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Microhabitat Conditions Influence Mesohabitat Associations and Distribution of Larval Salamanders in Headwater Streams

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Microhabitat conditions influence mesohabitat associations and distribution of larval salamanders in headwater streams

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Abstract Distribution patterns of stream biota are the result of complex interactions between individuals and their surrounding environment. Determining the spatial scale by which an organism is most influenced is paramount to understanding distribution patterns. Using a multi-scale approach, we investigated factors influencing habitat associations of larval *Ambystoma barbouri* (streamside salamander) and *Eurycea cirrigera* (southern two-lined salamander) in three Kentucky headwater streams. We used likelihood ratio *G* tests to identify associations between species and mesohabitat (i.e., runs, riffles, and pools), and we used microhabitat variables to predict the presence and abundance of salamanders via a priori multiple regression modeling. *Ambystoma barbouri* presence and abundance were influenced by conditions at micro-scales, which in turn dictated mesohabitat associations. *Eurycea cirrigera* were also influenced by microhabitat variables, but displayed associations to *A. barbouri* presence in late spring. Associations of larval salamanders to mesohabitat and microhabitat parameters shifted from early to late spring, likely in response to changes in developmental stage. The multi-scale approach of our study improved our understanding of complex relationships between larval salamanders and their surrounding environment in headwaters, and underscored the importance of (1) research investigating multiple spatial and temporal scales and (2) heterogeneous in-stream habitat to headwater biota.

Keywords Distribution · Headwater streams · Mesohabitat associations · Multi-scale research · Salamanders

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Introduction

The distribution of stream organisms is a result of complex interactions among factors operating at multiple spatial and temporal scales (Frissell et al., 1986; Vanni, 2002). Multi-scale drivers of distribution and abundance include direct and indirect effects of consumers (Vanni, 2002; McIntosh et al., 2004),

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spatiotemporal shifts in habitat availability and suitability (Torgersen et al., 1999; Smith & Grossman, 2003), and interactions of abiotic and biotic factors (Doi & Katano, 2008; McIntyre et al., 2008). Habitat within streams varies across spatial scales and processes at each scale interact to influence habitat characteristics (Frissell et al., 1986). Additionally, spatial heterogeneity shifts seasonally (Frissell et al., 1986). Thus, multi-scale approaches should be employed when investigating habitat associations and distribution patterns of stream organisms (Torgersen et al., 1999; Doi & Katano, 2008; Keitzer & Goforth, 2013).

In headwater streams lacking fishes, aquatic salamander larvae are often the dominant vertebrate predator (Davic & Welsh, 2004; McIntosh et al., 2004), but they also have important non-consumptive effects (reviewed in Wells, 2007). For example, salamanders play an important role in nutrient recycling in stream systems (Milanovich, 2010; Keitzer & Goforth, 2013; Munshaw et al., 2013). Therefore, stream salamanders are vital components of headwater ecosystems.

Distribution patterns of larval salamanders are subject to temporal shifts as a result of complex interactions with their environment (Smith & Grossman, 2003), syntopic species (Gustafson, 1994), or combinations of both abiotic and biotic factors (Barr & Babbitt, 2002). Individual body size also influences habitat associations (Lowe, 2005; Martin et al., 2012), and therefore contributes to how species are distributed across space and ontogeny. Thus, understanding habitat associations of larval salamanders is important to stream ecosystem dynamics.

Our objectives were to determine the effect of stream characteristics on the distribution of two common salamander species in our study region, *Ambystoma barbouri* (streamside salamander) and *Eurycea cirrigera* (southern two-lined salamander) across meso-scales (runs, riffles, and pools) and micro-scales (within 0.25-m^2 area). Larval *A. barbouri* have displayed negative associations to riffle mesohabitat, likely as a passive response to high-velocity turbulent stream flow (Petranka, 1984a; Holomuzki, 1991), and *E. cirrigera* larvae are generally found in slow-moving areas (Petranka, 1998). Petranka (1984a) suggested that because of lack of mobility, newly hatched *A. barbouri* individuals were displaced from high-velocity areas to low velocity, depositional habitats. Older *A. barbouri* and *E. cirrigera* are less likely to be displaced downstream (Bruce, 1986; Petranka et al., 1987). Larval *A. barbouri* typically do not utilize substrate cover when fish predators are absent (Sih et al., 1992) yet larval *E. cirrigera* use substrate diurnally (Petranka, 1984b). Studies on other aquatic organisms have shown habitat variables at meso-scales to be the most important predictors of distribution (i.e., Torgerson et al., 1999; Rabeni et al., 2002; Doi & Katano, 2008). Our study organisms are syntopic species that utilize similar mesohabitat yet differ in microhabitat use; therefore, we measured abiotic factors at meso- and micro-scales that could potentially influence their abundance and distribution at watershed scales. We sampled in early and late spring, which allowed us to determine if any ontogenetic shifts occurred in these relationships from early to late stages of aquatic development.

Materials and methods

Study site

We studied distributions of *E. cirrigera* and *A. barbouri* in three fishless headwater streams within the Inner Bluegrass Ecoregion of central Kentucky (Woods et al., 2002). While some of the headwaters are exposed to residential and pastureland areas, our study areas were surrounded by extended forest buffer (>100 m), which should be wide enough to support the core habitat requirements of amphibian species in the area (Semlitsch & Bodie, 2003) and likely remediate most negative effects of these landscape disturbances (Naiman & Décamps, 1997). Within our study streams, variable gradients, water velocities, channel widths, depths, bank slopes, and substrate result in a heterogeneous habitat that supports populations of *A. barbouri* (Storfer, 1999) and *E. cirrigera* (Petranka, 1984b). Larval *A. barbouri* in central Kentucky hatch in early spring and metamorphose after approximately 6–10 weeks (Petranka, 1984c; Petranka and Sih, 1986). Larval periods of *E. cirrigera* vary regionally (Petranka, 1998), but in Kentucky individuals metamorphose after 1–3 years of development (Barbour, 1971; Petranka, 1984b). In early spring, newly hatched *A. barbouri* and older *E. cirrigera* (hereafter second-year larvae) are both present in our study streams. Second-year *E. cirrigera*

larvae prey on *A. barbouri* as they hatch, but as *A. barbouri* larvae grow larger, they are no longer suitable prey for *E. cirrigera* (Petranka, 1984b). In late spring, recently hatched cohorts of *E. cirrigera* are present in the stream (hereafter first-year larvae) along with both second-year *E. cirrigera* larvae and *A. barbouri* that are approaching metamorphosis. The separate ontogenies of both species within our study timeframe (early to late spring) allowed us to investigate differences between habitat associations of both species across their aquatic life stages.

Sampling design

Within each of the study streams, we randomly selected a 100-m reach within the longest stretch of suitable habitat. We defined suitable habitat as areas of stream length that had substantial forest buffer, presence of multiple mesohabitat types, and no evidence of fishes. We sampled each 100-m reach twice in spring of 2012 (13–15 April and 18–20 May; hereafter referred to as early spring and late spring sampling events, respectively). Every 3 m, we established a 1-m-wide transect across the stream. Within each transect we arranged three 0.25-m^2 sampling plots. One plot bordered the left shoreline, one was in the midpoint of the stream channel, and one bordered the right shoreline. We overturned each substrate item within the 0.25-m^2 sampling plot and counted individuals, including substrate in contact with the border of the plot. To reveal population distributions and species coexistence within the stream, we documented the location of each observation as an x, y coordinate along the stream channel. We measured individuals after each capture to the nearest millimeter of total body length (TL), but we only attempted capture when it would not displace nearby larvae. Mean lengths were compared between mesohabitat type using one-way analysis of variance (ANOVA).

In each study stream, we mapped the 100-m reach according to mesohabitat type—run, riffle, or pool in early spring (Table 1). We modified the mesohabitat definitions of Montgomery and Buffington (1997) to apply to headwaters in central Kentucky primarily composed of bedrock, and characterizations of mesohabitat were based on geomorphology as well as hydrology. We incorporated geomorphology in order to strengthen the potential predictive power of our results during non-typical weather years. Runs generally had laminar flow and low gradients and were either dominated by limestone bedrock or composed of a variety of substrate. Riffles were characterized by relatively turbulent flow, moderate to low gradients, and a variety of substrate that caused non-laminar flow including undulating bedrock or multiple vertical incisions in bedrock. Pools had laminar flow and low gradients but were differentiated from runs based on slower water velocity caused by an obstruction in the stream channel or an abrupt incision in the stream bed. We represented natural availability of mesohabitat types by percentage of total sampling reach area, and we used these percentages to calculate the expected frequencies of captures within each mesohabitat type. To uncover mesohabitat associations of *A. barbouri* and *E. cirrigera*, we compared observed frequencies of captures within each mesohabitat type to expected frequencies using likelihood ratio *G* tests (Sokal & Rohlf, 1995; Lowe, 2005). We calculated likelihood ratio *G* values for each site from each sampling event and graphically represented overall habitat associations by combining sites as independent replicates.

Within each sampling transect, we randomly designated a 0.25-m^2 sampling plot for microhabitat sampling. Within each 0.25 m^2 plot, we visually estimated embeddedness as a percentage of total substrate area covered in fine sediment, debris and vegetative cover as the percent cover of the total surface area in the sampling plot, and percent cover of substrate for the following categories: pebble (<64 mm), cobble (64–256 mm), boulder (>256 mm with visible edges), and bedrock (>256 mm with no visible edges) (Bain, 1999). We noted the size class of substrate item under which an individual was captured (or reported as exposed if under no cover). We also characterized the microcondition (micro-pool, micro-run, or micro-riffle) within each plot. Microcondition only refers to habitat within the 0.25-m^2 sampling plot and was determined using the same criteria used to indentify mesohabitat types. It is a comprehensive, qualitative metric that attempts to incorporate variables difficult to quantify in first-order streams (e.g., water velocity). This was a novel measurement and it allowed us to empirically evaluate distinct microhabitats within a dominant mesohabitat.

Statistical analyses

We used Pearson's correlations to determine multicollinearity among predictive variables of salamander

Table 1 Percentages of each mesohabitat and microcondition in each study stream throughout our study of habitat associations of *Ambystoma barbouri* and *Eurycea cirrigera* (13–15 April and 18–20 May 2012)

Sampling session	Scale	Stream	Pool (%)	Run (%)	Riffle (%)
Early spring	Mesohabitat	1	16.82	20.59	62.59
		2	12.02	47.09	40.89
		3	6.09	36.02	57.89
	Microcondition	1	28.57	33.33	38.10
		2	57.89	26.32	15.79
		3	6.67	46.67	46.67
Late spring	Mesohabitat	1	16.82	20.59	62.59
		2	12.02	47.09	40.89
		3	6.09	36.02	57.89
	Microcondition	1	47.06	23.53	29.42
		2	70.59	17.65	11.76
		3	41.67	41.67	16.67

Percent mesohabitat represents the entire sampling area and microcondition represents only those samples we included in analyses

presence and abundance. If variables were correlated in either sampling session, we removed them from all analyses. Models used to predict response of *A. barbouri* in early spring were used in late spring as well. The similarity in candidate models promoted comparability between sampling sessions. Percent cobble and pebble cover were removed because they were highly correlated with percent bedrock cover. Location of transects along the stream sampling area was highly correlated with study stream and was removed. We could not compute detection probabilities because stream drying prevented a third sampling session. Therefore, implicit biases may be present in our data; however, we are confident that our detection rate was high because of the behavior of our study species, our plot sampling method, and the water clarity throughout our study streams.

We used multiple regression analyses to predict the presence and abundance of individuals in the 0.25-m^2 sampling plots. We excluded dry sampling plots from analyses because larval stages of *A. barbouri* and *E. cirrigera* are strictly aquatic. For all analyses, we performed model selection using second-order Akaike's Information Criterion (AIC_c) in R version 2.15.1 (R Development Core Team, Vienna, Austria) using the 'AICcmodavg' package (Burnham & Anderson, 2002; Mazerolle, 2013). We used logistic distribution models to predict the presence. We initially tested the assumptions of Poisson distribution on each of our abundance model sets. If Poisson distribution did not fit global models, we applied a negative binomial distribution. The estimated theta values of negative binomial distributed global models were used across all candidate models in the same model set (Mazerolle, 2013). We constructed candidate models a priori that evaluated combinations of stream and environmental factors at different spatial scales. Models for both species were based on several criteria: (1) measures of substrate complexity, (2) measures of habitat conditions, (3) habitat types at different scales, (4) sampling location, (5) spatial restrictions, and (6) combinations of these criteria (Appendix A—Supplementary Material). For each species, a single set of candidate models was used to predict the presence and abundance.

Some variables in top models can confound results, especially in the case of more than one model having $AIC_c < 2$ and only differing by one variable. Arnold (2010) reported that this is common in wildlife literature and that statistically competitive models are often erroneously considered biologically relevant. As a solution for potential uncertainty in making inferences from top models, we used model averaging to conduct multi-model inference (Burnham & Anderson, 2002). We used regression coefficients (β) and confidence intervals to represent effect sizes of continuous independent variables for both logistic and multiple regression analyses, and we interpreted values as the relative contribution of each variable to the response. We determined the effect sizes of categorical variables via dummy coding and interpreted values relative to a reference category (i.e., effect of micro-riffles on abundance compared to effect of micro-pools on abundance). We computed odds ratios of parameters from logistic regression by exponentiating the estimated β value. In the case of

categorical variables such as microcondition, we compared the odds between an individual occurring in one microhabitat type versus another. We used confidence intervals of 85% for parameter estimates. Arnold (2010) argued that using 85% confidence intervals when interpreting effects of AIC parameter estimates promotes compatibility between the information-theoretic approach and statistical inference. If 85% confidence intervals included zero, we interpreted the variable as having no effect on the response.

Results

We observed 672 *A. barbouri* larvae (pools = 137, runs = 369, riffles = 166) and 160 *E. cirrigera* larvae (pools = 11, runs = 75, riffles = 75) across all sites during this study. In early spring, we observed 453 *A. barbouri* (observed: pools = 120, runs = 214, and riffles = 119; expected: pools = 46, runs = 168, and riffles = 237), with densities reaching 90 individuals/m^2 ($\mu \pm 1$ SE = 8.11 ± 0.94). We captured and measured 274 *A. barbouri* in early spring ($\mu \pm 1$ SE = 18.52 ± 0.22 mm). We observed 17 second-year *E. cirrigera* (observed: pools = 6, runs = 5, and riffles = 6; expected: pools = 1, runs = 5, and riffles = 9) and captured and measured 12 individuals ($\mu \pm 1$ SE = 48.17 ± 1.93 mm). Due to low sample size, interpretations of *E. cirrigera* results for this sampling period were limited, but *A. barbouri* displayed clumped distribution throughout each sampling reach (Fig. 1). In late spring, we observed 219 *A. barbouri* (observed: pools = 17, runs = 155, and riffles = 47; expected: pools = 25, runs = 75, and riffles = 117), 110 first-year *E. cirrigera* (observed: pools = 5, runs = 70, and riffles = 69; expected: pools = 16, runs = 49, and riffles = 77), and 34 second-year *E. cirrigera* (observed: pools = 1, runs = 18, and riffles = 15; expected: pools = 4, runs = 11, and riffles = 17). Densities of *A. barbouri* reached 60 individuals/m^2 ($\mu \pm 1$ SE = 3.91 ± 0.54), and density of *E. cirrigera* reached 40 individuals/m^2 ($\mu = 2.52 \pm 0.38$ SE). We captured and measured 70 *A. barbouri* ($\mu \pm 1$ SE = 30.34 ± 1.05 mm) and 27 *E. cirrigera* (first-year larvae, $n = 19$, $\mu \pm 1$ SE = 17.33 ± 0.63 mm; second-year larvae, $n = 6$, $\mu \pm 1$ SE = 44.67 ± 4.03 mm). Both species displayed clumped spatial distribution and often shared the same habitat space (Fig. 2). Mean length of *A. barbouri* did not differ between mesohabitats in early or late spring (early spring ANOVA $P = 0.21$, late spring ANOVA $P = 0.19$). Mean length of *E. cirrigera* did not differ in early spring (ANOVA $P = 0.86$), and although low sample size restricted statistical analysis, mean length of first-year *E. cirrigera* was relatively similar between mesohabitats in late spring (pools: $\mu \pm 1$ SE = 17.50 ± 2.50 mm, runs: $\mu \pm 1$ SE = 18.57 ± 1.72 mm, and riffles: $\mu \pm 1$ SE = 16.56 ± 0.56 mm).

Habitat associations: early spring

Ambystoma barbouri displayed positive associations to runs and pools and a negative association to riffles (Fig. 3a). Observed frequencies of individuals within each mesohabitat type were not equal to expected frequencies based on natural availability (Site 1: $G = 9.91$, df = 2, $P = 0.007$; Site 2: $G = 135.00$, df = 2, $P < 0.0001$; and Site 3: $G = 73.73$, df = 2, $P < 0.0001$). *Eurycea cirrigera* were positively associated with pools (Fig. 3b), and observed proportions were different than expected at stream 2 ($G = 8.48$, df = 2, $P = 0.01$).

Percent bedrock, percent boulder, and microcondition best predicted the presence of *A. barbouri* (Table 2). The weight of the top model was less than 0.90; therefore, we performed model averaging on top predictive variables. An increase in 1% bedrock predicted an individual to be approximately $1.03x$ more likely to be present than absent (Table 3; Fig. 4). Bedrock cover was greatest in runs and likely had an influence on positive associations of individuals to this habitat (Fig. 5). *Ambystoma barbouri* abundance in early spring was best predicted by percent bedrock, percent boulder, and microcondition (Table 2). Micro-riffles had the greatest effect on abundance, with approximately 2.0 fewer individuals per sample predicted to be present in micro-riffles compared to micro-pools. The difference in effect of micro-runs compared to micro-pools was not different from zero. Compared to meso-scale riffles, both pools and runs contained fewer micro-riffles (Fig. 6). Sampling site also contributed considerably to *A. barbouri* abundance. Sample size of *E. cirrigera* precluded regression analysis.

Habitat associations: late spring

In late spring, *A. barbouri* displayed a strong positive association with runs and a strong negative association

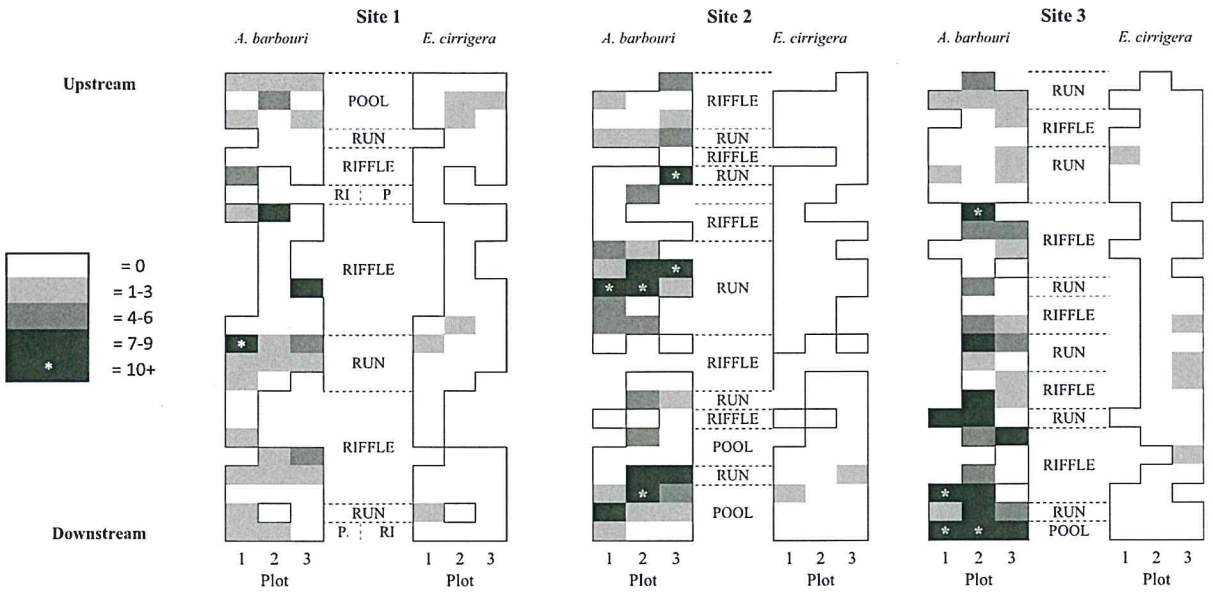

Fig. 1 Graphical representation of distribution patterns of *A. barbouri* and *E. cirrigera* during early spring (13–15 April 2012). *Solid outlines* represent areas of the stream that held water. *Shaded areas* represent number of observations in a sampling plot. *Dotted lines* represent boundaries between mesohabitat types within streams

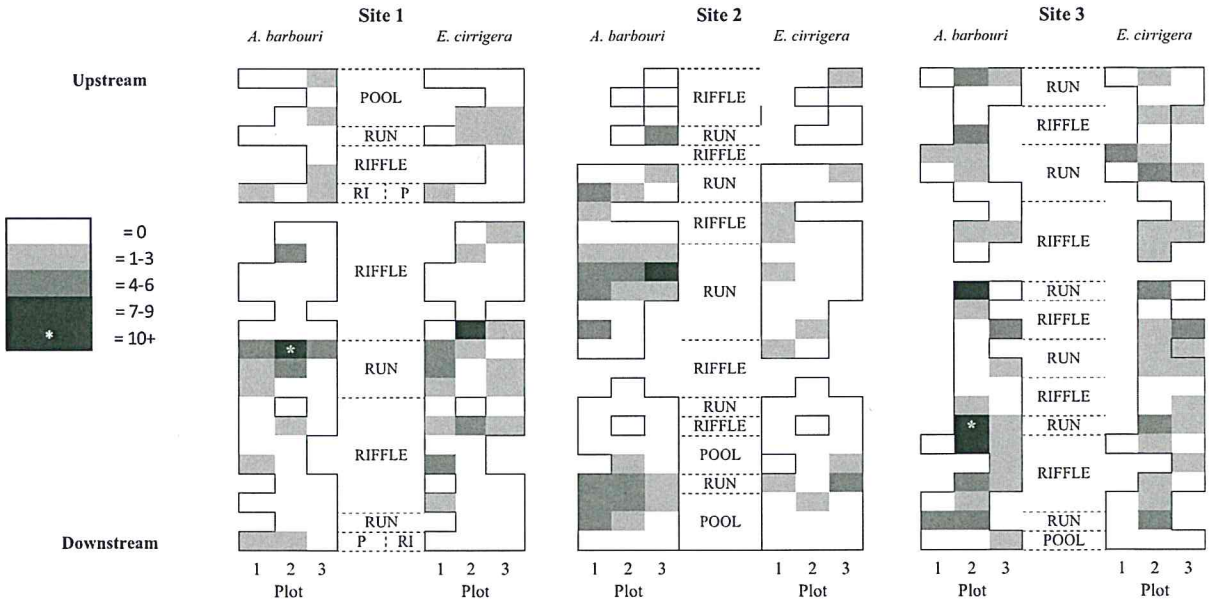

Fig. 2 Graphical representation of distribution patterns of *A. barbouri* and *E. cirrigera* during late spring (18–20 May 2012). *Solid outlines* represent areas of the stream that held water. *Shaded areas* represent number of observations in a sampling plot. *Dotted lines* represent boundaries between mesohabitat types within streams

with riffles and had no associations to pools (Fig. 3a). Observed frequencies of individual *A. barbouri* within each mesohabitat type were different than expected frequencies (site 1: $G = 33.85$, df $= 2$, $P < 0.0001$; site 2: $G = 105.42$, df $= 2$, $P < 0.0001$; and site 3: $G = 11.72$, df $= 2$, $P = 0.004$).

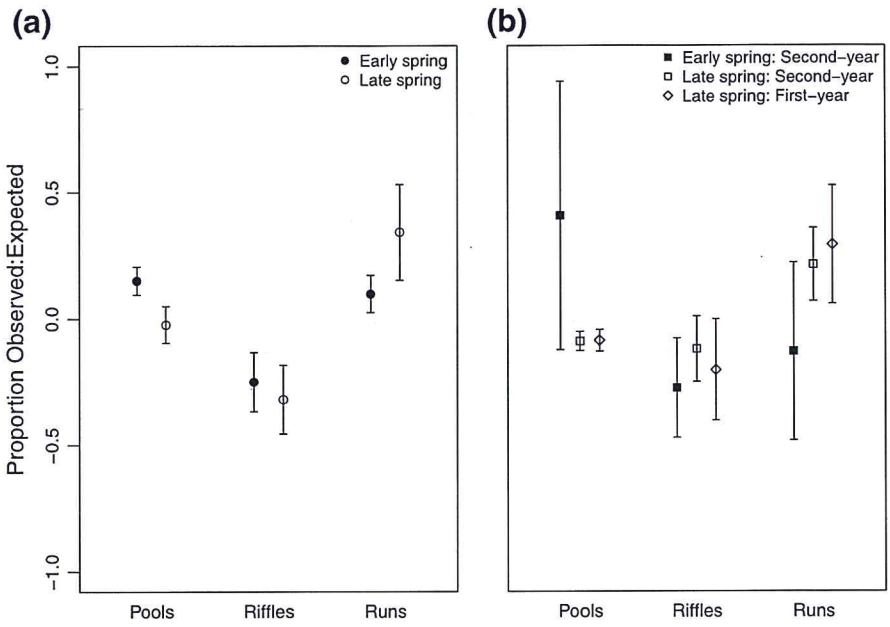

Fig. 3 Deviations of observed proportions of **a** *Ambystoma barbouri* and **b** *Eurycea cirrigera* from expected proportions in each mesohabitat in early (13–15 April) and late spring (18–20 May), 2012. *Error bars* represent 95% confidence intervals

Multiple predictive models of *A. barbouri* presence held similar weight (Table 2). Depth had positive effects on *A. barbouri* presence, with an increase in 1 cm of depth predicting an individual to be 1.5*x* more likely to be present than absent (Fig. 7; Table 3). The best model predicting abundance of *A. barbouri* was depth and microcondition (Table 2). An increase in approximately 5 cm in depth was predicted to result in the increase in 1.0 *A. barbouri* individual (Table 3). Micro-runs were predicted to contain approximately 1.0 more individual than micro-pools per sample, and micro-riffles were predicted to contain approximately 2.0 less individuals than micro-pools per sample. Depth was lowest in micro-riffles (Fig. 8), and riffle mesohabitat contained greater number of micro-riffles than other mesohabitats (Fig. 6).

In order to compare differences in habitat associations between *E. cirrigera* in different stages of aquatic development, we analyzed second- and first-year individuals separately. Low sample size precluded AIC_c modeling for second-year *E. cirrigera.* Both second- and first-year *E. cirrigera* were negatively associated with pools and riffles and positively associated with runs at meso-scales (Fig. 3b). There was a difference between observed and expected frequencies of second- and first-year *E. cirrigera* at one site (second-year, stream 1: $G = 8.63$, df $= 2$, $P = 0.01$; first-year, stream 1: $G = 10.01$, df $= 2$, $P = 0.007$), but these differences were not as pronounced as in *A. barbouri*. The model best predicting the presence of first-year *E. cirrigera* in late spring was sampling stream; however, multiple candidate models had relatively substantial weights (Table 2). Only 1 first-year *E. cirrigera* was sampled in stream 2 and this was likely driving model selection. First-year *E. cirrigera* were predicted to be approximately 4.0*x* more likely to be present in areas of *A. barbouri* presence than in areas of *A. barbouri* absence, and 1.24*x* more likely to be present with an increase in 1 cm of depth (Table 3). The model best predicting the abundance of first-year *E. cirrigera* in late spring was microcondition and depth (Table 2). An increase in 4.85 cm of depth was predicted to result in an increase in 1.0 *E. cirrigera* individual (Table 3). A decrease in 8.20% boulder cover was predicted to result in an increase in 1.0 *E. cirrigera* individual.

Discussion

The multi-scale approach of our study improved our understanding of the distribution of larval salamanders in relatively undisturbed headwater systems. Strong mesohabitat associations dictated locations of clumped individuals. Micro-scale environmental variables differentially predicted the presence and

Table 2 Top models predicting the presence and abundance of *Ambystoma barbouri* and *Eurycea cirrigera* in early (13–15 April) and late spring (18–20 May) 2012

Species	Sampling session	Response	Model[a]	K[b]	Log-likelihood	Δ_i	w_i
Ambystoma barbouri	Early spring	Presence	Bedrock + boulder + microcon	5	−19.16	0	0.73
			Microcon	3	−23.31	3.54	0.13
		Abundance	Bedrock + boulder + microcon	6	−114.12	0	0.54
			Global	14	−101.97	0.46	0.43
	Late spring	Presence	Bedrock + depth	3	−23.80	0	0.28
			Depth	2	−25.05	0.20	0.25
			Depth + microcon	4	−22.87	0.54	0.21
			Embed	2	−25.91	1.93	0.11
			Bedrock + depth + debris + Veg	5	−23.17	3.66	0.04
		Abundance	Depth + microcon	5	−66.99	0	0.64
			Microcon + embed	5	−67.73	1.48	0.30
Eurycea cirrigera	Late spring	Presence	Stream	3	−22.99	0	0.23
			A. barbouri presence	2	−24.62	0.97	0.14
			Depth	2	−24.94	1.61	0.10
			Bedrock + depth	3	−24.02	2.05	0.08
			Intercept	1	−26.40	2.34	0.07
			Depth + embed	3	−24.22	2.45	0.07
			Depth + bedrock + boulder	4	−23.38	3.19	0.05
			Microcon + *A. Barbouri* presence	4	−23.44	3.30	0.04
			Debris + depth	3	−24.93	3.89	0.03
			Bedrock	2	−26.09	3.90	0.03
			Bedrock + boulder	3	−24.98	3.97	0.03
		Abundance[c]	Depth + microcon	5	−28.84	0	0.53
			Depth + embed	4	−30.69	1.17	0.29
			Bedrock + boulder + embed	5	−30.76	3.85	0.08

Cutoff for top models was $\Delta_i < 4$

[a] Microcon (microcondition: micro-run, micro-riffle, micro-pool), bedrock (%bedrock cover within 0.25-m^2 plot), boulder (%boulder cover within 0.25-m^2 plot), embed (% of substrate embedded within 0.25-m^2 plot), debris (%debris cover within 0.25-m^2 plot), veg (%vegetation cover within 0.25-m^2 plot), depth (depth at midpoint of sampling plot)

[b] Includes error term and intercept for abundance models and intercept for presence models

[c] Indicates Quasi Akaike's Information Criterion ($QAIC_c$) results

abundance of both species within mesohabitats, therefore influencing overall distribution patterns.

Micro-scale environmental variables effectively predicted distribution of *A. barbouri* across their ontogeny. Micro-riffles had a strong negative influence on *A. barbouri* abundance in early and late stages of development. The high frequency of micro-riffles within riffle mesohabitat likely dictated the negative association of *A. barbouri* to these areas throughout their aquatic stage. The frequency of *A. barbouri* observed in riffles throughout our study contrasted the literature, however (Petranka, 1984a; Holomuzki, 1991). We observed 166 *A. barbouri* in riffle mesohabitat throughout this study. The presence of low velocity, laminar microhabitats (i.e., micro-pools and micro-runs) within meso-scale riffles resulted in *A. barbouri* inhabiting normally unsuitable mesohabitat. Positive mesohabitat associations were also driven by a prevalence of micro-pools and micro-runs. Our results indicate that the distribution of micro-pools, micro-runs, and micro-riffles within headwaters dictates *A. barbouri* in-stream distribution. Distributions of our study organisms were also

Table 3 Effects of top predictive parameters (i.e., different from zero) on the presence and abundance of *Ambystoma barbouri* and *Eurycea cirrigera* in early (13–15 April) and late spring (18–20 May) 2012

Species	Sampling session	Response	Parameter[a]	β	85% CI lower	85% CI upper	Odds ratio
Ambystoma barbouri	Early spring	Presence	Bedrock	0.029	0.010	0.047	1.029
		Abundance	Bedrock	0.010	0.003	0.018	–
			Veg	−0.019	−0.035	−0.004	–
			Boulder	−0.044	−0.075	−0.013	–
			Debris	−0.047	−0.078	−0.016	–
			Stream 3 versus stream 1	1.410	0.785	2.034	–
			Stream 2 versus stream 1	0.584	0.012	1.156	–
			Micro-riffle versus micro-pool	−1.955	−2.942	−0.968	–
	Late spring	Presence	Depth	0.433	0.212	0.655	1.542
			Embed	0.036	0.016	0.056	1.037
		Abundance	Depth	0.220	0.141	0.299	–
			Embed	0.026	0.015	0.038	–
			Micro-run versus micro-pool	0.930	0.362	1.498	–
			Micro-riffle versus micro-pool	−1.843	−3.572	−0.114	–
Eurycea cirrigera	Late spring	Presence	Depth	0.194	0.037	0.352	1.214
			A. barbouri presence	1.408	0.291	2.525	4.087
			Stream 2 versus stream 1	−2.167	−3.821	−0.513	0.115
		Abundance	Depth	0.206	0.096	0.317	–
			Boulder	−0.122	−0.196	−0.048	–

Parameter estimates were averaged across top models if the top model $w_i < 0.90$. Results are presented by species, sampling session, response, and type of parameter (continuous or categorical)

[a] Bedrock (%bedrock cover within 0.25-m^2 plot), boulder (%boulder cover within 0.25-m^2 plot), embed (% of substrate embedded within 0.25-m^2 plot), depth (depth at midpoint of sampling plot), veg (%vegetation cover within 0.25-m^2 plot), debris (%debris cover within 0.25-m^2 plot), stream (sampling stream 1, 2, or 3), microcondition (micro-pool, micro-riffle, or micro-run), mesohabitat (pool, riffle, or run)

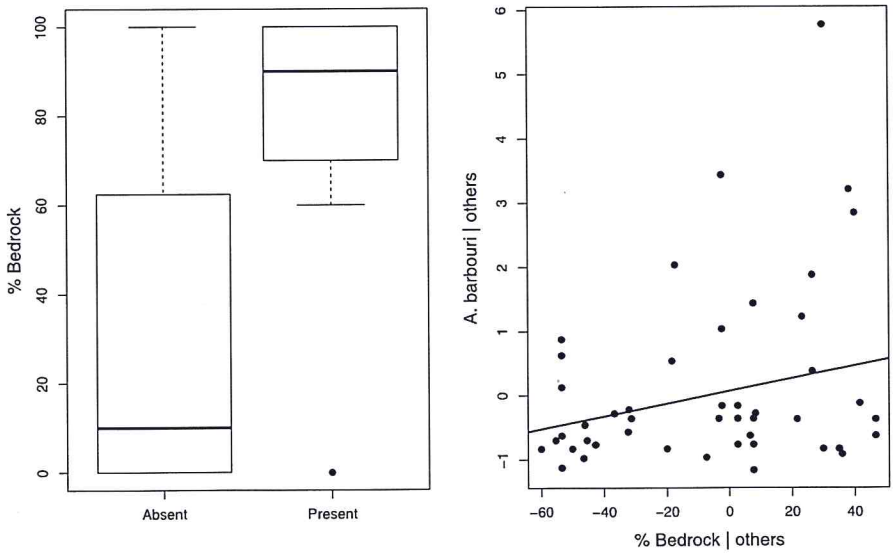

Fig. 4 *Box plot* of %bedrock and *Ambystoma barbouri* presence (*left*) and adjusted variable plot of the influence of %bedrock on abundance compared to other predictive variables (*right*) in early spring (13–15 April 2012)

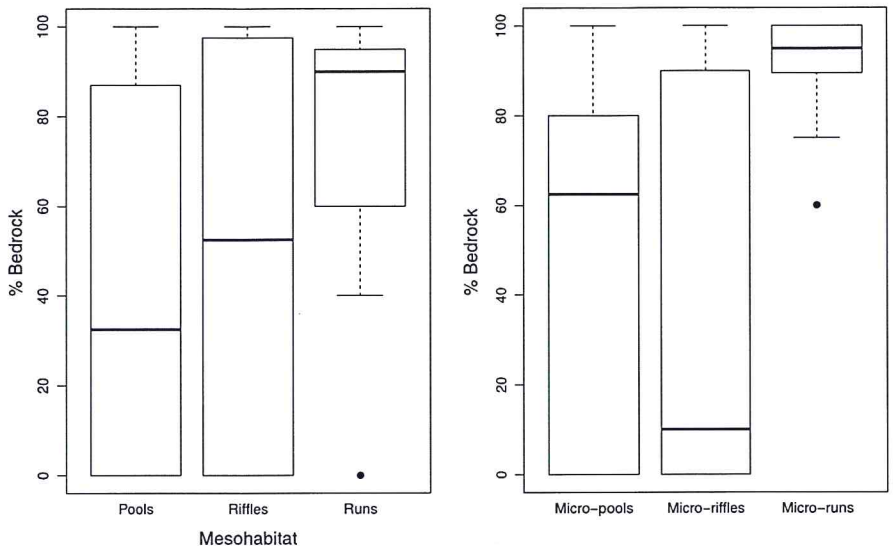

Fig. 5 Distribution of %bedrock within meso-scale (*left*) and micro-scale (*right*) habitat types in early spring (13–15 April 2012)

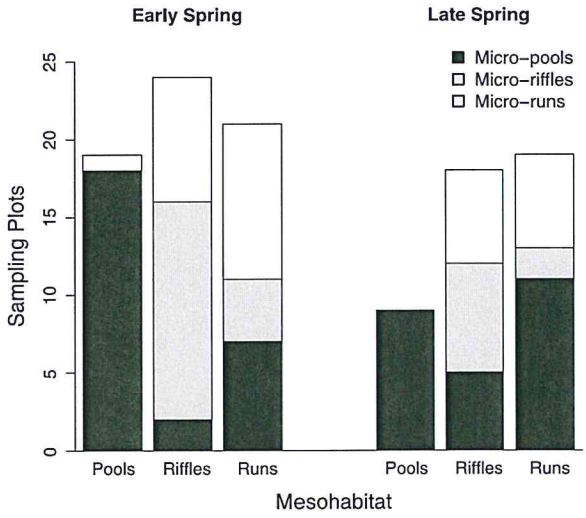

Fig. 6 Abundance of micro-pools, micro-riffles, and micro-runs within each mesohabitat type in early (13–15 April) and late spring (18–20 May) 2012

influenced by stream depth, and this was not unexpected because our study streams began to dry in late spring, and both species are restricted to aquatic habitats during their larval stage (Petranka, 1998).

Evolutionary and life history of *A. barbouri* likely contributed to their micro-scale associations in early spring (Holomuzki, 1991; Petranka, 1998). We observed the majority of *A. barbouri* exposed in the water column shortly after hatching ($\mu \pm 1$ SE $= 83.2 \pm 5.9\%$), indicating that individuals would have little resistance to downstream displacement. Body size–interstitial space relationships did not influence *A. barbouri* but negative associations of first-year *E. cirrigera* to large substrate reflected what is reported in the literature and likely influenced their distribution (Gustafson, 1994; Lowe, 2005; Martin et al., 2012).

The low abundance of *E. cirrigera* in our streams in early spring indicates that predation risk to *A. barbouri* is minimal and likely does not influence distribution of *A. barbouri* (Petranka, 1984b). The presence of *E. cirrigera* was influenced by the presence of *A. barbouri* in late spring, and the introduction of a new cohort of *E. cirrigera* may have initiated inter-specific interactions between our study organisms that influenced their distributions. Alternatively, similar mesohabitat associations of both species could be a relic of life history requirements of all aquatic larval salamanders. Our modeling reflects differing

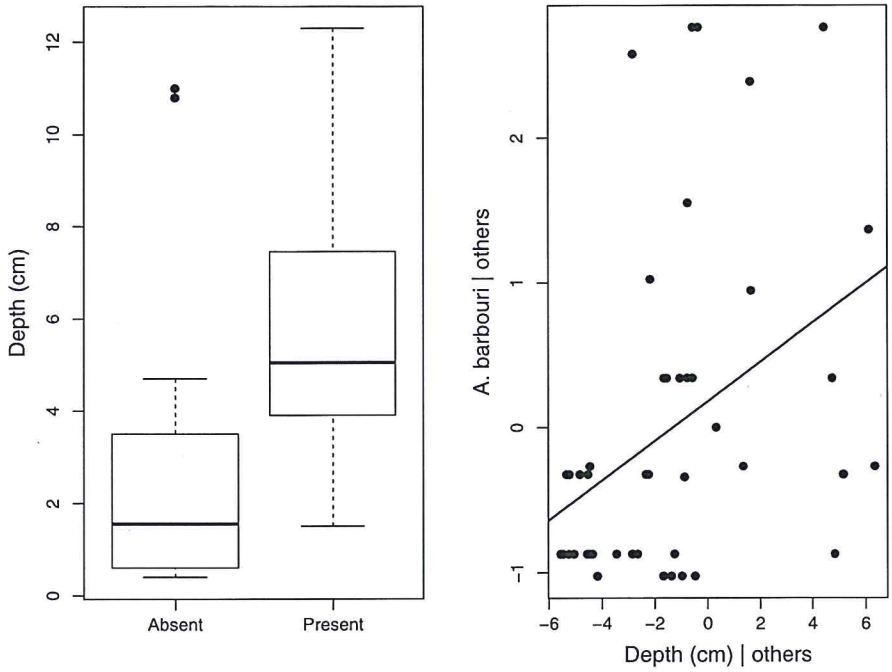

Fig. 7 *Box plot* of depth and *Ambystoma barbouri* presence (*left*) and adjusted variable plot of the influence of depth on abundance compared to other predictive variables (*right*) in late spring (18–20 May 2012)

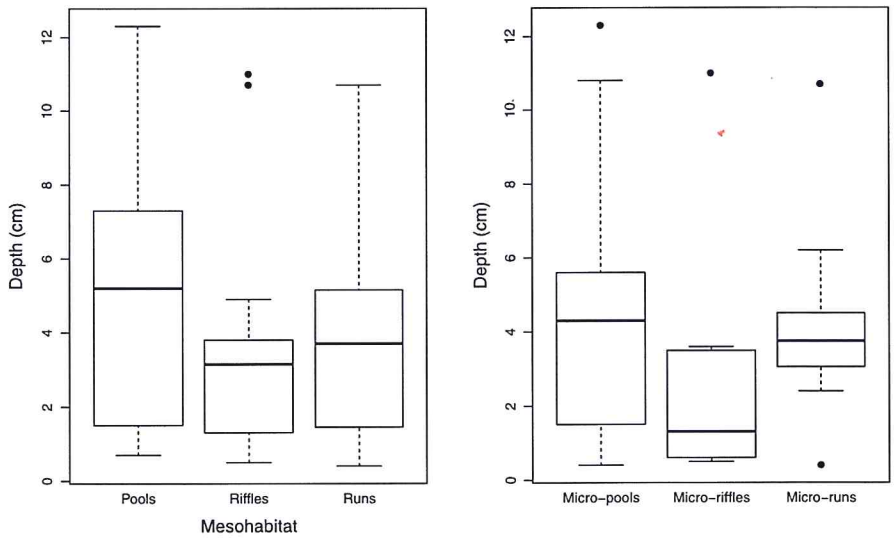

Fig. 8 Distribution of depth within meso-scale (*left*) and micro-scale (*right*) habitat types in late spring (18–20 May 2012)

associations to microhabitat parameters between species, and this suggests that mechanisms behind their similar mesohabitat associations differ.

Habitat associations of our study organisms shifted in response to ontogeny. The top predictive model of abundance and the presence of *A. barbouri* in early spring held no weight in late spring. Lack of associations to pool habitat at meso- and micro-scales suggests that displacement from high velocity to low velocity areas was likely not influencing habitat associations of larval salamanders in late spring. Active selection of areas with high densities of some prey species or differences in time to metamorphosis between individuals in different mesohabitats could have contributed to this shift in habitat associations of *A. barbouri* (Holomuzki, 1991). Our data do not address the mechanism behind shifts in habitat associations, but an important implication of our findings is the differing influence of microhabitat parameters on a species across ontogeny.

We attempted to acknowledge extrinsic variability by including stream sampling location as a blocking factor in our predictive regression model sets. The influence of stream location on abundance and presence of salamanders was not unexpected, as natural variability in habitat structure both within and outside of our stream channels was present. Strong mesohabitat associations and associations to micro-scale conditions indicate that our top models are effective predictors of the presence and abundance of *A. barbouri* across large landscapes. Relatively weak associations to mesohabitat and lack of strong abiotic predictors in top models suggest that our models likely would not effectively predict *E. cirrigera* distribution within headwater streams across their geographic range.

Our study demonstrated that multi-scale research is vital to understanding complex relationships between aquatic organisms and their surrounding environment. Our model predictions paired with observed patterns indicated habitat at micro-scales dictated mesohabitat associations of larval salamanders, which in turn contributed to their spatial distribution throughout headwaters. Our study highlights the complexity of interactions between aquatic organisms and their abiotic and biotic surroundings across spatial and temporal scales, and reinforces the use of hierarchical habitat classifications for aquatic research (Frissell et al., 1986; Poff, 1997).

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