

Potential Influence of Competition on Local Occurrence of the Cow Knob Salamander (*Plethodon punctatus*) and White-Spotted Slimy Salamander (*Plethodon cylindraceus*)

William D. Flint^{1,*}, Donald J. Brown^{2,3}, Amelia M. Hansen¹, and Jena M. Staggs³

Abstract - The Appalachian Mountains in eastern North America are a global biodiversity hotspot for woodland salamanders (genus *Plethodon*) and contain many range-restricted species endemic to areas of high elevation. Previous research indicates that interspecific competition could exert a strong influence on geographic distribution boundaries and local occurrence probabilities for high-elevation salamanders, but competition dynamics have not been assessed for most species. We investigated co-occurrence and competition dynamics between 2 large woodland salamanders that occur on Shenandoah Mountain in western Virginia and eastern West Virginia: the range-restricted *Plethodon punctatus* (Cow Knob Salamander) endemic to high elevations and the widespread *P. cylindraceus* (White-spotted Slimy Salamander). We performed salamander surveys along elevational transects across the zone of sympatry to estimate the relationship between elevation and relative abundance of each species and to assess if co-occurrence of the 2 species was random. We also performed laboratory experiments to characterize territorial behavior and determine factors that influenced probability of territory defense and invasion success. We found that relative abundance of Cow Knob Salamander and White-spotted Slimy Salamander increased and decreased with elevation, respectively, and the species were less likely to co-occur within the zone of sympatry than expected by chance. Our laboratory experiment indicated that White-spotted Slimy Salamanders were competitively dominant. Climatic changes on Shenandoah Mountain could increase competition pressure between the 2 species if the distribution and density of the White-spotted Slimy Salamander increases at the high elevations where the Cow Knob Salamander is currently most abundant.

Introduction

The Appalachian Mountains of eastern North America represent a global biodiversity hotspot for woodland salamanders (genus *Plethodon*; Kozak 2017). Many *Plethodon* species have highly restricted and disjunct geographic distributions, particularly species endemic to high elevations such as *P. shenandoah* Highton & Worthington (Shenandoah Salamander), *P. nettingi* Green (Cheat Mountain Salamander), and *P. hubrichti* Thurow (Peaks of Otter Salamander) (Petranka 1998, Powell et al. 2016). The potential influence of interspecific competition dynamics on fine-scale occurrence probabilities and geographic distribution boundaries of woodland salamanders has long been of interest to ecologists (e.g., Adams 2007,

¹Department of Biology, James Madison University, MSC 7801, Harrisonburg, VA 22807.

²Pacific Northwest Research Station, USDA Forest Service, Amboy, WA 98601. ³School of Natural Resources, West Virginia University, 1145 Evansdale Drive, 322 Percival Hall, Morgantown, WV 26506. *Corresponding author - flintwd@jmu.edu.

Grant et al. 2018, Hairston 1951). Previous studies have supported both competition (Adams 2007, Anthony et al. 1997, Deitloff et al. 2008, Marshall et al. 2004) and environmental conditions (Grant et al. 2018, Jaeger 1970, Jaeger et al. 2016) as likely factors influencing spatial distributions. The potential influence of interspecific competition dynamics on woodland salamander occurrence is likely species- and context-specific (Clay and Gifford 2016, Lyons et al. 2016).

Many woodland salamanders are known to establish surface territories and defend cover objects, such as rocks and coarse woody debris, within those territories (Anthony et al. 2017, Marvin 1998, Thurow 1976). A variety of factors have been found to influence competition dynamics, such as defender and competitor species, sex, body size, reproductive status, residency status, resource quality, and weather (e.g., Gabor and Jaeger 1995, Mathis et al. 2000, Ovaska 1988, Tornick 2010). Residents define and defend territories through several means, including marking territories with pheromones and performing aggressive acts towards intruders, such as raising their trunk, lunging, and biting (Anthony et al. 1997; Deitloff et al. 2008; Jaeger 1981, 1986; Marvin 1998; Nishikawa 1985). While competition has been widely observed in small *Plethodon* spp., large *Plethodon* spp. generally appear to be more aggressive (Thurow 1976), and highly aggressive competitor species could influence occurrence patterns of less aggressive species (Marshall et al. 2004).

Two large woodland salamanders, *Plethodon punctatus* Highton (Cow Knob Salamander; a member of the *P. wehrlei* Fowler and Dunn [Wherle's Salamander] species complex) and *P. cylindraceus* (Harlan) (White-spotted Slimy Salamander; a member of the *P. glutinosus* (Green) [Northern Slimy Salamander] species complex), occur in sympatry at mid-elevations on portions of Shenandoah Mountain in western Virginia and eastern West Virginia. Cow Knob Salamander is a mid- to high-elevation species with a highly restricted distribution primarily on Shenandoah Mountain with small disjunct populations on adjacent ridges (Highton 1988, Jacobsen et al. 2020), whereas White-spotted Slimy Salamander is a low- to high-elevation species with a comparatively large geographic distribution that extends from northern Virginia to South Carolina (Highton 1972, Powell et al. 2016). While the White-spotted Slimy Salamander is known to reach high elevations throughout much of its distribution (Downer 2009, Highton 1972), it appears to be absent from high elevations on Shenandoah Mountain where Cow Knob Salamander is present (Highton 1972, Tucker 1998), potentially indicating that competition with Cow Knob Salamander restricts the elevational distribution of White-spotted Slimy Salamander within their narrow band of sympatry. However, potential competition between these 2 species has not been investigated.

The purpose of this study was to investigate co-occurrence and competition dynamics of Cow Knob Salamander and White-spotted Slimy Salamander. We performed salamander surveys along elevational transects across the zone of sympatry on Shenandoah Mountain to estimate the relationship between elevation and relative abundance for each species and to assess if local co-occurrence of the 2 species was random. We performed laboratory experiments with adult males of each species to characterize territorial behavior and determine factors

that influenced probability of territory defense and invasion success. The results of this study further our knowledge of woodland salamander competition dynamics and improve our understanding of spatial occurrence relationships for these understudied species.

Field-Site Description

All our salamander transect surveys and collection of salamanders for competition trials occurred within the George Washington National Forest on Shenandoah Mountain in Rockingham County, VA, and Pendleton County, WV. Shenandoah Mountain is an ~110-km-long ridge within the Valley and Ridge physiographic province in western Virginia and Eastern West Virginia. The sandstone that makes up this mountain is often exposed along the ridges, and vast deposits of broken sandstone form talus slopes which are concentrated along steep drainages that cut deep coves into the sides of the mountain. These mid- to high-elevation talus slopes, particularly those that are the least exposed to the drying effects of the sun and wind, are the primary habitat for Cow Knob Salamander (Flint and Harris 2005, Jacobsen et al. 2020, Tucker 1998). White-spotted Slimy Salamanders occur throughout the low to mid-elevations of this mountain and can be found in a variety of habitats, but seem to also prefer rocky areas (W.D. Flint, pers. observ.).

Methods

Transect surveys

We conducted visual-encounter surveys between May and October of 2019 along north-facing talus slopes perpendicular to Forest Road 72 (Fig. 1). We chose this road because it bisects multiple northerly facing talus slopes from elevations starting at 600 m and extending to over 1000 m, which encompasses the elevational zone of overlap for Cow Knob Salamander and White-spotted Slimy Salamander (Downer 2009). We surveyed a total of 17 transects: 14 upslope from the road and 3 downslope from the road.

We conducted all surveys at night and either during or within 24 hrs of receiving at least 0.25 cm of rain as measured by the nearest National Oceanic and Atmospheric Administration rain gauge (<4.8 km from all transects surveyed) in order to maximize detection probability of the focal species (Flint and Harris 2005). Each survey began at least 1 hour after sunset and was completed by 0200 hrs. Surveyors walked in pairs upslope or downslope until they either reached the top of the ridge or the slope became too steep to continue, they reached the bottom of the ridge if traveling downslope, or they reached a terminal point for the talus (mean transect length = 320 m). The surveyor pairs attempted to stay within 5 m of one another and recorded their survey paths using handheld GPS units. We used headlamps to illuminate the ground and locate salamanders active on the surface. We identified to species and photographed all salamanders observed and recorded coordinates at the points of observation using Garmin GPSMAP 64S handheld GPS units (Olathe, KS).

Competition experiment

We conducted experimental trials to observe territorial behaviors and outcomes of pairing resident Cow Knob Salamanders and White-spotted Slimy Salamanders with both conspecific and heterospecific intruders. We collected 30 adult male salamanders of each species between May and July of 2008 from Shenandoah Mountain. We collected only adult males to avoid possible differences in territorial behavior related to sex and life stage (Jaeger and Forester 1993). The mean body mass of salamanders collected for this study (6.25 ± 1.51 g for White-spotted Slimy Salamander and 3.68 ± 0.81 g for Cow Knob Salamander) were representative of those observed from previous measurements of each species (WD. Flint, unpubl. data; P. Sattler, Liberty University, Lynchburg, VA, and J. Gibson, Averett University, Danville, VA, unpubl. data). We collected salamanders ~1 month prior to the competition experiments to avoid potential effects of stress related to capture and transportation, and to allow the salamanders time to acclimate to the controlled environment in which they were maintained. We held salamanders in individual plastic containers (15 cm x 16 cm x 10.5 cm) lined with moist filter paper and with a crumpled, unbleached paper towel moistened with tap water treated with Stress

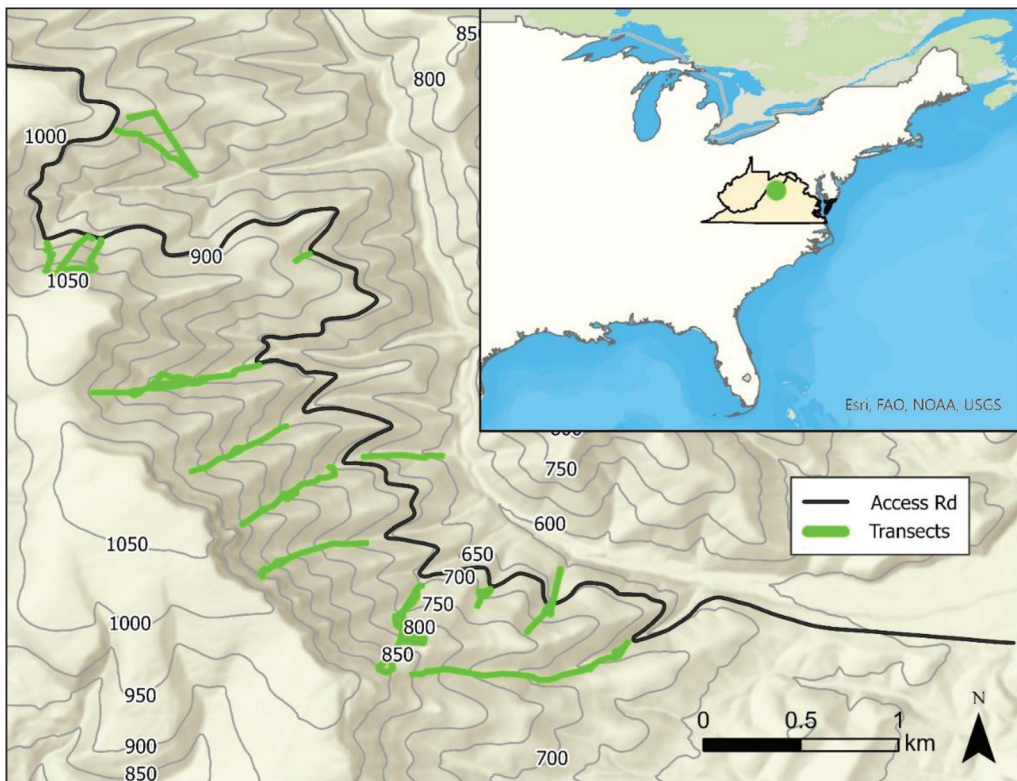


Figure 1. Locations of survey transects used to assess occurrence and co-occurrence dynamics of 2 large woodland salamanders that occur on Shenandoah Mountain in western Virginia and eastern West Virginia: *Plethodon punctatus* (Cow Knob Salamander) and *P. cylindraceus* (White-spotted Slimy Salamander). A nighttime visual-encounter survey was performed for each transect between May and October of 2019.

Coat[®] (Mars Fishcare, McLean, VA) to remove chlorine to serve as a cover object. We stored the salamanders in a temperature-controlled room at 16 °C with a natural light cycle and fed them 5–8 small crickets weekly.

We generally followed the approach of Marshall et al. (2004) for laboratory competition experiments; however, we only include the species-interaction trials here that are relevant to the objectives of this manuscript (Hansen 2009). Experimental chambers included 1 box (20 cm x 20 cm x 5 cm) placed into the center of a larger box (35 cm x 25 cm x 5 cm) (Fig. 2). We lined the bottom of the boxes with dampened filter paper that contained Stress Coat[®] to help the salamanders maintain their slime layer. The inner residential chamber had two 2.5-cm holes placed on 2 walls opposite of one another that acted as escape passages. We randomly selected salamander pairs without replacement. We used each salamander as a resident and an invader for a total of 81 trials. We did not pair salamanders based on body size so as to imitate encounters as they might arise in natural habitats.

For each trial, we placed the assigned resident inside of the residential chamber and covered the escape doors. The salamander was given 3 days to establish its territory. On the third day, we introduced the intruder. We first covered the resident with a petri dish and then placed the intruder inside the chamber and covered it with a petri dish. They remained covered for a period of 3 minutes to minimize stress caused during the intruder transfer. After opening the escape holes, we removed

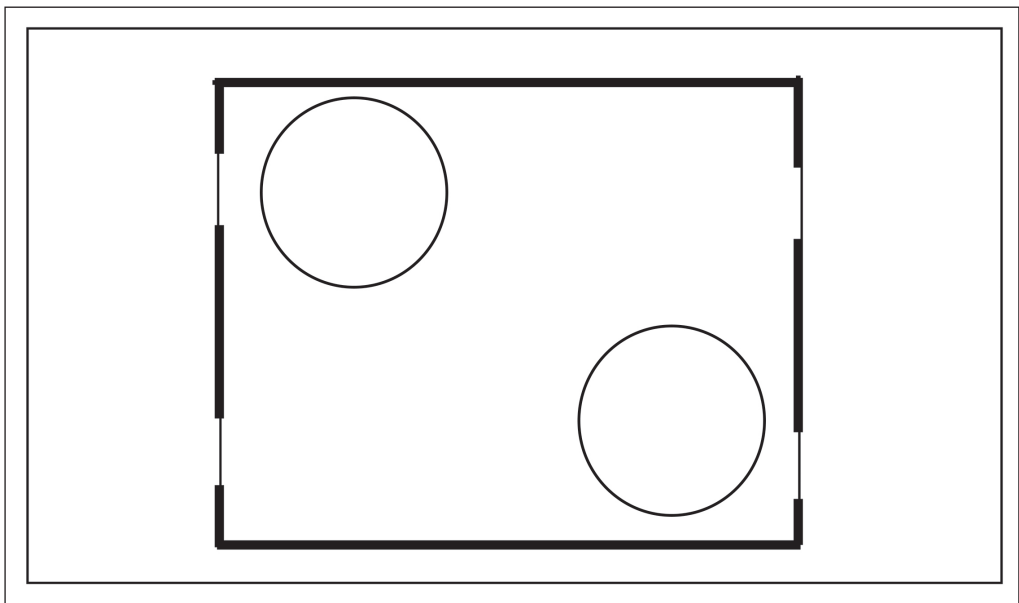


Figure 2. Diagram of the experimental chambers for competition trials between 2 large woodland salamanders that occur on Shenandoah Mountain in western Virginia and eastern West Virginia: *Plethodon punctatus* (Cow Knob Salamander) and *P. cylindraceus* (White-spotted Slimy Salamander). Experimental chambers included a residency chamber box (20 cm x 20 cm x 5 cm) with escape doors to a larger box (35 cm x 25 cm x 5 cm). The circles indicate the petri dishes used to cover salamanders prior to initiating each competition trial.

both petri dishes simultaneously to initiate the trial. For each trial, we recorded aggressive and submissive behaviors of the salamanders following Jaeger (1984), as well as the identity of the escaping salamander and time to escape (up to 15 min). We released all salamanders at their capture site following the completion of laboratory experiments.

Statistical analyses

Transect surveys. We used the GPS tracks from surveys to divide surveyor transects into a series of “sites” for modeling occurrence–elevation relationships and assessing whether species co-occurrence was random, negative, or positive. For the elevation data set, sites consisted of 15-m elevation bins within each of the 17 transects, resulting in a total of 226 sites. We summed the number of encounters for each species within each site as a metric of relative abundance. For the co-occurrence data set, sites consisted of 5-m linear-search-distance bins within each of the 17 transects. We treated the paired surveyor GPS tracks as the same site when the paths were ≤ 5 m from one another (84.2% of sites) and as separate sites when the paths were > 5 m from one another (15.8% of sites), resulting in a total of 1234 sites. We computed observed presence–absence of each species within each site to estimate occurrence and co-occurrence probabilities. We acknowledge that smaller distance bins could improve our ability to detect fine-scale co-occurrence relationships, but further reducing the bin size would greatly increase the potential for incorrect site designations due to GPS measurement error.

We used generalized additive models (GAM) to estimate the relationship between elevation and relative abundance for Cow Knob Salamanders and White-spotted Slimy Salamanders (Wood 2017). We chose to use relative abundance rather than presence–absence (i.e., non-detection) as our response variable to better reflect how prevalence of each species changed across the elevation gradient. We were unable to use contemporary models that explicitly account for detection probability because surveys were not replicated within sites and observers did not search the same area during surveys. We included elevation as a smoothed continuous predictor and included transect as a random effect. We modeled the smooth parameter using restricted maximum likelihood with Laplace approximation, which has been shown to reduce undersmoothing compared to generalized cross-validation (Reiss and Ogden 2009, Wood 2011). We assessed support for the elevation predictor using Wald tests (Zuur et al. 2009).

We tested whether co-occurrence of Cow Knob Salamanders and White-spotted Slimy Salamanders among sites was random, negative, or positive using the probabilistic model of species co-occurrence developed by Veech (2013). This model compares the observed frequency of co-occurrence to an expected frequency assuming random occurrence distributions for each species and computes the probability that observed species co-occurrence was greater and less than what could be observed by chance (Griffith et al. 2016, Veech 2013).

Competition experiment. We used logistic regression models to assess the influence of species and size (i.e., body mass) on territory defense/invasion success for Cow Knob Salamanders and White-spotted Slimy Salamanders. For analyses, we

used all trials where either the resident or invader were evicted from the territory (i.e., 95% of trials) to assess if species and size influenced eviction probability. We performed separate analyses with Cow Knob Salamander and White-spotted Slimy Salamander as the resident species, and coded successful trials (i.e., 1) as those in which the invader was evicted from the territory. For each analysis, we included invader species, invader size, and resident size as candidate predictors. We tested all possible additive and interaction model combinations and used a model-selection approach with Akaike information criterion corrected for small-sample size (AICc) to rank candidate models and assess relative variable importance (RVI) based on the sum of Akaike weights (Burnham et al. 2011, Giam and Olden 2016). For successful defenders and invaders, we also assessed the influence of species and size on time to eviction from the territory. For these analyses, we used linear regression models and followed the same analysis approach as above, except we included both resident species and invader species as candidate predictors and restricted the candidate model set to all possible additive combinations.

We assessed model assumptions using quantile–quantile and residual plots (Zuur et al. 2010) and removed 5 observations for the invader time-to-eviction analyses to satisfy the assumption of normality. We considered models with $\Delta\text{AICc} < 2$ to have strong support among the candidate model set (Symonds and Moussalli 2011). For the most-supported models, we computed the 85% confidence intervals (CI) of the beta coefficients (Arnold 2010) and considered there to be strong support for individual coefficient effects when CIs did not overlap zero (Halsey 2019). We performed all statistical analyses using program R (v. 4.2.1; The R Foundation for Statistical Computing, Vienna, Austria). We used the package ‘mgcv’ (v. 1.8-36; Wood 2017) to construct GAMs, the package ‘cooccur’ (v. 1.3; Griffith et al. 2016) to compute co-occurrence probabilities, the package ‘MuMIn’ (v. 1.46.0; Bartoń 2022) to perform model selections and compute RVI values, the packages ‘MASS’ (v. 7.3-57; Venables and Ripley 2002) and ‘AICmodavg’ (v. 2.3-1; Mazerolle 2023) to compute model CIs, and the packages ‘ggplot2’ (v. 3.3.6; Wickham 2023) and ‘cowplot’ (v. 1.1.1; Wilke 2019) to create graphs.

Results

Transect surveys

Across the 226 elevational sites, we documented a total of 246 Cow Knob Salamanders and 163 White-spotted Slimy Salamanders. We documented Cow Knob Salamanders at elevations of 682–1030 m (the maximum elevation surveyed), and White-spotted Slimy Salamanders at elevations of 613–980 m. The elevation predictor was strongly supported for both Cow Knob Salamanders ($P < 0.0001$) and White-spotted Slimy Salamanders ($P < 0.0001$). The GAM models estimated that relative abundance of Cow Knob Salamanders and White-spotted Slimy Salamanders increased and decreased with elevation, respectively (Fig. 3).

Across the 1234 linear-search-distance sites, we documented Cow Knob Salamanders and White-spotted Slimy Salamanders at 164 and 146 sites, respectively,

with co-occurrence at 10 sites. The model indicated that co-occurrence probability of the 2 species was negative, with an expected random co-occurrence frequency of 19 sites. The probability of greater and less co-occurrence by chance was 0.997 and 0.007, respectively.

Competition experiment

Of the 81 trials conducted, 77 (95%) resulted in an eviction, with 32 evicted residents and 45 evicted invaders. We did not observe biting or lunging behaviors in any of the trials; however, some aggressive contact and postures were observed. In a trial containing 2 White-spotted Slimy Salamanders, the invader displayed the “all raised” aggressive posture and walked over the resident before exiting the chamber. In another trial, containing 2 White-spotted Slimy Salamanders, the resident contacted the invader’s tail prior to the invader exiting the chamber. In a trial containing 2 Cow Knob Salamanders, the invader contacted the resident’s tail prior to the invader exiting the chamber. In another trail, a resident Cow Knob Salamander displayed a “head raised” posture prior to an invader White-spotted Slimy Salamander exiting the chamber.

Territory defense/invasion success. For resident Cow Knob Salamanders, invader size had the greatest RVI (0.67), followed by invader species (0.40) and resident size (0.33). Two candidate models received strong support for predictors influencing territory defense/invasion success (Table 1). The most supported model

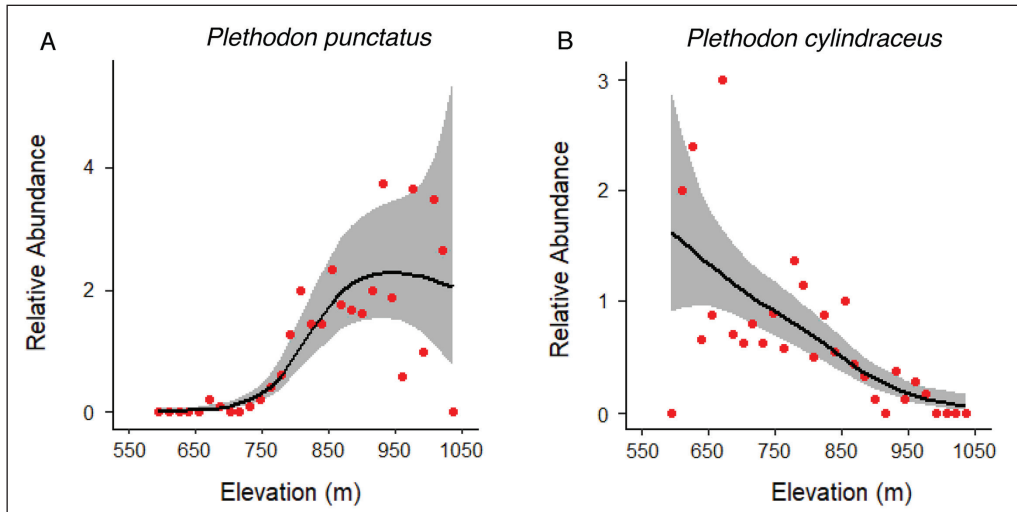


Figure 3. Estimated relationship between elevation and relative abundance of (A) *Plethodon punctatus* (Cow Knob Salamander) and (B) *Plethodon cylindraceus* (White-spotted Slimy Salamander) on Shenandoah Mountain, VA. We conducted elevational transect surveys across 17 sampling areas between 11 May and 8 Oct 2019. For this analysis, we split each transect into 15-m elevation bins and computed the total number of unique salamanders documented during the survey within each bin. We estimated relationships using generalized additive models with elevation included as a smoothed continuous predictor. Lines represent the expected salamander count per survey, gray bands represent 85% confidence intervals, and points represent the mean number of individuals observed for each elevation bin.

contained invader size ($w_i = 0.31$), and the second most supported model contained invader species ($w_i = 0.13$, $\Delta AICc = 1.83$). Both models received more support than the null model ($w_i = 0.09$, $\Delta AICc = 2.30$). For the invader size model, the probability of successful territory defense decreased as invader body mass increased (Fig. 4A), and the coefficient CI did not overlap 0 ($\beta = -0.38$, CI = -0.79, -0.03). For the invader species model, the probability of successful territory defense was 0.71 if the invader was a Cow Knob Salamander and 0.44 if the invader was a White-spotted Slimy Salamander (i.e., resident Cow Knob Salamanders were more successful defending against conspecific invaders). However, the coefficient CI overlapped 0 for this model ($\beta = -1.14$, CI = -2.62, 0.20).

For resident White-spotted Slimy Salamanders, resident size had the greatest RVI (0.50), followed by invader species (0.35) and invader size (0.31). Five candidate models received strong support for predictors influencing territory defense/invasion success (Table 1). However, the most supported model was the null model ($w_i = 0.24$), and the coefficient CIs overlapped 0 for all strongly supported models. Thus, estimated relationships for resident White-spotted Slimy Salamanders should

Table 1. Model-selection results for the influence of species and size (i.e., body mass) on territory defense/invasion success for *Plethodon punctatus* (Cow Knob Salamander) and *P. cylindraceus* (White-spotted Slimy Salamander), and on time to eviction for successful territory defenders and invaders. We included invader species (Species [I]), invader size (Size [I]), resident species (Species [R]; time to eviction analyses only), and resident size (Size [R]) as candidate predictors and tested all possible additive and interaction model combinations. We ranked models using Akaike information criterion corrected for small-sample size (AICc). Models with $\Delta AICc < 2$ are included in the table. Null models are shown as (.)

Model	AICc	$\Delta AICc$	w_i
Territory defense/invasion success			
Resident <i>Plethodon punctatus</i>			
Size (I)	56.33	0.00	0.31
Species (I)	58.16	1.83	0.13
Resident <i>Plethodon cylindraceus</i>			
(.)	49.21	0.00	0.24
Size (R)	49.52	0.31	0.21
Species (I)	50.58	1.37	0.12
Size (I)	51.04	1.83	0.10
Size (R) + Species (I)	51.08	1.87	0.09
Time to eviction			
Successful defenders			
Size (I)	475.74	0.00	0.31
Size (I) + Species (R)	476.62	0.87	0.20
Successful invaders			
Species (R)	298.14	0.00	0.19
Size (R)	298.37	0.23	0.17
Species (R) + Species (I)	299.06	0.92	0.12
Size (R) + Size (I)	299.72	1.59	0.09
Species (R) + Size (I)	299.79	1.65	0.08
Species (I) + Size (R)	299.82	1.68	0.08

be interpreted with caution. The most supported predictor model contained resident size ($w_i = 0.21$, $\Delta AICc = 0.31$) and estimated that the probability of successful territory defense decreased as resident mass increased ($\beta = -0.34$, $CI = -0.91, 0.13$). The second most supported predictor model contained invader species ($w_i = 0.12$, $\Delta AICc = 1.37$) and estimated that the probability of successful territory defense was 0.70 if the invader was a Cow Knob Salamander and 0.54 if the invader was a White-spotted Slimy Salamander ($\beta = -0.67$, $CI = -2.11, 0.74$).

Time to eviction. For successful territory defenders, invader size had the greatest RVI (0.89), followed by resident species (0.41), invader species (0.29), and resident size (0.25). Two candidate models received strong support, and both models were more supported than the null model ($w_i = 0.01$, $\Delta AICc = 6.93$; Table 1). The most supported model contained invader size ($w_i = 0.31$) and estimated that time to eviction increased with invader body mass ($\beta = 22.52$, $CI = 8.03, 37.00$) (Fig. 4B). The second most supported model contained invader size + resident species and estimated that time to eviction increased with invader body mass ($\beta = 22.52$, $CI = 8.03, 37.00$) and increased when the invader was White-spotted Slimy Salamander ($\beta = 28.80$, $CI = -18.79, 76.38$; note invader species coefficient CI overlapped 0).

For successful territory invaders, resident species had the greatest RVI (0.55), followed by resident size (0.49), invader species (0.32), and invader size (0.27). Six candidate models received strong support, and all 6 models were more supported than the null model ($w_i = 0.05$, $\Delta AICc = 2.54$; Table 1). The most supported model contained resident species ($w_i = 0.19$) and estimated that time to eviction increased by 52.9 seconds when the resident was a White-spotted Slimy Salamander ($\beta = 52.91$, $CI = 5.03, 100.78$). The second most supported model contained resident

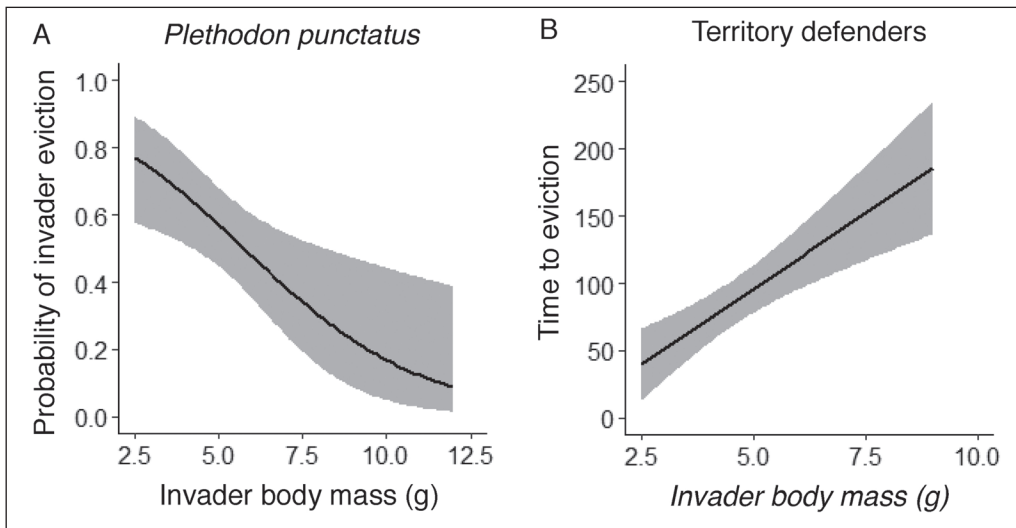


Figure 4. Influence of invader size (body mass) on (A) probability of invader eviction from territories held by resident *Plethodon punctatus* (Cow Knob Salamander) and (B) time to eviction for successful territory defenders. The estimated relationships were derived from logistic regression (A) and linear regression (B) models using results from laboratory trials conducted in 2008.

size ($w_i = 0.17$, $\Delta AICc = 0.23$) and estimated that time to eviction increased with resident body mass ($\beta = 14.60$, $CI = 1.05, 28.16$). Coefficient confidence intervals overlapped 0 for the remaining 3 models with strong support.

Discussion

We investigated whether interspecific competition may be influencing the local distributions of 2 large woodland salamander species on Shenandoah Mountain. We verified empirically that relative abundance of Cow Knob Salamanders and White-spotted Slimy Salamanders increased and decreased with increased elevation, respectively, consistent with our expectations based on previous studies (Highton 1972, Jacobsen et al. 2020). Our study also confirmed that the 2 species are sympatric across a portion of their elevation gradients (682–980 m), with the area of greatest overlap being at 750–850 m. We note that our study was conducted on north-facing talus slopes, and both the elevational ranges and zone of sympatry between Cow Knob Salamanders and White-spotted Slimy Salamanders may shift for more southerly aspect slopes and areas devoid of talus.

For woodland salamanders, it is not uncommon for the elevational distributions of species endemic to high elevations to partially overlap with lower-elevation congeners (e.g., Grant et al. 2018, Kroschel et al. 2014). Generally, the extent of the zone of sympatry is reflective of the intensity of the competition between the 2 species (Clay and Gifford 2016, Hairston 1980). Our co-occurrence analysis results suggest that Cow Knob Salamanders and White-spotted Slimy Salamanders may be competing for space, and our laboratory experiment results suggest that White-spotted Slimy Salamander is competitively dominant. Like other woodland salamanders endemic to high elevations, physiological constraints likely influence the lower distribution boundary of Cow Knob Salamander (Gifford and Kozak 2012, Lyons et al. 2016, Markle and Kozak 2018). However, our study indicates that competition with White-spotted Slimy Salamander may also be a factor limiting the lower-elevation boundary of Cow Knob Salamander.

Our laboratory competition experiment revealed that adult males of both White-spotted Slimy Salamander and Cow Knob Salamander may exhibit territorial behavior and will defend territories both intra- and interspecifically. Overall, White-spotted Slimy Salamanders were more successful than Cow Knob Salamanders at both defense and invasion of territories. This advantage may be due to differences in size since White-spotted Slimy Salamanders included in our study were larger on average than Cow Knob Salamanders, and we found that size was one of the most important predictors for successfully defending or invading territories. However, while woodland salamanders endemic to high elevations are usually smaller in size than their lower-elevation congeners, they are often more aggressive, especially when their zone of sympatry is narrow (Anthony et al. 1997, Arif et al. 2007, Gifford and Kozak 2012, Nishikawa 1985). Given that we have not observed White-spotted Slimy Salamanders at high elevations where Cow Knob Salamanders are present, we were surprised that White-spotted Slimy Salamander was the dominant competitor in this study. However, White-spotted

Slimy Salamander was also found to be more aggressive than the high-elevation woodland salamander *P. montanus* Highton and Peabody (Northern Gray-cheeked Salamander) in another lab study (Clay and Gifford 2016), although White-spotted Slimy Salamanders and Northern Gray-cheeked Salamanders occupy a much wider elevational zone of sympatry (~800 m; Clay and Gifford 2016) than White-spotted Slimy Salamanders and Cow Knob Salamanders. We speculate that while the White-spotted Slimy Salamander appears to be the dominant competitor, Cow Knob Salamanders may be able to exclude White-spotted Slimy Salamanders from high elevations through interference competition due to being numerically dominant, as was suggested by Hairston (1980) for *P. jordani* Blatchley (Jordan's Salamander) and Northern Slimy Salamander. In addition, our elevational transect data suggests that higher elevations in our study area could represent suboptimal climatic conditions for White-spotted Slimy Salamanders, but additional research in the study region is needed to confirm this relationship in the absence of potential competition with Cow Knob Salamanders.

In conclusion, our study assessed spatial and behavioral relationships between 2 narrowly sympatric large woodland salamanders, Cow Knob Salamander and White-spotted Slimy Salamander. We found that local co-occurrence of the species was non-random with a negative co-occurrence probability, and the species were behaviorally competitive in laboratory trials, indicating that interspecific competition may influence the elevational distributions and local density of the 2 species within their zone of sympatry. As the climate warms, the elevational distribution of Cow Knob Salamander is projected to constrict (Jacobsen et al. 2020), and based on the elevational relationship estimated in this study, we expect the occurrence and density of White-spotted Slimy Salamanders at high elevations will likely increase, as is being documented with other primarily mid-elevation species (e.g., Moskwik 2014, Rucker et al. 2022). As the Cow Knob Salamander is a species of conservation concern that is patchily distributed along a single mountain range, the species would benefit from creation of a long-term monitoring program that can detect changes to the distribution and abundance of both species, thus providing the information needed to determine if and when active management actions are needed to maximize probability of long-term persistence for Cow Knob Salamanders.

Acknowledgments

We thank students at James Madison University for assistance with field data collection, especially Ashley Hodge and Jack Doss. Donald Brown received support from the Northern Research Station and Pacific Northwest Research Station of the USDA Forest Service. We thank Adrienne Brand, 2 anonymous reviewers, and editor Val Titus for providing suggestions that improved the quality of this manuscript. Salamander sampling, handling, and laboratory experiments were approved by the Virginia Department of Game and Inland Fisheries, West Virginia Division of Natural Resources, and James Madison University Institutional Animal Care and Use Committee (protocol 08.04.18). Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the US Government. The findings and conclusions in this publication are those of the authors and should not be construed to represent any official US Department of Agriculture or US Government determination or policy.

Literature Cited

- Adams, D.C. 2007. Organization of *Plethodon* salamander communities: Guild-based community assembly. *Ecology* 88:1292–1299.
- Anthony, C.D., J.A. Wicknick, and R.G. Jaeger. 1997. Social interactions in two sympatric salamanders: Effectiveness of a highly aggressive strategy. *Behaviour* 134:71–88.
- Anthony, C.D., K. Jaworski, M. Messner, and C.-A.M. Hickerson. 2017. Differences in prey availability within the territories of striped and unstriped Eastern Red-backed Salamanders (*Plethodon cinereus*). *Herpetological Review* 48:509–514.
- Arif, S., D.C. Adams, and J.A. Wicknick. 2007. Bioclimatic modelling, morphology, and behaviour reveal alternative mechanisms regulating the distributions of two parapatric salamander species. *Evolutionary Ecology Research* 9:843–854.
- Arnold, T.W. 2010. Uninformative parameters and model selection using Akaike's information criterion. *Journal of Wildlife Management* 74:1175–1178.
- Bartoń, K. 2022. MuMIn: Multi-model inference. Version 1.46.0. Available online at <<https://cran.r-project.org/web/packages/MuMIn/index.html>>. Accessed 1 June 2026.
- Burnham, K.P., D.R. Anderson, and K.P. Huyvaert. 2011. AIC model selection and multi-model inference in behavioral ecology: Some background, observations, and comparisons. *Behavioral Ecology and Sociobiology* 65:23–35.
- Clay, T., and M. Gifford. 2016. Thermal sensitivity of aggression in two terrestrial salamanders, *Plethodon cylindraceus* and *P. montanus*. *Ethology* 122:127–133.
- Deitloff, J., D.C. Adams, B.F.M. Olechnowski, and R.G. Jaeger. 2008. Interspecific aggression in Ohio *Plethodon*: Implications for competition. *Herpetologica* 64:180–188.
- Downer, H.R. 2009. Distribution of woodland salamanders of the Valley and Ridge in West Virginia. M.Sc. Thesis. Marshall University, Huntington, WV. 137 pp.
- Flint, W.D., and R.N. Harris. 2005. The efficacy of visual encounter surveys for population monitoring of *Plethodon punctatus* (Caudata: Plethodontidae). *Journal of Herpetology* 39:578–584.
- Gabor, C.R., and R.G. Jaeger. 1995. Resource quality affects the agonistic behaviour of territorial salamanders. *Animal Behaviour* 49:71–79.
- Giam, X., and J.D. Olden. 2016. Quantifying variable importance in a multimodel inference framework. *Methods in Ecology and Evolution* 7:388–397.
- 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.
- Grant, E.H.C., A.B. Brand, S.F.J. De Wekker, T.R. Lee, and J.E.B. Wofford. 2018. Evidence that climate sets the lower elevation range limit in a high-elevation endemic salamander. *Ecology and Evolution* 8:7553–7562.
- Griffith, D.M., J.A. Veech, and C.J. Marsh. 2016. cooccur: Probabilistic species co-occurrence analysis in R. *Journal of Statistical Software* 69:1–17.
- Hairston, N.G. 1951. Interspecies competition and its probable influence upon the vertical distribution of Appalachian salamanders of the genus *Plethodon*. *Ecology* 32:266–274.
- Hairston, N.G. 1980. The experimental test of an analysis of field distributions: Competition in terrestrial salamanders. *Ecology* 61:817–826.
- Halsey, L.G. 2019. The reign of the *P*-value is over: What alternative analyses could we employ to fill the power vacuum? *Biology Letters* 15:article 20190174.
- Hansen, A.M. 2009. Potential competition between two narrowly sympatric salamander species (*Plethodon punctatus* and *Plethodon cylindraceus*). Unpublished report. James Madison University, Harrisonburg, VA. 32 pp.

- Highton, R. 1972. Distributional interactions among eastern North American salamanders of the genus *Plethodon*. Pp. 139–188, *In* P.C. Holt (Ed.). The Distributional History of the Biota of the Southern Appalachians. Part III: Vertebrates. Virginia Polytechnic Institute and State University Research Division Monograph, Blacksburg, VA. 306 pp.
- Highton, R. 1988. Catalogue account of *Plethodon punctatus*. Catalogue of American Amphibians and Reptiles 414:1–2
- Jacobsen, C.D., D.J. Brown, W.D. Flint, T.K. Pauley, K.A. Buhlmann, and J.C. Mitchell. 2020. Vulnerability of high-elevation endemic salamanders to climate change: A case study with the Cow Knob salamander (*Plethodon punctatus*). Global Ecology and Conservation 21:article e00883.
- Jaeger, R.G. 1970. Potential extinction through competition between two species of terrestrial salamanders. Evolution 24:632–642.
- Jaeger, R.G. 1981. Dear enemy recognition and costs of aggression between salamanders. American Naturalist 117:962–974.
- Jaeger, R.G. 1984. Agonistic behavior in the Red-backed Salamander. Copeia 1984:309–314.
- Jaeger, R.G. 1986. Pheromonal markers as territorial advertisement by terrestrial salamanders. Pp. 191–203, *In* D. Duvall, D. Muller-Schwarze, and R.M. Silverstein (Eds.). Chemical Signals in Vertebrates 4: Ecology, Evolution, and Comparative Biology. Plenum Press, New York, NY. 754 pp.
- Jaeger, R.G., and D.C. Forester. 1993. Social behavior of plethodontid salamanders. Herpetologica 49:163–175.
- Jaeger, R.G., B. Gollman, C.D. Anthony, C.R. Gabor, and N. Kohn. 2016. Behavioral Ecology of the Eastern Red-backed Salamander: 50 Years of Research. Oxford University Press, New York, NY. 248 pp.
- Kozak, K.H. 2017. What drives variation in plethodontid salamander species richness over space and time? Herpetologica 73:220–228.
- Kroschel, W.A., W.B. Sutton, C.J.W. McClure, and T.K. Pauley. 2014. Decline of the Cheat Mountain Salamander over a 32-year monitoring period and the potential influence of competition from a sympatric species. Journal of Herpetology 48:415–422.
- Lyons, M.P., D.B. Shepard, and K.H. Kozak. 2016. Determinants of range limits in montane woodland salamanders (genus *Plethodon*). Copeia 104:101–110.
- Markle, T.M., and K.H. Kozak. 2018. Low acclimation capacity of narrow-ranging thermal specialists exposes susceptibility to global climate change. Ecology and Evolution 8:4644–4656.
- Marshall, J.L., C.D. Camp, and R.G. Jaeger. 2004. Potential interference competition between a patchily distributed salamander (*Plethodon petraeus*) and a sympatric congener (*Plethodon glutinosus*). Copeia 3:488–495.
- Marvin, G.A. 1998. Territorial behavior of the plethodontid salamander *Plethodon kentucki*: Influence of habitat structure and population density. Oecologia 114:133–144.
- Mathis, A., D.W. Schmidt, and K.A. Medley. 2000. The influence of residency status on agonistic behavior of male and female Ozark Zigzag Salamanders, *Plethodon angusticlavius*. American Midland Naturalist 143:245–249.
- Moskwick, M. 2014. Recent elevational range expansions in plethodontid salamanders (Amphibia: Plethodontidae) in the southern Appalachian Mountains. Journal of Biogeography 41:1957–1966.
- Mazerolle, M.J. 2023. AICcmodavg: Model selection and multimodel inference based on (Q)AIC(c). Version 2.3-1. Available online at <<https://cran.r-project.org/web/packages/AICcmodavg/index.html>>. Accessed 1 June 2026.

- Nishiwaka, K.C. 1985. Competition and the evolution of aggressive behavior in two species of terrestrial salamanders. *Evolution* 39:1282–1294.
- Ovaska, K. 1988. Spacing and movements of the salamander *Plethodon vehiculum*. *Herpetologica* 44:377–386.
- Petranka, J.W. 1998. Salamanders of the United States and Canada. Smithsonian Institution Press, Washington, DC. 587 pp.
- Powell, R., R. Conant, and J.T. Collins. 2016. Peterson Field Guide to Reptiles and Amphibians of Eastern and Central North America. Fourth Edition. Houghton Mifflin Harcourt, New York, NY. 512 pp.
- Reiss, P.T., and R.T. Ogden. 2009. Smoothing parameter selection for a class of semiparametric linear models. *Journal of the Royal Statistical Society B: Statistical Methodology* 71:505–523.
- Rucker, L.E., D.J. Brown, M.B. Watson, and T.K. Pauley. 2022. Long-term occupancy dynamics of the threatened Cheat Mountain Salamander and its competitors in relation to linear habitat fragmentation. *Forest Ecology and Management* 505:article 119847.
- Symonds, M.R.E., and A. Moussalli. 2011. A brief guide to model selection, multimodel inference, and model averaging in behavioural ecology using Akaike's information criterion. *Behavioral Ecology and Sociobiology* 65:13–21.
- Thurrow, G. 1976. Aggression and competition in eastern *Plethodon* (Amphibia, Urodela, Plethodontidae). *Journal of Herpetology* 10:277–291.
- Tornick, J.K. 2010. Factors affecting aggression during nest guarding in the Eastern Red-backed Salamander (*Plethodon cinereus*). *Herpetologica* 66:385–392.
- Tucker, R.B. 1998. Ecology and natural history of the Cow Knob Salamander, *Plethodon punctatus*, in West Virginia. M.Sc. Thesis. Marshall University, Huntington, WV. 119 pp.
- Veech, J.A. 2013. A probabilistic model for analysing species co-occurrence. *Global Ecology and Biogeography* 22:252–260.
- Venables, W.N., and B.D. Ripley. 2002. Modern Applied Statistics with S. Fourth edition. Springer, New York, NY. 495 pp.
- Wickham, H. 2016. ggplot2: Elegant graphics for data analysis. Springer-Verlag, New York, NY. 260 pp.
- Wilke, C.O. 2019. Fundamentals of Data Visualization: A Primer on Making Informative and Compelling Figures. O'Reilly Media Inc., Sebastopol, CA. 387 pp.
- Wood, S.N. 2011. Fast stable restricted maximum likelihood and marginal likelihood estimation of semiparametric generalized linear models. *Journal of the Royal Statistical Society B: Statistical Methodology* 73:3–36.
- Wood, S.N. 2017. Generalized Additive Models: An Introduction with R. 2nd Edition. Chapman and Hall/CRC Press, Boca Raton, FL. 496 pp.
- Zuur, A.F., E.N. Ieno, N.J. Walker, A.A. Saveliev, and G.M. Smith. 2009. Mixed-effects Models and Extensions in Ecology with R. Springer, New York, NY. 596 pp.
- Zuur, A.F., E.N. Ieno, N.J. Walker, A.A. Saveliev, and G.M. Smith. 2010. A protocol for data exploration to avoid common statistical problems. *Methods in Ecology and Evolution* 1:3–14.