0.017	0.035 ± 0.023
Emergence	0.237 ± 0.073	0.152 ± 0.073	0.234 ± 0.065	0.155 ± 0.080
Mortality	0.188 ± 0.117	0.125 ± 0.117	0.063 ± 0.105	0.250 ± 0.127
Crayfish				
Refuge-use	0.600 ± 0.084	0.462 ± 0.083	0.495 ± 0.074	0.566 ± 0.092
Body Condition	-0.094 ± 0.264	0.364 ± 0.281	0.152 ± 0.260	0.119 ± 0.284

Table 2-2. Effects of refuge density, species identity, the interactive effect on salamander refuge use, body condition, frequency of cohabitation, frequency of emergence from the water, and mortality in artificial stream experiments.

	df	***t***	***P***
Salamanders			
Refuge-use			
Refuge Density	1	0.643	0.521
Subject Identity	1	-0.165	0.869
Interaction	1	-1.678	0.096
Residuals	145		
Body Condition			
Refuge Density	1	-0.739	0.939
Subject Identity	1	-0.857	0.960
Interaction	1	1.291	0.228
Residuals	9		
Cohabitation			
Refuge Density	1	1.531	0.128
Subject Identity	1	-0.205	0.838
Interaction	1	-1.412	0.1601
Residuals	145		
Emergence			
Refuge Density	1	-1.265	0.208
Subject Identity	1	0.595	0.553
Interaction	1	0.469	0.640
Residuals	144		
Mortality			
Refuge Density	1	0.000	1.000
Subject Identity	1	-0.536	0.602
Interaction	1	-0.379	0.711
Residuals	12		

Table 2-3. Effects of refuge density, species identity, the interactive effect on body condition and refuge-use of crayfish in artificial stream experiments.

	df	***t***	***P***
Crayfish			
Refuge-use			
Refuge Density	1	-2.483	0.014
Subject Identity	1	0.375	0.708
Interaction	1	0.393	0.695
Residuals	149		
Body Condition			
Refuge Density	1	-0.739	0.479
Subject Identity	1	-0.857	0.414
Interaction	1	1.291	0.229
Residuals	9		

Table 2-4. Estimates from logistic regression models of interspecific cohabitation between salamanders and crayfish in response to environmental variables during field surveys conducted in Caldwell County, NC. 'Substrate composition' represents the proportion of silt, sand, gravel, cobble, boulder, and bedrock within a 0.5-m radius of the center of the refuge; 'Stream characteristics' represents the depth, width (bankfull and wetted), and flow of the stream measured every 50 m along site; 'Rock diameter' is the largest diameter of the refuge; and 'Length of individual' is the SVL or CPL of the cohabitating subjects.

	df	**Estimate ± SE**	***z***	***P***
Salamanders				
Substrate composition				
Silt	112	0.068 ± 0.040	1.709	0.087
Sand	112	0.220 ± 0.226	0.971	0.332
Gravel	112	0.435 ± 0.504	0.862	0.389
Cobble	112	0.400 ± 0.493	0.811	0.418
Boulder	112	-0.081 ± 0.044	-1.851	0.064
Bedrock	112	0.012 ± 0.063	0.191	0.849
Stream characteristics				
Depth	112	1.469 ± 5.610	0.262	0.793
Bank	112	-0.199 ± 0.226	-0.882	0.378
Wet	112	-0.175 ± 0.365	-0.480	0.631
Flow	112	0.658 ± 0.561	1.174	0.24
Rock diameter	109	-0.010 ± 0.050	-0.196	0.845
Length of individual	104	-0.042 ± 0.030	-1.417	0.156
Crayfish				
Substrate composition				
Silt	68	0.009 ± 0.043	0.206	0.837
Sand	68	0.129 ± 0.148	0.872	0.383
Gravel	68	0.286 ± 0.388	0.737	0.461
Cobble	68	0.619 ± 0.652	0.949	0.343
Boulder	68	-0.050 ± 0.046	-1.107	0.268
Bedrock	68	0.111 ± 0.084	1.327	0.185
Stream characteristics				
Depth	68	0.016 ± 6.086	0.003	0.998
Bank	68	-0.148 ± 0.220	-0.675	0.500
Wet	68	-0.130 ± 0.385	-0.338	0.735
Flow	68	0.812 ± 0.710	1.145	0.252
Rock diameter	67	-0.089 ± 0.058	-1.517	0.129
Length of individual	62	-0.011 ± 0.035	-0.327	0.743

Table 2-5. Mean probabilities of interspecific cohabitation for salamanders and crayfish across macro-habitat types during field surveys conducted in Caldwell County, NC.

	Mean	**SE**
Salamanders		
Riffle	0.036	0.036
Run	0.146	0.051
Pool	0.081	0.045
Crayfish		
Riffle	0.100	0.100
Run	0.346	0.095
Pool	0.139	0.058

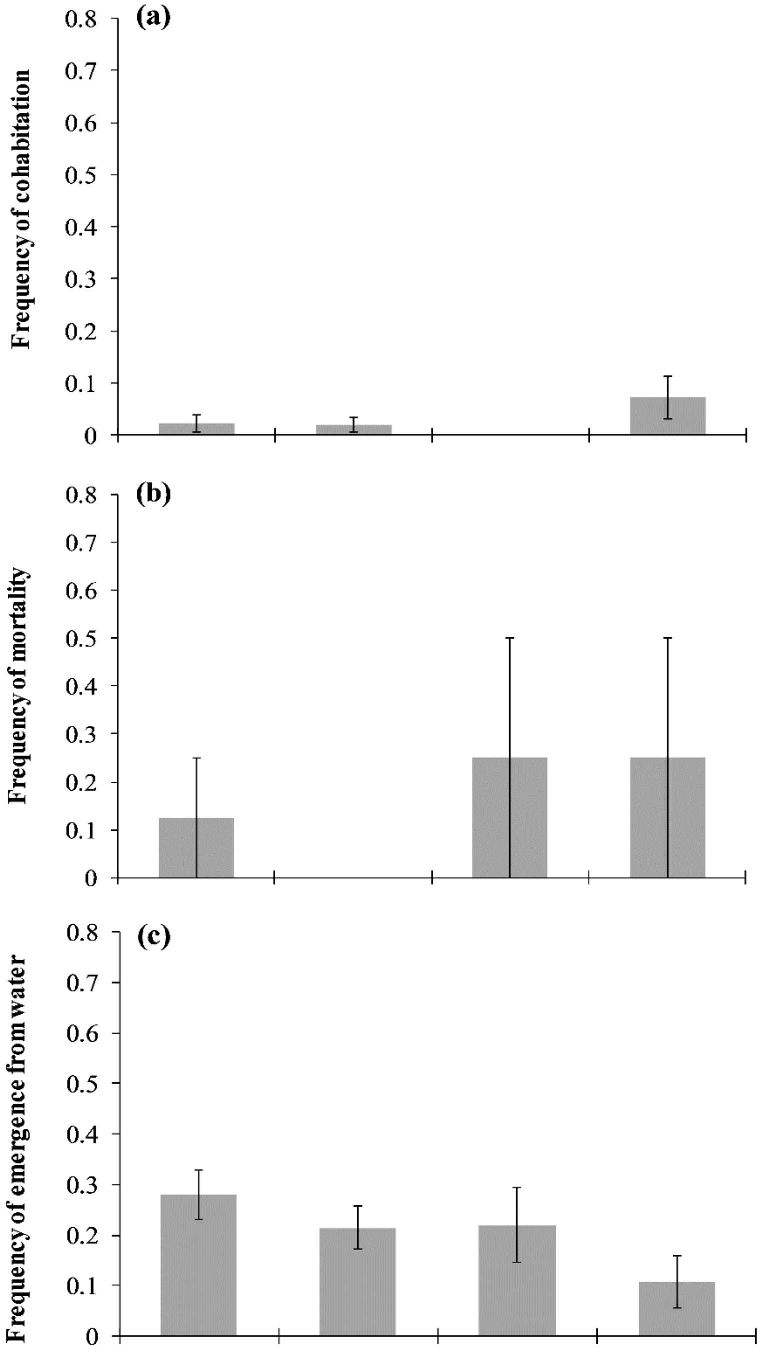

Figure 2-1. *D. quadramaculatus* mean frequency of cohabitation (a), mean frequency of mortality (b), and mean frequency of emergence of from water (c) across refuge density and species identity treatment combinations during artificial stream experiments. SSH: only salamander subjects with high refuge densities, SSL: only salamander subjects with low refuge densities, SCH: salamander and crayfish subjects with high refuge densities, and SCL: salamander and crayfish subjects with low refuge densities.

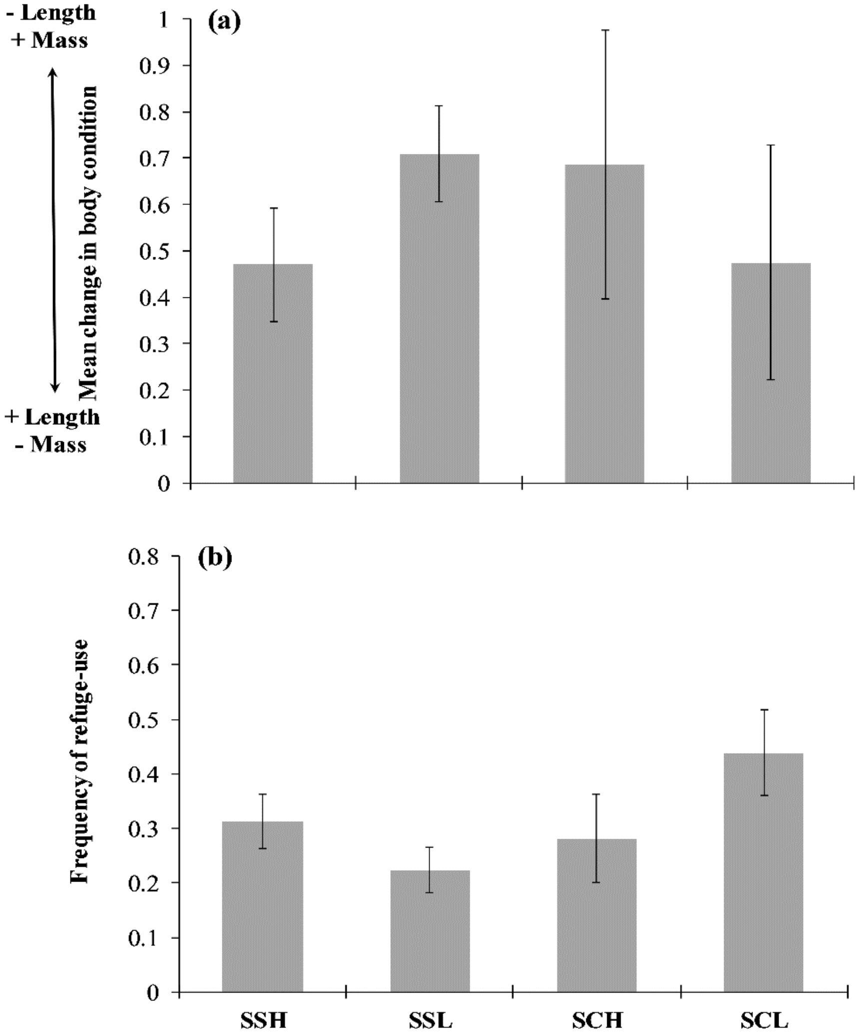

Figure 2-2. *D. quadramaculatus* mean change in body condition (a) and mean frequency of refuge use (b) across refuge density and species identity treatment combinations during artificial stream experiments. SSH: only salamander subjects with high refuge densities, SSL: only salamander subjects with low refuge densities, SCH: salamander and crayfish subjects with high refuge densities, and SCL: salamander and crayfish subjects with low refuge densities.

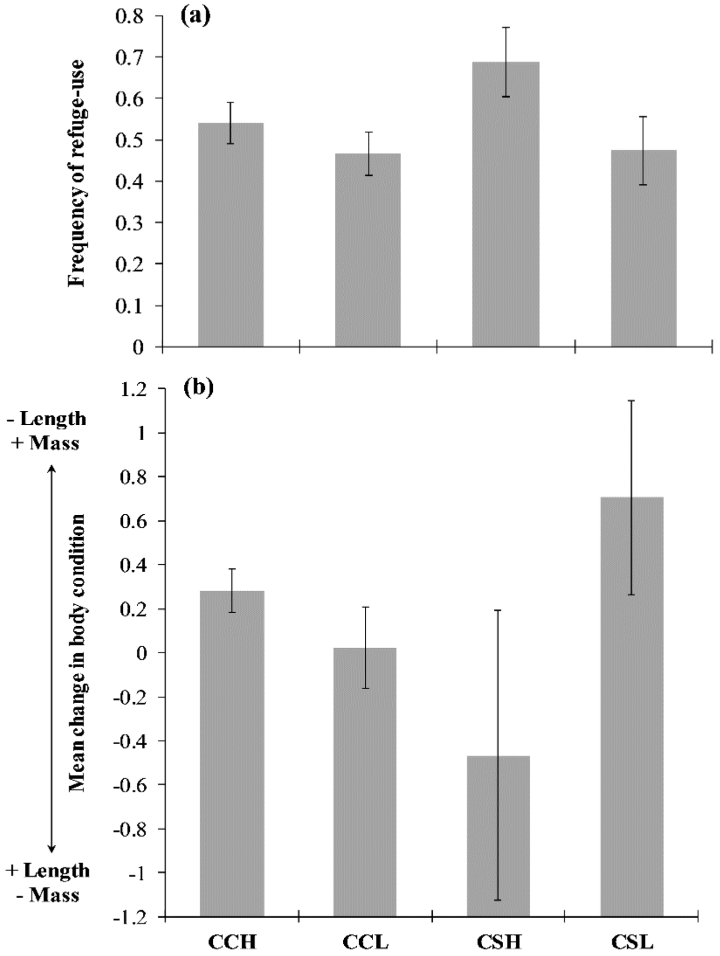

Figure 2-3. *C. bartonii* mean frequency of refuge use (a) and mean change in body condition (b) across refuge density and species identity treatment combinations during artificial stream experiments. CCH: only crayfish subjects with high refuge densities, CCL: only crayfish subjects with low refuge densities, CSH: crayfish and salamander subjects with high refuge densities, and CSL: crayfish and salamander subjects with low refuge densities.

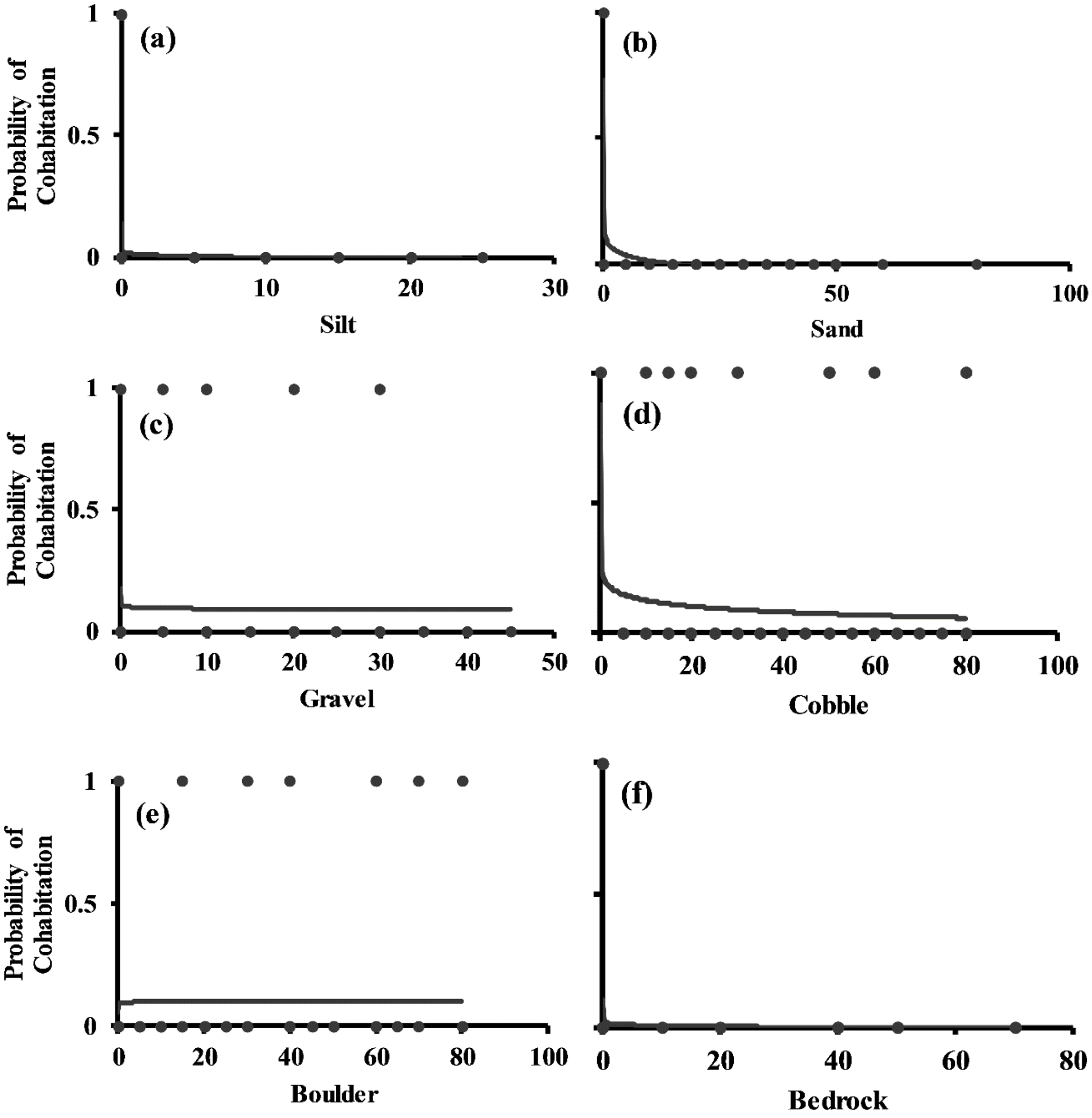

Figure 2-4. The effect of substrate composition on the probability of cohabitation of salamanders during spring field surveys conducted among 4 streams in Caldwell County, NC between May 28 and July 7, 2019: (a) silt, (b) sand, (c) gravel, (d) cobble, (e) boulder, and (f) bedrock.

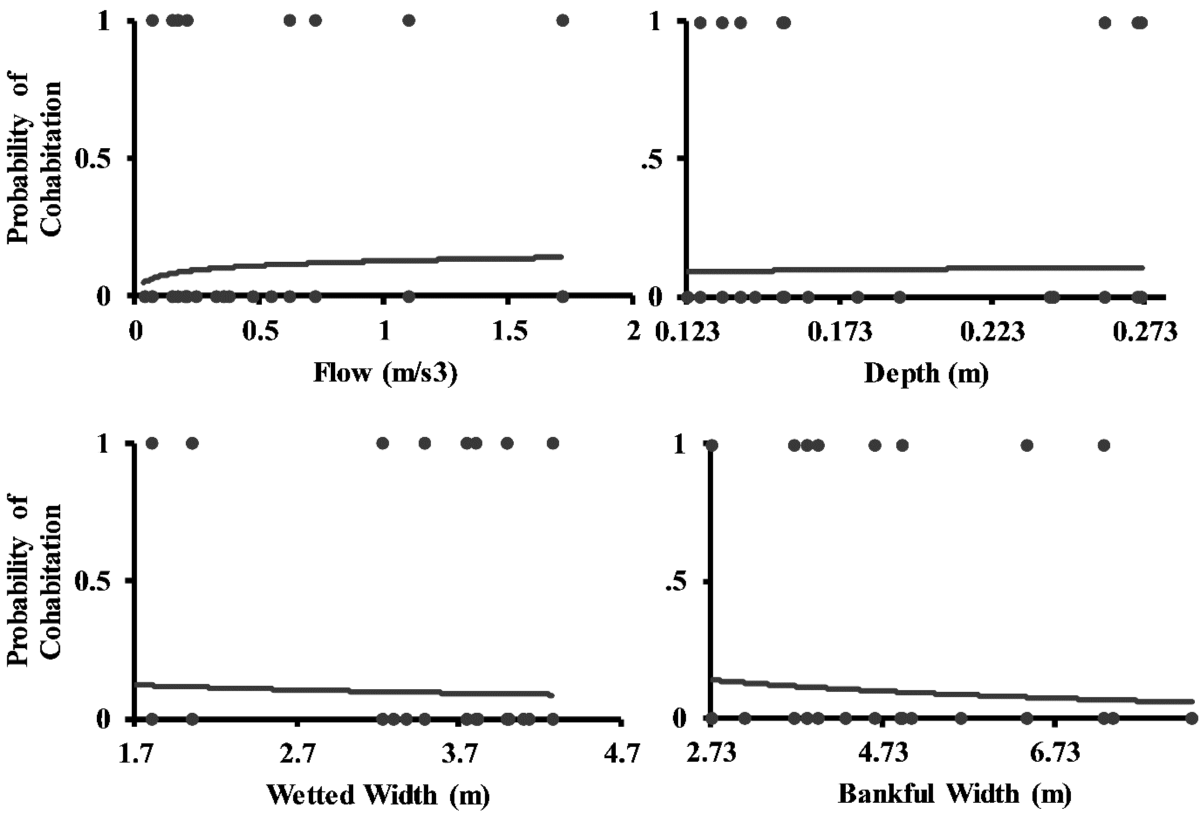

Figure 2-5. The effect of stream characteristics on the probability of cohabitation of salamanders during field surveys conducted among 4 streams in Caldwell County, NC between May 28 and July 7, 2019: (a) flow, (b) depth, (c) wetted width, and (d) bankfull width.

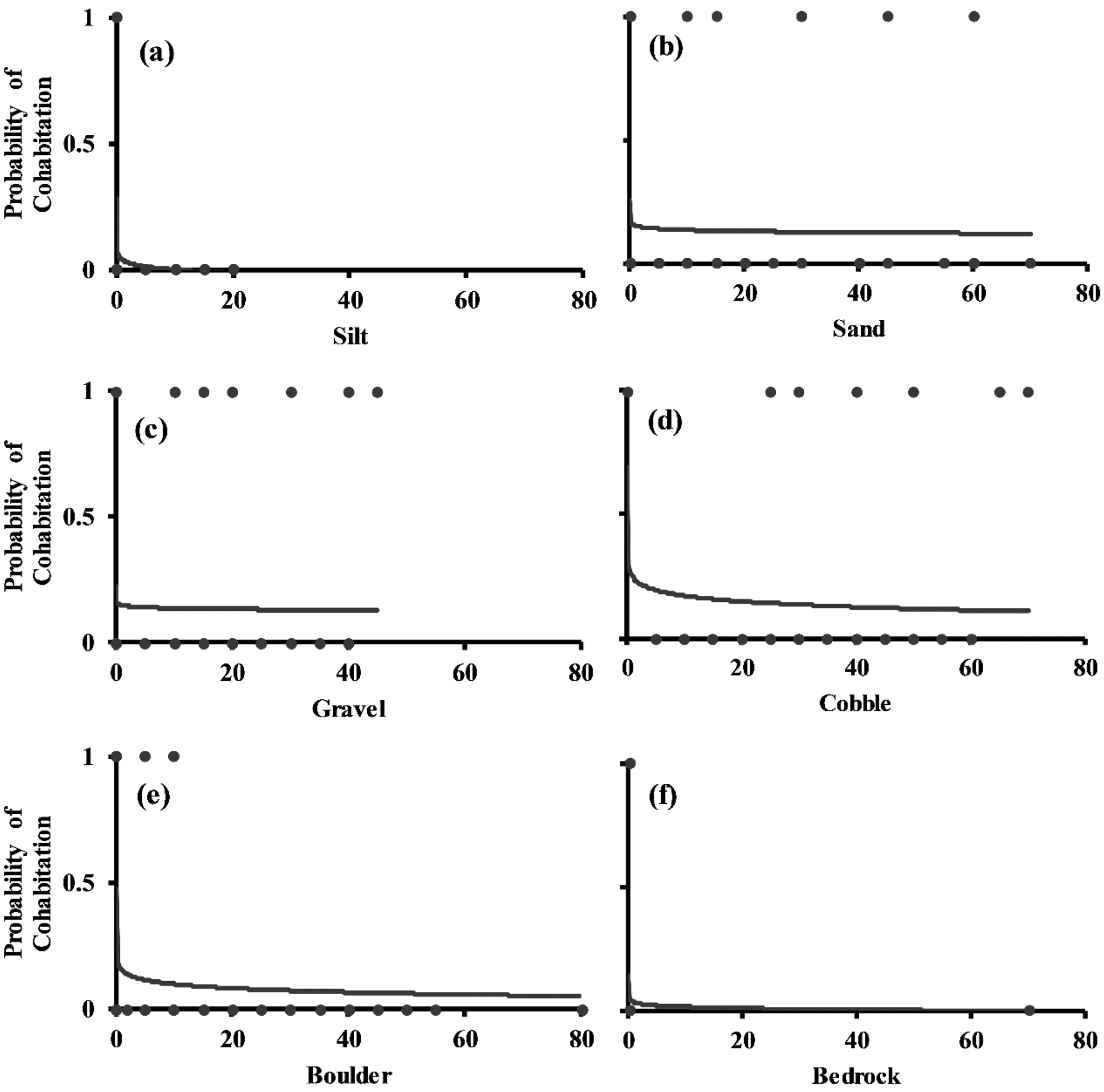

Figure 2-6. The effect of substrate composition on the probability of cohabitation of crayfish during field surveys conducted among 4 streams in Caldwell County, NC between May 28 and July 7, 2019: (a) silt, (b) sand, (c) gravel, (d) cobble, (e) boulder, and (f) bedrock.

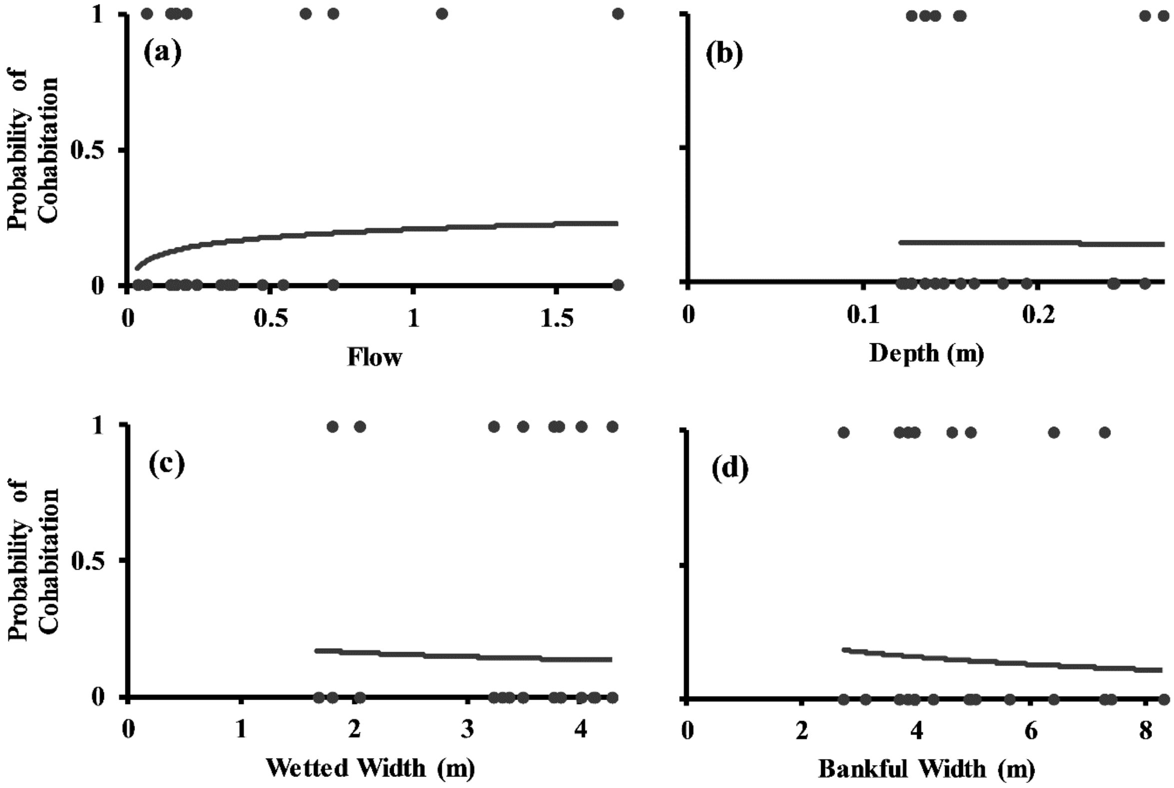

Figure 2-7. The effect of stream characteristics on the probability of cohabitation of crayfish during field surveys conducted among 4 streams in Caldwell County, NC between May 28 and July 7, 2019: (a) flow, (b) depth, (c) wetted width, and (d) bankfull width.

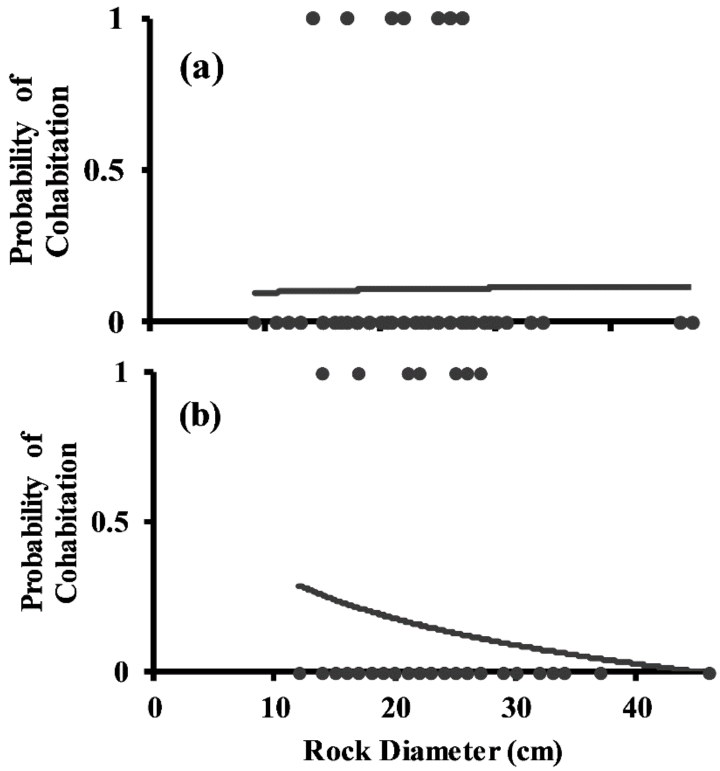

Figure 2-8. The effect of refuge size (rock diameter) on (a) salamander and (b) crayfish probability of cohabitation during field surveys conducted among 4 streams in Caldwell County, NC between May 28 and July 7, 2019.

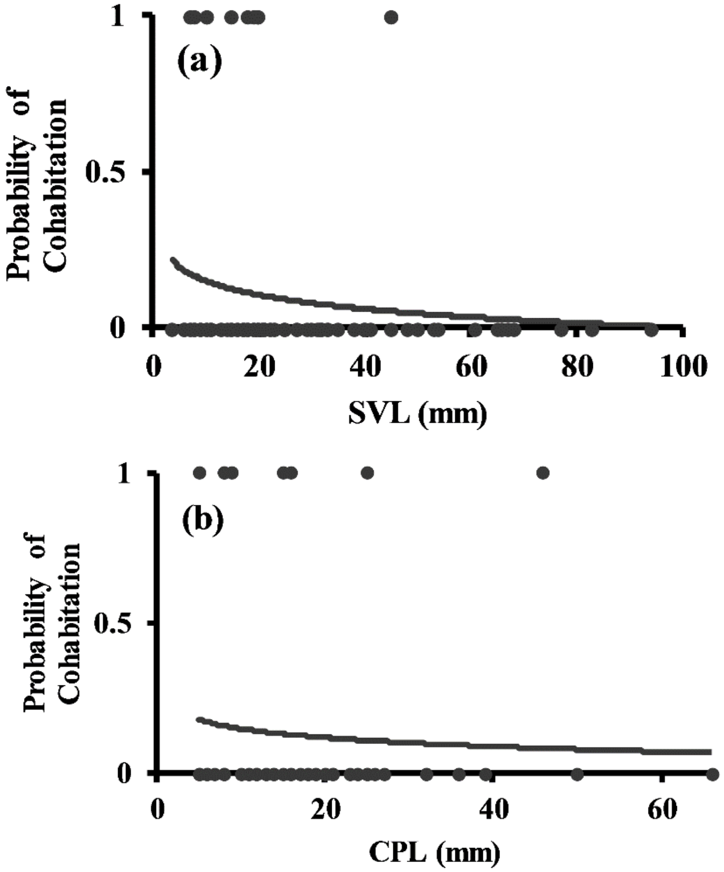

Figure 2-9. The effect of an individual's length (SVL or CPL) on (a) salamander and (b) crayfish probability of cohabitation during field surveys conducted among 4 streams in Caldwell County, NC between May 28 and July 7, 2019.

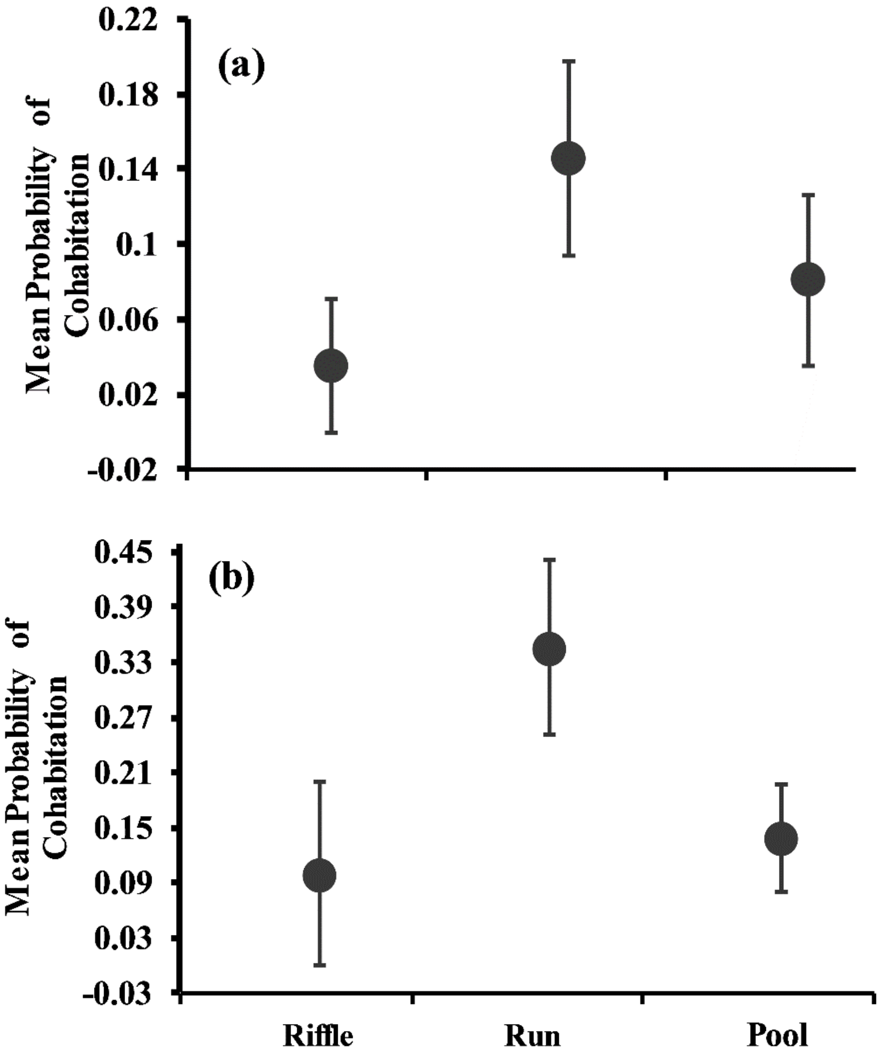

Figure 2-10. Differences in mean probability of interspecific cohabitation for (a) salamander and (b) crayfish across macro-habitat types (riffle, run, and pool) during field surveys conducted among 4 streams in Caldwell County, NC between May 28 and July 7, 2019.

VITA

Susan Lee Cragg was born in Annapolis, Maryland to Philip Cragg and Dorothy Kallman. She graduated from East Bay Arts High School in Hayward, California in June 2010. In the fall of 2011, she attended Paul Smith’s College in upstate New York to study Wildlife Biology. Ms. Cragg transferred to Iowa State University in fall 2014 and was awarded the Bachelor of Science degree in Biology in fall 2015. Immediately following graduation, Ms. Cragg was employed as a biological technician until she accepted a graduate teaching assistantship in Biology at Appalachian State University in 2018. She was awarded a Master of Science degree in 2020.

Ms. Cragg resides in Boone, North Carolina, where she spends her free time searching for salamanders and painting the local fauna that fascinate her.