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# AN EXPERIMENTAL APPROACH TO UNDERSTANDING ELEVATION LIMITS IN THE NORTHERN GRAY-CHEEKED SALAMANDER, *PLETHODON MONTANUS*

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**Abstract.**—As global temperatures rise, there is an urgency to understand the limiting factors of ranges of species and how those factors vary across the range to develop predictions of shifts in spatial distributions. A long-standing hypothesis postulates that, for Northern Hemisphere species, abiotic factors, such as temperature, limit northern or higher elevation extents, while biotic factors like competition limit the southern or lower elevation range edges. Recent evidence, however, suggests that salamanders may follow the opposite of this general trend, although experimental tests of this hypothesis are lacking. Therefore, we determined the role of origin population (i.e., where individuals were captured) and transplant location (i.e., where individuals were placed) on survival, growth, and reproduction using a reciprocal transplant experiment across an elevational gradient with the Northern Gray-cheeked Salamander (*Plethodon montanus*). We collected juveniles from each of three elevations and transplanted them within mesocosms such that each origin population was represented within each transplant location. We found that individuals transplanted to low elevations had lower survival and growth rates compared to those moved to mid or high elevations regardless of origin location; whereas, individuals originating from low elevations had the highest survival regardless of their transplant location. Our study provides experimental support that conditions found at lower elevations limit the distribution of *P. montanus* but populations at the lower edge may feature adaptive traits that increase survival. Although climate change is predicted to reduce environmental suitability for this species especially at lower elevations, these traits may be important to mitigating extirpations.

**Key Words.**—abiotic; growth; maturation; range edges; reciprocal transplant; survival

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## INTRODUCTION

The North-South Hypothesis is a long-standing macroecological hypothesis (Darwin 1859; MacArthur 1972), which posits that abiotic conditions determine higher latitude or elevation range limits of a species, while biotic conditions determine the equator-ward or lower elevation range limit and has been shown across a range of taxa (Dobzhansky 1950; MacArthur 1972; Parmesan et al. 2005; reviewed in Schemske et al. 2009; Hargreaves et al. 2014). Lower elevations or equatorial habitats are often species-rich, and the interactions that develop among species, such as competition or parasitism, can exclude some species; whereas, physiological restrictions, such as freeze tolerance or growing days, can limit a species from habitats in either higher elevations or higher latitudes (Gaston 2003). For species in the Northern Hemisphere, this means that northern or higher elevation populations should be more constrained by abiotic factors, such as temperature; whereas, southern or lower elevation populations

should be more constrained by biotic factors, such as competition. However, recent studies suggest that at least some species of salamanders may not conform to this general trend and that the opposite is more likely (Gifford and Kozak 2012; Cunningham et al. 2016; Lyons et al. 2016; but see Hairston 1980; Crespi et al. 2003).

Understanding the mechanisms responsible for range limits is also of concern for conservation biologists given the current rate of global climate change (Loarie et al. 2009). Mean global temperatures have increased by 0.6° C during the last century and are expected to increase 2–4° C by 2100 (Intergovernmental Panel on Climate Change 2014). As a response to contemporary changes in climate, amphibians have shown shifts in breeding phenology (Beebee 1995; Reading 1998; Gibbs and Breisch 2001; Green 2016; Sheridan et al. 2018), geographic range limits (Pounds et al. 1999; Seimon et al. 2007), and body size (Reading 2007; Caruso et al. 2014; but see Connette et al. 2015). Environmental suitability models predict 50–100% reduction in suitable



FIGURE 1. A Northern Gray-cheeked Salamander (*Plethodon montanus*) in Pisgah National Forest, North Carolina, USA. (Photographed by Nicholas Caruso).

climate for salamanders in the southern Appalachian Mountains (Milanovich et al. 2010), which is a major amphibian hotspot (Rissler and Smith 2010). Although potentially suitable future habitat may exist, many species lack the ability to disperse through the intervening lower suitability habitat, especially crossing lower elevation regions, in the face of climate change (Bernardo and Spotila 2006; Gifford and Kozak 2012; Lyons et al. 2016).

Although studies examining range limits among different species are not uncommon (Schemske et al. 2009), relatively few examine factors that limit boundaries of a species at different portions its range (reviewed in Cahill et al. 2014; but see Cunningham et al. 2009). Determining how and why the demography of a species changes along gradients of environmental conditions across their range can offer insights into potential future responses to changes in climate (e.g., Swab et al. 2015). Ecological gradients can result in lowered resource availability or quality at edges of the distribution of a species. As a consequence, populations that are not adapted to conditions found at the periphery of the distribution of a species typically suffer from lower densities and increased spatial isolation or *de facto* fragmentation (Brown et al. 1995; Thomas and Kunin 1999) because of diminished condition or death of individuals (Hutchins 1947; Gaston 2003). Under future warming, expectations are that distributions of species will likely shift towards higher latitudes and elevations (Parmesan and Yohe 2003). For example, warmer and drier conditions currently exclude montane salamanders from low elevations (Gifford and Kozak 2012), and environmental suitability models for the Appalachian region predict the greatest loss of biodiversity at lower elevations and southern latitudes for these amphibians (Milanovich et al. 2010). However, salamanders found at the lower elevations can minimize the energetic costs associated with warmer, drier conditions

through locally adapted traits, including physiological acclimatization and shifts in behavior (Riddell and Sears 2015; Riddell et al. 2018). If these local adaptations allow populations to persist in warmer climates, then immediate losses under future warming predicted by suitability models are likely overestimates (Riddell et al. 2018). Therefore, as global climates continue to shift, predicting the persistence of populations, especially those that are isolated, will require an understanding of the relationship between climate and population vital rates (e.g., survival; Buckley et al. 2010; Urban et al. 2016) as well as understanding the extent to which populations can respond to changes via local adaptation or plasticity (Parmesan 2006; Merilä and Hendry 2014; Urban et al. 2014).

Studies conducted across elevational gradients provide a tractable way to understand how and why the demography of a species varies along gradients of current environmental conditions. Furthermore, by translocating individuals from a given populations to other elevations along the gradient, we can test the extent to which populations are adapted to their local environment and are able to respond to different conditions. As such, we used a reciprocal transplant experiment across an elevational gradient to explore the role of the elevation-specific environmental conditions on the range limits of the Northern Gray-cheeked Salamander (*Plethodon montanus*; Fig. 1). We investigated the role of origin population (i.e., where individuals were captured) and transplant location (i.e., where individuals were placed) on three relevant population responses: survival, growth, and maturation rate, to test the hypothesis that conditions at the warmer range edge (i.e., lower elevations) limits the distribution of montane salamanders. We asked the following question: How do the origin location, transplant location, and initial body condition of individuals influence survival, growth rate, and maturation? If conditions found at the lower and warmer range edge limits the range of *P. montanus* we expected to find lowest growth, survival, and maturation at the lower elevation sites whereas these demographic rates would be highest at the higher elevation sites. If montane salamanders do best at their origin population, we would expect to find increased survival, growth, and maturation for individuals at those origin locations compared to individuals transplanted to a non-origin location.

## MATERIALS AND METHODS

**Focal species.**—*Plethodon montanus* is terrestrial salamander that is found in montane (generally above 1,000 m and have been collected at elevations as high as 1,670 m) regions of western North Carolina and southwestern Virginia, USA. This species, like other

plethodontids, lacks lungs, and as such requires cool, moist conditions to facilitate gas exchange across its skin (Feder 1983). Not surprisingly, temperature and precipitation patterns have been shown to be associated with various aspects of plethodontid ecology and demography, such as foraging (Ovaska and Gregory 1989), fecundity (Milanovich et al. 2006), and survival and growth (Caruso and Rissler 2019). *Plethodon montanus* is currently classified as a species of Least Concern by the International Union for Conservation of Nature (Amphibian Specialist Group 2014), but deforestation (Petranka et al. 1993) and future changes in climate (Milanovich et al. 2010) threaten populations.

**Reciprocal transplant experiment.**—From 6 June to 1 October 2015, we conducted a transplant experiment in Pisgah National Forest, which is located in the Southern Appalachian mountains in western North Carolina. We collected salamanders from low (about 1,000 m; 82.4258°W, 36.0328°N), mid (about 1,250 m; 82.1417°W, 36.1372°N), and high (about 1,450 m; 82.0917°W, 36.0928°N) elevations and reciprocally transplanted them to the same low, mid, and high elevation sites. Thus, each origin population was represented within each transplant location. Average annual temperatures varied predictably among these three elevations; the low site was the warmest (10.8° C), followed by the mid (9.6° C) and high elevation sites (8.8° C); whereas, annual precipitation was lowest at the mid elevation site (1,346.8 mm) and higher at the low (1,463.8 mm) and high (1,394.1 mm) elevation sites (Parameter-elevation Regressions on Independent Slopes Model [PRISM] Climate Group, Oregon State University. Available from <http://prism.oregonstate.edu> [Accessed 26 March 2017]). We established two replicate sites within each transplant elevation, with each replicate site containing 18 mesocosms (36 per elevation) for a total of 108 mesocosms. We chose replicate sites based on proximity to established mark-recapture sites (Caruso and Rissler 2019), were approximately 200–400 m apart, and were on northwestern aspects with similar (about 85–92%) canopy coverage. Each mesocosm consisted of a single 53 × 43 × 30 cm polyethylene tub that had the same number and size of holes drilled along the bottom and side for drainage (Cunningham et al. 2009). We filled each mesocosm with a layer of approximately 10 cm of soil, then a layer of approximately 2 cm of leaf litter (each gathered from the respective transplant site) and one 30 × 15 × 5 cm untreated pine cover board in the middle of each mesocosm. To maintain consistency among mesocosms within each experimental site, we homogenized the collected soil and leaf litter separately prior to adding to each mesocosm.

After establishing the mesocosms, we collected

36 juvenile salamanders (30–45 mm snout-vent length [SVL]) from each origin population. We chose salamanders of this size because they could potentially reach reproductive maturity by the end of the experiment based on the time to maturation for Jordan's Salamander (*P. jordani*; Hairston 1983). Because the sex of juvenile salamanders is impossible to determine without dissection, we assumed a 1:1 sex ratio. We kept animals in a cooler, maintained between 15–20° C, for approximately 36 h before the start of the experiment. Immediately before beginning the experiment, we measured the SVL (tip of the snout to posterior margin of the vent) and tail length using calipers (to the nearest 0.1 mm), and weighed each animal using a spring scale (to the nearest 0.1 g). We took all measurements while the animal was secured in a new plastic bag to ensure consistent measurements and reduce probability of disease transmission from potentially contaminated equipment. We randomly assigned an equivalent number of animals to a transplant elevation (low, mid, and high) and replicate site within transplant location (1 or 2) such that each transplant location contained an equal number of individuals from each origin population and vice-versa. After adding a single salamander to each mesocosm, we covered the mesocosm with window screen, which we secured with zip ties and waterproof caulk to prevent animal escape. The start date of the experiment varied from 6–16 June 2015, and the end date of the experiment varied from 29 September to 1 October 2015 (106–115 d). At the end of the experiment, we thoroughly searched the leaf litter and soil of all mesocosms; salamanders were assumed dead if not found.

We measured, euthanized (using 20% liquid Benzocaine), and dissected all animals to determine sex and assess reproductive maturity. For males with pigmented testes, we assessed reproductive maturity by removing both testes and photographing them using a Leica M165C stereo microscope (Leica Microsystems, Wetzlar, Germany). We took all photographs on the same day, under identical lighting conditions, and using the same field of view. We used Leica Application Suite version 4.1 (Leica Microsystems, Wetzlar, Germany) to determine the average area of both testes and ImageJ version 1.49 (Schneider et al. 2012) to determine mean pigmentation. We standardized testes area and pigmentation by SVL at the end of the experiment, and we took the inverse of the mean standardized brightness of testes, such that darker testes would be scored as a higher number than lighter testes. More densely pigmented testes in male plethodontids has been shown to be an indicator of sperm production and reproductive condition (Sayler 1966; Brodie Jr. 1968). We scored males with unpigmented testes as zero for standardized area and the inverse of mean testis brightness. Because

testis area and inverse of pigmentation were correlated ( $r = 0.65$ ;  $t = 4.033$ ;  $df = 22$ ,  $P < 0.001$ ), we used testis pigmentation (scaled and centered by dividing by subtracting the mean and dividing by the standard deviation) for further analyses. Although we designed our experiment to assess reproductive maturity for both sexes, we found no females with mature follicles; therefore, we did not assess female reproductive condition any further.

**Analyses.**—To determine if mass or SVL differed among origin populations or transplant locations at the start of the experiment, we used an Analysis of Variance (ANOVA) in which the responses (mass and SVL) were square root-transformed responses. This transformation was necessary to meet the assumption of normally distributed residuals, which we assessed graphically both before and after transformation. To estimate body condition, we regressed log transformed mass against the total length (i.e., SVL + tail length) for all individuals (both beginning and end of the experiment) and extracted the residuals from the linear model; hereafter, we will refer to this as body condition index (BCI). A positive BCI indicates individuals with a greater mass for a given SVL; whereas, a negative BCI indicates individuals with a lesser mass for a given SVL (e.g., Reading 2007; Băncilă et al. 2010). To determine if survival varied among origin, transplant, or initial body condition, we used a Generalized Linear Mixed Effect Model with a binomial error distribution; our model contained two random intercepts, transplant location and replicate nested within transplant location. Similarly, we used Linear Mixed Effects Models, with the same random intercepts as above, to determine if growth rates (i.e., rate of change in SVL or mass) varied. For both SVL and mass rates of change, we standardized these measures by first dividing them by the starting SVL of the animal and then dividing by the number of days that the animal was in the experiment. Lastly, we used a Linear Model because of our smaller sample size to determine if maturation (measured as testes pigmentation) varied among origin location, transplant location, or initial body condition.

For our four response variables, we compared all 18 models using combinations of our three fixed effects (origin population, transplant location, and initial body condition) as well as interactions. Interactions were only included when the additive components of the interaction were present in the model. We were not able to fit the three-way interaction model for survival; therefore, it was not included. We fit all models using maximum likelihood and selected the best model based on lowest Akaike's Information Criteria corrected for small sample size (AICc; Hurvich and Tsai 1989). We determined significance of model parameters of

the top model using a likelihood ratio test; however, when competing models were within 2 AICc points of the top model (i.e., those that with substantial support; Burnham and Anderson 2002), we determined an average of model parameters, and we determined the significance of parameters as those with 95% confidence intervals that did not overlap zero; for brevity, we show visualizations for only significant results. For Linear Models (i.e., responses of SVL/mass change and male maturation), we graphically assessed models to ensure they met the assumptions of homoscedasticity and normality of residuals. We performed all statistical analyses in Program R version 3.3.1 (R Core Team 2015); we used the lme4 package (Bates et al. 2015) for analyzing mixed effects models, the Hmisc package (Harrell et al. 2016) to determine binomial confidence intervals of apparent survival, and the MuMIn package (Barton 2016) to compare models by AICc, estimate average model parameters, and determine predictions from the model sets.

## RESULTS

We recovered 70 (30 females and 40 males) of the 108 salamanders at the end of the experiment (apparent survival = 65%; 95% CI, 55–73%). Apparent survival was lowest for individuals originating from higher elevations (56%; 95% CI, 40–71%) with increasing survival for animals from mid (64%; 95% CI, 48–78%) and low elevations (75%; 95% CI, 59–86%) regardless of transplant location. Apparent survival, however, was highest for animals transplanted to higher and mid (72%; 95% CI, 56–84%) elevations compared to low elevation (50%; 95% CI, 34–66%) regardless of their origin population.

Overall, individuals at the start of the experiment were generally smaller (mean SVL = 38.2 mm; 95% CI, 37.3–39.1 mm; mean mass = 1.0 g; 95% CI, 0.9–1.1g) but had a higher BCI (0.1; 95% CI = 0.0–0.1) compared to the end of the experiment (mean SVL = 42.6; 95% CI, 41.9–43.4mm; mean mass = 1.1; 95% CI, 1.0–1.1g; mean and 95% BCI = -0.1). Additionally, at the start of the experiment, SVL and mass were not statistically different among origin populations (SVL:  $F_{2,103} = 0.046$ ;  $P = 0.955$ ; mass:  $F_{2,103} = 0.037$ ;  $P = 0.963$ ) and transplant locations (SVL:  $F_{2,103} = 0.399$ ;  $P = 0.678$ ; mass:  $F_{2,103} = 0.567$ ;  $P = 0.569$ ); however, individuals transplanted to the low elevation gained less SVL and had more negative change in mass compared to those transplanted to mid and high elevations (Supplemental Figs. 1, 2). Regardless of origin population and transplant elevation, individuals exhibited a negative change in BCI through the duration of the experiment (Supplemental Fig. 3) although we noted an abundance of prey items within the mesocosms and within the gut of individuals during

**Table 1.** Estimates for the mixed model parameters included in the top model for survival, rate of snout-to-vent length (SVL) change, and rate of mass change of the Northern Gray-cheeked Salamander (*Plethodon montanus*). Interactions are denoted by a colon (:) and bolded terms indicate significant variables (i.e., 95% CI do not overlap zero). Relative importance of each parameter is shown along with the number of models in the top model set that contain each variable (n). Model effects for Transplant and Origin are either Mid or High in reference to the Low elevation.

Response	Parameter	Estimate	Lower 95% CI	Upper 95% CI	Variable Importance (n)
Survival	<b>Intercept</b>	<b>0.501</b>	<b>0.014</b>	<b>0.988</b>	—
	<b>BCI</b>	<b>3.673</b>	<b>0.961</b>	<b>6.384</b>	<b>1.00 (5)</b>
	Transplant (Mid)	0.521	-0.094	1.136	0.67 (3)
	Transplant (High)	0.399	-0.297	1.095	
	<b>BCI:Transplant (Mid)</b>	<b>4.449</b>	<b>0.654</b>	<b>8.243</b>	<b>0.55 (2)</b>
	BCI:Transplant (High)	3.487	-0.393	7.367	
	<b>Origin (Mid)</b>	<b>-0.676</b>	<b>-1.338</b>	<b>-0.014</b>	<b>0.53 (3)</b>
Rate of SVL Change	Origin (High)	-0.390	-1.007	0.228	
	<b>Intercept</b>	<b>0.00091</b>	<b>0.00067</b>	<b>0.00114</b>	—
	<b>BCI</b>	<b>-0.00115</b>	<b>-0.00188</b>	<b>-0.00042</b>	<b>1.00 (2)</b>
	<b>Transplant (Mid)</b>	<b>0.00034</b>	<b>0.00010</b>	<b>0.00059</b>	<b>0.65 (1)</b>
	Transplant (High)	0.00001	-0.00022	0.00023	
	Intercept	0.00060	-0.00028	0.000147	—
	<b>BCI</b>	<b>-0.00662</b>	<b>-0.00942</b>	<b>-0.00382</b>	<b>1.00 (2)</b>
Rate of Mass Change	Origin (Mid)	-0.00059	-0.00130	0.00012	1.00 (2)
	Origin (High)	-0.00042	-0.00121	0.00037	
	<b>Transplant (Mid)</b>	<b>0.00144</b>	<b>0.00058</b>	<b>0.00230</b>	<b>1.00 (2)</b>
	Transplant (High)	0.00051	-0.00030	0.00132	
	BCI:Origin (Mid)	0.00274	-0.00084	0.00632	0.62 (1)
	BCI:Origin (High)	-0.00254	-0.00700	0.00192	

dissection. Most of the males (23/24; 96%) showed some degree of pigmentation; the only male that did not show pigmented testes originated from, and was transplanted to, the low elevation site. Lastly, we found that the size at the start of the experiment for those missing animals were generally smaller than individuals that were recovered (Supplemental Fig. 4).

The most parsimonious predictors of survival were a set of five models containing the parameters of starting BCI, transplant location, origin population and the interaction between transplant locations and starting BCI (Table 1; Supplemental Table 1). The probability of survival was greatest for animals that started with a higher BCI. Those salamanders with higher BCI who originated from low elevations and those that were transplanted to mid or high elevations had higher survival than other treatments (Fig. 2). For SVL, our top models included parameters of transplant location and starting BCI (Table 1; Supplemental Table 2). Animals that started off with a lower BCI had more positive rates of growth (SVL change), and this relationship was greatest for animals transplanted to mid elevations but lower for animals transplanted to high and low elevations (Fig. 3A). Though our model selection for mass change

included more parameters, results were similar to the change in SVL (Table 1; Supplemental Table 3); animals that began the experiment with a lower BCI and were transplanted to mid and high elevations had a more positive rate of mass change (Fig. 3B). Lastly, we found that starting BCI significantly predicted ( $\chi^2 = 8.713$ ,  $df = 1$ ,  $P < 0.001$ ) maturation in males (Supplemental Table 4) and that males that started the experiment with a more positive BCI had darker testes (Fig. 4) but was not dependent on the environment or origin population.

## DISCUSSION

How and why species range limits are set are fundamental questions that span the breadth of biological disciplines (Darwin 1859; MacArthur 1972). Amphibians, and especially salamanders, present an interesting case for answering these questions as recent evidence suggests that the influence of abiotic and biotic factors on their range limits are the opposite of what is expected based on general patterns (Gifford and Kozak 2012; Cunningham et al. 2016; Lyons et al. 2016; Grant et al. 2018). Moreover, answering these questions has become increasingly pressing under the threat of future

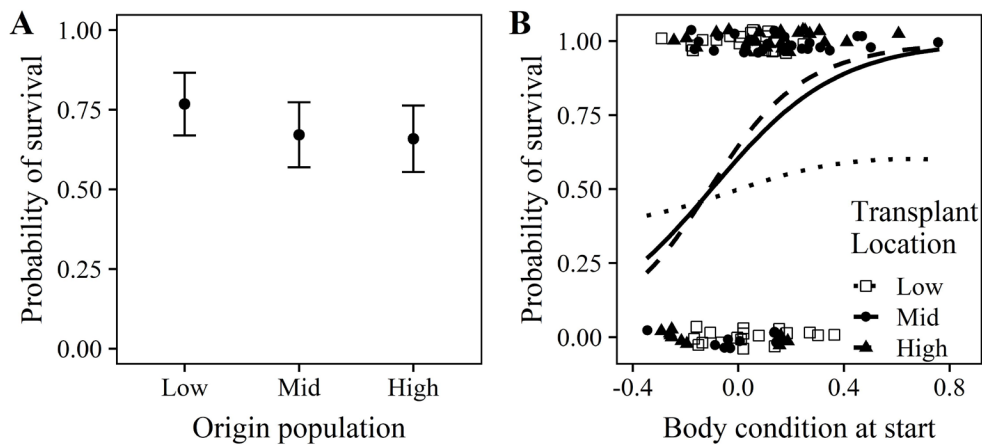


FIGURE 2. Relationship between survival of the Northern Gray-cheeked Salamander (*Plethodon montanus*) and (A) origin population, and (B) transplant location and BCI from binomial mixed effects model. In A, points show predicted probability of survival with 95% CI (error bars); whereas, in B, lines show predicted probability of survival. Points in B indicate data for low (dotted line, open squares), mid (solid line, closed circles) and high elevations (dashed line, closed triangles).

climate change (Milanovich et al. 2010) and developing mechanistic predictions will require an understanding of the extent to which populations can respond and adapt (Parmesan 2006; Merilä and Hendry 2014; Urban et al. 2014). Here we performed a reciprocal transplant experiment along an elevational gradient and found support for the abiotic environment limiting the lower elevation edge of *P. montanus* and that populations that currently exist at the lower range edge likely have adaptations to lower elevation conditions that are not shared by populations from higher elevations.

**Origin population.**—We expected *P. montanus* to have higher survival, growth, and maturation for

individuals that had a transplant location that matched their origin population, which would have indicated that these populations were adapted to local conditions. We did not find support for this hypothesis for any of our measured response variables, although we found that the elevation where an individual originated was a significant predictor of survival such that individuals from lower elevations had higher survival than individuals that originated from mid and high populations. Patterns of survival in our transplant experiment may suggest interacting effects of local adaption or phenotypic plasticity; conditions experienced by individuals originating from low elevations were either those to which they were locally adapted (i.e., their origin

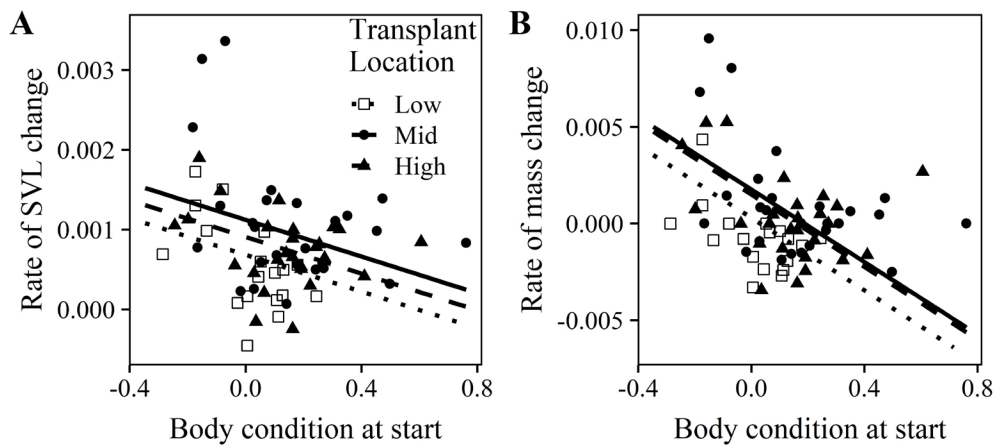


FIGURE 3. (A) Influence of transplant location and body condition on rates of snout-to-vent length (SVL) and (B) mass change of the Northern Gray Cheeked Salamander (*Plethodon montanus*) from linear mixed effects models. Lines show predicted fit, and dots indicate data points for low (dotted line, open squares), mid (solid line, closed circles) and high elevations (dashed line, closed triangles).

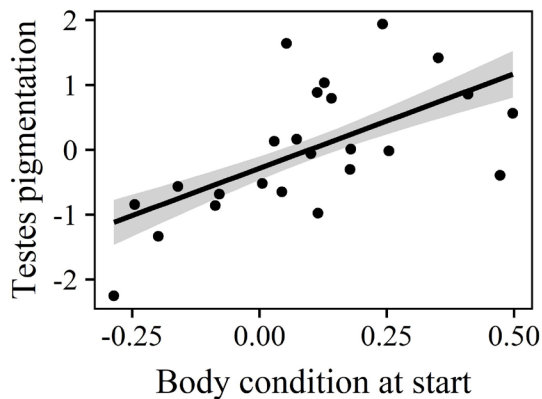


FIGURE 4. Reproductive condition (testes pigmentation) of the Northern Gray Cheeked Salamander (*Plethodon montanus*) and body condition. Shaded ribbon denotes 95% CI of predicted fit (solid line) from the linear model.

population at the low elevation) or potentially better (i.e., transplanted to higher elevations than origin) in terms of cooler temperatures. Individuals who originated from mid and high elevations, however, experienced locally adapted conditions (i.e., their origin populations) or better conditions (when transplanted from mid elevation to high elevation only), or worse conditions (i.e., when transplanted to elevations lower than origin).

One of the main limitations of our study is our inability to confirm if the individuals that were missing at the end of the experiment were the result of mortality or they had simply escaped. We found evidence to suggest that missing individuals may have been the result of escape as no remains were recovered and non-recovered animals were generally smaller than those that were recovered. However, these mesocosms have been used previously with no noted salamander escapes (Cunningham et al. 2009), but salamander decomposition is relatively rapid (Regester and Whiles 2006) and dead individuals possibly decomposed beyond recognition during our experiment. Additionally, the size disparity between the survivors and those that were missing are likely the result of size-specific survival rates as smaller individuals of *P. montanus* have lower survival rates than larger individuals (Caruso and Rissler 2019).

Future studies can refine hypotheses concerning local adaptation and phenotypic plasticity. For example, clutches could be split so that individual salamanders from the same clutch could be subjected to different treatments so as to control for genetic factors; however, this technique is logistically challenging for many species of *Plethodon*. Our study was limited as we were not able to control for relatedness, maternal effects, or genotype-environment interactions (e.g., Via and Lande 1985; Sinervo 1990; Bernardo 1996; Bronikowski 2000), all of which could have added unknown sources of variation to our data. Using animals with unknown

genetic relationships was out of necessity as it is not feasible to assess reproductive condition in a natural setting for salamanders that take at least three years to mature (Hairston 1983).

**Transplant location.**—We expected to find lower growth, survival, and maturation at the lower elevation compared to the higher elevations and our results for survival and growth support this hypothesis. Although multiple factors that can vary along an elevational gradient, such as prey availability (Lessard et al. 2011) or soil chemistry (Knoepp and Swank 1994), we suggest that temperature is the most likely factor to explain our observed variation in survival and growth. In Wood Frogs (*Rana sylvatica*), variation in life-history traits among populations are primarily the result of environmental conditions, especially temperature (Berven 1982a,b; Sheridan et al. 2018) and warmer summer temperatures have been associated with reduced growth rates in the Eastern Red-backed Salamander (*P. cinereus*; Muñoz et al. 2016) and in *P. montanus* (Caruso and Rissler 2019). We acknowledge that there are alternative explanations for the observed variation in survival and growth among transplant locations. A limitation of our experiment is our inability to separate soil and litter conditions within the mesocosms from the environmental conditions experienced at each location as we used local soil and leaf litter that may have differed in chemistry, microbial diversity, prey availability, or other factors. Unfortunately, use of local soil and leaf litter was necessary to prevent the potential spread of diseases among sites. Prey availability in the form of arthropod abundance and diversity has been shown to decrease with increasing elevations (Lessard et al. 2011). Because we did not modify or augment prey availability within the mesocosms, prey availability possibly varied among our transplant locations. Regardless, variation in prey abundance apparently is unimportant in explaining the relationship between *Plethodon* abundance and elevation (Gifford and Kozak 2012). Lastly, while prey were able to enter the mesocosms, an overall reduction in BCI for individuals at the end of the experiment compared to the start likely suggests that either prey availability or foraging conditions were limiting throughout our experiment (Jaeger 1979). Therefore, an explicit manipulation of prey availability along with the abiotic conditions experienced along a climate gradient (e.g., elevation) would be necessary to refine these hypotheses.

**Initial body condition.**—Body condition is an important factor for explaining variation in life-history traits among individuals (Wheeler et al. 2003; Karraker and Welsh 2006; Reading 2007; Janin et al. 2011). We controlled for body condition of each individual at the

start of the experiment in all analyses and found that animals with higher BCI had higher survival rates but lower growth rates, and males had higher reproductive condition (females did not have mature follicles). At the population level, body condition can be an important determinant for the outcome of population growth. For example, Reading (2007) found that warmer temperatures reduced BCI in Common Toads (*Bufo bufo*), which led to lower survival and egg production and ultimately decreased population growth. While by itself, body condition may not predict the fate of a population (i.e., persisting or not), these data are relatively easy to collect and combined with other demographic or physiological data (e.g., corticosterone) provide an effective tool for identification of populations at risk (Janin et al. 2011).

By using a reciprocal transplant experiment, we were able to test the relative influence of origin population and transplant location simultaneously; our results suggest that transplant location has more influence on survival and growth than origin population. Warmer or drier conditions can result in reduced surface activity, increased metabolism, increased water loss, as well as reductions in growth and survival in plethodontids (Connette et al. 2015; Riddell and Sears 2015; Catenazzi 2016; Muñoz et al. 2016; Caruso and Rissler 2019). Continued trends towards warmer and drier climates in the Appalachian region will reduce environmental suitability and likely salamander biodiversity (Milanovich et al., 2010) unless compensated by an increase in immigration or reproduction (Gaston 2009; Tavecchia et al. 2016) or populations that can adapt rapidly to these unique conditions (Riddell et al. 2018). We provide experimental support for the hypothesis that conditions found at the lower elevation edge of *Plethodon montanus* limits its range, which is consistent with predictions for montane salamanders (e.g., Gifford and Kozak 2012; Cunningham et al. 2016; Lyons et al. 2016; Grant et al. 2018).

*Acknowledgments.*—Thanks to Peter Scott and Sarah Duncan for assistance setting up the mesocosms and to Christina Staudhammer, Gregory Starr, Dean Adams, Peter Scott, Susanne Wiesner, Sujit Kunwor, and Satyra George for comments that improved this manuscript. This research was funded through Graduate Research Fellowship, E.O. Wilson Fellowship, and the Herpetologists' League E.E. Williams Research Grant awarded to NMC. We had North Carolina state and Pisgah National Forest permits to conduct this research. All animal work was conducted as outlined by national guidelines (University of Alabama IACUC approval 15-02-0098).

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