Role of abiotic factors and habitat heterogeneity in the interactions between stream salamanders and crayfish in the southern Appalachians

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Abstract: Species interactions are important for determining the biological organization of natural communities. Presumably, as interacting organisms become more similar in resource niches, the magnitude of competition strength increases. However, the strength of interactions can be context dependent and mitigated by abiotic factors. In western North Carolina, headwater-stream salamanders and crayfish coexist across broad and fine spatial scales throughout their ranges. These dissimilar taxa occupy similar ecological niches within streams, yet there is limited understanding of the role that species interactions play in their local coexistence at fine spatial scales. We used both in-situ and ex-situ methods to evaluate the mechanisms promoting coexistence between stream salamanders (*Desmognathus quadramaculatus* [Holbrook, 1840] and *Desmognathus marmoratus* [Moore, 1899]) and Appalachian Brook Crayfish (*Cambarus bartonii* [Fabricius, 1798]). We conducted field surveys and documented factors potentially associated with refuge cohabitation between crayfish and salamanders within natural streams. We also used a stream mesocosm experiment to test if refuge density and competitor identity affected salamander and crayfish growth and behavior. In our in-situ approach, we found low interspecific cohabitation among desmognathan salamanders and *C. bartonii* with no detected effects of abiotic or biotic factors. Similarly, in our ex-situ experiment, we found that neither refuge density nor the presence of hetero- or conspecifics influenced the frequency of cohabitation and refuge use, growth, and mortality of *D. quadramaculatus* and *C. bartonii*. Although it is possible that local adaptation facilitates coexistence between our focal species, it is more likely that other abiotic (e.g., flow or temperature) and biotic factors (e.g., predators and other community members) in headwater streams besides the presence or absence of our focal taxa affect their distributions, or their interactions are size structured. These results support existing concepts that distantly related species are less likely to compete because of their morphological and phylogenetic dissimilarities, but future evaluations of interactions through time, space, and ontogeny would be useful to fully understand how these 2 taxa interact in headwater streams.

Key words: headwaters, refuge, competition, Desmognathus, Cambarus

Competitive species interactions are important for organizing communities, but the strength and outcome of interactions are highly variable. Variability is introduced through the type of interaction, ranging from predation to interference or exploitative competition, as well as phenotypes (e.g., body size, jaw size, appendage types). Presumably, as interacting organisms become more similar, the magnitude of competition strength should increase and result in alpha selection for diverging phenotypes (Aarssen 1983, Abrams 1983). This is a well-studied phenomenon in a variety of organisms across major taxonomic divisions (Gatz 1979, Schoener and Spiller 1987, Morin et al. 1988, Davenport and Riley 2017). Less understood is how organisms with widely divergent phenotypes interact (Violle et al. 2011). Though exploitative competition has been documented between organisms as divergent as plants and fish (Davenport and Riley 2017), the adaptive outcomes or effects on species ecology have received far less study.

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Highly dynamic stream systems have communities that can be strongly structured by abiotic factors (Power et al. 1988), yet biotic interactions can influence the habitat selection, distribution, and success of community members (Creed 2006, Fortino and Creed 2007, Cecala et al. 2020). Within headwater streams, many distantly related species occupy similar ecological niches (e.g., use of stream cover objects and shared food resources) with common life-history requirements and adaptations to dynamic habitats (Connell 1980, Davic and Welsh 2004). For example, both stream-breeding salamanders and headwater crayfish use stream cover objects to protect themselves from downstream displacement and predation and have overlapping diets (Resetarits 1991). Species with similar ecological niches, theoretically, should not coexist, and yet these taxa coexist throughout much of their ranges at both broad and fine scales (Abrams 1983). The few studies that have considered competitive interactions between salamanders and crayfish have yielded conflicting results about whether or how these species interact. Within native ranges, Appalachian Brook Crayfish (*Cambarus bartonii* [Fabricius, 1798]) had no effect on the survival or growth of a stream salamander (*Gyrinophilus porphyriticus* [Green, 1827]), yet when invasive, the Red Swamp Crayfish (*Procambarus clarkii* [Girard, 1852]) negatively affected habitat use by California Newts (*Taricha torosa* [Rathke, 1833]) (Gamradt and Kats 1996). Because stream salamanders and crayfish can be significant members of headwater-stream communities in the eastern United States, their interactions could help explain natural variation in their abundances throughout their ranges.

Adult salamanders and crayfish consume similar prey items and are known to be territorial, actively defending burrows and other features in space (Usio and Townsend 2004, Richmond and Lasenby 2006, Cruz and Rebelo 2007). Because in-stream refuges serve as sources of prey, protection from predators, and protection from high- and low-flow events, it is possible that in-stream refuge is the limiting resource governing interactions between these taxa (Benvenuto et al. 2008, Keitzer et al. 2013). Variation in abiotic (e.g., refuge availability or substrate size) and biotic factors (e.g., predators) influences their competitive interactions and can presumably weaken or strengthen their competitive relationship. However, at large size differences, a transition from competitive interactions to intraguild predation is likely between crayfish and stream salamanders. In both competitive and predatory interactions, we would expect each species to exhibit different microhabitat preferences, yet anecdotally, we have found them using the same refuge at fine spatial scales, suggesting that neither competitive nor predatory interaction strengths are large enough to drive habitat partitioning or differential habitat selection.

This study aimed to understand cohabiting behavior and interactions between co-occurring stream salamanders and crayfish and the biotic and abiotic factors affecting those interactions. We had 2 primary research objectives. First, we asked if the frequency of cohabitation, defined as an event where multiple individuals occupy the same refuge, is associated with macrohabitat and microhabitat characteristics of headwater streams. We predicted that 1) cohabitation rates between salamanders and crayfish would be positively associated with greater refuge size and 2) cohabitation patterns would be associated with mesoscale stream types (i.e., riffles and runs) that affect predation risk and refuge availability (i.e., increased predation risk with smaller refuges). Second, we investigated the competitive and predatory interactions between stream salamanders and crayfish. We predicted that growth, survival, and frequency of cohabiting a refuge would be lower in interspecific treatments relative to intraspecific treatments as a result of agonistic interactions.

METHODS

To understand the context, type, and strength of interactions between desmognathan salamanders and crayfish, we used both in-situ and ex-situ approaches. Our in-situ approach consisted of stream surveys to assess the cohabitation frequency between the large, aquatic desmognathan salamanders *Desmognathus quadramaculatus* (Holbrook, 1840) and *D. marmoratus* (Moore, 1899) and Appalachian Brook Crayfish (*C. bartonii*) and to determine whether cohabitation patterns are associated with macrohabitat and microhabitat characteristics of headwater streams. To test our predictions that cohabitation would be related to macro- and microhabitat, we used logistic regression modeling. Next, we performed an ex-situ stream mesocosm study to experimentally explore the type and strength of competition and predation interactions between *D. quadramaculatus* and *C. bartonii* and to determine whether those interactions were dependent on refuge availability. We characterized refuge use and interactions between experimental individuals, and we used mixed modeling to test our predictions that individuals would experience more positive outcomes in intraspecific than interspecific treatments.

In-situ field surveys

We conducted field surveys between 28 May and 7 July 2019 in Watauga County, North Carolina, USA. We established four 150-m reaches, 1 each at 4 sites, in the Watauga River watershed and sampled each 4× with a minimum of 8 d and a maximum of 22 d between sampling events. Following established protocols (Lowe et al. 2018), we standardized our effort across sites by haphazardly selecting 1 rock/m ($n = 150$) to sample during each survey. All rocks were ≥8 cm and were rotated parallel to the stream bed while a net was positioned downstream of the rock. We dipnetted or hand captured any salamander or crayfish that moved away from the disturbance. We identified all captured individuals to species and life stage based on expert assessment, and we measured their lengths (carapace length [CPL] or snout-to-vent length [SVL]). We removed all captured individuals from the stream until the entire site was surveyed and then released individuals at their specific point of capture. We placed all disturbed rocks back in their initial location.

We collected macrohabitat and microhabitat variables when an individual salamander or crayfish was present underneath a sampled rock. We categorized macrohabitats, defined as 1 m^2 around the rock's center, as either riffles, runs, or pools (referred to as channel unit type; Grant et al. 2009, Cecala et al. 2014, Lowe et al. 2018). Microhabitat variables included the widest diameter (cm) of the sampled rock and distance to the closest rock ≥8 cm (referred to as rock proximity). Microhabitat also included the proportions of different substrate types in the surrounding substrate within a 50-cm radius of the center of each flipped rock. 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 was assessed within the wetted boundaries of the entire sub-reach. The values for substrate proportions were correlated; therefore, we used the prcomp function in R (version 4.0.1; R Project for Statistical Computing, Vienna, Austria) to conduct principal component analyses (PCA) on the combined datasets to produce 2 statistically independent substrate variables for further statistical tests, which are described below.

For these analyses, we focused on captures of *C. bartonii* and 2 large and mostly aquatic desmognathans—*D. quadramaculatus* and *D. marmoratus*. We chose these desmognathans because of their similarity of appearance (they can be difficult to distinguish without genetic data; Jones and Weisrock 2018), unresolved phylogenetic relationships (Jones and Weisrock 2018), and probability of interacting with *C. bartonii.* We developed separate datasets for each species to characterize their refuge use. We developed separate logistic regression models (glm function) for salamanders and crayfish with cohabitation as the response variable and body size of the focal species, microhabitat variables (rock proximity, rock size, and 2 PCA axes for substrate), and macrohabitat (channel unit type) as fixed effects. We used a binomial distribution in the models. We used the *car* package (Fox and Weisberg 2019) in R to perform a Wald chi-squared test with a type-III analysis-of-variance table to determine if explanatory variables contributed to the variance explained by the model as a whole.

Ex-situ experiment

We used stream mesocosms to evaluate interactions between *D. quadramaculatus* and *C. bartonii* at the Tennessee Aquarium Conservation Institute in Chattanooga, Tennessee, USA. We conducted a 3 × 2 factorial experiment manipulating species identity of competitors (i.e., 3 levels of species pairs: 2 *D. quadramaculatus* [intraspecific], 2 *C. bartonii* [intraspecific], or 1 each of *D. quadramaculatus* and *C. bartonii* [interspecific]) and refuge density (i.e., 2 levels: 2 or 4 objects). Only adults were used of all species. Sex was not determined for salamanders, but all crayfish were males. We replicated the 6 treatments 4×, and the density of individuals within each replicate was constant at 2 individuals/mesocosm. Species pairs were size matched and randomly assigned to treatments. The experiment ran for 62 d between 21 December 2018 and 23 February 2019.

The 24 artificial streams were 68-L fiberglass mesocosms (1.20 × 0.60 × 0.53 m). Mesocosms had independent recirculating systems that contained the same substrate composition (3-cm-deep layer of sand followed by a single layer of gravel [0.3 × 1.5 cm]) to mimic natural stream conditions (Ennen et al. 2016, Liles et al. 2017, Hoffacker et al. 2018). We added dechlorinated water to the streams, with a depth of 11.5 cm between the substrate and water surface. We used pumping systems made from aquarium pumps, standpipes, and PVC tubing to keep water at a constant flow rate of 40.5 L/min. We covered the artificial streams with window screening secured with binder clips to prevent escape. Each stream received 2 or 4 artificial refuges, consisting of 15 × 15-cm semi-transparent plexiglass squares that were elevated from the substrate by bolts inserted through the corners (McNeely et al. 1990). We randomly placed each of the artificial refuges throughout the mesocosms. We provided all species pairs with equal quantities of prey items (8 live mayfly nymphs) every 15 d (Ennen et al. 2016, Liles et al. 2017, Hoffacker et al. 2018). We also added 3 leaf-litter pieces (12 × 12 mm) to all artificial streams during each feeding event for *C. bartonii* to supplement their diet with detritus (Huryn and Wallace 1987). We observed the specific locations of subjects, on average, every 3 d in the morning to evaluate refuge use and cohabitation.

All study organisms were collected from 6 streams within the Tellico River watershed in Monroe County, Tennessee. From 14 to 16 December 2018, we collected 24 *D. quadramaculatus* (SVL = 50 mm, range = 45–55 mm) and 24 *C. bartonii* (CPL = 35 mm, range = 30–40 mm). All individuals were collected outside the known range of *D. marmoratus*, according to recent phylogenetic analyses (Jones and Weisrock 2018), to ensure species identity of *D. quadramaculatus*. All study organisms were housed individually in plastic containers (1-L deli cups) at Tennessee Aquarium Conservation Institute until the experiment was initiated. We used a U-100 insulin syringe and 28-gauge needle to mark all experimental animals with visible implant elastomers (VIE) (Northwest Marine Technologies, Shaw Island, Washington, USA). Salamanders were marked ventrally above their hindlimbs, whereas crayfish were marked on their ventral abdominal tissue. We measured experimental animals for total length (in cm for size matching) and mass (g). Additionally, we measured SVL and CPL for *D. quadramaculatus* and *C. bartonii*, respectively. We used these measurements to estimate growth with the scaled mass index calculation (Peig and Green 2009, 2010, Davenport and Lowe 2016, 2018), which accounts for both changes in SVL/CPL and mass. Next, we calculated proportional change in growth (i.e., scaled mass) between the beginning and end of the experiment; hereafter, we refer to this variable as body condition. Other response variables, recorded as a binary data, included frequency of cohabitation, mortality, and refuge use. Refuge use was noted when most of the body was found under the refuge.

We used mixed-effects models with the *nlme* package (Pinheiro et al. 2020) in R to evaluate how refuge density and species identity (intraspecific or interspecific) affected outcomes of *D. quadramaculatus* and *C. bartonii*. We evaluated each species separately and included random effects accounting for stream mesocosm number and observation day. We used linear mixed-effects models to evaluate body condition, and we used a mixed-effects logistic regression with a logit link function to evaluate frequency of cohabitation, mortality, and refuge use.

RESULTS

In-situ field surveys

We used a PCA to reduce the dimensionality of the substrate types. The first 2 PCA axes explained 71.8% of the variance in the substrate datasets (Table 1). The 1^{st} PCA axis represented a rock-size gradient. This axis was positively associated with cobble and negatively associated with boulders. The 2^{nd} PCA axis represented a sediment-size gradient. This axis was positively associated with larger rocks (i.e., cobble and boulders) and negatively associated with sand.

Salamanders and crayfish infrequently cohabited the same refuges in field surveys. Of the 2400 rocks rotated across 4 streams, we recorded 117 rocks that were occupied by at least 1 salamander, and only 11 rocks were occupied by both a salamander and crayfish. The probability of interspecific cohabitation for salamanders with crayfish was not dependent on any microhabitat variable, macrohabitat, or body size (Table S1). The mean SVL of salamanders cohabitating with crayfish was 17.4 mm (range = 7–45 mm), and the mean SVL of salamanders not cohabitating with crayfish was 25.8 mm (range = 6–94 mm). We recorded 74 rocks occupied by at least 1 crayfish, and of those rocks, only 10 had both a salamander and crayfish. The probability of interspecific cohabitation for crayfish with salamanders was also not influenced by microhabitat, macrohabitat, or body size (Table S1). The mean CPL of crayfish cohabitating rocks with salamanders was 16.5 mm (range = 5–46 mm), and the mean CPL of crayfish not cohabitating with salamanders was 17.5 mm (range = 5–66 mm). Totals include all size classes of individuals for each focal species (*C. bartonii*, *D. quadramaculatus*, and *D. marmoratus*). The number of cohabitations for salamanders (11) vs crayfish (10) differs because 1 rock had 2 salamanders cohabitating with 1 crayfish.

Table 1. Loading scores from a principal component (PC) analysis on the 6 sediment-type microhabitat variables in our dataset. Percentages represent the variance explained by each axis (PC1 and PC2).

Substrate	PC1 (40.8%)	PC2 (31.0%)
Silt	0.04	−0.12
Sand	0.12	−0.75
Gravel	0.09	0.08
Cobble	0.58	0.56
Boulder	−0.80	0.31
Bedrock	−0.04	−0.08

Ex-situ experiment

Contrary to our predictions, our mixed-modeling results showed little to no association of mortality, refuge use, and body condition with competitor identity, refuge density, or their interaction for either species. Mortality, refuge use, and body condition of *D. quadramaculatus* were not affected by species identity, refuge density, or the interaction term (Tables 2, S1. Salamander mortality was independent of refuge density and species identity (salamander–crayfish, low refuge: $n = 1$ replicate with mortality; salamander–crayfish, high refuge: $n = 1$ replicate with mortality; salamander–salamander, low refuge: $n = 0$ replicates with mortality; salamander–salamander, high refuge: $n = 1$ replicate with mortality; Table S1). Body condition of *C. bartonii* was not affected by species identity, refuge density, or the interaction term (Table S2). Refuge density was marginally associated with the frequency of refuge use for *C. bartonii* ($F = 3.125$, df = 1, $p = 0.079$). The mean frequency of refuge use was 1.3× greater in high refuge density (mean ± 1 standard error; 0.60 ± 0.08) than in the low density treatments (0.46 ± 0.08). Neither species identity nor the interaction term for this model was an important driver of refuge use (Table S1). Because of 100% survival and individuals never leaving water, we did not analyze mortality for crayfish.

Salamanders and crayfish did not frequently use the same refuges in our experiment. On average, salamanders and crayfish were found together under refuges 10.7 ± 2.9% ($n = 4$ total cohabitations out of 38 observations across 3 replicates) of the time in low refuge-density treatments and 3.3 ± 0.3% ($n = 1$ total cohabitation out of 31 observations across 3 replicates) of the time in high refuge-density treatments. Salamanders cohabitated with other salamanders on average 2 ± 2.4% ($n = 2$ total cohabitations out of 98 observations across 4 replicates) of the time in low refuge-density treatments and 3.1 ± 3.6% ($n = 2$ total cohabitations out of 87 observations across 4 replicates) of the time in high refuge-density treatments. Crayfish never cohabitated with another crayfish during any observation across all replicates and refuge treatments.

DISCUSSION

Our study is one of the 1^{st} to use both in-situ and ex-situ approaches to examine potential agonistic interactions between salamanders and crayfish. Using this combined approach, we evaluated the potential abiotic and biotic factors driving the microhabitat use and spatial distributions

Table 2. Mean responses of salamanders (*Desmognathus quadramaculatus*) and crayfish (*Cambarus bartonii*) to refuge density and species identity in artificial stream experiments. Body condition represents the change in body condition (differences in scaled mass derived from length and mass measurements). Mortality represents changes in individual survival only for salamanders because no crayfish died during the experiment. Cohabitation indicates the mean frequency of organisms sharing the same refuge with another individual. Species identity indicates whether subjects in a treatment were of the same species (intraspecific) or of different species (interspecific). All values are means ± 1 standard error. Crayfish did not cohabit with one another.

		Refuge density		Species identity	
Taxon	Dependent variable	High	Low	Intraspecific	Interspecific
Salamander	Body condition	0.58 ± 0.13	0.59 ± 0.12	0.59 ± 0.10	0.58 ± 0.14
Salamander	Mortality	0.19 ± 0.12	0.13 ± 0.12	0.06 ± 0.11	0.25 ± 0.13
Salamander	Refuge use	0.31 ± 0.08	0.32 ± 0.07	0.28 ± 0.07	0.35 ± 0.08
Salamander	Cohabitation	0.01 ± 0.21	0.05 ± 0.02	0.02 ± 0.02	0.04 ± 0.02
Crayfish	Body condition	−0.09 ± 0.26	0.36 ± 0.28	0.15 ± 0.26	0.12 ± 0.28
Crayfish	Refuge use	0.60 ± 0.08	0.46 ± 0.08	0.49 ± 0.07	0.57 ± 0.09

of salamanders and crayfish in natural stream systems while also testing for the effects of specific variables in a controlled experimental setting. Our results indicate that, despite having overlapping resource requirements and numerical dominance in headwater streams, competition for spatial refuges between larger species of *Desmognathus* and *C. bartonii* is weak. This finding supports previous studies proposing that distantly related species are less likely to compete because of local adaptation and morphological differences that minimize competition (Case and Gilpin 1974, Gatz 1979, Connell 1980). Specifically, the claws of crayfish can be used to construct burrows or chimneys outside of stream channels to reduce cohabitation (Guiasu 1997, Hale et al. 2016). This morphological adaptation (i.e., claws) would allow crayfish to exploit alternative refuges while also defending them from intruders (Guiasu 1997, Hale et al. 2016). Because of 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.

We expected that, although crayfish and salamanders are distantly related, there could be significant overlap in resource usage (e.g., prey and refuges) and potentially strong competitive interactions. However, we did not find evidence for agonistic interactions. Because both *D. quadramaculatus* and *C. bartonii* are native to southern Appalachian headwater streams, it is likely that these 2 species coexist through evolutionary adaptations (e.g., behavioral and morphological) that reduce the costs associated with competition. The lack of change in body condition for both of our focal species is consistent with the results of Resetarits (1991), which concluded that the presence of *C. bartonii* did not influence the growth of 2 salamander species (*G. porphyriticus* and *Eurycea bislineata* [Green, 1818]) and salamander presence did not affect crayfish growth. We observed no differences in salamander habitat use or wounds when in the presence of a native crayfish, suggesting that either *C. bartonii* is a less aggressive species than the salamanders or that *Desmognathus* spp. are able to avoid aggressions (e.g., by leaving aquatic environments).

We found very low rates of cohabitation between desmognathan salamanders and *C. bartonii* in the wild, which was similar to our experimental results. Our results from the field component indicate that neither biotic nor abiotic factors influenced the probability of cohabitation for stream salamanders and crayfish. It is possible that cohabitation rates will vary based on focal species. For example, some studies have implicated a slightly positive association between finer substrates and stream salamander abundance (Barr and Babbitt 2002, Keitzer and Goforth 2012), but this work was not done with *D. quadramaculatus*. It is also possible that the streams used in our study consisted of high enough proportions of larger rocks (i.e., cobble and boulder) that refugia were not a limiting factor. Another possible reason for our results could be lack of variability in abiotic factors (e.g., flow variability, water depth, and temperature) over the 1-y period of our in-situ surveys. Occupancy of stream salamanders is known to be dependent on multiple abiotic and biotic factors and the spatial extent of the study (Cecala et al. 2018). Thus, future studies should consider incorporating stream sites that have greater variation in habitat characteristics over longer time scales to evaluate whether greater variation influences interspecific cohabitation rates.

Because of the dynamic nature of headwater streams, abiotic factors have been hypothesized to be responsible for structuring communities and interactions therein. Our findings indicate that abiotic factors, when measured over the course of a single field season, may not predict cohabitation of some species pairs. Moreover, our results also suggest that stream species have likely adapted to this dynamic environment to minimize similar resource use and avoid agonistic interactions. At least with our focal species, the divergent phenotypes of crayfish and stream salamanders may preclude competitive interactions despite similar resource use. Even when forced to cohabitate, we detected no negative consequences in our ex-situ experiment. Therefore, additional abiotic factors measured in situ over longer periods along with tests of interactions with additional community members (e.g., larger crayfish species, smaller salamander species and individuals, and fish) may reveal the causal mechanisms responsible for shaping headwater communities.

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