1   An experimental approach to understanding elevation limits in a montane terrestrial salamander,

2   *Plethodon montanus*

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16   Survival

17

18 **Abstract**

19   Understanding the abiotic and biotic factors that determine the limits to species' range is an
20   essential goal in ecology, biogeography, evolutionary biology, and conservation biology.
21   Moreover, predictions of shifts in species' distributions under future changes in climate can be
22   improved through understanding the spatial variation in survival, growth, and reproduction. A
23   long-standing hypothesis postulates that, for Northern Hemisphere species, abiotic factors like
24   temperature limit northern and/or higher elevation extents, while biotic factors like competition
25   limit the southern and/or lower elevation range edges; though amphibians may not follow this
26   general trend. Therefore, we combined environmental suitability models and a reciprocal
27   transplant experiment across an elevational gradient to explore the role of the abiotic
28   environment on the range limits of a montane salamander (*Plethodon montanus*). We first
29   determined suitability of the abiotic environment for *P. montanus*, under current (1960 – 2000)
30   and future (2050) climate scenarios. Second, we collected juveniles from each of three elevations
31   and transplanted them within mesocosms such that each origin population was represented
32   within each transplant location and vice-versa. We found that environmental suitability in 2050
33   decreased throughout the range compared to current predictions, especially at lower elevations.
34   Additionally, we found that individuals' starting body condition and transplant location were
35   important predictors of survival, growth, and reproduction condition; importantly, individuals
36   transplanted to low elevation had lower survival and growth rates compared to those moved to
37   mid or high elevations. Our study provides experimental support that the abiotic environment
38   limits the lower elevation distribution of *P. montanus* and, unfortunately, our results also paint a
39   possible bleak future for this species and likely other montane terrestrial plethodontids. The
40   abiotic environment, which will become increasingly limited under future changes in climate,
41   was found to have more influence on survival and growth than population identity.

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Introduction

Fundamentally, a species persists when the numbers of individuals entering the population (birth and immigration) are at least equal to those leaving (death and emigration) the population (Gaston 2009). However, the numbers and fitness of individuals across a species' range vary due to spatial and temporal variation in abiotic (e.g., temperature, precipitation) or biotic (e.g., predation, food availability) factors. Limits to a species' range often arise because of lowered resource availability or quality at range edges that can result in diminished condition or death of individuals (Hutchins 1947; Gaston 2003). For example, in the Southern Appalachians, warmer and drier conditions at lower elevations physiologically constrains the montane endemic salamander *Plethodon jordani* to higher elevations, while *P. teyahalee* is excluded from high elevations by the superior competitor, *P. jordani* (Gifford and Kozak 2012). Understanding how abiotic and biotic factors result in a population becoming a sink or extirpated is essential in determining the role of the environment on species' range limits. As such it is both a fundamental question in ecology, biogeography, and evolutionary biology (Brown 1984; Gaston 2003; Case et al. 2005; Parmesan et al. 2005; Gaston 2009; Lee-Yaw 2016), and also critical for effective conservation and predictive modeling (Gaston 2003; Hampe and Petit 2005; Gaston 2009; Urban et al. 2016).

The North-South Hypothesis is a long-standing macroecological hypothesis (Darwin 1859; MacArthur 1972) which posits that abiotic conditions determine a species' pole-ward or higher elevation range limit, but biotic conditions determine the equator-ward or lower elevation range limit (Dobzhansky 1950; MacArthur 1972; Brown et al. 1996; Gaston 2003; Parmesan et al. 2005; reviewed in Schemske et al. 2009; Hargreaves et al., 2014). For species in the Northern Hemisphere, this means that northern and/or higher elevation populations should be more constrained by abiotic factors like temperature, while southern and/or lower elevation populations should be more constrained by biotic factors like competition. However, several studies have shown that amphibians may not conform to this general trend (Hairston 1980; Nishikawa 1985; Gifford and Kozak 2012; Cunningham et al. 2016; Lyons et al. 2016). While studies examining range limits across different species are not uncommon (Schemske et al. 2009), there are fewer that examine what limits the different portions of a single species' range (reviewed in Cahill et al. 2014; but see Cunningham et al. 2009).

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 $0.6^{o}C$ over the last century and are expected to increase 2 – $4^{o}C$ more by 2100 (IPCC 2014). As a response to contemporary changes in climate, amphibians have shown shifts in breeding phenology (Beebee 1995; Reading 1998; Gibbs and Breisch 2001; Chadwick et al. 2006; Green 2016), geographic range limits (Pounds et al. 1999; Seimon et al. 2007), and body size (Reading 2007; Caruso et al. 2014). Environmental suitability models predict 50-100% reduction in suitable climate space for Southern Appalachians salamanders (Milanovich et al. 2010) – 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 in the face of climate change (Bernardo and

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85 Spotila 2006, Gifford and Kozak 2012; Lyons et al. 2016). Therefore, as global climates continue
86 to shift, predicting species' persistence will require an understanding of the relationship between
87 climate and population vital rates (e.g., survival; Buckley et al. 2010; Urban et al. 2016) as well
88 as decoupling local adaptation and plastic responses to climate (Parmesan 2006; Merilä and
89 Hendry 2014; Urban et al. 2014).

90   Improvement to predictions of shifts in species' distributions under future changes in
91 climate can be accomplished through understanding how survival, growth, and reproduction vary
92 spatially. Therefore, we combine environmental suitability models and a reciprocal transplant
93 experiment across an elevational gradient to explore the role of the abiotic environment on the
94 range limits of a montane salamander (*Plethodon montanus*). Using environmental suitability
95 models we explore the variation in current and future suitability of the abiotic environment and
96 compare models to identify how future ranges will be affected by changes in climate. Moreover,
97 we used a reciprocal transplant experiment to investigate the role of origin population (i.e.,
98 where individuals were captured) and transplant location (i.e., where individuals were raised) on
99 three relevant population responses: survival, growth, and maturation, to test the hypothesis that
100 abiotic conditions limit the warmer range edge (i.e., lower elevations) of montane salamanders.
101 Therefore, we asked the following questions, 1) how does environmental suitability vary across
102 the current range of *P. montanus,* and how will suitability change (e.g., among elevations) under
103 a future climate scenario? and 2) How do the origin location, transplant location, and initial body
104 condition of individuals influence survival, growth rate, and maturation? Thus, if abiotic
105 conditions limit the lower, warmer range edge, we expected to find lowest growth, survival, and
106 maturation at the lower elevation sites and highest growth, survival, and maturation at the higher
107 elevation sites. Moreover, if montane salamanders do best at their origin population we would
108 expect to find increased survival, growth, and maturation for individuals at those locations
109 compared to individuals transplanted to a non-origin location.

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Methods

Environmental Suitability Models

113   We created predictive Ecological Niche Models (ENMs) for *P. montanus*, under current
114 (1960 – 2000) and future (2050) climate scenarios, using program Maxent version 3.3.3k
115 (Phillips et al. 2006). We obtained 262 unique high accuracy (at least four decimal points; ~11 m
116 resolution) geographic coordinates (latitude and longitude) using GBIF (http://www.gbif.org/) on
117 17 March 2016. We used the 11 bioclimatic variables that Rissler and Apodaca (2007) and
118 Milanovich et al. (2010) used in distribution models of other plethodontid species (30 sec
119 resolution; Hijmans et al. 2005) clipped to North America. We bootstrapped our ENMs by using
120 75% of the data for training and 25% of the data to test the models with 100 replicates. We used
121 area under the curve (AUC) of the receiver operating characteristic (ROC) plots to evaluate
122 model fit (i.e., values closer to 1 indicate a better fit; Swets 1988; Elith 2002), and determined if
123 our current ENM models performed better than 262 random points with 999 replicates (Raes and
124 ter Steege 2007). We used the default settings in Maxent for all other parameters and projected

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125   our current ENM model onto a future climate scenario (Hadley GEM2-ES model; RCP4.5
126   greenhouse gas scenario; 30 second resolution; Hijmans et al. 2005). For brevity, we report the
127   average and standard deviation AUC, and data from the average and standard deviation of
128   current and future ENM of bootstrapped models.

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130   *Reciprocal Transplant Experiment*

131   From 6 June – 1 October 2015, we conducted this transplant experiment in Pisgah
132   National Forest, in which we collected salamanders who originated from low (~1,000 m;
133   82.4258°W, 36.0328°N), mid (~1,250 m; 82.1417°W, 36.1372°N), and high (~1,450 m;
134   82.0917°W, 36.0928°N) elevations and reciprocally transplanted them to the same low, mid, and
135   high elevation sites. Thus, each origin population was represented within each transplant location
136   and vice-versa. Average annual temperatures varied predictably among these three elevations;
137   the low site was the warmest (10.79°C), followed by the mid (9.59°C) and high elevation sites
138   (8.78°C), whereas annual precipitation was lowest at the mid elevation site (1346.76mm) and
139   higher and the low (1463.81mm) and high (1394.13mm) elevation sites (PRISM 2017). We
140   established two replicate sites within each transplant elevation, with each replicate site
141   containing 18 mesocosms (36 per elevation) for a total of 108 mesocosms. Replicate sites were
142   chosen based on proximity to established mark-recapture sites, were approximately 200 – 400 m
143   apart, and were on northwestern facing slopes with similar canopy coverage. Each mesocosm
144   consisted of a single 53 x 43 x 30 cm polyethylene tub (Cunningham et al. 2009) that had the
145   same number and size of holes drilled along the bottom and side for drainage. We filled each
146   mesocosm with a layer of approximately 10 cm of soil, then a layer of approximately 2 cm of
147   leaf litter (each gathered from the respective transplant site) and one 30 x 15 x 5 cm untreated
148   pine cover board. The soil and leaf litter were collected and homogenized, separately, prior to
149   adding equal amounts to each mesocosm in order to maintain consistency among mesocosms
150   within each experimental site.

151   After establishing the mesocosms, we collected 36 juvenile salamanders that ranged from
152   30 – 45 mm SVL from each origin population. This size class was chosen because it represented
153   the range of animals that could potentially reach reproductive maturity by the end of the
154   experiment (N.M.C. *unpublished data*). Because the sex of juvenile salamanders is currently
155   impossible to determine without dissection, we assumed a 1:1 sex ratio, which is consistent with
156   museum specimens (N.M.C. *unpublished data*). Animals were kept in a cooler, maintained
157   between 15 – 20°C, for approximately 36 hours before the start of the experiment. Immediately
158   before beginning the experiment, we measured the snout-to-vent length (tip of the snout to
159   posterior margin of the vent; SVL), tail length, and weighed each animal. All measurements
160   were taken while the animal was secured in a new plastic bag to ensure consistent measurements
161   and reduce probability of disease transmission from potentially contaminated equipment. We
162   randomly assigned animals to a transplant elevation (low, mid, and high) and replicate site within
163   transplant location (1 or 2). After adding a single salamander to each mesocosm, the mesocosm
164   was covered with window screen and secured by both zip ties and waterproof caulk to prevent
165   animal escape. Because we could not logistically collect all animals on a single day, the start date

166   of the experiment varied from 6 June 2015 – 16 June 2015, and the end date of the experiment
167   varied from 29 September 2015 – 1 October 2015 (106 – 115 days). At the end of the
168   experiment, we thoroughly searched the leaf litter and soil of all mesocosms; salamanders were
169   assumed dead if not found.

170   All animals were measured, euthanized (20% liquid Benzocaine), and dissected to
171   determine sex and assess reproductive maturity. For males with pigmented testes, we assessed
172   reproductive maturity by removing both testes and photographing them using a Leica M165C
173   stereo microscope. All photos were taken on the same day, under identical lighting conditions,
174   and using the same field of view. We used Leica Application Suite version 4.1 (Wetzlar,
175   Germany) to determine the average area of both testes and ImageJ version 1.49 (Schneider et al.
176   2012) to determine mean pigmentation; we standardized testes area and pigmentation by the
177   SVL at the end of the experiment, and we took the inverse of the mean standardized brightness
178   of testes, such that darker testes would be scored as a higher number than lighter testes. Males
179   with unpigmented testes were scored as 0 for standardized area and the inverse of mean testis
180   brightness. Because testis area and inverse of pigmentation were correlated (0.65; t_{22}=4.027; P <
181   0.001), we used testis pigmentation (scaled and centered) for further analyses. Although we
182   designed our experiment to assess reproductive maturity for both sexes, no females were found
183   with mature follicles; therefore, we did not assess female reproductive condition any further.

184   *Analyses*

185   To determine if survival varied among origin, transplant, or initial body condition, we
186   used a generalized linear mixed effect model with a binomial error distribution; our model
187   contained two random intercepts, transplant location and replicate nested within transplant
188   location. Similarly, we used linear mixed effects models, with the same random intercepts as
189   above, to determine if growth rates (i.e., rate of change in SVL or mass) varied. To estimate body
190   condition, we regressed log transformed mass against the total length (i.e., SVL + tail length) for
191   all individuals (both beginning and end of the experiment) and extracted the residuals from the
192   linear model; hereafter, we will refer to this as body condition index (BCI). A positive BCI
193   indicates individuals with a greater mass for a given SVL, while a negative BCI indicates
194   individuals with a lesser mass for a given SVL (e.g., Reading 2007; Băncilă et al. 2010). For
195   both SVL and mass rates of change, we standardized these measures by first dividing them by
196   the animal's starting SVL and then dividing by the number of days that the animal was in the
197   experiment. Lastly, we used a linear model, because of our smaller sample size, to determine if
198   maturation (measured as testes pigmentation) varied among origin location, transplant location,
199   or initial body condition. For our four response variables, we compared all 18 models using
200   combinations of our three fixed effects (origin population, transplant location, and initial body
201   condition) as well as interactions. Interactions were only included when the additive components
202   of the interaction were present in the model. We were not able to fit the three-way interaction
203   model for survival; therefore, it was not included. We fit all models using maximum likelihood
204   and selected the best model based on lowest Akaike's Information Criteria corrected for small
205   sample size (AICc; Hurvich and Tsai 1989). We determined significance of model parameters of
206   the top model using a likelihood ratio test. However, when competing models were within 2

207   AICc points of the top model (i.e., those that with "substantial support"; Burnham and Anderson
208   2002), we determined an average of model parameters, and the significance of parameters was
209   determined as those with 95% confidence intervals that did not overlap zero; for brevity, we
210   show visualizations for only significant results. For linear models (i.e., responses of SVL/mass
211   change and male maturation), we graphically assessed models to ensure they met the
212   assumptions of homoscedasticity and normality of residuals. All statistical analyses were
213   performed in Program R version 3.3.1 (R Core Team 2016); we used the *dismo* package
214   (Hijmans et al. 2016) to simulate random ENM models, the *lme4* package (Bates et al. 2015) for
215   analyzing mixed effects models, the *Hmisc* package (Harrell Jr. et al. 2015) to determine
216   binomial confidence intervals of apparent survival, and the *MuMIn* package (Barton 2016) to
217   compare models by AICc, estimate average model parameters, and determine predictions from
218   the model sets.

219

Results

221   *ENM Modeling*

222   Our ENM model provided an excellent fit (AUC = 0.992; SD = 0.001) to the locality data
223   and was better than random (rAUC = 0.580 – 0.690). Within the current range of *P. montanus*,
224   we found that the mean environmental suitability was 0.430 (SD = 0.044). Additionally, the
225   occurrence data used to train and test our model had a mean ENM suitability of 0.518 (SD =
226   0.077). As expected, environmental suitability in 2050 decreased throughout the range (Fig. 1;
227   Supplemental Fig 1), averaging 0.056 (SD = 0.034), while mean predicted ENM suitability was
228   0.112 (SD = 0.097) for the occurrence data used to train and test our models. Moreover, we
229   found that the change in mean environmental suitability showed a linear or logistic trend
230   associated with elevation while the difference in current and future suitability showed a quadratic
231   or no relationship with both latitude and longitude (Fig. 1). Lastly, while current mean ENM
232   suitability was similar for our focal sites (low = 0.570 [SD = 0.011); mid = 0.564 [SD = 0.007];
233   high = 0.572 [SD = 0.009]), predicted ENM suitability for 2050 is depreciated, especially at
234   lower elevations (low = 0.075 [SD = 0.063); mid = 0.065 [SD = 0.054]; high = 0.202 [SD =
235   0.112]).

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237   *Reciprocal Transplant*

238   We recovered 70 of the 108 salamanders at the end of the experiment (apparent survival
239   = 65%; 95% CI = 55 – 73%). Apparent survival was lowest for individuals originating from
240   higher elevations (56%; 95% CI = 40 – 71%) with increasing survival for animals from mid
241   (64%; 95% CI = 48 – 78%) and low elevations (75%; 95% CI = 59 – 86%); however, apparent
242   survival was highest for animals transplanted to higher and mid (72%; 95% CI = 56 – 84%)
243   elevations compared to low elevation (50%; 95% CI = 34 – 66%). Individuals at the start of the
244   experiment were generally smaller (mean SVL = 38.17 mm; 95% CI = 37.26 – 39.08 mm; mean
245   Mass = 0.998 g; 95% CI = 0.933 – 1.062 g) but had a higher BCI (0.07; 95% CI = 0.03 – 0.11)
246   compared to the end of the experiment (mean SVL = 42.64; 95% CI = 41.87 – 43.42 mm; mean

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247   Mass = 1.059; 95% CI = 1.002 – 1.115 g; mean BCI = -0.11; 95% CI =-0.13 – -0.08).

248   Additionally, at the start of the experiment, SVL and mass were similar among origin

249   populations and among origin populations and transplant locations. However, individuals

250   transplanted to the low elevation gained less SVL and had more negative change in mass

251   compared to those transplanted to mid and high elevations (Supplemental Figs. 2, 3). Regardless

252   of origin population and transplant elevation individuals exhibited a negative change in BCI

253   through the duration of the experiment (Supplemental Fig. 4). Lastly, most of the males (23/24;

254   96%) showed some degree of pigmentation and increase in size of their testes; the only male that

255   did not show pigmented testes originated from, and was transplanted to, the low elevation site.

256   The most parsimonious predictors of survival were a set of five models containing the

257   parameters of starting BCI, transplant location, origin population and the interaction between

258   transplant locations and starting BCI (Table 1; Supplemental Table 1). The probability of

259   survival was greatest for animals that started with a higher BCI, which is not unexpected.

260   Interestingly, those salamanders with higher BCI who originated from low elevations and those

261   that were transplanted to mid or high elevations had higher survival than other treatments (Fig.

262   2). For SVL, our top models included parameters of transplant location and starting BCI (Table

263   1; Supplemental Table 2). Animals that started off with a lower BCI had more positive rates of

264   growth (SVL change), and this relationship was greatest for animals transplanted to mid

265   elevations but lower for animals transplanted to high and low elevations (Fig. 3a). Though our

266   model selection for mass change included more parameters, results were similar to the change in

267   SVL (Table 1; Supplemental Table 3); animals that began the experiment with a lower BCI and

268   were transplanted to mid and high elevations had a more positive rate of mass change (Fig. 3b).

269   Lastly, we found that starting BCI significantly predicted ($\chi^2_1 = 8.713$; P < 0.001) maturation in

270   males (Supplemental Table 4); males that started the experiment with a more positive BCI had

271   darker testes (Fig. 4) and was not dependent on the environment or origin population.

272

Discussion

274   Understanding how survival, growth, and reproduction vary spatially, leading to limits to

275   species' distributions, can inform population models and improve predictions of species' range

276   distributions under future changes in climate. Therefore, we determined the effect of origin

277   population, transplant location, and initial BCI on juvenile salamander survival, growth, and

278   reproductive condition. We found that individuals transplanted to mid and high elevations

279   typically had higher survival and higher growth (SVL and mass) rates, while individuals that

280   originated from low elevations had higher overall survival, irrespective of their transplant

281   location (Figs. 2 – 4). Lastly, salamander body condition at the beginning of the summer is an

282   important driver of survival, growth, and maturation; juveniles with a more positive BCI were

283   more likely to survive, but had slower growth rates, and males had larger, darker testes (Figs. 2 –

284   4) at the end of the experiment.

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286 *ENM Models and predicting future changes in suitability of sites and consequences to*
287 *salamanders*

288   Our current ENM models showed high and similar levels of suitability across our three
289 transplant sites; however, ENM suitability for *P. montanus* decreased for forecasted 2050
290 climate, especially at lower elevations (Fig 1; Supplemental Fig. 1). Notably, mean predicted
291 ENM suitability for 2050 was lower than 99.6% (261/262) of the *P. montanus* occurrence sites
292 that we used to train and test our models. While our experiment was not conducted to explicitly
293 test the role of environmental suitability per se on salamander growth and survival, which would
294 require multiple transplant locations across many more sites across the environmental suitability
295 landscape, our results do support predictions that lower elevations will become more limiting for
296 montane salamanders as temperatures increase (Milanovich et al. 2010; Gifford and Kozak 2012;
297 Lyons et al. 2016). Moreover, changes in climate do not affect species independently, and can
298 result in shifts in the distribution of competitors, prey, or predators, which could alter
299 interspecific interactions and potentially compound the negative effects of changes in climate on
300 population growth (Blois et al. 2013; Liles et al. 2017).

301

302 *Initial Body Condition*

303   Initial BCI was an important factor predicting observed trends in survival, growth, and
304 reproductive condition in males; animals with higher BCI had higher survival rates, and males
305 had higher reproductive condition. Our results for survival and reproductive condition agree with
306 other studies that have examined the importance of body condition for individuals as well as
307 populations (Wheeler et al. 2003; Karraker and Welsh Jr. 2006; Reading 2007; Janin et al. 2011).
308 For example, Reading (2007) found that decreasing BCI in common toads, caused by warming
309 climate, led to a decrease in survival and egg production. Our study shows that healthier
310 individuals (i.e., those with higher body conditions) are afforded a greater probability of survival;
311 however, for montane salamanders in low elevation habitats, this higher body condition may not
312 be enough. As climate is expected to become less favorable for montane salamanders, especially
313 at lower elevations, monitoring these low elevations populations is of increasing importance
314 because at least 55% of montane plethodontids (lower elevation limit is greater than or equal to
315 1,000m) are threatened with extinction (IUCN 2016). Moreover, key life history characteristics
316 such as body condition are relatively easy to collect and can provide an effective tool for
317 identification of populations at risk (Janin et al. 2011).

318   Interestingly, animals that started the experiment with a greater BCI had lower rates of
319 SVL changes and more negative changes in mass yet those same males had larger, darker testes
320 indicative of greater reproductive condition (Sayler 1966; Peacock and Nussbaum 1973). This
321 suggests that males with a greater BCI may put more energy into reproduction rather than growth
322 during this life stage; this trend was strong for the males in our experiment (Supplemental Fig.
323 5). Tradeoffs between growth and reproduction in wild animals have been well-documented
324 (e.g., Reznick 1983, 1985); for example, in *P. cinereus*, brooding females allocated less

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325   resources to growth compared to non-brooding females regardless of food availability (Yurewicz
326   and Wilbur 2004).

327

328   *Origin Population*

329   We found that where an individual originated was a significant predictor of survival;
330   individuals from lower elevations had higher survival than individuals from mid and high
331   populations (Fig. 2) irrespective of their transplant location. One of the limitations in our data
332   was our inability to control for relatedness, maternal effects, or genotype-environment
333   interactions (e.g., Via and Lande 1985; Sinervo 1990; Bernardo 1996; Bronikowski 2000), all of
334   which could have added unknown sources of variation to our data. This was out of necessity as it
335   would not have been feasible to assess reproductive condition in a natural setting for salamanders
336   that take at least three years to mature (N.M.C. *unpublished data*). However, patterns of survival
337   in our transplant experiment may suggest interacting effects of local adaption and phenotypic
338   plasticity; individuals originating from low elevations were transplanted to elevations that
339   represent either locally adapted conditions (i.e., low elevation) or better conditions (i.e.,
340   transplanted to higher elevations than origin). On the other hand, individuals who originated
341   from mid and high elevations experienced locally adapted conditions or better conditions (when
342   transplanted from mid elevation to high elevation only), or worse conditions (i.e., when
343   transplanted to elevations lower than origin). This variation in survival may be explained, at least
344   in part, by temperature, which decreased with increasing elevation (PRISM, 2017). Although low
345   and mid elevations show similar ENM suitability, the scale at which these variables were
346   measured (~1 km) may not have been fine enough to adequately characterize these habitats. For
347   example, Gifford and Kozak (2012) found that lower elevations, though they appeared to have
348   identical habitats to higher elevations, contained microclimates that constrained *P. jordani*, a
349   montane salamander. Future studies can refine hypotheses concerning local adaption and
350   phenotypic plasticity in salamanders by splitting clutches or otherwise controlling for genetic
351   factors; however, this technique is logistically challenging for many *Plethodon* species.

352

353   *Transplant Location*

354   The abiotic environment, represented by transplant location in this experiment, was an
355   important predictor for both growth and survival; salamanders that were transplanted to low
356   elevations responded with the lowest survival and lowest growth (both mass and SVL) rates (Fig.
357   3). These results also conform to *in situ* observations; warmer summer temperatures were
358   associated with reduced growth rates in *P. cinereus* (Muñoz et al. 2016) and lower elevations had
359   lower survival than higher elevations in *P. montanus* (Caruso and Rissler 2017). Our results
360   support the hypothesis that amphibian distributions are influenced at the southern or lower
361   elevation range limits by abiotic variables (Buckley and Jetz 2007; Gifford and Kozak 2012;
362   Cunningham et al. 2016; Lyons et al. 2016), although here we did not explicitly test the relative
363   influence of biotic factors. It should be noted, however, that climatic barriers at the lower
364   elevational limit are not universal for plethodontids and biotic variables may be more important.

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365 For example, *Desmognathus wrighti* is precluded from suitable lower elevations via predation by
366 larger and more aquatic desmognathines (Organ 1961; Crespi et al. 2003), while *P. shendadoah*
367 is outcompeted from non-talus slopes by *P. cinereus* (Jaeger 1970). Nonetheless we demonstrate
368 here, that for the montane endemic *P. montanus*, the abiotic environment, specifically hotter and
369 drier conditions (Fig. 1; Supplemental Fig. 1), likely limits its lower elevation distribution,
370 similar to *P. jordani* (Gifford and Kozak 2012).

371 Though we did not modify or augment prey availability, we believe that prey availability
372 was an unlikely source of variation in our results. First, all mesocosms had mesh screen and
373 holes drilled along the side and bottom that were large enough to allow for smaller arthropods to
374 enter the mesocosms. Second, local soil and leaf litter, which contained prey sources, were added
375 to each mesocosm; thereby, minimizing variation among mesocosms within a transplant
376 replicate. Lastly, we noted an abundance of prey items within the mesocosms when extracting
377 the resident salamander at the end of the experiment, and during dissection to assess reproductive
378 condition animals had prey items in their gut, indicating recent feeding (gut-passage time in *P.*
379 *cinereus* is 1 – 2 weeks; Merchant 1970; Gabor and Jaeger 1995). While we do not suppose that
380 prey populations affected our experiment, future shifts in salamander abundance could have big
381 consequences for ecosystem function. Due to their large numbers in forest ecosystems (Burton
382 and Likens 1975; Milanovich and Peterman 2016), salamanders can exhibit strong top-down
383 effects on invertebrate populations; their presence is associated with reduced leaf-litter
384 decomposition and overall carbon retention (Wyman 1998; Rooney et al. 2000; Walton et al.
385 2006; Best and Welsh 2014; but see Walton and Steckler 2005; Homyack et al. 2010; reviewed
386 in Walton 2013). Therefore, declines of salamander populations may have a negative feedback,
387 in which the loss of salamander biomass reduces forest carbon sequestration, potentially
388 accelerating anthropogenic climate change, resulting in further reductions in areas of suitable
389 climate for salamander persistence.

390 In conclusion, we first provide experimental support for the hypothesis that the abiotic
391 environment constrains the lower elevation limits of *Plethodon montanus*, which is consistent
392 with predictions for amphibians, and more specifically, montane salamanders (e.g., Gifford and
393 Kozak 2011; Cunningham et al., 2016; Lyons et al. 2016. Importantly, by using a reciprocal
394 transplant experiment, we were able to test the relative influence of origin population and
395 transplant location simultaneously; our results (i.e., AICc-selected variables and variable
396 importance; Table 1; Supplemental Tables 1-3), suggest that the abiotic environment (transplant
397 location) has more influence on survival and growth than population identity (origin population).
398 Warmer and/or drier conditions can result in reduced surface activity, increased metabolism,
399 increased water loss, as well as reductions in growth and survival in plethodontids (Caruso et al.
400 2014; Riddell and Sears 2015; Catenazzi 2016; Connette et al. 2015; Muñoz et al. 2017; Caruso
401 and Rissler 2017). Continued trends towards warmer and drier climates in the Appalachian
402 region will likely lead to reductions in population growth unless compensated by an increase in
403 immigration or reproduction (Tavecchia et al. 2016; Gaston 2009). These data are important not
404 just because they add to the growing body of literature seeking to understand what determines
405 the limits of a species' range (Gaston 2003), but they further suggest a worrisome forecast for
406 montane salamanders under predicted future climate changes.

11

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614

615 **Table 1:** Estimates for the parameters included in the top model set (ΔAICc < 2) for each
616 response (Supplemental Tables 1-4). Interactions are denoted by ":", and bolded terms indicate
617 significant variables (i.e., 95% CI do not overlap zero). Relative importance of each parameter is
618 shown along with the number of models in the top model set that contain each variable (n).

Response	Parameter	Estimate	Lower 95% CI	Upper 95% CI	Variable Importance (n) [619]
Survival	**Intercept**	**0.501**	**0.014**	**0.988**	—
	BCI	**3.673**	**0.961**	**6.384**	**1.00 (5)**
	Transplant (Mid)	0.521	-0.094	1.136	0.67 (3)
	Transplant (High)	0.399	-0.297	1.095	
	BCI:Transplant (Mid)	**4.449**	**0.654**	**8.243**	**0.55 (2)**
	BCI:Transplant (High)	3.487	-0.393	7.367	
	Origin (Mid)	**-0.676**	**-1.338**	**-0.014**	**0.53 (3)**
	Origin (High)	-0.390	-1.007	0.228	
Rate of SVL Change	**Intercept**	**0.00091**	**0.00067**	**0.00114**	—
	BCI	**-0.00115**	**-0.00188**	**-0.00042**	**1.00 (2)**
	Transplant (Mid)	**0.00034**	**0.00010**	**0.00059**	**0.65 (1)**
	Transplant (High)	0.00001	-0.00022	0.00023	
Rate of Mass Change	Intercept	0.00060	-0.00028	0.000147	—
	BCI	**-0.00662**	**-0.00942**	**-0.00382**	**1.00 (2)**
	Origin (Mid)	-0.00059	-0.00130	0.00012	1.00 (2)
	Origin (High)	-0.00042	-0.00121	0.00037	
	Transplant (Mid)	**0.00144**	**0.00058**	**0.00230**	**1.00 (2)**
	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	

620   **Figure 1:** Relationship between ENM suitability and latitude, longitude, and elevation. Current
621   (blue squares), future (red triangles) and the difference between future and current (purple
622   circles) are shown.

623

624   **Figure 2:** Relationship between survival of *P. montanus* and A) origin population, and B)
625   transplant location and BCI. Error bars denote 95% CI, lines show predicted probability of
626   survival and dots indicate data points for low (dotted line, open squares), mid (solid line, closed
627   circles) and high elevations (dashed line, closed triangles).

628

629   **Figure 3:** Influence of transplant location and BCI on rates A) of SVL and B) mass change.
630   Lines show predicted fit, and dots indicate data points for low (dotted line, open squares), mid
631   (solid line, closed circles) and high elevations (dashed line, closed triangles).

632

633   **Figure 4:** Reproductive condition (testes pigmentation) of *P. montanus* and BCI. Shaded ribbon
634   denotes 95% CI of predicted fit (solid line).

A — Rate of SVL Change vs BCI at Start. Transplant Location: Low (dotted, open square), Mid (solid, filled circle), High (dashed, filled triangle).

B — Rate of Mass Change vs BCI at Start.