

Effects of Elevation on Plethodontid Salamander Body Size

William E. Peterman¹, John A. Crawford², and Daniel J. Hocking³

The size of an organism is perhaps its most overt physical characteristic, and variation in body size has long been of interest to biologists. Bergmann's rule has been actively studied and debated for more than 150 years. Despite this long history, the generality and applicability of Bergmann's rule to ectothermic organisms generally, and to plethodontid salamanders specifically, has resulted in an extensive and conflicting literature. Regardless of mechanism, clinal variation in body size has been widely observed in plethodontid salamanders and other ectothermic vertebrates. In this study, we assessed the change in adult body size of four plethodontid salamanders (*Desmognathus imitator*, *D. ocoee*, *D. wrighti*, and *Plethodon jordani*) across a 1,350 m elevational gradient in Great Smoky Mountains National Park. Using 1,293 observations of salamanders at 25 sites, we found clear and significant patterns of increasing adult body size with elevation in all four species. Average rates of increase ranged from 1.09% to 3.98% per 100 m elevation gain. We found that elevation significantly covaried with maximum and mean temperature, as well as average annual precipitation. Our study reinforces previous research describing increases in plethodontid salamander body size with elevation, but also extends these findings to fully terrestrial, direct-developing species. However, the mechanisms underlying the observed pattern are still unclear and highlight an important area for future research. As a critical life history characteristic, an understanding of geographic variation in body size is important for assessing current population dynamics, as well as the potential effects of future climate changes.

ECOLOGISTS have long been drawn to describing biogeographic patterns in the context of “biological rules” (e.g., Allen's rule, Rensch's rule, Rapoport's rule). Perhaps one of the most widely studied and debated of these biological rules is Bergmann's rule (Bergmann, 1847), which states that organisms tend to be larger in cooler climates. Originally developed from observations of interspecific differences in endothermic organisms with heat conservation as the underlying mechanism, Bergmann's rule has since been widely interpreted (e.g., Mayr, 1956; Blackburn et al., 1999) and assessed across taxa and taxonomic levels (Ray, 1960; Cushman et al., 1993; Ashton, 2002; Ashton and Feldman, 2003; Adams and Church, 2008). The generality of Bergmann's rule varies widely among taxa, and the mechanisms underlying clinal variation in body size are often unclear and debated (Pincheira-Donoso, 2010; Olalla-Tárraga, 2011). Despite the uncertainty concerning the validity or mechanisms of clinal variation in body size, there are clear and pressing needs to better understand this macroecological process, especially in light of global climate change (Gardner et al., 2011; Ohlberger, 2013). There is increasing evidence that the average body size of many species is changing as a result of contemporary climate change (Yom-Tov and Yom-Tov, 2005; Daufresne et al., 2009; Gardner et al., 2011). While climate change has been implicated in reductions in plethodontid salamander body size (Caruso et al., 2014), these findings have been questioned by Connette et al. (2015) and Grant (2014).

Changes in body size have the potential to affect amphibian populations in a number of ways. First, fecundity is positively correlated with body size in amphibians (reviewed by Wells, 2007). If this critical life history attribute changes, future population dynamics of species may be significantly affected. Additionally, changes in body size will affect rates of water loss (Tracy et al., 2010; Peterman et al., 2013), which can affect surface activity time (Peterman and Semlitsch, 2014). Changes in activity time can subsequently

affect foraging, dispersal, mating, and possibly alter future species' distributions (Gifford and Kozak, 2012). The direct and indirect effects that changes in body size may have are complex and unclear. However, this further underscores the need to understand current patterns of body size distribution so that future changes in behavior, ecology, population dynamics, and distribution can be appropriately interpreted as potential responses to physical changes in size.

Plethodontid salamanders have been the focus of demographic and behavioral studies in the southern Appalachian Mountains for more than six decades (e.g., Hairston, 1949; Organ, 1961; Tilley, 1968; Bruce, 1988; Beachy, 1993; Crawford and Semlitsch, 2007; Peterman et al., 2011). More recently, there has been increased concern over how climate change will affect the distribution and demography of plethodontid salamanders. Milanovich et al. (2010) predicted distributional range contractions for the majority of plethodontid species within the Appalachian Highlands, identifying species with small, southerly ranges as those most likely to experience declines in range size and to be most at risk of extinction. They also predicted significant range contractions by the year 2020 compared with distributions from 1960–1990 (Milanovich et al., 2010). Gifford and Kozak (2012) hypothesized that species that belong to clades that originated in cool climates (such as plethodontids) will have their lower elevational range margins set by physiological stress. In warmer climates, metabolic rates will increase and surface foraging activity will decrease due to increased rates of evaporative water loss (Gifford and Kozak, 2012; Peterman and Semlitsch, 2014), which may lead to reduced fecundity (Maiorana, 1976; Milanovich et al., 2006) or a reduction in overall body size (Gardner et al., 2011). Therefore, in order to assess any long-term demographic changes in plethodontid salamanders, it is important to establish how body size naturally varies over existing environmental gradients.

In this study, we assessed average adult body size over an elevational gradient in four plethodontid salamanders.

¹ School of Environment and Natural Resources, The Ohio State University, 2021 Coffey Road, 210 Kottman Hall, Columbus, Ohio 43210; Email: Peterman.73@osu.edu. Send reprint requests to this address.

² National Great Rivers Research and Education Center, One Confluence Way, East Alton, Illinois 62024; Email: joacrawford@lc.edu.

³ U.S. Geological Survey, S.O. Conte Anadromous Fish Research Center, P.O. Box 796, One Migratory Way, Turners Falls, Massachusetts 01376; Email: dhocking@usgs.gov.

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Specifically, we sought to answer three main questions: (1) Does average adult body size of plethodontid salamanders increase with elevation? (2) Does body condition change over an elevational gradient? (3) How do temperature and precipitation change with elevation?

MATERIALS AND METHODS

Study species.—Four species were the focus of this study: *Desmognathus imitator* (Imitator Salamander), *D. ocoee* (Ocoee Salamander), *D. wrighti* (Pygmy Salamander), and *Plethodon jordani* (Red-cheeked Salamander). Both *D. imitator* and *D. ocoee* lay eggs near water, with larvae taking 7–10 months to metamorphose (Petranka, 1998), while *D. wrighti* and *P. jordani* are fully terrestrial. Within Great Smoky Mountains National Park (GSMNP), all four of these species are widely distributed throughout terrestrial forested habitats and are generally high elevation specialists occurring from ~750–2025 m in elevation (Dodds, 2004).

Field sampling.—We conducted nighttime visual encounter surveys to sample surface-active plethodontid salamanders in terrestrial habitats within GSMNP. Sample sites ($n = 25$) were located off of Route 441 (both sides of the continental divide) and Clingman's Dome Spur Road. Sites ranged in elevation from 669–2019 m a.s.l. (Table 1). All surveys were conducted in July 2012 and occurred within 24 hrs of a rain event. Upon capture, salamanders were identified to species, sexed by candling (Gillette and Peterson, 2001), measured (snout–vent length [SVL]) to the nearest 0.01 mm with digital calipers, and weighed to the nearest 0.01 g with a portable digital balance. During candling, an individual was designated as a female if ova were visible and a male if testes were present. We considered males to be mature if the testes were pigmented (Burger, 1937; Saylor, 1966). In male desmognathan salamanders, we also quantified the number of testis lobes present (Organ, 1961; Tilley, 1973, 1977). All salamanders were released at the site of capture following measurement.

Statistical analysis.—To assess how mean adult SVL of salamanders changed with elevation, we used linear mixed effects models (Bates et al., 2014). Species were analyzed separately, with SVL as the response variable. Elevation, sex, and elevation by sex interaction were predictor variables, and site was modeled as a random effects intercept. Models were fit using restricted maximum likelihood. Sites with fewer than three observations for a particular species and sex were omitted from analysis. Confidence intervals of model parameter estimates were obtained through 10,000 parametric bootstrap iterations. We used generalized linear mixed effects models with a Poisson error distribution to determine if the number of testis lobes differed across the elevational gradient; site was again modeled as a random effect. Additionally, describing a change in mean adult SVL necessitates that an adequate representation of juveniles are sampled to define the lower threshold of when individuals become sexually mature. To evaluate this, we used generalized linear models with a binomial error distribution to determine if the proportion of juveniles collected at each site differed in relation to elevation.

If significant changes in mean adult SVL occur, it is of interest to know if allometric differences exist. For all species that exhibited significant body size changes with elevation, we calculated a body mass index (BMI) following methods

Table 1. Descriptions of site location, elevation, and species collected. All species observed at a site are listed, but some species-by-site combinations have been omitted from analysis due to sample size. Species acronyms are DIMI = *Desmognathus imitator*, DOCO = *D. ocoee*, DWRI = *D. wrighti*, PJOR = *Plethodon jordani*.

Elevation (m)	Latitude	Longitude	Species
669	35.5540	–83.3113	DIMI, DWRI, PJOR
714	35.5632	–83.3305	DIMI, PJOR
766	35.5759	–83.3429	PJOR
778	35.6424	–83.5062	DWRI, PJOR
843	35.5868	–83.3582	PJOR
860	35.5886	–83.3638	DIMI, DOCO, PJOR
970	35.5921	–83.3801	DIMI, DOCO, DWRI, PJOR
1020	35.5927	–83.3846	DWRI, PJOR
1048	35.6342	–83.4704	DWRI, PJOR
1057	35.6353	–83.4683	DIMI, PJOR
1060	35.5941	–83.3919	DWRI, PJOR
1124	35.5989	–83.4019	DIMI, DOCO, DWRI, PJOR
1168	35.6001	–83.4085	DOCO
1169	35.6295	–83.4503	DIMI, PJOR
1257	35.5986	–83.4136	DIMI, DOCO, DWRI, PJOR
1329	35.6209	–83.4246	PJOR
1383	35.5890	–83.3989	DIMI, DWRI, PJOR
1416	35.5851	–83.3988	DWRI, PJOR
1499	35.6078	–83.4376	DIMI, DOCO, PJOR
1572	35.6097	–83.4485	DIMI, DOCO, DWRI, PJOR
1717	35.5952	–83.4596	DIMI, DOCO, DWRI, PJOR
1771	35.5667	–83.4785	DIMI, DOCO, DWRI, PJOR
1787	35.5901	–83.4700	DIMI, DOCO, DWRI
1860	35.5562	–83.4971	DIMI, DOCO, DWRI, PJOR
2019	35.5633	–83.4986	DIMI, DOCO, DWRI, PJOR

described by Peig and Green (2009) and then assessed BMI using linear mixed effects models as described above for the analysis of SVL. Finally, because elevation is only a proxy for differences in local climate, we obtained 800 m resolution, 30 yr normal data for precipitation and mean, minimum, and maximum temperature from the PRISM Climate Group (Oregon State University, <http://prism.oregonstate.edu>, accessed 1 September 2014). We assessed pairwise correlations between elevation and climate using Pearson's product moment correlation. All analyses were conducted with R (R Core Team, 2015).

RESULTS

Survey summaries.—In total, we collected 1,635 salamanders, 1,293 of which were determined to be adults (112 *D. ocoee*, 425 *D. imitator*, 250 *D. wrighti*, and 506 *P. jordani*; Table 2). The mean number of observations for each species at each site ranged from 16.0–38.6 (± 12.4 –31.2 SD; Table 2).

Changes in mean adult SVL.—The mean adult SVL of all species increased significantly with elevation (Table 3, Fig. 1). In no species was the sex by elevation interaction significant, so models were refit without this term to obtain better parameter estimates. On average, mean adult SVL increased 2.19% (range = 1.09–3.98%) per 100 m elevation gain (Fig. 1). *Desmognathus ocoee* showed the greatest rate of SVL increase; however, we note that the mixed effects model for *D. ocoee* exhibited the most uncertainty in parameter estimates. In all species except *D. ocoee*, females were significantly larger than males (Table 3; Fig. 1).

Table 2. Capture summaries for each species evaluated in this study. Sites are the number of sites with three or more samples for each sex; total is the total number of each species collected across all sites; adult is the number of adult salamanders collected across all sites; the number of female, male, and juvenile salamanders collected across all sites; the mean number of adult salamanders collected at each site; and the standard deviation of the number of adults collected at each site.

Species	Sites	Total	Adult	F/M/J	Mean	SD
<i>Desmognathus ocoee</i>	7	154	112	40/72/42	24.4	11.0
<i>D. imitator</i>	11	539	425	175/250/114	61.5	28.6
<i>D. wrighti</i>	13	338	250	86/166/86	26.6	12.0
<i>Plethodon jordani</i>	22	612	506	148/360/104	32.5	13.8

Number of testis lobes.—We found no evidence that the number of testis lobes in any of the male desmognathan species differed across the sampled elevation range (all P -values ≥ 0.15 ; Supplement 1).

Proportion of juveniles sampled.—For three of the four species, there was not a significant relationship between elevation and the proportion of juveniles collected at a site (Supplement 2). However, there was a moderate trend for fewer juvenile *P. jordani* as elevation increased. However, we note that juveniles still comprised $\sim 10\%$ of our total sample at the highest elevations.

Body mass index.—Despite the significant increase in mean adult SVL with elevation for all species, there were no significant elevation, sex, or elevation by sex relationships for BMI in any of the species (Supplement 3).

Relationship between elevation and climate.—Elevation at each site was highly correlated with annual precipitation ($r = 0.96$), average maximum temperature ($r = -0.97$), and average mean temperature ($r = -0.93$). Elevation was weakly correlated with average minimum temperature ($r = -0.22$).

DISCUSSION

Our survey of four plethodontid salamander species over an elevational transect in GSMNP clearly and unambiguously found that these species are larger at higher elevations. While this finding is not altogether novel (see Hairston, 1949; Martof and Rose, 1963; Bruce, 1972; Tilley, 1973, 1980), our

Table 3. Summary tables of linear mixed effects models predicting changes in mean adult snout-vent length in relation to elevation and sex. The elevation by sex interaction was not significant for any species. In all models, collection site was modeled as a random effects intercept.

	Estimate	SE	95% CI	t-value
<i>Desmognathus ocoee</i>				
Intercept	27.728	5.440	16.90–36.61	5.097
Elevation	0.013	0.003	0.008–0.020	3.931
Sex (Male)	1.160	1.432	–1.64 to 3.96	0.810
<i>D. imitator</i>				
Intercept	30.851	2.523	25.98–35.66	12.227
Elevation	0.008	0.002	0.005–0.011	4.623
Sex (Male)	–1.464	0.610	–2.70 to –0.29	–2.399
<i>D. wrighti</i>				
Intercept	22.428	1.041	20.43–24.50	21.540
Elevation	0.003	0.001	0.002–0.005	4.830
Sex (Male)	–2.788	0.272	–3.31 to –2.25	–10.240
<i>Plethodon jordani</i>				
Intercept	54.190	2.391	49.56–58.85	22.667
Elevation	0.006	0.002	0.003–0.010	3.499
Sex (Male)	–4.733	0.508	–5.73 to –3.74	–9.324

study represents one of the most intensive fine-scale assessments of this pattern and the first (to our knowledge) to demonstrate this pattern in the direct-developing *D. wrighti* and *P. jordani*. We found no obvious biases in our sample of individuals at each site: male *Desmognathus* spp. had similar numbers of testis lobes and juveniles were included in samples at all sites. Interestingly, the BMI of salamanders did not change, suggesting that as salamanders increase in mean adult SVL with elevation, they are maintaining the same body mass relative to their length. It is important to note that the elevation of a site itself likely has no direct effect on salamanders, but it is the concomitant climatological features of a site that are affecting growth, survival, and reproduction. We found that elevation was highly correlated with the maximum and mean temperature of a site, as well as the average annual precipitation. As such, it is impossible to tease apart the individual or additive effects of climate on body size, and we only assess elevation as this can be much more precisely measured.

Our findings that salamanders are larger at higher elevations are in contrast to Olalla-Tárraga et al. (2006) and Adams and Church (2008) who found evidence of converse Bergmann's rule and no Bergmann's rule in North American *Plethodon* spp., respectively. It is becoming increasingly apparent that body size clines in ectothermic organisms can result from a multitude of processes, which are manifest in idiosyncratic body size clines within and among taxa (Shelomi, 2012; Berke et al., 2013). Research with freshwater fishes has shown that body size can be strongly predicted by the thermal niche of a species. Rypel (2014) found that species dependent on cool-water habitats demonstrated Bergmann's rule, while those dependent upon warm waters demonstrated converse Bergmann's rule. This finding is of potential interest to this study because all of the species we assessed are considered high elevation specialists (Petranka, 1998; Dodd, 2004), which are dependent on cool, moist, and relatively stable environments. Further reinforcing our finding was that temperatures decreased and moisture increased with elevation. Despite the potential for a more limited active season, the cool, moist conditions of high elevation sites should allow for more frequent and extended surface activity, which could increase overall food intake while minimizing metabolic expenditures (Gifford and Kozak, 2012). Bernardo (1994) demonstrated that growth rate is an extremely plastic trait in *D. ocoee*; greater food resources resulted in increased rates of growth.

While the underlying mechanisms responsible for clinal variation in body size are varied and debated (Meiri et al., 2007; Adams and Church, 2008; Berke et al., 2013), detailed life history studies of plethodontid salamanders have shown that individuals in high elevation populations tend to mature later and at a larger size (Bruce, 1972; Tilley, 1980).

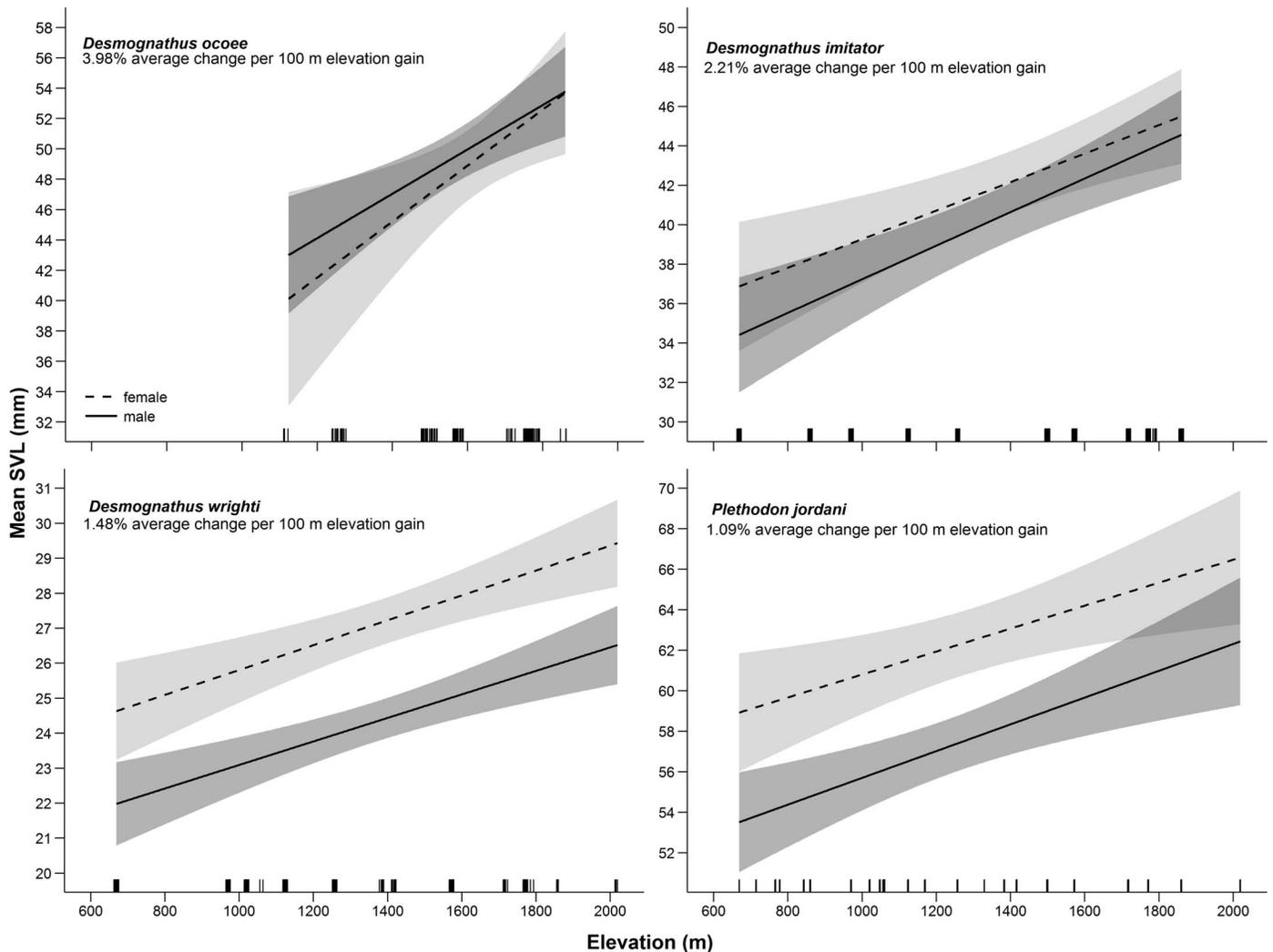


Fig. 1. Mean snout–vent length of adult salamanders estimated from linear mixed effects models. Shading around fit lines represents 95% confidence intervals. Males are indicated by the solid lines and dark gray shading. Females are indicated by the dashed lines and light gray shading. Ticks along the x-axes indicate the elevation of sites included in the analysis.

Tilley (1973, 1980) found that most growth occurs prior to sexual maturity in *D. carolinensis* and *D. ocoee*. As such, delayed time to maturity allows for additional growth and larger maximal body size. Such an increase in body size has tremendous potential to increase female fecundity. As table 2 of Tilley (1973) demonstrates for *D. carolinensis* (averaged over all populations), a 1 mm increase in SVL is expected to increase clutch size by 0.68 eggs (± 0.23 SD). However, Tilley (1980) also found that growth rates were higher in high elevation populations prior to sexual maturity; therefore, size differences are not merely a consequence of additional resources allocated to growth prior to first reproduction. Using the same high and low elevation populations studied by Tilley (1980), Bernardo (1994) conducted a common garden experiment to assess the environmental and genetic contributions to size and age at maturity. This experiment found evidence that timing of maturation (low elevation populations mature earlier than high elevation populations) was rooted in genetics but that growth potential did not differ. From these findings, Bernardo (1994) concluded that low elevation *D. ocoee* are capable of greater growth rates than observed in the field, and that growth appears to be limited by extrinsic ecological factors.

The detailed work of Tilley (1973, 1980) and Bernardo (1994) has revealed that growth and age at maturity is a result of both genetic and environmental factors. These studies provide a foundation for the interpretation and context for our findings, especially those of *D. imitator* and *D. ocoee*, which are ecologically similar to populations of *D. ocoee* and *D. carolinensis* studied by Tilley and Bernardo. It is of interest, however, that we observed an identical body size trend in the direct-developing, terrestrial *D. wrighti* and *P. jordani*. The lack of detailed life history studies on either of these, or closely related, species precludes detailed assessment of the underlying mechanisms and life history trade-offs. Ultimately, the data collected in our study falls short of being able to rigorously assess whether the increase in adult body size is a result of variation in size at maturity or variation in adult age distributions (see violin plots of SVL distributions, Supplement 4). To adequately address these questions, detailed mark–recapture studies would be necessary to measure growth rates, estimate survival, and more accurately delineate the size and age when sexual maturity is reached. Future research should seek to understand the mechanisms shaping body size clines in these direct-developing species.

From a more mechanistic perspective, it can be argued that salamanders inhabiting warmer, lower elevation sites have higher metabolic rates (necessitating greater food intake), but also that their surface activity is more constrained due to increased evaporative water loss. Larger salamanders will also have higher metabolic rates (Gifford et al., 2013). As such, it would appear that large-bodied salamanders would be particularly disadvantaged at low elevations. However, Riddell and Sears (2015) found that *P. metcalfi*, a sister species to *P. jordani*, have increased resistance to water loss at low elevations. These findings suggest either acclimatization or physiological adaptation to warmer, drier conditions, which has the potential to increase surface activity time. Mechanistic approaches have been utilized to accurately describe the distribution of *P. jordani* (Gifford and Kozak, 2012) and to predict the abundance and population dynamics of *P. albagula* across the landscape (Peterman and Semlitsch, 2014). The incorporation of mechanistic models in future research should facilitate detailed exploration of physiological and ecological constraints on salamander body size, as well as potential responses to climate change.

Despite our clear and congruent findings among divergent species of plethodontid salamanders, we must address and acknowledge the caveats and limitations present in our data. First, all data used in this study were collected over a short time period. The obvious effect of this can be seen in the biased sex ratio of our samples (Table 2), which is likely a consequence of some females tending to, or preparing to lay eggs. Second, there is increasing evidence that different sized salamanders are more likely to be active under different conditions (Connette et al., 2015); larger salamanders tend to be more detectable under wetter survey conditions. As such, surveys conducted under suboptimal (i.e., dry) conditions could underestimate the actual size of salamanders. We note, however, that all of our surveys were conducted under similar, near ideal nocturnal conditions. As such, we feel that we have equitably surveyed the salamanders at each site studied and have adequately sampled the adult population. In this study, we also counted and report the number of testis lobes in desmognathan salamanders. While the number of testis lobes has been used to estimate age (Organ, 1961; Tilley, 1973), Tilley (1977) found these counts to be inaccurate and to only loosely correspond to minimum age. We report our finding that testis lobe count did not differ across the elevational gradient sampled but note that this does not necessarily indicate that we have sampled similarly aged individuals. Finally, we acknowledge that our sampling was not exhaustive of a pre-defined or constrained area. Such a study design would allow us to much more rigorously delineate the size and age when individuals become sexually mature (as previously done by Tilley, 1973, 1974). We note that our inclusion of juveniles at every site gives us confidence that the smallest possible adults were being incorporated into the analyses. However, without more detailed information concerning the ages of individuals measured at each elevation, it is impossible to ascertain the mechanisms underlying the variation in body sizes that we observed.

Our study adds to the existing literature describing elevational differences in plethodontid salamander body size, reinforcing previous findings (Bruce, 1972; Tilley, 1973, 1980) and extending this pattern to species with direct development. The research of Tilley (1980) and Bernardo (1994) exemplify the nuanced effects that genetics and resources can have on growth rate, size at maturity, and

survival, factors that ultimately affect population growth and stability. With the uncertain effects of climate change looming, it is critical that we not only identify how life history traits vary across the landscape but also understand the mechanisms shaping them. In doing so, we can better understand how species will respond to future temperature and precipitation regimes, potentially facilitating more targeted monitoring and conservation efforts.

DATA ACCESSIBILITY

The supplemental materials referenced in this manuscript are available from the Figshare Digital Repository: <https://dx.doi.org/10.6084/m9.figshare.2076223.v1>

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