BioOne COMPLETE

Factors Facilitating Co-occurrence at the Range Boundary of Shenandoah and Red-Backed Salamanders

Authors: Amburgey, Staci M., Miller, David A. W., Brand, Adrianne, Dietrich, Andrew E., and Campbell Grant, Evan H.

Factors Facilitating Co-occurrence at the Range Boundary of Shenandoah and Red-Backed Salamanders

Staci M. Amburgey,[1,2,3] David A. W. Miller,[1] Adrianne Brand,[4] Andrew E. Dietrich,[5] and Evan H. Campbell Grant[4]

[1]*Department of Ecosystem Sciences and Management, The Pennsylvania State University, University Park, Pennsylvania, USA*
[2]*Intercollege Graduate Ecology Program, The Pennsylvania State University, University Park, Pennsylvania, USA*
[4]*U.S. Geological Survey Patuxent Wildlife Research Center, S.O. Conte Anadromous Fish Research Center, Turners Falls, Massachusetts, USA*
[5]*U.S. Geological Survey Patuxent Wildlife Research Center, Laurel, Maryland, USA*

Abstract.—The transition from species in allopatry to sympatry, that is, the co-occurrence zone, allows for investigation of forces structuring range limits and provides evidence of the evolutionary and population responses of competing species, including mechanisms facilitating co-occurrence (e.g., character displacement). The Shenandoah Salamander (*Plethodon shenandoah*), an endangered plethodontid, is limited to three mountaintops in Shenandoah National Park, Virginia, USA. This species' distributional limits are attributed to competitive exclusion by the Red-Backed Salamander (*Plethodon cinereus*). Recent work showed range overlap between these species is greater than previously thought, requiring investigation of species morphology, behavior, and demographic measures in single-species and co-occurrence zones that might facilitate such overlap. We analyzed individual characteristics from 2 yr of transect surveys to see whether traits differed within and outside co-occurrence zones. Measures showed species- and zonal-specific differences, but we found limited support for character displacement. Both species were larger in the co-occurrence zone, indicating larger salamanders might better compete for resources or that symmetric competition restricts dispersal or recruitment processes at the co-occurrence zone. Microhabitat use also differed by species across transects, with Red-Backed Salamanders using more rock microhabitats in the co-occurrence zone, potentially because of competition for microclimates that minimize physiological stress. The lack of strong evidence of differentiation in situ at the range edge suggests weaker character displacement and potentially weaker competition than previously thought, with other factors contributing to the range limits of Shenandoah Salamanders.

Understanding factors that shape species' current range limits is important to identifying conditions that may lead to local extirpation or range expansion in response to shifting abiotic and biotic conditions (Thomas et al., 2004; Hijmans and Graham, 2006). Species interactions can significantly shape species distributions (Araújo and Luoto, 2007; Gilman et al., 2010; Pigot and Tobias, 2012) and may provide a mechanistic explanation of distributional patterns (Pearson and Dawson, 2003; Soberón, 2007) and the formation of range boundaries (HilleRisLambers et al., 2013; Yackulic et al., 2014).

In particular, antagonistic interactions such as interspecific competition have long been suspected as important in influencing species distributions (MacArthur, 1958; Strong, 1980; Sexton et al., 2009) through their modification of local species occurrence and population demographic processes that create and maintain range limits (Bengtsson, 1989; Gilman et al., 2010). Variation in species traits and behaviors in co-occurrence zones, the location where two allopatric species shift to sympatry, are key in understanding the role of species interactions in the creation and maintenance of range boundaries. The gradient from allopatry to sympatry allows for study of the altered evolutionary and population processes that may structure range limits at the junction of species ranges (Grether et al., 2009). Co-occurrence zones provide opportunities to see the manifestation of competition through changes in species morphology, behavior, and population dynamics (Adams and Rohlf, 2000; Grether et al., 2009).

The principle of competitive exclusion states that species with similar ecological niches will not be able to coexist because of competition for resources (Gause, 1934). This antagonism can

form a range boundary for one or both species over a gradient in the limiting resources (Case and Taper, 2000). The width of the co-occurrence zone between species with similar niches can be related to the presence and strength of competitive exclusion (Tobias et al., 2014). Co-occurrence of ecologically similar species is possible if individuals in overlapping portions of the range develop differentiated physical traits or behaviors (i.e., character displacement; Brown and Wilson, 1956; Adams and Rohlf, 2000; Adams, 2004, 2010). Population processes such as dispersal or demographic measures (e.g., sex ratios) may also change in response to competitive interactions with the other species (Carrete et al., 2005). Alternatively, co-occurrence may be possible with no trait differentiation or even with trait convergence if other shared environmental or biotic selective pressures exist (e.g., thermal or predation pressure) that constrain species occurrence more than competition (Cavender-Bares et al., 2009; Tobias et al., 2014).

To understand the role of species interactions in setting species' range limits, we studied the co-occurrence zone of two salamanders of genus *Plethodon* in a montane ecosystem with a range boundary largely attributed to competitive interactions (but see Grant et al., 2018b). The Shenandoah Salamander (*Plethodon shenandoah* Highton and Worthington, 1967) is a federally endangered terrestrial salamander (54 CFR 34464; Jacobs, 1994) endemic to Shenandoah National Park, Virginia, USA. This species occurs on only three mountaintops >850 m in elevation (Jaeger, 1970). Competitive exclusion by the broadly distributed Red-Backed Salamander (*Plethodon cinereus* Green, 1818) has been considered the primary driving force shaping the distribution of this species (Jaeger, 1970, 1971b; Griffis and Jaeger, 1998). The Red-Backed Salamander occurs across most of the northeastern United States and southern Canada (Moore and Ouellet, 2015), preferring moist soil and leaf litter in forested habitats (Jaeger, 1970). Although north-facing slopes are generally more moist and cooler, Shenandoah Salamanders

are limited within northwestern- and northeastern-facing slopes to sparsely vegetated, rocky habitats with thinner soils where moisture and temperature regimes are less tolerable for the Red-Backed Salamanders (Jaeger, 1971a). In field and laboratory-based experiments, Red-Backed Salamanders excluded Shenandoah Salamanders in habitats with deeper soil (Griffis and Jaeger, 1998) that both species appeared to prefer (Jaeger, 1971a,b). Competition for microhabitat (Jaeger, 1971b; Griffis and Jaeger, 1998) and food (Jaeger, 1972) were cited as potential limiting factors structuring these interspecific interactions. Laboratory and field manipulations, however, do not satisfactorily explain the observed range boundary for these two species (Jaeger et al., 2016), and further clarification as to the role of species competition in this system is required.

Recent work using an occupancy probability modeling framework that accounts for imperfect species detection indicated the range boundary of these two species is characterized by a 60- to 170-m zone of overlap (Amburgey et al., 2019). This is wider than previously characterized (Jaeger, 1970, 1972) but still relatively narrow compared with the range extent of the Shenandoah Salamander. Amburgey et al. (2019) defined areas of predominantly single-species occurrence (PSHE = *P. shenandoah* and PCIN = *P. cinereus* zones) and areas where both species could be found (co-occurrence zone). To identify mechanisms facilitating these broader than expected areas of species co-occurrence, we investigated how 1) morphological traits, 2) behaviors, and 3) demographic metrics of the two species differ among these three zones. For example, larger adult males may be better able to compete for resources (Jaeger, 1972; Wrobel et al. 1980; Mathis, 1991; but see Lang and Jaeger, 2000), resulting in morphological (size) and demographic (sex ratio) differentiation in the co-occurrence zone compared with single-species zones. Other morphological traits such as variation in dorsal stripe and coloration may result in differential predation rates (Fitzpatrick et al., 2009; Grant et al., 2018a), which can facilitate co-occurrence by reducing abundances and decreasing competition. In addition, microhabitat selection may alter antagonistic interactions between these lungless salamanders that must breathe via diffusion across the skin, making microhabitat temperature and moisture critical to survival (Jaeger, 1971a,b; Griffiths and Mylotte, 1987; Hagey et al., 2016). Differences in physiological tolerances exist between the Shenandoah and Red-Backed Salamanders (Griffis and Jaeger, 1998), and escape of suboptimal conditions may lead to competition over optimal microhabitat (Griffis and Jaeger, 1998). Shenandoah Salamanders tolerate warmer and drier conditions better than Red-Backed Salamanders, although both preferentially select wetter conditions (Jaeger, 1971a). We also investigated tail damage (i.e., partial or complete tail autotomy), not as a sign of character displacement but as an indicator of aggressive behaviors (potentially inter- or intraspecific; Mathis, 1991) that might corroborate any observed differences in microhabitat use. Tail autotomy may also indicate predation attempts but has been used as an indicator of territorial interactions (Mathis, 1991).

We investigated this suite of morphological, behavioral, and population-level characteristics (Supplementary Table S1 and S2) to evaluate five hypotheses (H1–H5; Fig. 1). Although trait differentiation could occur only in zones of species co-occurrence (H1: character displacement) or between species across the entire area surveyed (H2: species differentiation), trait convergence could also occur but only in certain zones along the range boundary (H3: character convergence by zone). Traits could also be similar between the two species but vary across

the area surveyed (H4: character convergence with zonal differences) or be similar between both species across the entire area surveyed (H5: complete character convergence). Importantly, we emphasize that character convergence may be the product of evolution over longer time spans but may also occur because of nongenetic changes (e.g., phenotypic plasticity; Losos, 2011). We predicted that if interspecific competition is important in structuring range limits of the Shenandoah Salamander (as posited by Jaeger, 1970, 1972), co-occurrence should be explained by some differentiation in species traits and behaviors to alter competitive interactions between these species (Jaeger, 1972; Mougi and Nishimura, 2005).

MATERIALS AND METHODS

Field Sampling.—We established and repeatedly surveyed 11 transects at four study areas at the confluence of the Shenandoah and Red-Backed Salamander ranges ($n = 6$–9 surveys per transect). We established transects based on historical occurrence records in addition to modern monitoring by the U.S. Geological Survey (USGS) and National Park Service. Because the Shenandoah Salamander is federally protected, location names and geographic coordinates are omitted in this article. Methods to establish and survey transects are detailed in Amburgey et al. (2019); in general, observers checked all microhabitat objects within 2 m of both sides of transect tape extending laterally from one species' territory through an area of predicted co-occurrence and into the other species' territory. We surveyed transects in 50-m segments and continued surveying for 100 m past the last detection of the other species. We assigned each captured salamander to a 5×4-m (2 m on each side of the transect tape) site on the transect. Captured salamanders were placed in plastic sandwich bags until subsequent species identification and measurement.

We recorded morphological data on every captured salamander including dorsal surface (striped or leadbacked), stripe color (if stripe was present; red-orange, yellow, brown, or white), and snout–vent length (SVL). Population-parameter measures included life stage (adult or juvenile) and sex (male or female). All salamanders <35 mm in total length were considered juveniles (Sayler, 1966), and sex determination is considered unreliable at this stage. The two species are similar in physical appearance (Highton and Worthington, 1967), but we identified each salamander to species by using a combination of stripe width and shape (when present) and ventral surface pigmentation (sensu Highton and Worthington, 1967). Any uncertainty in species identification required verification by a second observer, and we eliminated salamanders from the analysis where species was uncertain (<10 salamanders removed).

We recorded all microhabitat types (rock, log, or leaf litter) and measured microhabitat temperatures for objects found in the first 15 m of the lower side of each 50-m transect segment via Fluke (Everett, Washington, USA) 62 MAX infrared thermometer. Outside of the first 15 m, we measured the microhabitat type and temperature where each detected salamander was found, along with five additional surrounding microhabitats to account for temporal fluctuations in temperature while surveying and to sample unused microhabitats. To avoid altering temperatures when displacing microhabitat, we took temperatures immediately after flipping objects or objects were replaced immediately until a temperature could be taken (preserving the original temperature with little to no change, Amburgey, pers. obs.). For salamanders found wandering on the surface of leaf

Shenandoah Range (PSHE zone)	Range Overlap (Coocurrence zone)	Red-backed Range (PCIN zone)

H1. Character Displacement

H2. Species Differentiation

H3. Character Convergence by Zone

H4. Zonal Differences
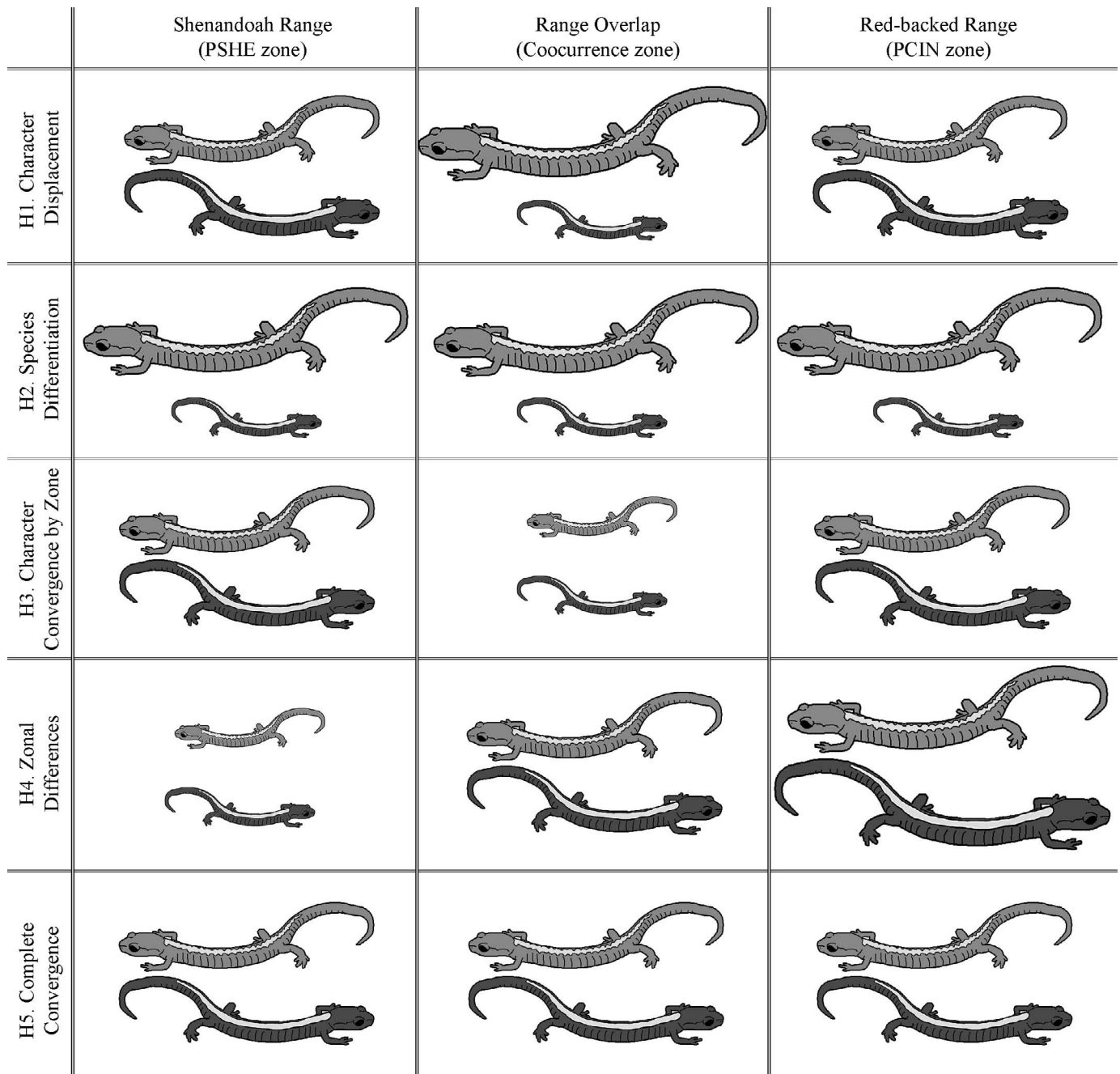
H5. Complete Convergence

FIG. 1. Hypotheses to test for measured traits and behaviors of Shenandoah (gray) and Red-Backed (dark gray) Salamanders across the Shenandoah Salamander (PSHE), co-occurrence, and Red-Backed Salamander (PCIN) zones. For the pictured example of salamander body size (SVL), larger Shenandoah and smaller Red-Backed Salamanders in only the co-occurrence zone would support H1. Species differentiation (H2) indicates species are consistently different across all zones. Character convergence by zone (H3) occurs when both species are similar in only certain zones. Zonal differences (H4) indicate size differs by zone but similarly for both species. Last, complete convergence (H5) indicates no difference across zones or species. Shenandoah Salamanders are depicted as the larger species in this example based on Jaeger (1972).

litter, we took temperatures directly beneath where they were captured. In addition, we noted tail damage on every individual as a proxy for behavioral aggression. We processed salamanders immediately after capture and returned them to the location at which they were detected. We collected individual trait data in 2015 and 2016 and microhabitat type and temperature data in 2015.

Data Analysis.—In a previous study (Amburgey et al., 2019), we estimated site-specific occupancy probabilities for Shenandoah and Red-Backed Salamanders. We corrected for heterogeneity in salamander surface activity (Feder, 1983) and other

factors resulting in imperfect detection (MacKenzie et al., 2006) by using repeat surveys to get robust estimates of species occupancy (i.e., the probability that a species was present at a given site). Using our best-supported model, we calculated co-occurrence probabilities (i.e., the probability of occupancy of resident individuals of both species) for every site in each transect, allowing for delineation of the co-occurrence zone vs. single-species zones. We defined co-occurrence zones as areas where the likelihood of co-occurrence summed across three 5×4-m sites (60 m^2, based on habitat-use estimates in Muñoz et al., 2016b) was ≥ 1. Individuals of both species occurred in all

transect zones; however, the core areas of occurrence for each species (and thus those with the hypothesized highest abundances) correlated to their single-species zones. We expected that peak interspecific competitive interactions occur in the co-occurrence zone, and salamanders in the opposite species' zone are nonresident salamanders and that intraspecific interactions may be more important in resource disputes in single-species zones. We used these transect zones (PSHE zone, co-occurrence zone, and PCIN zone) to test our hypotheses regarding the differentiation of measures across the range edge.

We indicated life stage, sex, dorsal surface, and tail damage as a binary measure (1/0) and analyzed each of these traits via logistic regression in Program R (R Core Team 2018). We analyzed stripe color and microhabitat type by splitting each category into a binary response (e.g., red stripe vs. not red stripe) and running logistic regressions on each category with a Bonferroni correction for multiple comparisons (Bonferroni, 1936). This is similar statistically to a multinomial logistic regression with the added benefit of increased interpretability for all zone and species–trait combinations. We checked SVL data for normality and log transformed all measurements to correct for skewness before running a linear regression. For each physical and behavioral trait analysis, we ran four models using the trait or behavior as the response variable and species, zone, species and zone (additive), or species and zone (interaction) as fixed-effect explanatory variables.

Last, we analyzed microhabitat temperature using logistic regression to investigate whether each species differentially used microhabitats at different temperatures in co-occurrence vs. single-species zones. We analyzed the binomial response of habitat use, indicated as a 0 (no salamander found under object) and 1 (salamander found). We standardized temperature by the mean and SD across all sites and study areas. We ran five models with the response variable of microhabitat–temperature use and an additive explanatory variable of temperature with species, zone, species and zone (additive), species and zone (interaction), or a three-way interaction of temperature, species, and zone. We modeled microhabitat temperature as a quadratic relationship because we expected salamanders to prefer some optimal temperature with fewer salamanders found at temperatures outside this optimum (Muñoz et al., 2016a).

We performed model selection to choose a top-supported model for each measure based on smallest quasi–Akaike information criterion (QAICc; Akaike, 1973, Burnham and Anderson, 2002). This is a quasi-likelihood form of AIC that corrects for small sample sizes and introduces a correction (ĉ) to the penalty term, traditionally accounting for overdispersion. We used ĉ to correct for the potential resampling of individuals during different surveys, which would result in fewer unique samples and a need to account for the additional deviance from these repeat samples (Burnham and Anderson, 2002). To calculate ĉ, we started by assuming an individual detection probability of 0.1. This is fairly conservative as detection probability is likely much lower based on capture rates from current studies (Grant, pers. obs.) and the fact that linear transect sampling only surveys a part of a salamander's home range. In addition, the probability of capturing a salamander at all is estimated to be fairly low (Sutherland et al., 2016), and this probability does not account for death or emigration. Using this detection probability over a total of visits, we estimated an average of 1.48 captures per individual given it was captured at least once (Supplementary Code). We used this average number of captures as our ĉ to account for potential repeat observations.

For models with similar QAICc values (ΔQAICc < 1), we selected the top model with the smallest QAICc value that minimized the number of model parameters (Arnold, 2010) and that also changed the −2 log-likelihood value (Anderson, 2008). Significance (α) was set at 0.05 for all regression analyses except stripe color and microhabitat type analyses that required Bonferroni correction for multiple comparisons. Instead, stripe color was set at 0.013 (four logistic regressions) and microhabitat type at 0.017 (three logistic regressions). To better understand differences in measures by pairwise comparison, we conducted post hoc chi-square tests for our logistic regressions and Tukey honestly significant difference (HSD) tests for our linear regression where needed. We again used a Bonferroni correction when conducting multiple chi-square tests on a binomial trait, but Tukey HSD already uses a family-wise error rate.

RESULTS

We detected 858 Shenandoah Salamanders and 4470 Red-Backed Salamanders. Sample size differed by analysis because observers occasionally did not record all trait information for every salamander (Supplementary Tables S1 and S2). For Shenandoah Salamanders, we captured 36 and 62.4% of salamanders in the PSHE zone and co-occurrence zone, respectively, and only 1.6% of salamanders in the PCIN zone. We found 61.5 and 37.5% of Red-Backed Salamanders in the PCIN zone and co-occurrence zone, respectively, with only 1% of salamanders found in the PSHE zone. On average, transect length was 263 m, with 116 m of co-occurrence zone. Ability to collect salamanders to measure is connected to the number of available microhabitat objects, but the amount of microhabitat objects varied primarily by visit and not consistently by zone. This would potentially change the frequency of measured traits within a single visit, but not when summed over the course of all surveys as was done for our analyses. We checked an average of 81 ± 36 SD microhabitat objects per 50 m of transect. Beta estimates, *P* values from top-supported models, and results of post hoc tests are in Supplementary Tables S4 and S5.

Morphological Traits.—Our top model included only species as an explanatory variable for dorsal surface (*w* = 0.69; Supplementary Table S3), supporting only species differentiation in this trait (Fig. 1, H2). Red-Backed Salamanders were more often striped compared to Shenandoah Salamanders (Fig. 2A; Supplementary Table S4). When stripe was present, all top-supported models for stripe colors contained only species (*w* ≥ 0.53; Supplementary Table S3), again supporting species differentiation (Fig. 1, H2). Red-Backed Salamanders were more likely to be red-orange striped than Shenandoah Salamanders, whereas Shenandoah Salamanders were more likely to have a white or brown stripe (Fig. 3A). Both species were equally likely to have a yellow stripe, supporting complete convergence in this trait (Fig. 1, H5).

The best-supported model for differences in log-transformed SVL was the full model including species, zone, and their interaction (*w* = 0.85; Supplementary Table S3). Shenandoah Salamanders became increasingly larger from their own zone to that of Red-Backed Salamanders, whereas Red-Backed Salamanders increased in size only in the co-occurrence zone (Fig. 4; Supplementary Table S4). Shenandoah Salamanders were larger than Red-Backed Salamanders in the co-occurrence and PCIN zones. Species and zonal differences were similar to, but not fully consistent with, character displacement (Fig. 1, H1) as we
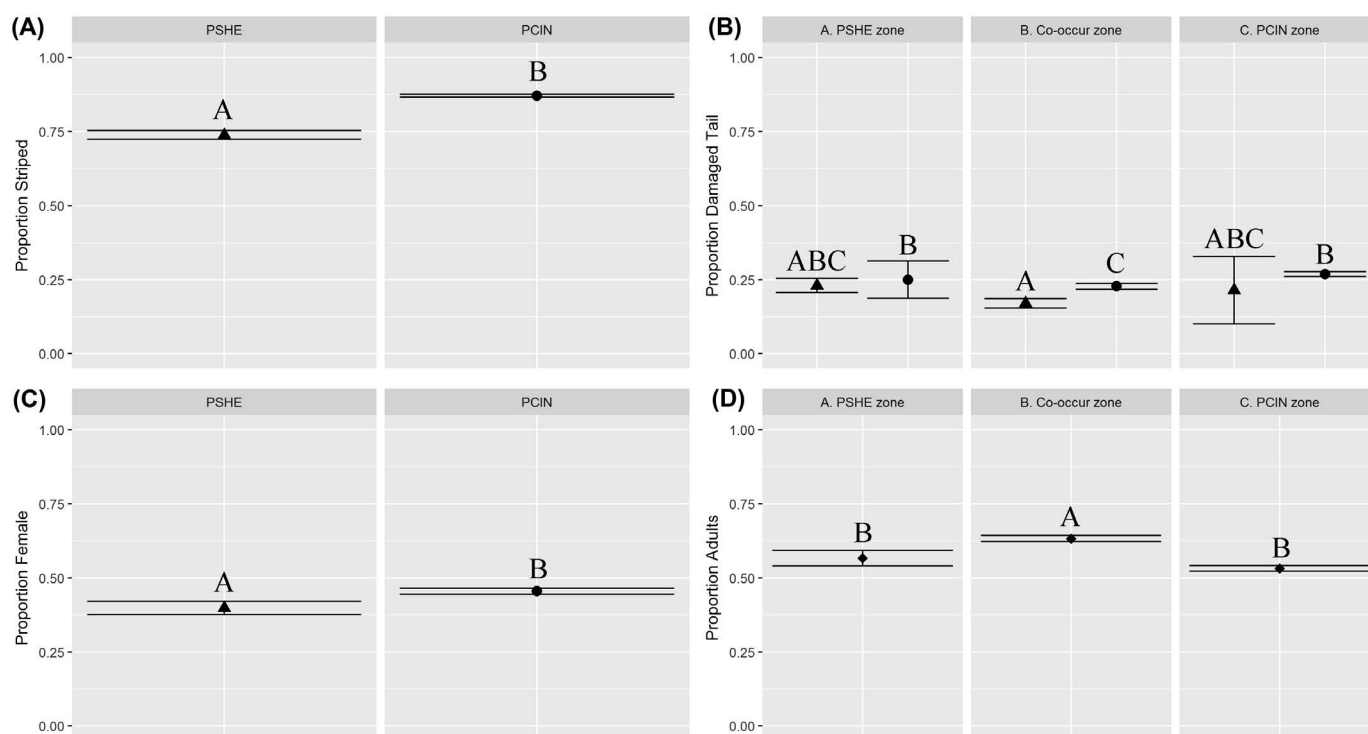
FIG. 2. Morphological traits and population parameters differed by species (Shenandoah [PSHE, triangles] and Red-Backed Salamanders [PCIN, circles]), transect zone (diamonds), or both. The co-occur zone indicates the co-occurrence zone where there was a high probability of both species vs. the single-species zones. Shown are proportions with SEs of salamanders (A) with a striped dorsal surface, indicating differences by species (B) with damaged tails, indicating differences by species by zone, (C) that are female, indicating differences by species, and (D) that are adults, indicating differences by zone.

predicted traits would become more dissimilar where the two species overlapped. Instead, both species became larger, although different in SVL from one another.

Behaviors.—For tail damage (i.e., our competitive aggression proxy), we found the additive model containing species and zone was best supported ($w = 0.71$; Supplementary Table S3), indicating species-level differentiation that varied across transect zone (Fig. 1, H2, H4). Red-Backed Salamanders in the co-occurrence zone had less tail damage than those in the PSHE and PCIN zones (Fig. 2B; Supplementary Table S4). Although tail damage of Shenandoah Salamanders did not differ across the zones, fewer Shenandoah Salamanders possessed damaged tails compared with Red-Backed Salamanders in the co-occurrence zone.

The top supported models for microhabitat type included either the interaction of species and zone (log, $w = 0.99$) or the additive effect of species and zone (leaf litter and rock, $w \geq 0.62$; Supplementary Table S3). For leaf-litter microhabitat, our results indicated species-level differentiation that varied across transect zone (Fig. 1, H2, H4). We found Red-Backed Salamanders used a similar proportion of leaf litter in all transect zones (Fig. 3B; Supplementary Table S5). Shenandoah Salamanders were most often on leaf litter in the PSHE zone but used the leaf litter less often in the co-occurrence and PCIN zones. Shenandoah Salamanders used the leaf litter more often than Red-Backed Salamanders in all zones except the PCIN zone.

For log microhabitat, our results were congruent with character convergence and zonal differences (Fig. 1, H3, H4). Log microhabitat use was highest for Red-Backed Salamanders and lowest for Shenandoah Salamanders in the PSHE zone. In the co-occurrence and PCIN zones, Shenandoah and Red-Backed Salamanders used the same proportion of log micro-

habitats as each other. Red-Backed Salamanders used slightly more log microhabitats in the PCIN zone compared with the co-occurrence zone.

For rock microhabitat, our results indicated species-level differentiation that varied across transect zone (Fig. 1, H2, H4). Shenandoah Salamanders used an equivalent amount of rock microhabitats across most transects, except in the co-occurrence zone where they used more rocks compared with the PSHE zone. Red-Backed Salamanders used a similar amount of rock microhabitats to Shenandoah Salamanders, except in the co-occurrence zone where they increased their use. Red-Backed Salamanders used the most rock microhabitats in the co-occurrence zone and the least in the PSHE zone.

We found 19.5% of detected salamanders shared microhabitat with another salamander, and 4.8% of these individuals were Shenandoah Salamanders. Shenandoah Salamanders were found with a Red-Backed Salamander 21% of the time and with a Shenandoah Salamander 20% of the time. There were several instances where the other salamander escaped before species identification (18%). When microhabitat sharing occurred in Shenandoah Salamanders, we found a maximum of one other Shenandoah Salamander, but one to five Red-Backed Salamanders present.

For microhabitats where salamanders were detected, mean temperature for leaf litter was warmer ($17.3 \pm 0.63°C$) than that of log ($15.6 \pm 0.16°C$) and rock ($14.6 \pm 0.11°C$) microhabitats. The best-supported model for temperature differences in microhabitat use included the additive effect of the interaction between species and zone ($w = 0.98$; Supplementary Table S3). We found increased use of microhabitats at cooler temperatures for both species (Fig. 5; Supplementary Table S5). In the co-occurrence zone, we detected more Red-Backed Salamanders
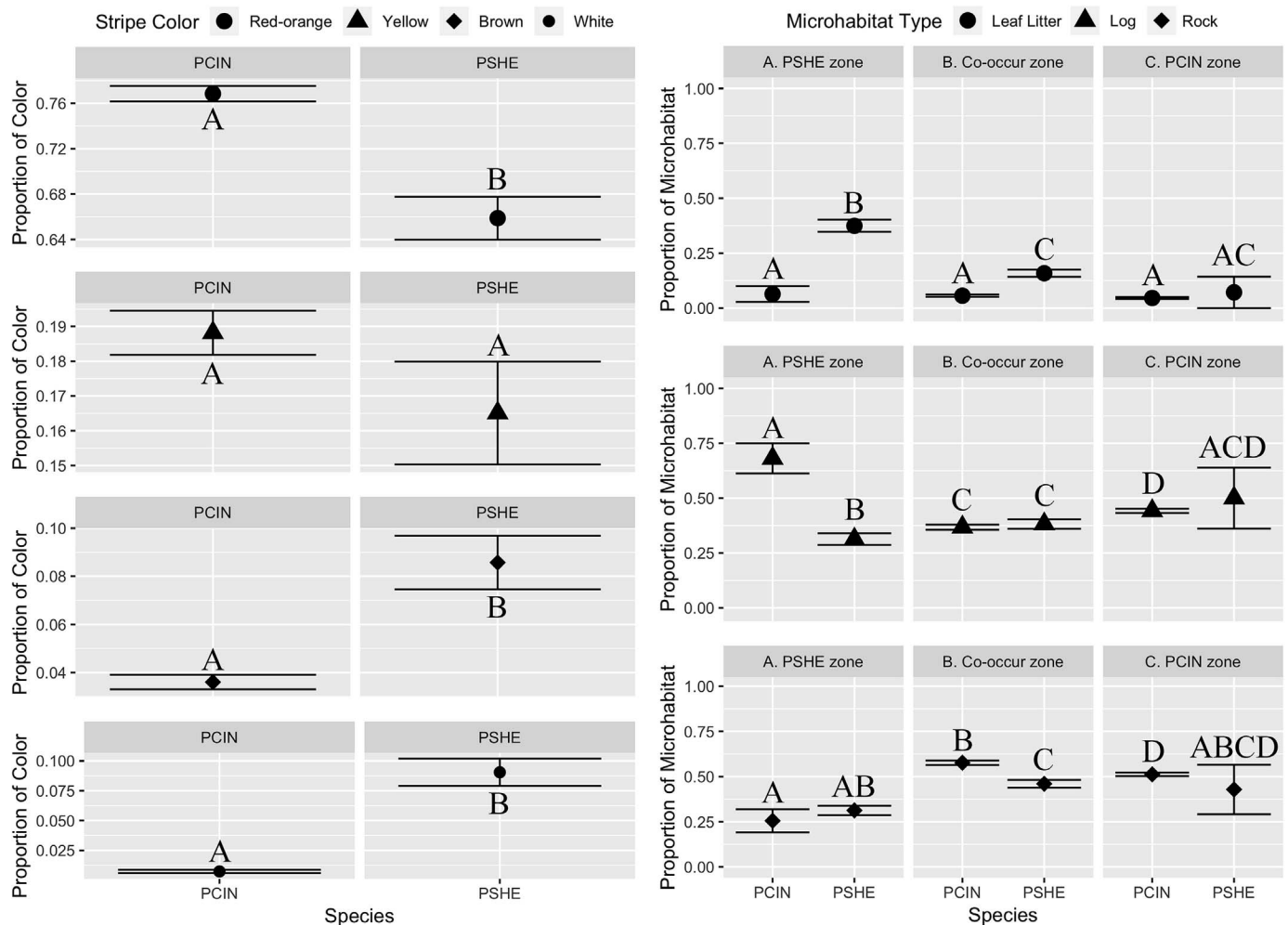
FIG. 3. Morphological stripe traits and microhabitat selection behavior differed either by species (Shenandoah [PSHE] and Red-Backed Salamanders [PCIN]) or species by transect zone. The co-occur zone indicates the co-occurrence zone where there was a high probability of both species vs. the single-species zones. Percentages of salamanders (left panels) with different stripe colors, indicating differences by species (except in the case of yellow stripes where differences were non-significant) and (right panels) using microhabitat types, indicating differences by zone by species (log) or by zone and species (leaf litter, rock).

than Shenandoah Salamanders. Unsurprisingly, Shenandoah Salamanders were detected most in the PSHE zone and Red-Backed Salamanders were detected most in the PCIN zone. There was no support for the interaction of temperature by species ($w = 0.00$; Supplementary Table S3), indicating convergence in microhabitat temperature use by species (Fig. 1, H5; Supplementary Fig. S1). Red-Backed Salamanders appeared to use microhabitats at similar temperatures to Shenandoah Salamanders, except for warmer leaf litter in the co-occurrence zone (Supplementary Fig. S2). Average microhabitat temperature differed more by day of survey than by season from spring into summer (Supplementary Fig. S3).

Demographic Traits.—The top model for life stage included only zone ($w = 0.54$; Supplementary Table S3), with adults of both species found more often in the co-occurrence zone compared with the PSHE and PCIN zones (Fig. 1, H3; Fig. 2D; Supplementary Table S4). We found support for species differences in explaining the probability of male vs. female salamanders ($w = 0.63$; Fig. 1, H2; Supplementary Table S3), with significantly fewer female Shenandoah Salamanders compared with Red-Backed Salamanders in all zones (Fig. 2C; Supplementary Table S4).

DISCUSSION

We predicted physical, behavioral, and population-level differences between Shenandoah and Red-Backed Salamanders would explain the ability of these ecologically similar species to co-occur more than previously expected (Amburgey et al., 2019). Differentiation in measures in the co-occurrence zone (i.e., character displacement; Brown and Wilson, 1956) could allow for niche differentiation between the species and thus present a mechanism to reduce competitive interactions (Adams and Rohlf, 2000; Adams, 2004, 2010). However, most of the characteristics investigated did not demonstrate distinct character displacement for either or both species in the co-occurrence zone (i.e., Fig. 1, H1). We observed several important species-specific and zonal differences, which are potentially interesting for understanding the ecology of these species. Only differences in log microhabitat use, however, might indicate behavioral differentiation as a potential means to reduce competitive interactions. Body size differences by species and zone could also be the result of altered population processes such as dispersal or recruitment driven by competition, but further investigation is required to understand current population demography.
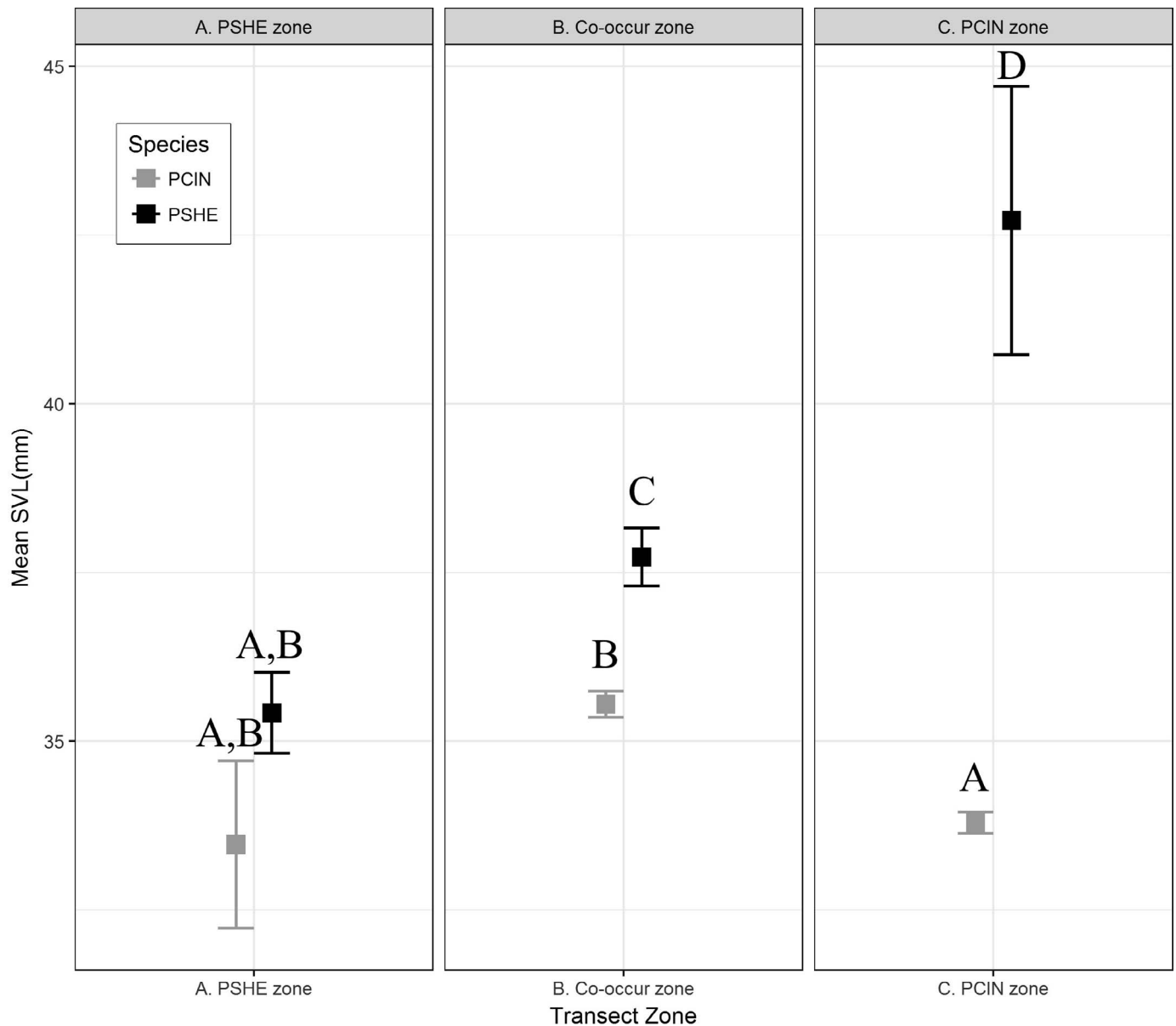
FIG. 4. Mean SVL of captured salamanders. Bars represent SEs. An interaction between species (Shenandoah [PSHE] and Red-Backed [PCIN] Salamander) and zone best explained differences in body size for both metrics. The co-occur zone indicates the co-occurrence zone where there was a high probability of both species vs. the single-species zones.

Instead of minimizing overlap in trait frequencies (i.e., character displacement; Fig. 1, H1), both species were larger in the co-occurrence zone compared with their home range, although Shenandoah Salamanders were the largest in the PCIN zone (similar to Jaeger, 1972, who found exclusion of the smallest individuals of both species when co-occurring). We suggest this could be because of two manifestations of how species respond to competition. First, an increased presence of adults in the co-occurrence zone could explain differences in body size (Fig. 2D), indicating these are larger, dispersing salamanders at the range edge or that recruitment by smaller adults and juveniles is prohibited at the range edge. Second, increased body size can be an important determinant of competitive species interactions (Jaeger, 1972; Mathis, 1991). Shenandoah Salamanders in the co-occurrence and PCIN-only zones may encounter increasingly more interspecific competition as Red-Backed Salamander abundance increases; however,

Red-Backed Salamanders in the PSHE-only range may not encounter as many of the less abundant Shenandoah Salamanders (Jaeger, 1970). In the co-occurrence zone, abundance of both species may cause increased inter- and intraspecific competition that exclude all but the largest Red-Backed Salamanders. Observed differentiation in size across the range boundary could also indicate some character displacement via differences in size-based prey consumption ability that may lessen resource competition between species (Adams and Rohlf, 2000). In addition, because differences in SVL were asymmetrical, this may indicate Shenandoah Salamanders are able to occur farther into Red-Backed Salamander territory and that Red-Backed Salamanders are less able to persist in the drier, shallower soil habitats.

We observed species differentiation in microhabitat use, although not completely consistent with character displacement (Fig. 1, H1). Both Red-Backed and Shenandoah Salamanders
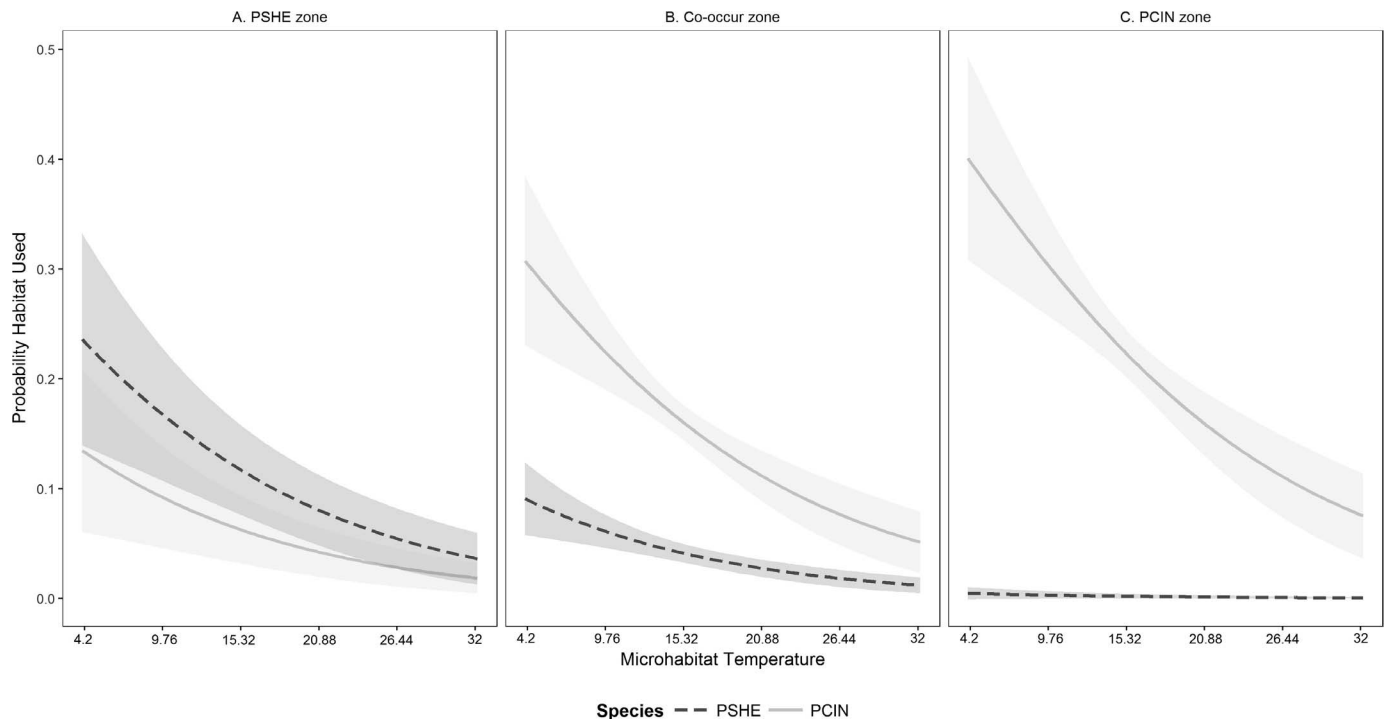
FIG. 5.   General linear model results from our best-supported microhabitat temperature-use model indicated support for an interaction of species (Shenandoah [PSHE] and Red-Backed [PCIN] Salamander) and zone. The co-occur zone indicates the co-occurrence zone where there was a high probability of both species vs. the single-species zones. The 95% confidence intervals are shown in gray. Each salamander species used microhabitats more frequently in each species' respective home range, but microhabitat use similarly declined as temperature increased for both species.

used more rock microhabitats in the co-occurrence zone compared with their own species' zones. In the co-occurrence zone, however, Red-Backed Salamanders were found under rocks more often than Shenandoah Salamanders. This increased use of rock microhabitat in the co-occurrence zone by Red-Backed Salamanders could be indicative of competitive exclusion of Shenandoah Salamanders from potentially cooler, wetter microhabitats (those that were, on average, 1°C cooler than log microhabitats and generally had increased contact with the ground to retain soil moisture). However, an interaction of species by zone was not significant, indicating species-level differences varied similarly by zone. Red-Backed Salamanders used more log microhabitats than Shenandoah Salamanders in the PSHE zone, but log-microhabitat use converged in the co-occurrence and PCIN zones. Competition over rock microhabitats may force Shenandoah Salamanders to find alternate cover beneath logs along transects. We also detected more Shenandoah Salamanders on the leaf litter in nearly all areas of the transect, possibly resulting from their resilience to drier and hotter conditions (Griffis and Jaeger, 1998). Increased salamander surface activity may occur after rain events, but Red-Backed Salamanders were still not as surface active as Shenandoah Salamanders. Differences in microhabitat availability may explain some differences in microhabitat use as the lateral range boundary goes from rockier to deeper soil habitats. Amburgey et al. (2019) observed rock microhabitats were generally more available in Shenandoah Salamander territory (41% of microhabitat was rock) and decreased into Red-Backed Salamander territory (11% of microhabitat was rock). Salamander use of microhabitat temperatures did not differ by species overall but instead showed temperature-dependent surface activity characteristic of plethodontid salamanders (Feder,

1983), with warmer temperatures resulting in fewer detected salamanders.

Species-specific differences in sex ratios, presence of dorsal stripe, and stripe color when present (except for yellow coloration) indicate potentially interesting species life-history differences. The assumption that these two taxonomically, geographically, and behaviorally similar species would display similar sex ratios (and thus mating behaviors, reproductive strategies, and population dynamics) may not be supported. For coloration, Red-Backed Salamanders sported the red stripe more often, whereas Shenandoah Salamanders had rarer white and brown stripes. Coloration may be related to immune system functioning (Venesky et al., 2015), aggressive behaviors (Reiter et al., 2014), and physiological tolerances (Lotter and Scott, 1977, but see Petruzzi et al., 2006). More would be required to test whether these colors align with differences in these species' thermal and moisture constraints (Griffis and Jaeger, 1998). Coloration can also influence predation (Fitzpatrick et al., 2009; Grant et al., 2018a), which can ease competition and facilitate co-occurrence by reducing abundances.

Last, we found tail condition, our measure for interspecific aggression, indicated no heightened interspecific aggression in the co-occurrence zone. The theorized competitively inferior Shenandoah Salamander (Griffis and Jaeger, 1998) had no difference in tail condition across the range edge. Increased tail damage in the Red-Backed Salamander in its home range could be because of increased intraspecific territorial defense as conspecific densities increase, whereas Shenandoah Salamanders may avoid combative interactions or simply be less abundant (Jaeger, 1970). However, it is important to recognize that tail autotomy may also result from predation and is not conclusive evidence of competition.

Our results do not conclusively find character displacement as a means of facilitating co-occurrence in the face of competition, and we found little support for potential alternative strategies by which species avoid interspecific competition (e.g., changes in morphology; see Myers and Adams, 2008). This may indicate that additional stressors outside of interspecific competition are important in setting this range boundary. Grant et al. (2018b) found that climate was more important than the presence of Red-Backed Salamanders in explaining the lower range limit of Shenandoah Salamanders (sensu Gifford and Kozak, 2012). Resources may also vary spatially across the range edge and may structure competitive relationships, facilitating co-existence of the two species with no observable character differentiation (MacArthur and Levins, 1964; Camp et al., 2013). Similar abiotic (e.g., environmental) or biotic (e.g., predation) pressures could result in trait convergence (Abrams and Chen, 2002). Last, similarities in certain traits may evolve so that species may recognize another species and respond with territorial defense (e.g., similar bird songs; Cody, 1969). Further sampling of individual salamanders farther away from the range edge, measuring additional morphology or nutrition (e.g., jaw structure or gut contents to investigate resource acquisition; Adams and Rohlf, 2000; Myers and Adams, 2008), and measuring climate gradients (e.g., humidity) across the range boundary and through time will help clarify the role of antagonism and environmental conditions in maintaining these two species' range limits. In addition, our methods required potentially resampling individuals throughout this study (although because of the territorial nature of both species, resampling would have captured resident salamanders with equal probability across both species and all transect zones). To avoid potential inflation of counts and to gain more insight into potential competition in this system, mark–capture–recapture studies may provide useful insights (Muñoz et al., 2016b).

Endemic species are especially vulnerable to shifting abiotic and biotic conditions because many are range-restricted and threatened species (Thuiller et al., 2005; Isaac et al., 2009), making it easier for conditions to shift beyond their evolutionary history. Limited variation in conditions across a small range reduce the potential adaptive capacity of a species (e.g., through limited thermal tolerance; Thuiller et al., 2005; Shah et al., 2017), and limited geographic space can physically hinder movement necessary for distributional shifts (Dirnböck et al., 2011). Montane species (and therefore doubly montane endemics such as the Shenandoah Salamander) are at particular risk from shifting biotic and abiotic conditions because of an upward limit on geographic space in which to respond (e.g., Raxworthy et al., 2008, but see Elsen and Tingley, 2015). For example, Jaeger (1980) predicted a range contraction for Shenandoah Salamanders because of extreme climate events exacerbated by competition with Red-Backed Salamanders. If competition is weaker than previously expected or is altered with changing climate (Dallalio et al., 2017), this will change projections of Shenandoah Salamander range shifts and risk.

Gradients where allopatric species transition into sympatry (i.e., co-occurrence zones) offer a chance to study the processes by which species interactions shape species distributions. By studying species in situ, we can attain a better understanding of the role interspecific interactions such as competition play in limiting species occurrence or altering morphological, behavioral, or population processes. Shifts in frequencies of morphological traits (i.e., character displacement) or altered behaviors or population parameters such as dispersal can indicate the presence and strength of competition, which characteristics are under selection pressure, and the means by which these traits facilitate spatially overlapping ranges of ecologically similar species. A lack of evidence of these altered traits, behaviors, or population parameters may indicate interspecific competition is not the sole force structuring species occurrence and can lead to a better understanding of additional biotic and abiotic factors driving species range limits.

LITERATURE CITED

ABRAMS, P. A., AND X. CHEN. 2002. The evolution of traits affecting resource acquisition and predator vulnerability: character displacement under real and apparent competition. American Naturalist 160: 962–704.

ADAMS, D. C. 2004. Character displacement via aggressive interference in Appalachian salamanders. Ecology 85:2664–2670.

———. 2010. Parallel evolution of character displacement driven by competitive selection in terrestrial salamanders. BMC Evolutionary Biology 10:72.

ADAMS, D. C., AND F. J. ROHLF. 2000. Ecological character displacement in *Plethodon*: biomechanical differences found from a geometric morphometric study. Proceedings of the National Academy of the Sciences of the United States of America 97:4106–4111.

AKAIKE, H. 1973. Information theory and an extension of the maximum likelihood principle. Pp. 267–281 in B. N. Petrov and F. Caski (eds.), Proceedings of the Second International Symposium on Information Theory. Akademiai Kiado, Hungary.

AMBURGEY, S. M., D. A. W. MILLER, A. BRAND, A. DIETRICH, AND E. H. C. GRANT. 2019. Knowing your limits: estimating range boundaries and co-occurrence zones for two competing plethodontid salamanders. Ecosphere 10:e02727.

ANDERSON, D. R. 2008. Model based inference in the life sciences. Springer Science, New York.

ARAÚJO, M. B., AND M. LUOTO. 2007. The importance of biotic interactions for modelling species distributions under climate change. Global Ecology and Biogeography 16:743–753.

ARNOLD, R. W. 2010. Uninformative parameters and model selection using Akaike's information criterion. Journal of Wildlife Management 74:1175–1178.

BENGTSSON, J. 1989. Interspecific competition increases local extinction rate in a metapopulation system. Nature 340:713–715.

BONFERRONI, C. E. 1936. Teoria statistica delle classi e calcolo delle probabilità, Pubblicazioni del R Istituto Superiore di Scienze Economiche e Commerciali di Firenze 8:3–62.

BROWN, W. L., AND E. O. WILSON. 1956. Character displacement. Systematic Zoology 5:49–64.

BURNHAM, K. P., AND D. R. ANDERSON. 2002. Model selection and multimodel inference. A practical information-theoretic approach. Springer-Verlag, New York.

CAMP, C. D., J. A. WOOTEN, C. M. CORBET, E. A. DULKA, J. A. MITCHEN, AND T. J. KRIEGER. 2013. Ecological interactions between two broadly sympatric, cryptic species of dusky salamander (genus *Desmognathus*). Copeia 2013:499–506.

CARRETE, M., J. A. SÁNCHEZ-ZAPATA, J. F. CALVO, AND R. LANDE. 2005. Demography and habitat availability in territorial occupancy of two competing species. Oikos 108:125–136.

CASE, T. J., AND M. L. TAPER. 2000. Interspecific competition, environmental gradients, gene flow, and the coevolution of species' borders. American Naturalist 155:583–605.

CAVENDER-BARES, J., K. KOZAK, P. V. A. FINE, AND S. W. KEMBEL. 2009. The merging of community ecology and phylogenetic biology. Ecology Letters 12:693–715.

CODY, M. L. 1969. Convergent characteristics in sympatric species: a possible relation to interspecific competition and aggression. Condor 71:222–239.

DALLALIO, E. A., A. B BRAND, AND E. H. C. GRANT. 2017. Climate-mediated competition in a high-elevation salamander community. Journal of Herpetology 51:190–196.

DIRNBÖCK, T., F. ESSL, AND W. RABITSCH. 2011. Disproportional risk for habitat loss of high-altitude endemic species under climate change. Global Change Biology 17:990–996.

ELSEN, P. R., AND M. W. TINGLEY. 2015. Global mountain topography and the fate of montane species under climate change. Nature Climate Change 5:772–776.

FEDER, M. E. 1983. Integrating the ecology and physiology of plethodontid salamanders. Herpetologica 39:291–310.

FITZPATRICK, B. M., K. SHOOK, AND R. IZALLY. 2009. Frequency-dependent selection by wild birds promotes polymorphism in model salamanders. BMC Ecology 9:12.

GAUSE, G. F. 1934. The Struggle for Existence. Williams and Wilkins, USA.

GIFFORD, M. E., AND K. H. KOZAK. 2012. Islands in the sky or squeezed at the top? Ecological causes of elevational range limits in montane salamanders. Ecography 35:193–203.

GILMAN, S. E., M. C. URBAN, J. TEWKSBURY, G. W. GILCHRIST, AND R. D. HOLT. 2010. A framework for community interactions under climate change. Trends in Ecology and Evolution 25:325–331.

GRANT, A. H., T. S. RANSOM, AND E. B. LIEBGOLD. 2018a. Differential survival and effects of predation on a color polymorphic species, the red-backed salamander (*Plethodon cinereus*). Journal of Herpetology 52:127–135.

GRANT, E. H. C., A. B. BRAND, S. F. J. DE WEKKER, T. R. LEE, AND J. E. B. WOFFORD. 2018b. Evidence that climate sets the lower elevation range limit in a high-elevation endemic salamander. Ecology and Evolution 2018:1–10.

GREEN, J. 1818. Descriptions of several species of North American Amphibia, accompanied with observations. Journal of the Academy of Natural Sciences Philadelphia 1:348–359.

GRETHER, G. F., N. LOSIN, C. N. ANDERSON, AND K. OKAMOTO. 2009. The role of interspecific interference competition in character displacement and the evolution of competitor recognition. Biological Reviews 84:617–635.

GRIFFIS, M. R., AND R. G. JAEGER. 1998. Competition leads to an extinction-prone species of salamander: interspecific territoriality in a meta-population. Ecology 79:2494–2502.

GRIFFITHS, R. A., AND V. J. MYLOTTE. 1987. Microhabitat selection and feeding relations of smooth and warty newts, *Triturus vulgaris* and *T. cristatus*, at an upland pond in mid-Wales. Holarctic Ecology 10:1–7.

HAGEY, T. J., N. COLE, D. DAVIDSON, A. HENRICKS, L. L. HARMON, AND L. J. HARMON. 2016. Temporal variation in structural microhabitat use of *Phelsuma* geckos in Mauritius. Journal of Herpetology 50:102–107.

HIGHTON, R., AND R. D. WORTHINGTON. 1967. A new salamander of the genus *Plethodon* from Virginia. Copeia 1967:617–626.

HIJMANS, R. J., AND C. H. GRAHAM. 2006. The ability of climate envelope models to predict the effect of climate change on species distributions. Global Change Biology 12:2272–2281.

HILLERISLAMBERS, J., M. A. HARSCH, A. ETTINGER, K. R. FORD, AND E. J. THEOBALD. 2013. How will biotic interactions influence climate change-induced range shifts? Annals of the New York Academy of Sciences 1297:112–125.

ISAAC, J. L., J. J. VANDERWAL, C. N. JOHNSON, AND S. E. WILLIAMS. 2009. Resistance and resilience. Quantifying relative extinction risk in a diverse assemblage of tropical mammals. Diversity and Distributions 15:280–288.

JACOBS, J. 1994. Shenandoah salamander (*Plethodon shenandoah*) recovery plan. U.S. Department of the Interior, Fish and Wildlife Service, Region 5, USA.

JAEGER, R. G. 1970. Potential extinction through competition between two species of terrestrial salamanders. Evolution 24:632–642.

———. 1971a. Moisture as a factor influencing the distributions of two species of terrestrial salamanders. Oecologia 6:191–207.

———. 1971b. Competitive exclusion as a factor influencing the distributions of two species of terrestrial salamanders. Ecology 52:632–637.

———. 1972. Food as a limited resource in competition between two species of terrestrial salamanders. Ecology 53:535–546.

JAEGER, R. G., B. GOLLMANN, C. D. ANTHONY, C. R. GABOR, AND N. R. KOHN. 2016. Interspecific territoriality between *P. cinereus* and *P. shenandoah*. In Behavioral Ecology of the Eastern Red-Backed Salamander: 50 Years of Research. Oxford University Press, USA.

LANG, C., AND R. G. JAEGER. 2000. Defense of territories by male-female pairs in the red-backed salamander (*Plethodon cinereus*). Copeia 2000:169–177.

LOSOS, J. B. 2011. Convergence, adaptation, and constraint. Evolution 65:1827–1840.

LOTTER, F., AND N. J. SCOTT, JR. 1977. Correlation between climate and distribution of the color morphs of the salamander *Plethodon cinereus*. Copeia 4:681–690.

MACARTHUR, R. H. 1958. Population ecology of some warblers of northeastern coniferous forests. Ecology 39:599–619.

MACARTHUR, R., AND R. LEVINS. 1964. Competition, habitat selection, and character displacement in a patchy environment. Proceedings of the National Academy of Sciences of the United States of America 51:1207–1210.

MACKENZIE, D. I., J. D. NICHOLS, J. A. ROYLE, K. H. POLLOCK, L. L. BAILEY, AND J. E. HINES. 2006. Occupancy Estimation and Modeling: Inferring Patterns and Dynamics of Species Occurrence. Academic Press, USA.

MATHIS, A. 1991. Territories of male and female terrestrial salamanders: costs, benefits, and intersexual spatial associations. Oecologia 86:433–440.

MOORE, J. D., AND M. OUELLET. 2015. Questioning the use of an amphibian colour morph as an indicator of climate change. Global Change Biology 21:566–571.

MOUGI, A., AND K. NISHIMURA. 2005. Coexistence of competitive species with a stage-structured life cycle. Ecological Research 20:581–589.

MUÑOZ, D. J., K. M. HESED, E. H. C. GRANT, AND D. A. W. MILLER. 2016a. Evaluating within-population variability in behavior and fitness for the climate adaptive potential of a dispersal-limited species, *Plethodon cinereus*. Ecology and Evolution 6:8740–8754.

MUÑOZ, D. J., D. A. W. MILLER, C. SUTHERLAND, AND E. H. C. GRANT. 2016b. Using spatial capture-recapture to elucidate population processes and space-use in herpetological studies. Journal of Herpetology 50:570–581.

MYERS, E. M., AND D. C. ADAMS. 2008. Morphology is decoupled from interspecific competition in *Plethodon* salamanders in the Shenandoah Mountains, USA. Herpetologica 64:281–289.

PEARSON, R. G., AND T. P. DAWSON. 2003. Predicting the impacts of climate change on the distribution of species: are bioclimatic envelope models useful? Global Ecology and Biogeography 12:361–371.

PETRUZZI, E. E., P. H. NIEWIAROWSKI, AND F. B.-G. MOORE. 2006. The role of thermal niche selection in maintenance of a colour polymorphism in redback salamanders (*Plethodon cinereus*). Frontiers in Zoology 3:10.

PIGOT, A. L., AND J. A. TOBIAS. 2012. Species interactions constrain geographic range expansion over evolutionary time. Ecology Letters 16:330–338.

R CORE TEAM. 2018. R: a language and environment for statistical computing. Available from: http://www.R-project.org/.

RAXWORTHY, C. J., R. G. PEARSON, N. RABIBISOA, A. M. RAKOTONDRAZAFY, J. RAMANAMANJATO, A. P. RASELIMANANA, S. WU, R. A. NUSSBAUM, AND D. A. STONE. 2008. Extinction vulnerability of tropical montane endemism from warming and upslope displacement: a preliminary appraisal for the highest massif in Madagascar. Global Change Biology 14:1703–1720.

REITER, M. K., C. D. ANTHONY, AND C.-A. M. HICKERSON. 2014. Territorial behavior and ecological divergence in a polymorphic salamander. Copeia 3:481–488.

SAYLER, A. 1966. The reproductive ecology of the red-backed salamander, *Plethodon cinereus*, in Maryland. Copeia 1966:183–193.

SEXTON, J. P., P. J. MCINTYRE, A. L. ANGERT, AND K. J. RICE. 2009. Evolution and ecology of species range limits. Annual Review of Ecology, Evolution, and Systematics 40:415–436.

SHAH, A. A., B. A. GILL, A. C. ENCALADA, A. S. FLECKER, W. C. FUNK, J. M. GUAYASAMIN, B. C. KONDRATIEFF, N. L. POFF, S. A. THOMAS, K. R. ZAMUDIO, ET AL. 2017. Climate variability predicts thermal limits of aquatic insects across elevation and altitude. Functional Ecology 31:2118–2127.

SOBERÓN, J. 2007. Grinnellian and Eltonian niches and geographic distributions of species. Ecology Letters 10:1115–1123.

STRONG, D. R., JR. 1980. Null hypotheses in ecology. Synthese 43:271–285.

SUTHERLAND, C., D. J. MUNOZ, D. A. W. MILLER, AND E. H. CAMPBELL GRANT. 2016. Spatial capture-recapture: a promising method for analyzing data collected using artificial cover objects. Herpetologica 72:6–12.

THOMAS, C. D., A. CAMERON, R. E. GREEN, M. BAKKENES, L. J. BEAUMONT, Y. C. COLLINGHAM, B. F. N. ERASMUS, M. FERREIRA DE SIQUEIRA, A. GRAINGER, L. HANNAH, ET AL. 2004. Extinction risk from climate change. Nature 427:145–148.

THUILLER, W., S. LAVOREL, AND M. B. ARAÚJO. 2005. Niche properties and geographical extent as predictors of species sensitivity to climate change. Global Ecology and Biogeography 14:347–357.

TOBIAS, J. A., C. K. CORNWALLIS, E. P. DERRYBERRY, S. CLARAMUNT, R. T. BRUMFIELD, AND N. SEDDON. 2014. Species coexistence and the dynamics of phenotypic evolution in adaptive radiation. Nature 506:359–363.

VENESKY, M. D., A. HESS, J. A. DEMARCHI, A. WELL, J. MURONE, C.-A. M. HICKERSON, AND C. D. ANTHONY. 2015. Morph-specific differences in disease prevalence and pathogen-induced mortality in a terrestrial polymorphic salamander. Journal of Zoology 295:279–285.

WROBEL, D. J., W. F. GERGITS, AND R. G. JAEGER. 1980. An experimental study of interference competition among terrestrial salamanders. Ecology 61:1034–1039.

YACKULIC, C. B., J. REID, J. D. NICHOLS, J. E. HINES, R. DAVIS, AND E. FORSMAN. 2014. The roles of competition and habitat in the dynamics of populations and species distributions. Ecology 95:265–279.

SUPPLEMENTARY DATA

Supplementary data associated with this article can be found online at http://dx.doi.org/10.1670/15-040.s1.