

Spatial variation in the abundance, demography, and physiology of the montane endemic  
salamander, *Plethodon shermani*, and the consequences of climate change

Dissertation

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## Abstract

As climate change marches on, it is imperative to understand how species respond via distribution, abundance, physiology, and behavior to their environment present-day in order to inform possible responses in the future. Populations of species exist across heterogeneous environments that may differentially influence particular responses. The scale at which these responses are assessed must be considered because patterns can emerge at a local scale that may not be detectable at broader scales. Yet, only assessing local-scale patterns and responses neglects the broader landscape patterns that ultimately shape fine-scales. Montane biodiversity experience extreme abiotic variation over small spatial scales. To increase our understanding of how these gradients influence wildlife across scales, my research examines the patterns of distribution, abundance, demographic life-history traits, behavior, and physiology in the red-legged salamander, *Plethodon shermani*, across multiple abiotic gradients in the Southern Appalachian Mountains. The primary objectives of my research are to (1) understand the effects of spatial patterns on ecological responses of *P. shermani* and (2) use that knowledge to predict how responses will change in the future.

Terrestrial *Plethodon* salamanders lack lungs and depend on cool and moist microhabitat to facilitate gas exchange across their skin surface. As such, salamanders are restricted to specific habitats. We know that salamander abundance increases with

elevation due to the cool and moist regional climate at high elevations. However, my research investigates the role of both elevation and stream distance gradients as broad and fine-scale abiotic gradients, respectively, that salamanders may be responding. Although low elevations are regionally warm and dry, microhabitats near streams are buffered and tend to be cooler and wetter. I found that salamander distribution and abundance track these landscape patterns such that at low elevations, animals are distributed in their highest abundance near stream sides but become less dependent on stream-side habitat at high elevations due to the regionally cool and moist habitat. Salamander life history also varies across these gradients; survival decreases with elevation but reproductive rates and growth increase with elevation. Both survival and reproduction decrease with stream distance at low elevations, but growth and movement increase with stream distance. I also found that a metric for stress, dermal Corticosterone, (dCORT) was lowest in animals at low elevations and stream distance had no effect. Salamander dCORT additively increased in response to experimental conditions reflective of future climate change. I also found that the surface activity probability of salamanders will likely increase in the future in response to rising temperatures. Using a highly integrative approach, my research collectively shows that salamanders have multi-faceted responses to spatial variation of landscapes and will be impacted by future climate change.

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## Fields of Study

Major Field: Environment and Natural Resources

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## Chapter 1. Introduction

Every ecological process occurs over both space and time. The scale at which a process is assessed is an important consideration to ensure relevant measures to a focal organism (Turner and Gardner 2015). Conservation has increasingly considered the importance of spatial scale as it can drive dynamic management decisions aimed towards mitigating the effects of environmental change on species that interact with the environment at different scales (Fletcher and Fortin 2018). Against the backdrop of climate change, the need to understand not only the patterns but also the scale of effect of ecological outcomes including abundance, distribution, and life history is hastened. Because of the inherent biotic and abiotic heterogeneity of a landscape, the effects of climate change are not uniform across taxa, species, or even populations of species across a given landscape. Thus, evaluation of scale-relevant ecological processes across landscapes is necessary to inform strategies that promote species survival and persistence in the future.

Mountains provide a particularly valuable landscape to not only evaluate spatial variation in ecological processes, but also the effects of climate change. Globally (excluding Antarctica), mountains cover just 25% of Earth's surface but harbor 87% of avian, mammal, and amphibian diversity (Rahbek et al. 2019). Mountains are excellent 'natural laboratories', offering environmental conditions that cannot easily be replicated

in controlled laboratory-based experiments. A major reason for the usefulness of mountains are the highly heterogeneous abiotic conditions over small spatial scales (Körner 2007). Montane regions have steep gradients of temperature, moisture, solar radiation, soil properties, among others that allow for evaluating species responses across these gradients. Certain ends of an abiotic gradient are more representative of expected conditions with climate change, and thus can provide an assessment of how species will respond in the future. Mountains offer the rare ability to substitute “space-for-time” in predicting climate change responses (Pickett 1989; Blois et al. 2013).

Mountains host high species diversity of many taxa, and the Southern Appalachian Mountains are a biodiversity hotspot for the lungless salamander family Plethodontidae. Without lung-based respiration, species in this family rely on cutaneous respiration for gas exchange. As such, they must inhabit cool and moist microhabitat to facilitate skin-breathing. While the broad elevational distributions are well documented (Dodd and Dorazio 2004; Kozak and Wiens 2010; Hocking et al. 2020), the fine-scale habitat associations across multiple environmental gradients are less well known. Further, surprisingly little is known about how plethodontids interact with their environment from a demographic and physiological perspective. Given the high density and abundance of terrestrial *Plethodon* in forests, with estimates ranging up to 90,000 individuals/hectare (Gade and Peterman 2019), their contributions to ecosystem function and food webs (Davic and Welsh 2004; Best and Welsh 2014; Hocking and Babbitt 2014), and their sensitivity to habitat alteration given their strict physiological requirements, the need to

better understand the basic ecology of these animals is imperative for current and future vulnerability to climate change.

In this dissertation, I integrate field observations, laboratory experiments, and quantitative modelling to better understand current and future spatial patterns of abundance, distribution, demography, physiology, and behavior in terrestrial Plethodontid salamanders. I organized my dissertation such that Chapters 2–5 represent separate manuscripts that have either been published or are intended for publication in the near future. As such, some content and methodologies may appear redundant. I have used plural pronouns throughout because I have coauthors on all manuscripts, but I take full responsibility for the content of each chapter. Chapter 2 has been published in *Landscape Ecology*, Chapter 3 has been prepared for publication in *Journal of Animal Ecology*, Chapter 4 is currently under review at *Copeia*, and Chapter 5 has been published in *Ecology*.

The primary objectives of my dissertation research are to (1) understand the effect of spatial patterns on processes in *Plethodon* salamanders across scales and (2) use that information to predict responses to climate change. Chapter 2 provides foundational research that the remaining chapters are built upon. In Chapter 2, I determined the spatial variation in the distribution and abundance of the Red-Legged Salamander, *Plethodon shermani*, across elevation and stream distance gradients. Broadly, high elevations are cooler and wetter than low elevations. However, stream distance provides an additional gradient that salamanders may respond. For example, microhabitats near stream sides, especially at low elevation, are cooler and wetter than ridgelines. I found that salamander

distribution and abundance tracked these landscape patterns such that at low elevation, salamanders were distributed in highest abundance near stream sides while at high elevations, abundance was not tied to stream sides, and animals were distributed more uniformly across the landscape.

In Chapter 3, I present the results of a 4- year spatial mark-recapture study of *P. shermani* to characterize baseline population demographic estimates including survival, growth, movement, and reproduction across elevation and stream distance gradients. I used a combination of quantitative models to estimate these demographic parameters across multiple scales. I found that survival was highest at low elevations but decreases with stream distance and remains somewhat constant at high elevations. Per capita reproduction rates increased with elevation but declined with stream distance at low elevation. Further, low elevation animals appear to move more and with higher variance which may be related to higher temperatures increasing metabolic demands and nutrient uptake. I also considered the role of hybridization in the population dynamics of this system, as my sampling plots were within a known hybridization zone between the high elevation *P. shermani* and low elevation *P. teyahalee*. I show that hybridization may be a unique strategy for future persistence since hybrids had higher survival probability.

In Chapter 4, I evaluated how physiological stress varies across the abiotic gradients of interest and experimentally tested how stressful weather conditions may affect stress physiology. This chapter used dermal swabs for obtaining a measure of stress (dermal corticosterone, dCORT), a method that could be valuable for conservation and management to obtain rapid and repeatable measures of amphibian population health. I

took baseline dCORT measures on animals from across the landscape and found that low elevation animals had lower dCORT levels than high elevations, but stream distance did not affect dCORT at any elevation. I then experimentally exposed salamanders to hot, dry, or the combination of hot and dry conditions and dry conditions lead to lowering dCORT levels, hot conditions lead to no difference, and the combination of stressful conditions lead to an additive increase in dCORT. I conclude that salamanders may downregulate dCORT to compensate for the more chronically stressful conditions at low elevations and salamanders may be able to tolerate the effect of one abiotic stressor, but in combination, multiple abiotic stressors may surpass the ability to maintain hormone regulation.

Finally, in Chapter 5 I used data from five independent datasets on three species of closely related *Plethodon* spanning 13 years to assess surface activity probability under various climate change scenarios. Surface activity is an important fitness-related parameter for salamanders as it dictates foraging and mating opportunities. I determined the environmental parameters that drive surface activity at a fine scale in the present which I then used to then estimate future activity probability over the next 80 years at a broad scale. I found that average temperature and vapor pressure deficit (VPD) were the strongest predictors of current activity and future activity probability will likely increase due to increasing temperatures and VPD. This study applies a relatively common ecological process model to a new framework that allowed for the estimation of surface activity behavior that is otherwise exceptionally challenging to measure due to the secretive nature of *Plethodon*.

Together my research identifies scale-specific patterns to ecological processes in terrestrial *Plethodon* salamanders and demonstrates fine-scale variation in distribution, abundance, growth, survival, reproduction, physiology, and behavior. I use a highly integrative approach to assess my research questions and provide innovative methods for estimating and quantifying population dynamics and health today and in the future. This work helps to inform potential management and conservation efforts and promotes that should be dynamic with respect to where on the landscape these efforts are taking place.

## Chapter 2: Multiple environmental gradients influence the distribution and abundance of a key forest-health indicator species in the Southern Appalachian Mountains

### Abstract

The effects of global climate change are threatening biodiversity, with particular concern for amphibians, whose survival often depends on specific abiotic conditions. To predict how future climate change will affect wildlife populations, it is first necessary to understand how patterns of abundance are shaped by multiple environmental conditions at both local and regional scales. Plethodontid salamanders are a group of lungless ectotherms that require cool and moist habitats to survive. While the broad elevational distribution and abundance patterns are well-understood, other important abiotic gradients exist within montane systems that are contributing to fine-scale spatial abundance patterns. We aim to assess the fine-scale spatial abundance of a plethodontid salamander across two key environmental gradients: temperature and moisture. We conducted area-constrained repeated point-count surveys at plots situated across temperature and moisture gradients in western North Carolina. Each plot was surveyed on 4 occasions, and site and survey-level covariates were measured. We found heterogeneous abundance patterns across these two gradients whereby warmer low elevations contain the greatest abundance near stream sides, where conditions are cooler and wetter than the regional landscape. At cooler, higher elevations, salamanders are

distributed more uniformly across the broader landscape, likely as a result of the suitable regional climate. Our study shows that fine-scale habitat associations of plethodontids are driven by temperature and moisture, and the spatial patterns of suitable microhabitats drive regional scale patterns. Incorporating multiple environmental gradients provides a more biologically relevant prediction of abundance patterns, which will help inform conservation and management strategies especially in the context of climate change.

## Introduction

Understanding the distribution and abundance of organisms across the landscape is paramount not only for attaining greater knowledge of the basic ecology of a species, but also for gaining insight into species' potential for persistence in the face of environmental change (Brown 1984). However, the scale at which we assess these relationships is a critical consideration because patterns can emerge at local scales that may be undetectable at landscape scales, while only assessing local scale relationships may neglect the broader ultimate factors influencing patterns (Miguet et al. 2016; Suárez-Castro et al. 2018). Local-scales typically represent the abiotic conditions individuals are directly experiencing, but the surrounding habitat matrix may have steep abiotic gradients that differentially influence adjacent local patches (Steen et al. 2012; Suárez-Castro et al. 2018). The hierarchical nature of different scales influencing and constraining each other is key and integrating both landscape and local scale conditions is needed to better ascertain the drivers of spatial distribution and abundance.

The scale by which species' spatial patterns emerge is a key concern when predicting responses to climate change and developing effective conservation strategies. With climate change, temperatures are expected to rise and there will be greater spatial and temporal climate and habitat heterogeneity (Nadeau et al. 2017). Thus, knowing how species relate to local abiotic features, as well as how the broader landscape gradients influence local-scale microhabitats is necessary (Lenoir et al. 2017). Often, studies use broad, macroclimate covariates to predict species vulnerability to climate change (e.g., Randin et al. 2009; Warren and Chick 2013). However, landscape-scale data overlook microhabitats embedded within a landscape, which often experience decoupled abiotic conditions from the regional conditions, and are more strongly driving spatial patterns (Nadeau et al. 2017; Scheffers et al. 2014a). Montane systems have particularly complex topographic and climatic variation which drives a wide range of microclimatic conditions across the landscape (Ashcroft et al. 2009). As such, landscape level assessments of species distribution and abundance are often broad, spatially inaccurate generalizations of fine-scale spatial patterns (Nadeau et al. 2017b; Chase et al. 2018). To provide more biologically relevant predictions of species persistence in the face of change, we must first assess abundance relationships with local-scale environmental conditions to understand the drivers of regional scale spatial patterns (Lenoir et al. 2017).

Amphibians are an ideal taxa to consider the effect of scale and climate change because of their ubiquity across landscapes and their strict abiotic requirements for survival (Blaustein et al. 2010). Because of their physiological limitations and subsequent sensitivity, amphibians have experienced unprecedented declines in the wake of climate

change as a result of these requirements (Blaustein et al. 2010; Grant and al. 2016).

Recent evidence suggests that amphibians have responded to climate change through shifting ranges, changes in physiology such as metabolic depressions and slowed growth, and overall reduced fitness (Bernardo and Spotila 2006; Catenazzi 2016; Muñoz et al. 2016). It is likely that amphibian's vulnerability to climate change will be exacerbated from the increased spatial and temporal habitat heterogeneity which limits physiologically suitable habitat (Milanovich et al. 2010; Nadeau et al. 2017). However, many climate change impact assessments of amphibians use coarse climatic data, which may misrepresent risks relative to more local-scale assessments (Nadeau et al. 2017). The fine-scale habitat associations of many amphibians, and how broader environmental gradients influence microhabitat associations are not well understood (but see Peterman and Semlitsch 2013). Such lack of knowledge significantly limits our capacity to predict the effects of future climate change on species.

Terrestrial plethodontid salamanders are a group of amphibians that may be particularly sensitive to change due to their lungless anatomy and highly specialized physiological requirements (Dodd and Dorazion 2004). Plethodontid distribution and abundance is strongly driven by their associations with cool and moist habitats to facilitate cutaneous respiration (Spotila 1972, Peterman and Semlitsch 2013). These salamanders are widely distributed throughout the Southern Appalachian mountains, and the broad elevational distributions are generally well-documented (Dodd and Dorazio 2004; Kozak and Wiens 2010; Peterman et al. 2016). However, broad scale patterns typically do not represent environmental conditions salamanders directly experience

(Clark et al. 2011). Thus, examining the micro-habitat associations with local-scale environmental gradients is of utmost importance. Elevation is typically used as a proxy for temperature, whereby low elevations exhibit warmer and drier conditions than higher elevations, which are cooler and wetter (Barry 2008). Therefore, salamanders inhabiting low elevations may experience a more challenging climate than their high elevation conspecifics. However, there are multiple other abiotic gradients in montane systems that are likely influencing spatial patterns of salamander distribution and abundance. For example, stream sides provide a microrefugia with cool temperatures and moist conditions amenable to plethodontid habitat requirements (Petranka and Smith 2005). Salamanders inhabiting harsher regional climates such as lower elevations are therefore likely utilizing microhabitats shaped by multiple abiotic gradients to persist in these suboptimal regions (Feder and Londos 1984; Crawford and Semlitsch 2008).

Terrestrial salamanders play a key role in ecosystem functioning and serve as forest health indicators due to their high densities, long life-span, low fecundity, and high sensitivity to disturbance (Hairston 1983; Welsh and Droege 2001). As such, it is important to garner a better understanding of their physiological constraints and habitat relationships across relevant abiotic gradients to develop effective management strategies. Herein, we assess how two abiotic gradients, temperature and moisture, across a montane landscape drives the variation in distribution and abundance of terrestrial plethodontid salamanders. We specifically focus on the red-legged salamander, *Plethodon shermani*. We hypothesized that salamanders at low elevation will be more abundant closer to streams, due to the cool and moist microhabitat provided by streams,

relative to the regional climate. Conversely, salamanders at higher elevations will be less restricted to streams due to the cooler and wetter regional climate at higher elevations. By assessing fine-scale spatial patterns of *P. shermani* across these two dimensions of environmental variation, our ultimate goal is to refine our understanding of the local processes shaping landscape-scale patterns.

## Methods

### Field Methods

Our study was conducted in the vicinity of Wayah Bald (35.158, -83.574) in the Nantahala National Forest in western North Carolina (Figure 2.1). Forest composition consisted primarily of upland oak-hickory, cove hardwood, and northern hardwood forest, with forest stand age ranging from 15 – 165 years, with an average age of  $78 \pm 49$  years. Elevation ranged from 700 m – 1600 m and distance from stream, ranging from <5 m to ~300 m was used as a proxy for moisture. Eighty-seven locations were generated in ArcGIS v.10.2 in a stratified random design to incorporate the full range of available elevation and stream distances. We incorporated elevation and stream distance to independently assess temperature and moisture. Each of the 87 plots were at least 30-m from each other to insure independence of plots (Connette and Semlitsch 2013). Once each plot location was determined in the field, a second, paired plot was established 10 m in a random direction from the original plot, to maximize survey areas due to other logistical constraints, resulting a total of 174 survey plots. Before establishing each plot,

we ground-truthed the presence of streams to ensure our GIS-derived hydrological surfaces were accurate. Each plot was 3 m x 3 m in size.

Southern Appalachian plethodontid activity is restricted to the summer months when climactic conditions are optimal (Hairston 1949). As such, plots were surveyed on four separate occasions between 4 June – 2 August 2017, during peak activity time between 2130 – 0230 EST. Two observers walked through the center of each plot looking for surface active salamanders. Surveys took approximately 3 – 5 minutes and, in addition to recording the number of observed salamanders, the following environmental covariates were recorded: surface soil temperature using an infrared thermometer (Raytek MT4), soil temperature at 10 cm below the surface using a digital pocket thermometer (Thermoworks RT600), and air temperature and relative humidity using a Ketsrel 5200. We also deployed 23 environmental data loggers (HOBO U23-003 Pro v2 [n = 8] and Thermochron DS1920 iButton [n= 14]) at 23 of the survey plots ensuring that the wide range of elevation and moisture (stream distances) gradients were captured. The environmental loggers were placed ~12 in from the forest floor to collect near-ground measurements realistic to those experienced by the salamanders (Riddell and Sears 2015), and continually monitored temperature and relative humidity at 30-minute intervals for the duration of the study period.

#### Site and Landscape Covariates

Site-level covariates including percent canopy cover, percent ground cover, and leaf litter depth (cm) were assessed prior to nocturnal surveys to avoid effects of potential site

disruption from surveys. Canopy cover and percent ground cover were collected using the iPhone app Caneopo (Patrignani and Ochsner 2015), which estimates fractional green canopy cover from a photograph taken at the center of each plot. Leaf litter depth was determined by the average of three haphazard measurements taken within each plot. Site topographic variables including elevation, slope, aspect, and topographic position index (TPI) were obtained from a 9 m<sup>2</sup> - resolution digital elevation model obtained from EarthExplorer ASTER Global DEM ([earthexplorer.usgs.gov](http://earthexplorer.usgs.gov)). TPI was calculated as the slope position relative to the surrounding 90 m, with negative values indicating ravines and positive values indicating ridges. Aspect was transformed from degrees (1-360) to two linear measures, indicating eastness and northness by taking the sine and cosine of aspect, respectively. Streams were delineated by generating a flow accumulation layer using a 450-pixel cell threshold from the 9 m<sup>2</sup> -resolution DEM resulting in a 0.405 hectare drainage area. The 450-pixel cell threshold was selected as the best representation of actual stream networks based on visual inspection of the map, comparison with other maps, and ground-truthing. Stream distance was then assessed using the Euclidean distance tool in ArcGIS v.10.2. Although not a direct part of the sampling design, we also included forest stand age as a landscape covariate using a 2018 compartment map provided by the US Forest Service.

### Temporal Covariates

Because salamander detection can be highly dependent on weather conditions (Peterman and Semlitsch 2013a; Connette et al. 2015), we obtained a series of hourly temperature

and precipitation data from the Wayah Bald Mountain WINE weather station using the North Carolina Climate Retrieval and Observation Network of the Southeast Database (CRONOS). The Wayah Bald Mountain WINE weather station is the closest continual weather station to our sites, with all sites at least 3km from the top of the mountain and is the closest approximation of precipitation across our sites. We used moving window analyses to assess the total precipitation in the 24-hours preceding a survey (hereafter 24-hr precipitation), total precipitation in the three days preceding surveys (hereafter 3-day precipitation), the total number of days since rain (hereafter dry days), and average temperature over the preceding 24 hours (hereafter 24-hour temperature). The Julian date of each survey was also included as a detection covariate.

### Statistical Analyses

To assess the repeated count observations, and to account for site-level covariates and imperfect detection, we implemented a binomial mixture model analyzed in a Bayesian framework (Royle 2004). We included the following parameters as abundance predictors: elevation, elevation<sup>2</sup>, eastness, northness, TPI, forest stand age, canopy cover, leaf litter depth, stream distance, and the interaction of elevation and stream distance. The following parameters were included as predictors of detection probability: ground cover, 24-hour precipitation, 3-day precipitation, dry days, 24-hour temperature, relative humidity, surface soil temperature, belowground soil temperature, and Julian date. Plots were considered independent, but we included a random effect to account for the spatial autocorrelation between sampling plots in the abundance model. The detection model

included a random effect to account for the survey night. Uninformative, normally distributed priors with a mean of zero and a precision of 0.1 were assigned to each parameter. We included all of the aforementioned parameters in a global model, and subsequently removed covariates that had < 75% of their posterior distribution having the same sign as the mean estimate (overlap zero) to create the final, reduced model (see Appendix A: Table A.1). Posterior summaries were based on 372,500 Markov Chain Monte Carlo iterations, thinned at a rate of 5 following an adaption phase of 210,000 and a burn-in of 150,000 iterations. Models were assessed, and convergence was confirmed using Gelman-Rubin statistic ( $R_{hat} < 1.1$ ), a calculated Bayesian p-value (bpv) using the Freeman-Tukey statistic, and visual assessment of MCMC chain mixing. Analyses were conducted using the JagsUI packages in R version 3.4.1 (Kellner, 2017; Plummer, 2003; R Core Team, 2013). Model code is available at <https://github.com/meaghanregina/PlethodonLandscapeEcology>

We quantified the relationship of temperature and humidity across elevation and moisture gradients in our study using mixed effects models in a Bayesian framework using the brms package (Bürkner 2017). The iButton and HOBO logger data were combined, and data from 2100 – 0300 EST each night were averaged for each logger. We fit two models, one with temperature and the other with humidity as response variables. Both models used elevation, stream distance, and the interaction of elevation and stream distance as fixed effects, with the environmental logger ID number as a random effect. Both models used a Gaussian distribution with uninformative, flat priors (brms default), run for 2000 iterations after a burn-in phase of 1,000, thinned at a rate of 1. We assessed

model fit based on visual inspection of MCMC chain mixing and the Gelman-Rubin statistic ( $R_{\text{hat}} < 1.1$ ).

After fitting the hierarchical abundance model, we projected abundance across the landscape using 500 random posterior samples generated from the reduced Jags model using R version 3.4.1 (R Core Team 2013). We only included spatial layers generated from ArcGIS v.10.2 from which abundance estimates could be spatially extrapolated, including elevation, elevation<sup>2</sup>, stream distance, northness, TPI, and the interaction of elevation and stream distance. All other covariates (leaf litter, canopy cover) were predicted at their mean value. We then generated eighteen elevation rasters each comprising of a 50-m elevation interval ranging from <600 m to >1450 m (Table 2.1). We generated a multi-ring buffer with twenty-five, 10-m intervals, ranging from 10 m to 250 m, extending away from streams. These buffers were intersected with each elevation raster to determine the stream distance that encompassed both 50% and 95% of the total salamander abundance across the study site (as determined from the spatial projection) at each respective 50-m elevation range.

## Results

We observed a total of 713 *P. shermani* across the four survey periods. Both the global and reduced binomial mixture models fit the data (global bpv = 0.72, reduced bpv = 0.72). All results are reported for the reduced model. The average detection rate of an individual salamander was 0.059 (SD = 0.025), while the total abundance across all sites (total area = 1566 m<sup>2</sup>) is estimated to be 6244.34 (SD = 5571.98). After correcting for

imperfect detection, salamander abundance was positively associated with canopy cover and leaf litter depth, and negatively associated with northness (Table 2.2). Abundance also showed a quadratic relationship with elevation, peaking at ~1350 m (Figure 2.2). Further, there was a significant interactive effect between stream distance and elevation on salamander abundance (Figure 2.3). Detection was negatively associated with surface soil temperature and positively related with belowground soil temperature, ground cover, relative humidity, 24-hour temperature and 3-day precipitation (Table 2.2, Figure 2.4).

The mixed effect models of temperature and humidity showed adequate convergence (all Rhat = 1.00). Temperature was inversely related to elevation ( $\beta = -0.96$ , CRI = -1.32, -0.59), and positively associated with stream distance ( $\beta = 0.27$ , CRI = -0.13, 0.65) (Figure 2.5). There was no relationship between temperature and the interaction of stream distance and elevation ( $\beta = 0.001$ , CRI = -0.38, 0.35). Relative humidity showed a significant negative relationship with stream distance ( $\beta = -3.60$ , CRI = -7.84, 0.55) (Figure 2.5) and non-significant negative relationship with elevation ( $\beta = -1.34$ , CRI = -5.18, 2.58). We also found that salamander abundance varied across the landscape in relation to elevation and stream distance (Figure 2.6). At the lowest elevations (<650 m), 50% of the total abundance is predicted to be within 35 m of streams, and 95% of the abundance is predicted to be within 120 m from streams (Figure 2.7). Higher elevations (>1100 m) reach 50% of the total salamander abundance at >80 m from a stream and 95% of the total abundance >220 m away from streams (Figure 2.7).

## Discussion

The prevailing threat of climate change endangers the persistence and survival of many species, most especially those dependent on specific abiotic conditions. Understanding the relationship between abundance and environmental gradients at the finest possible scale is a critical first step in understanding the potential species responses to such change. By incorporating both fine-scale habitat associations as well as broader landscape-scale gradients, we provide a better understanding of spatial abundance patterns of terrestrial salamanders, and our results provide strong evidence showing variable distribution and abundance patterns across elevation and moisture gradients.

Plethodontid salamanders are generally known to be associated with cool and moist regions (Feder 1983; Grover 2000; Peterman and Semlitsch 2013). Our study demonstrates that these patterns are consistent in montane habitats, as well as showing predictable and repeatable patterns across various environmental gradients, emphasizing the importance of assessing multiple abiotic gradients relevant to the organism. Our results highlight the interaction between stream distance and elevation as a strong driver of the spatial patterns of *P. shermani*, particularly at low elevations where 50% of abundance is contained within 35 m of a stream (Figure 2.7). Higher elevations appear to support higher salamander abundance at greater distances from streams with a more uniform distribution, likely as a result of more optimal temperature and moisture conditions across the broader landscape. Our fitted models indicate that areas farthest from streams at high elevations support the highest abundance of salamanders (Figure 2.3). However, this result is likely due to model extrapolation as the uncertainty in

estimated abundance was greatest at high elevation in areas far from streams (Figure 2.6b).

Temperatures along streams are consistently decoupled from the regional climate and have historically experienced more stable temperature relative to other parts on the landscape (Fridley 2009; Scheffers et al. 2014b; Lesser and Fridley 2016; Caruso and Rissler 2018). Our results suggest that temperature increases and relative humidity decreases as the distance from a stream-side increases (Figure 2.5). Thus, regions close to streams at low elevation likely provide valuable microhabitat for salamanders, which experience hotter and drier conditions than salamanders at higher elevations (Barry 2008). Our results showed a weak inverse relationship between relative humidity and elevation. Other studies of the Southern Appalachian region, as well as other mountainous regions, have failed to identify a consistent pattern between humidity and elevation, likely as a result of the uniformly high humidity across the region (Busing et al. 2005; Duane et al. 2008; Fridley 2009). Further, montane regions often exhibit winds that cause an upslope moisture transport during the day, and downslope moisture transport at night (Barry 2008; Duane et al. 2008). We used temperature and humidity data from only a short nocturnal window, which may be capturing such patterns. Overall, we show that at a fine spatial scale, salamander abundance is linked to cool and moist microhabitats, but these microhabitats vary across a landscape given the abiotic gradients present.

We found salamander abundance to be highest in areas with greater canopy coverage and deeper leaf litter which are factors that influence soil moisture, temperature,

and foraging opportunities, subsequently leading to suitable salamander habitat (Crawford and Semlitsch 2008; Suggitt et al. 2011). *Plethodon shermani* were also marginally more abundant on south-facing slopes, a trend consistent with other observations (Connette and Semlitsch 2013). Previously, forest stand age was identified as an important covariate affecting salamander abundance (Connette and Semlitsch 2013; Dupuis, Smith, and Bunnell 1995). For example, Connette and Semlitsch (2013) found greater salamander abundance in older forests, and peak abundance was not reached until 100 years post- timber harvest. Although we included forest stand age in our global model, it was ultimately removed as it did not have a strong effect on abundance (see Table A.1). Our sampling design was focused on capturing elevation and moisture gradients without specific attention to stand age, potentially leading to the small effect of stand age on abundance in our model (see Appendix A: Figure A.1).

Terrestrial salamanders vertically migrate between belowground refugia and the surface depending on the available climate conditions during their active summer season (Spotila 1972; Feder and Londos 1984). Our study adds additional evidence that including survey-level environmental measures increases the precision of abundance estimates by accounting for variable detection probability based on environmental conditions (Dodd and Dorazio 2004, Connette et al. 2015). Additionally, we found that detection increases with increasing ground cover. It is possible this relationship is due to plethodontid salamander's propensity to climb on top of vegetation (McEntire 2016), subsequently becoming more detectable. It is also possible that increased ground cover has a buffering capacity on temperature and humidity creating more favorable conditions

for salamanders (Frey et al. 2016; Davis et al. 2018). Further, we found a positive effect of the total 3-day precipitation on detection probability (Figure 2.4). Higher rates of precipitation create conditions more suitable for salamander activity, which provides more foraging opportunities, likely leading to greater detectability (Petranka and Murray 2001; Connette and Semlitsch 2013; Peterman and Semlitsch 2013a).

Physiological constraints of plethodontid salamanders have been shown to be a primary mechanism driving abundance (Peterman and Semlitsch 2014). The requirement of salamanders to cutaneously respire limits the suitable habitat available to individuals, however despite such limitations, our study does not assess salamander physiology, body condition, or fitness in each habitat. As such, areas with greater abundance may not indicate greater habitat quality or relate to individual fitness. However, there are numerous interactions between climate conditions and salamander distribution, abundance, and demography. For example, salamander growth rates have been shown to increase with elevation and precipitation (Connette et al. 2015; Caruso and Rissler 2018), and higher temperatures are linked with higher energy assimilation and survival rates, but only to an optimal temperature, that when surpassed, results in declining rates (Clay and Gifford 2016; Caruso and Rissler 2018). Yet, these patterns were examined across broad elevation gradients, neglecting other abiotic gradients across the landscape. Our results show that incorporating fine-scale temperature and moisture gradients could be an important consideration that may affect local demographic rates of salamanders. For example, although it has been shown that growth rates increase with increasing elevation (Caruso and Rissler 2018), salamanders at low elevations in close proximity to streams

may experience similar microclimate conditions to salamanders at higher elevation, and therefore experience similar growth rates. The interactions of key demographic rates with multiple abiotic gradients across local and landscape spatial scales are an increasingly important consideration to fully understanding the responses and resilience of wildlife to climate change. Going forward, it will be important to assess direct measurements of individual fitness, behavior, and physiology in the environment including reproductive output, physiological stress, and overall survival (Ellis et al. 2012).

Relating abundance to local-scale abiotic factors will be most informative in accurately predicting the potential resilience of plethodontids to climate change (Ashcroft et al. 2009; Seo et al. 2009). For example, Gillingham et al. (2012) modeled habitat suitability for ground beetles in Wales under various warming scenarios at both fine (5 m<sup>2</sup>) and coarse (1 km<sup>2</sup>) resolution, and found that coarse resolutions underestimated suitable landscape relative to the fine scale assessment. However, the landscape-level gradients that influence microhabitats must also be considered to more completely ascertain drivers of spatial patterns. On a global scale, McCain and Colwell (2011) showed that extirpation risk of over 800 vertebrates increases ten-fold when precipitation gradients are included in models that previously only considered temperature. With species that are restricted to specific abiotic conditions and limited in movement and dispersal such as plethodontid salamanders, the incorporation of multiple relevant gradients, as well as local-scale conditions will provide the most biologically relevant information in assessing vulnerabilities and responses to climate change (Lenoir et al. 2017; Nadeau et al. 2017). Our results show that previous accounts of plethodontid

distribution have been overgeneralized, therein highlighting the importance of including numerous relevant abiotic gradients at the finest possible scale when assessing species distribution and abundance, especially in the context of assessing responses to global change.

The results from our study show that salamander abundance increases with elevation (Table 2.1), a trend consistent with other studies (Dodd and Dorazio 2004). However, we show that abundance varies with stream distances across elevational gradients (Figure 2.7). Such results lead to important implications for habitat management. Previous studies have offered habitat management recommendations for amphibians (Petranka et al. 1994; Demaynadier and Hunter 1995; Semlitsch and Bodie 2003; Crawford and Semlitsch 2007). For example, Semlitsch and Bodie (2003) recommended a 3-tiered model for conserving herpetofaunal biodiversity around wetland and riparian habitat whereby the region immediately adjacent to the wetland/riparian zone is fully protected, the area of core habitat extending beyond the first zone is conserved, and a buffer outside the core habitat to protect the wetland/riparian zone from surrounding land use is implemented (Semlitsch and Bodie 2003). We support the recommendation of conserved buffer regions surrounding riparian habitats. However, our results allow us to add a more dynamic management strategy in montane systems with respect to the incorporation of elevation gradients. For example, in low elevation (<900 m) regions, 50% of salamander abundance is found within 90 m of a stream, indicating a 90 m conservation buffer is of the utmost importance. At elevations >900 m, and the majority of salamander abundance is not constrained to riparian regions. Therefore, at higher elevation habitats, immediate

riparian conservation may be of less concern for terrestrial plethodontids, and instead managers can focus on promoting spatial habitat heterogeneity across the broader region (Petranka et al. 1994). Managing forests in the Southern Appalachians for terrestrial salamanders, while likely a low priority of forest managers, will provide benefit to the forest ecosystem as a whole and promote the integrity of such systems due to the critical role salamanders play to ecosystem function (Demaynadier and Hunter 1995; Welsh and Droege 2001; Semlitsch et al. 2014). Forest managers should consider the interactions between distance from stream and elevation when making decisions, especially in the context of changing climates.

The conservation of plethodontids is critical, as they contribute to nutrient cycling and storage, leaf-litter decomposition, and ultimately serve as indicators of forest health (Welsh and Droege 2001; Semlitsch et al. 2014). Herein, we showed that across a montane landscape, salamanders are tightly linked to cool and moist regions, leading to heterogeneous yet predictable patterns of abundance across the landscape. Evaluating the patterns of species' distribution and abundance in relation to local-scale habitat and climate variation can reveal critical relationships that may have been missed by assessing only landscape-scale patterns. We emphasize the importance of evaluating how key environmental gradients interact to create local-scale microhabitats that are critical to understanding spatial variation in abundance patterns. It will be important for to consider the dynamics of numerous abiotic gradients when making decisions for conserving biodiversity and ecosystem function in the context of climate change.

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Table 2.1 The total area and salamander abundance at each elevation interval across our study area at Wayah Mountain, NC. These values were used to determine the distance from a stream for which 50% and 95% of the total salamander abundance exists (Figure 2.7)

<b>Elevation Interval (m)</b>	<b>Total Area (hectares)</b>	<b>Total Abundance (millions)</b>	<b>Abundance/m<sup>2</sup></b>
<600	78.08	3.55	3.54
600 – 650	235.02	9.99	3.62
650 – 700	166.72	8.13	3.87
700 – 750	138.28	7.42	4.36
750 – 800	117.00	6.79	4.81
800 – 850	102.68	6.41	5.24
850 – 900	110.12	7.42	5.74
900 – 950	102.20	7.22	6.07
950 – 1000	88.84	6.64	6.47
1050 – 1100	81.44	6.45	6.93
1100 – 1150	73.76	6.16	7.36
1150 – 1200	81.48	7.14	7.76
1200 – 1250	94.09	8.52	8.06
1250 – 1300	100.21	9.28	8.26
1300 – 1350	95.18	9.00	8.46
1350 – 1400	81.58	7.49	8.62
1400 – 1450	59.95	5.86	8.78
>1450	87.99	8.86	9.06

Table 2.2 Parameter estimates for the reduced detection and abundance hierarchical n-mixture model. N effective refers to the effective sample size from posterior samples (total =178,000) and f refers to the proportion of the posterior with the same sign as the mean.

<b>Model</b>	<b>Parameter</b>	<b>Beta</b>	<b>Lower CI</b>	<b>Upper CI</b>	<b>f</b>	<b>n effective</b>
<b>Detection</b>	Intercept	-2.949	-4.449	-2.123	1.00	1,173
	Ground cover	0.042	-0.068	0.151	0.78	31,794
	3-day precipitation	0.082	-0.045	0.206	0.90	36,691
	24-hour temperature	0.098	-0.043	0.236	0.92	50,048
	Relative humidity	0.082	-0.010	0.174	0.96	44,372
	Surface soil temperature	-0.157	-0.338	0.021	0.96	20,129
	Belowground soil temperature	0.089	-0.034	0.214	0.92	52,766
<b>Abundance</b>	Intercept	2.098	-2.808	4.403	0.90	1,182
	Elevation	0.849	-0.164	1.545	0.96	5,768
	Elevation2	-0.733	-1.372	0.369	0.93	3,522
	Northness	-0.031	-0.118	0.055	0.58	16,870
	Canopy cover	0.070	-0.023	0.162	0.93	43,641
	Leaf litter depth	0.068	-0.032	0.164	0.91	172,474
	Stream distance	-0.321	-0.561	-0.029	0.98	26,000
	Elevation * Stream distance	0.294	-0.123	0.590	0.93	46,257

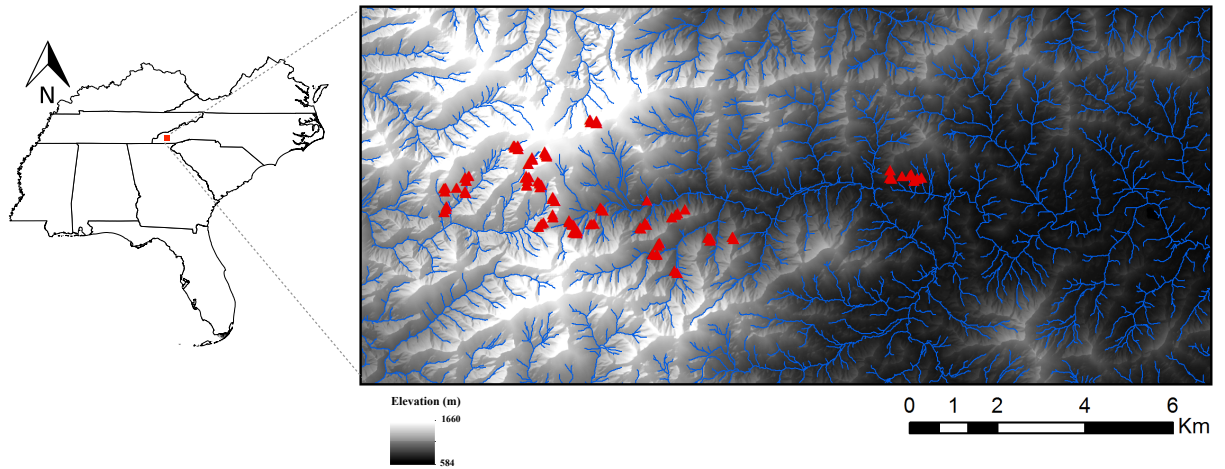


Figure 2.1 Locations of salamander survey plots (red triangles) on Wayah Mountain in the Nantahala National Forest, North Carolina, USA. Blue lines indicate streams.

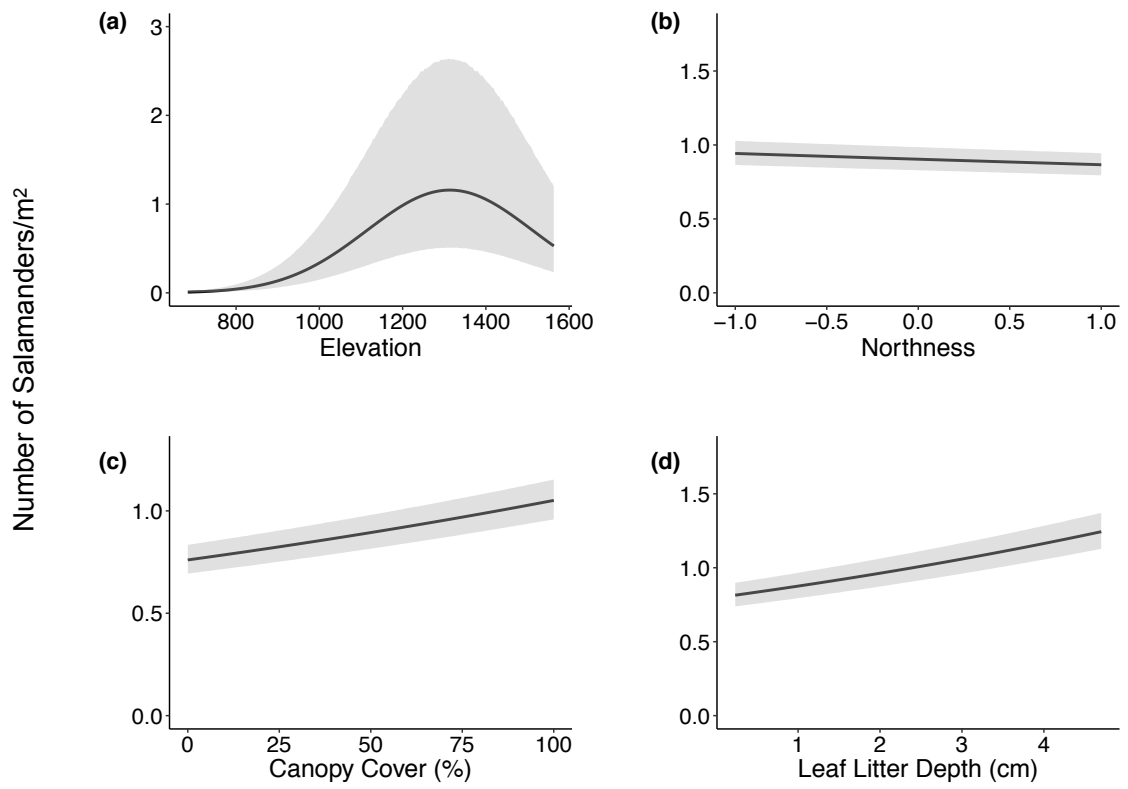


Figure 2.2 Marginal effect plots (+/- CRI) showing the relationship between salamander abundance and (a) elevation, (b) northness, (c) percent canopy cover, (d) leaf litter depth.

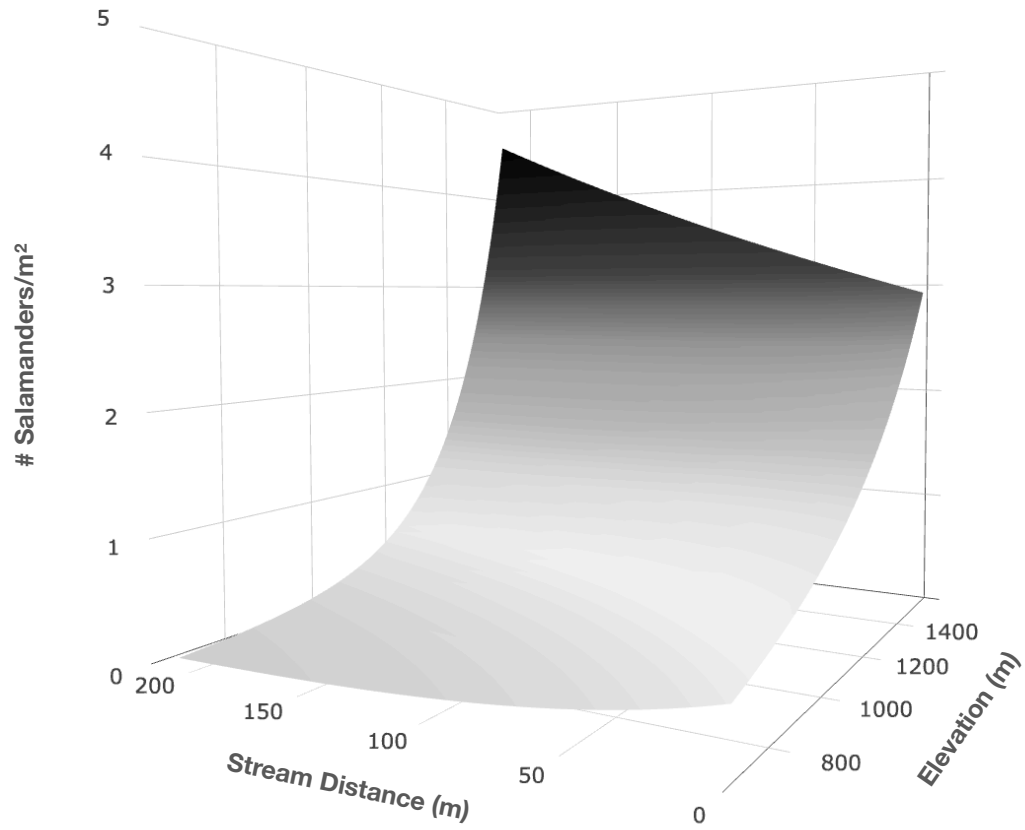


Figure 2.3 Interaction plot showing the relationship between elevation (m), stream distance (m), and *P. shermani* abundance.

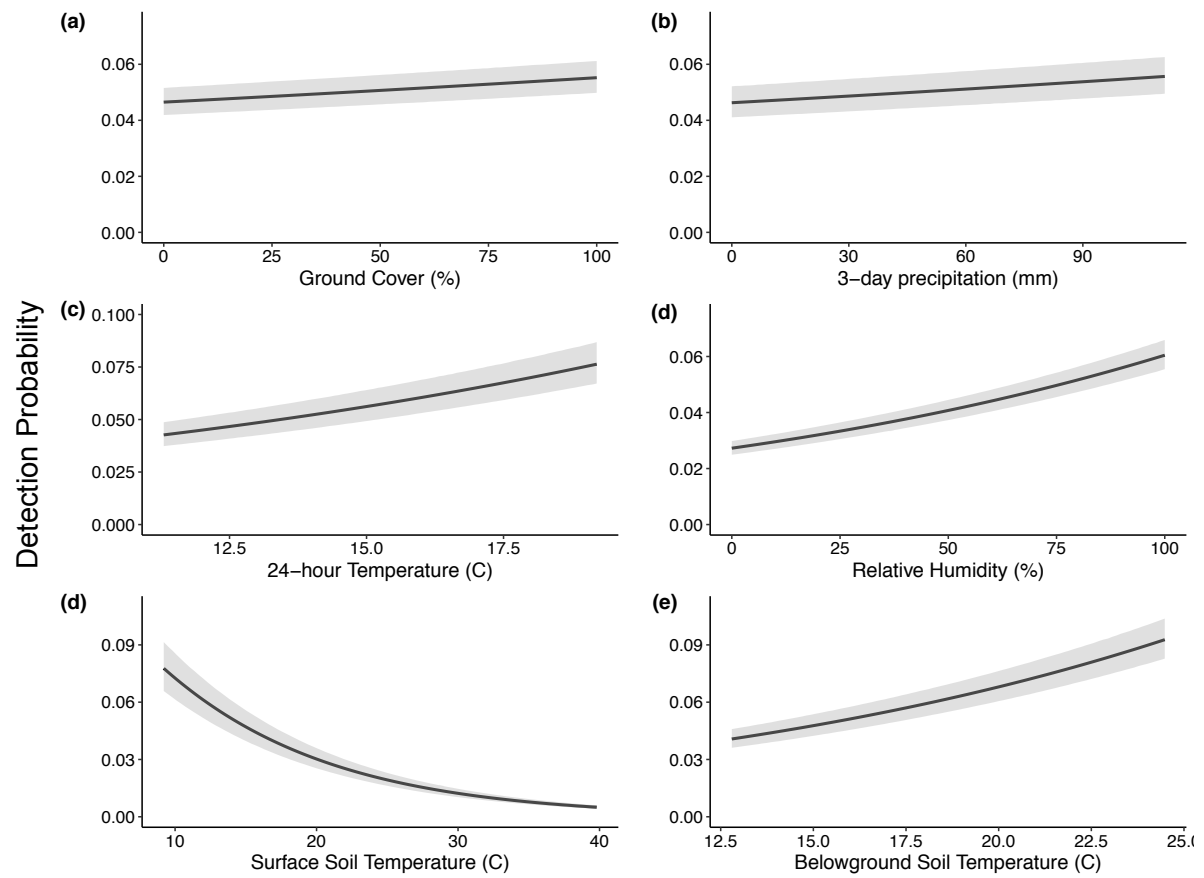


Figure 2.4 Marginal effect ( $\pm$  CRI) of the relationship between detection probability and (a) percent ground cover, (b) 3-day precipitation, (c) 24-hour temperature, (d) relative humidity, (e) surface soil temperature, and (f) belowground soil temperature in *P. shermani*.

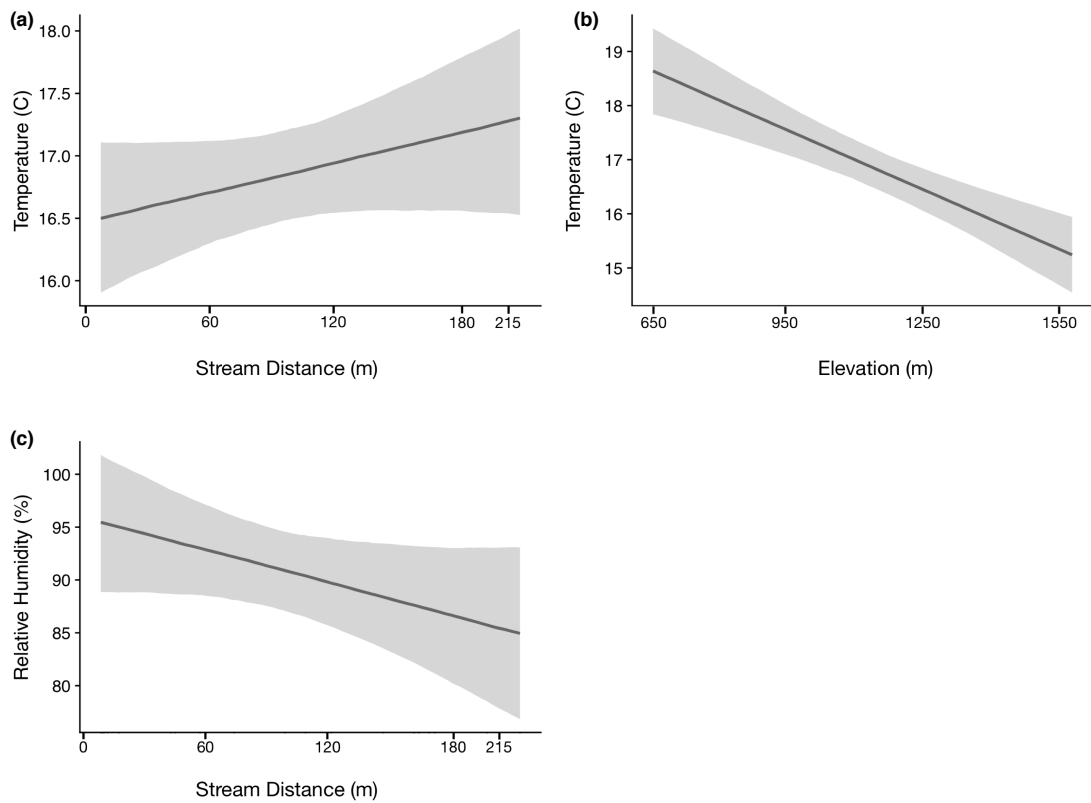


Figure 2.5 Marginal effects plots ( $\pm$  CRI) of a mixed effect model showing the predicted relationship between temperature and (a) stream distance and (b) elevation, and between relative humidity and (c) stream distance.

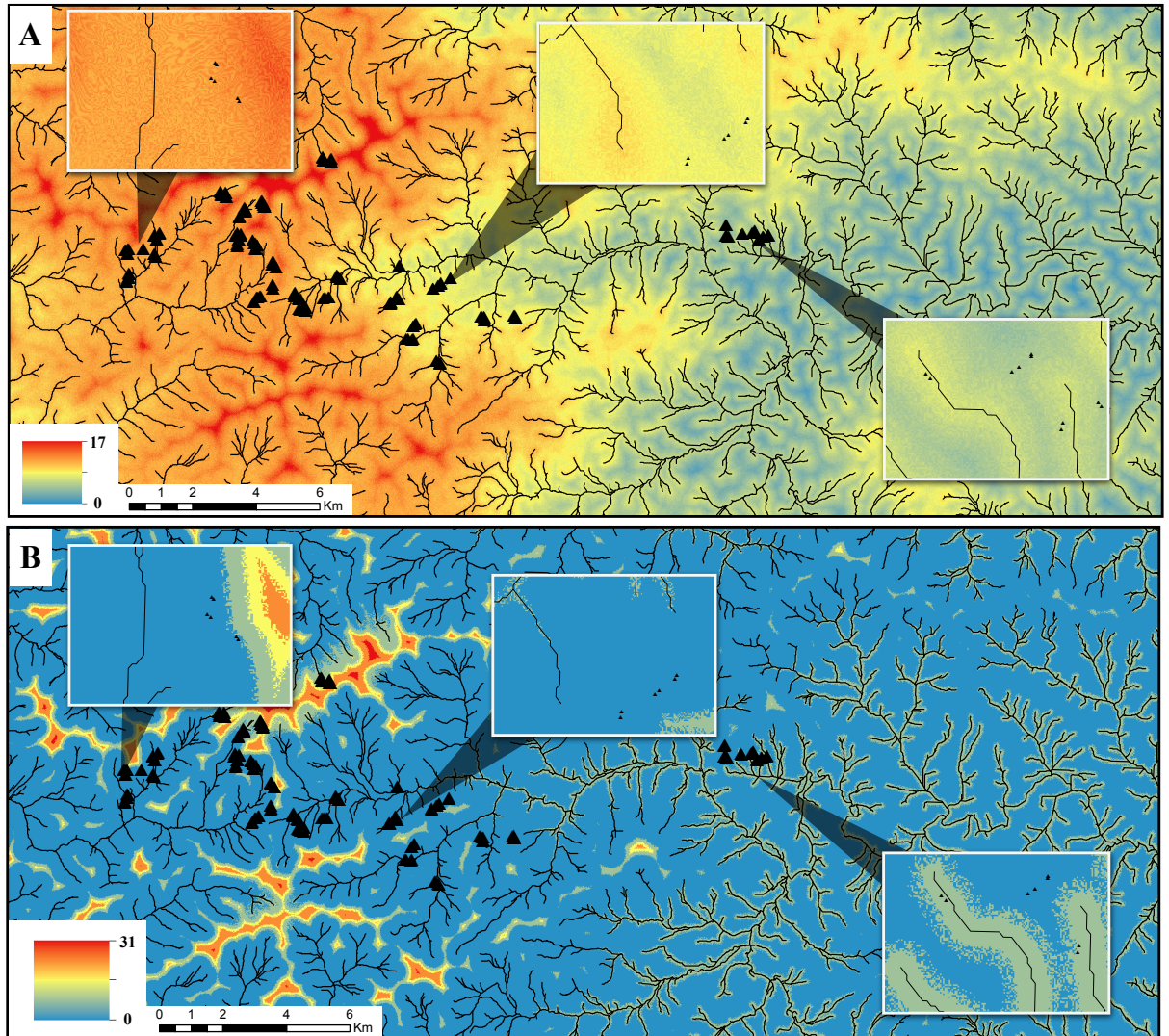


Figure 2.6 (a) Spatial estimate of mean salamander abundance across the Wayah Mountain region of the Nantahala National Forest (b) Standard deviation of the mean salamander abundance. Greater abundance is indicated with warmer colors. Inset maps are at a 120 m scale. Triangles represent sampling sites.

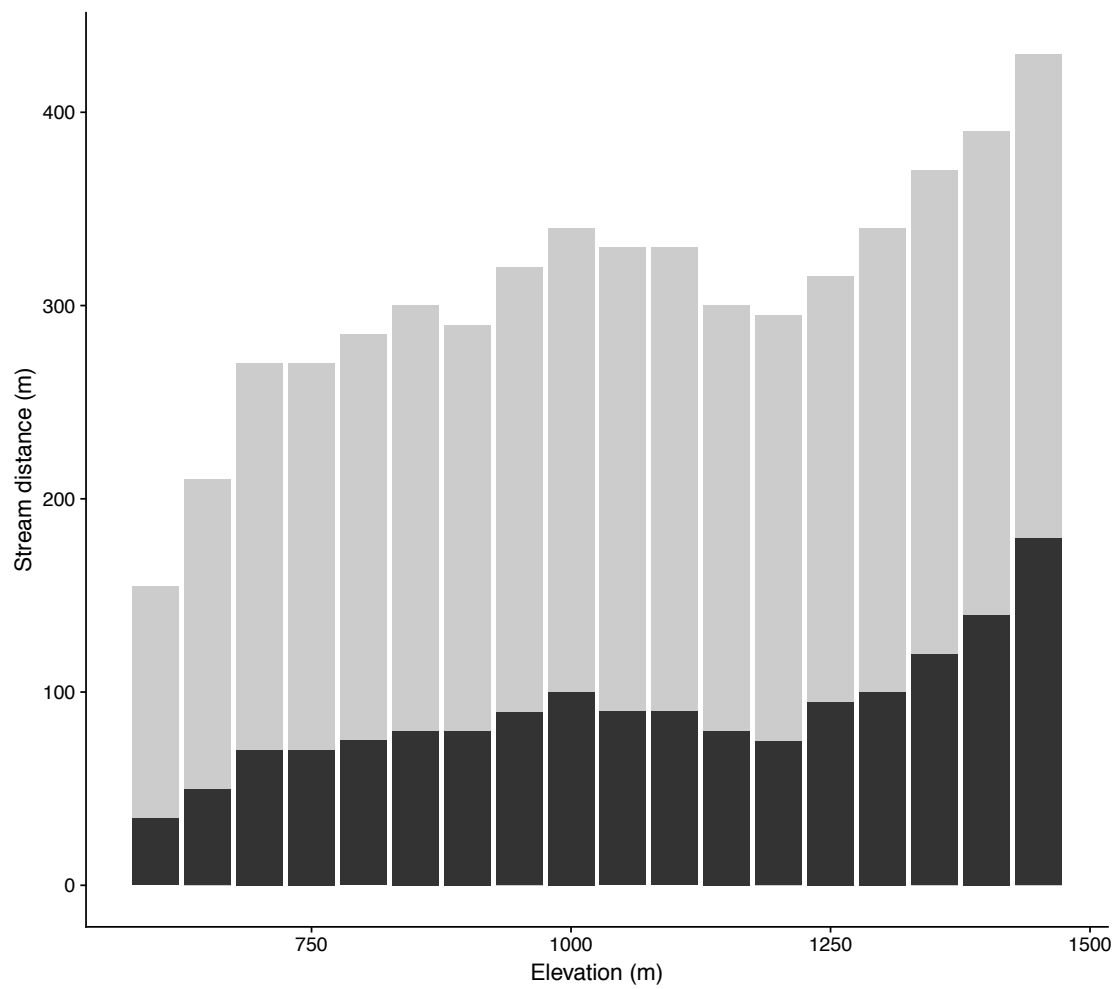


Figure 2.7 Depiction of the distance away from a stream at which 50% (black) and 95% (grey) of the total abundance of *P. shermani* is reached across an elevational gradient on Wayah Mountain, NC.

### Chapter 3. Spatial variation in demographic processes and the potential role of hybridization to future persistence

#### Abstract

Spatial variation in demography plays a crucial role in the structure and dynamics of populations. The demographic responses of local populations to fine-scale habitat heterogeneity have consequences for species at a broader scale. Yet, most previous demographic work focuses on a single to few populations, which may neglect important nuanced spatial heterogeneity. Here, we use multiple modelling approaches including spatially explicit Cormack-Jolly-Seber models and Integrated Population Models (SEIPM) to estimate growth, movement, survival, and reproduction of cryptic terrestrial salamanders across two primary abiotic gradients, elevation (i.e. temperature) and stream distance (i.e. moisture). Considering species of lungless salamanders form hybrid zones between low elevation and high elevation-specialist species, we also investigate the role of hybridization on demographic rates. We found that growth rates were equal across elevations, but larger animals were found at low elevations, likely because of a higher proportion of hybrid animals. We also estimated movement rates to be higher at low elevations, especially far from streams, likely as a result of increased temperatures. Survival was highest at low elevations and significantly declined with distance to stream, where recruitment also was lowest. High elevations exhibited the highest recruitment rates, and may suggest demographic compensation between survival and reproduction

across abiotic gradients. The harsh hot and dry habitats far from streams at low elevations may reflect what future habitats will look like under climate change, which could be a significant threat to the survival and persistence of salamanders. Yet, hybrid animals had higher survival probabilities, suggesting hybridization could be a mechanism for high-elevation, cool temperature-adapted populations to persist with warmer temperatures in the future. Together, our study reveals nuanced spatial variation in demographic patterns that differ in magnitude depending on the scale at which they are assessed. We also highlight the powerful ability of SEIPMs to leverage multiple data sources to provide continuous demographic estimates across environmental gradients without intensive sampling, a framework that future work would adapt to better predict population dynamics.

## Introduction

There is no singular window by which to view every ecological question and careful consideration of the scale of pattern is key to a robust evaluation of any ecological phenomenon (Turner and Gardner 2015). Since landscapes are inherently heterogeneous in both biotic and abiotic features across spatial scales, life history parameters are expected to covary in response to such heterogeneity (Wiens et al. 1993). Microhabitats are a strong driver of life history variation because environmental conditions experienced by individuals at a fine scale can be very different from conditions measured at a broader scale. For example, the breeding success of red billed choughs (*Pyrrhocorax pyrrhocorax*) differs between individual nest sites but not at across a larger spatial scale,

suggesting local-scale variation in habitat likely drives this demographic parameter (Reid et al. 2006). The role of microhabitats are particularly salient for small-bodied organisms like amphibians or small mammals, for example, which interact with the landscape at a fine-scale and experience an environment buffered from broader landscape conditions (Riddell et al., 2021). Understanding fine-scale spatial heterogeneity of demographic rates will provide a clearer understanding of species dynamics across a broader landscape and inform species monitoring and management efforts.

The growing threats from climate change hasten the need for local-scale demographic data. Climate change can have differential impacts on species throughout their range depending on habitat, resources, species adaptive capacity, among others (Urban et al. 2016). These effects may be particularly pronounced for organisms that are highly sensitive to habitat disturbances and have limited dispersal capacity, such as many amphibian species. In response to climate change, organisms may modify their behavior, physiology preferences, and/or alter their geographic ranges to track their optimal climate niche (MacLean and Beissinger 2017). In montane regions, upward range shifts may be limited for high elevation species due to already constrained geographic ranges and ever-decreasing habitat availability at the highest elevation (Elsen and Tingley 2015). For such high-elevation isolates, or “sky island” species, counteracting mortality from extreme conditions with recruitment and/or immigration from other populations is limited, increasing the threat to high elevation population declines (Brown and Kodric-Brown 1977). As a consequence of upward range shifts in montane habitats, some species have modified (e.g., Taylor et al. 2014) and/or formed novel (e.g., Garroway et al. 2010)

hybrid zones. Hybridization can provide a unique answer to climate change by providing genetic variation that may facilitate local adaptation within a single to few generations (Seehausen 2004). In fact, there are reports of hybrid taxa outperforming parental species, especially in extreme, marginal, or novel environments (Willis et al. 2006; Chunco et al. 2012). For organisms that lack evolutionary capacity to keep pace with climate change due to long generation times, low gene flow, or narrow physiological tolerances (Ficke et al. 2007), hybridization could be an important mechanism to allow population persistence in the future (Chunco 2014).

Amphibians are among the most endangered vertebrate taxa as a result of not only climate change, but also disease, habitat loss, and invasive species (Blaustein et al. 2011; Grant et al. 2016). Despite their clear importance to contributing to myriad ecosystem services including nutrient cycling, biological control, bioturbation, and decomposition (Valencia-Aguilar et al. 2013; Hocking and Babbitt 2014), we have little understanding of basic demographic rates for most amphibian species. Without such data, we are severely limited in our ability to effectively conserve and predict species persistence in the future. Amphibians in the family Plethodontidae are particularly susceptible to the effects of climate change in part because these salamanders lack lungs and rely on cool and wet conditions to facilitate gas exchange across the skin surface. As such, any disruptions to their microclimate and habitat may be potentially catastrophic to survival (Feder and Londos 1984). In the Southern Appalachian Mountains, where the highest abundance and species richness of terrestrial *Plethodon* exists, there are multiple hybrid zones between terrestrial *Plethodon* species. One of the most well-studied is that of *P.*

*teyahalee* and *P. shermani* in the Nantahala Mountains of North Carolina (Hairston et al. 1992; Walls 2009b). Hairston et al. (1992) showed that the hybrid zone between these two species has moved upward, with the proportion of individuals with traits of *P. teyahalee*, the low elevation species, increasing with elevation over a 16-year period. Walls (2009) attributed this upward shift to increasing temperatures across elevations. To understand the role hybridization may play in the survival of species requires a detailed knowledge of demographic vital rates of both parent and hybrid. Yet for many *Plethodon* salamanders, we know virtually nothing about their fine-scale demographic vital rates, especially as they relate to spatial variation (but see Caruso and Rissler 2018).

Many significant challenges for measuring demographic rates at fine scales exist including time, labor, and cost intensive sampling. A common method for estimating demographic rates is capture-mark-recapture (CMR) studies whereby individuals within a population are uniquely marked over a period of time and survival and recruitment are subsequently estimated. However, traditional CMR analyses including Cormack-Jolly-Seber (CJS) models cannot distinguish between emigration and mortality, thereby severely biasing apparent survival rates (Marshall et al. 2004; Schaub and Royle 2014). The development of spatially explicit CJS (sCJS) models ameliorate this bias by incorporating specific capture locations to identify when dispersal or emigration has occurred at each plot, allowing for the estimation of true, instead of the traditional apparent survival (Schaub and Royle 2014). However, with this statistical advancement comes the requirement for a large amount of CMR data to obtain reliable estimates. Also, the sCJS model still does not provide a comprehensive understanding of metapopulation

dynamics. Integrated population models (IPM) have been increasingly applied in ecological research as an elegant solution to intensive, long-term data collection as they can estimate latent parameters that have not been measured directly (Schaub and Abadi 2011). IPMs also leverage numerous data sources including CMR, repeated counts, reproduction, among others, to account for multiple sources of uncertainty to more accurately and precisely estimate demographic parameters (Schaub and Abadi 2011). Further, spatially explicit IPMs (SEIPM) allow for heterogeneity in reproduction and survival processes based on the spatial variation in the landscape, density dependent processes, and dispersal between habitat patches (Zhao 2020). SEIPMs also are more flexible in the data inputs and can accommodate large amounts of count data with smaller amounts of CMR data. Count data is typically far simpler to collect in space and time in comparison to the time-consuming and expensive CMR data collection, adding an additional benefit to SEIPM.

In this study, we use three different models to understand spatial patterns in demographic parameters for terrestrial salamanders across a montane landscape to understand how demographic variation at fine scales are constrained by broader scale patterns. The Southern Appalachian Mountains are characterized by stark abiotic gradients that shape the distribution and abundance of terrestrial *Plethodon* across scales. Gade and Peterman (2019) found that at low elevations, where the regional climate is hot and dry, salamanders are distributed and in the highest abundance near stream sides. Stream sides offer a microhabitat that is cooler and moister than the surrounding landscapes. However, at high elevations, salamanders are distributed more uniformly

across the landscape and are less tied to stream sides since the regional climate at high elevations is more broadly cool and wet (Gade and Peterman, 2019). The dynamics of broad-scale elevation and local-scale stream distance gradients interact to drive the distribution of these salamanders, yet we do not understand how population dynamics vary across these gradients. Using 4 years of CMR data and 3 years of count data, we attempt to fill this knowledge gap to provide an understanding of both fine-scale and landscape-scale spatial dynamics of terrestrial *Plethodon* salamander population demographics.

## Methods

### Data collection

#### Field Surveys

We initiated a long-term spatial capture-mark-recapture (sCMR) study in 2017 to assess salamander demographics and life history across elevation and moisture gradients.

Twelve plots, 10m x 10m in size, gridded off in 1-m<sup>2</sup> sections, were spatially arranged to capture an elevation and moisture gradient on Wayah Mountain, Macon County, North Carolina (35.158, -83.574). Specifically, six plots were situated at low elevation (~900 m) and six plots were at high elevation (~1500 m). At each elevation, two replicate sets of three plots were < 5 m of a stream (close), between ~100 – 150 m from a stream (medium), and >190 m from a stream (far) (Figure 3.1). Each plot was centered within a larger 20 m x 20 m plot that was sampled for captured animals only. To minimize

extraneous variation, forest stand, slope, aspect, and stream order were standardized to the best extent possible among plots (Appendix B: Table B.1).

We conducted nocturnal area-constrained surveys of each plot whereby at least two observers exhaustively searched plots and hand captured surface-active salamanders, recording their specific capture location to within 1 m<sup>2</sup>. We conducted at least 3 (maximum= 5) surveys each year from 2017–2020 during salamander active season, between May and August. Captured salamanders were placed in a sealable bag with moist leaf litter and transported to Highlands Biological Station, approximately 60 km away. At each plot during each survey, we measured environmental covariates including surface soil temperature, soil temperature at 10 cm below ground, air temperature, wind speed, and relative humidity using an infrared thermometer (Raytek MT4), soil temperature probe, and Kestrel 5200.

Salamanders were housed at Highlands Biological Station in a 10°C environmental chamber to limit their metabolism (Connette, 2014) until processing occurred within 24 hours of each survey. We anesthetized each salamander in an Orajel solution (1g/1L) and uniquely marked them with a visual implant elastomer (Northwest Marine Technologies, LLC). We also recorded morphometric data including snout-vent-length (SVL), tail length, sex, and mass, as well as a photograph of the dorsum of each individual using an iPhone 6S+. We then returned salamanders to their capture location (within 1 m) within 48 hours of initial capture.

### Hybrid quantification

Our study region was within a known hybridization zone of *P. teyahalee* and *P. shermani*, (Hairston et al., 1992; Highton & Peabody, 2000; Walls, 2009). *Plethodon teyahalee* is distinctly characterized by white spots across their body and *P. shermani* is characterized by red coloration on the legs. Thus, we quantified hybridization from photographs by estimating the percent of each leg covered with red coloration and counting the number of white dots on 4 body regions: (1) head, (2) anterior dorsum, (3) posterior dorsum, and (4) tail (Appendix B: Figure B.1). We loaded the percent red and spotting scores into a Principal Components Analysis to find a composite measure of ‘hybridization’.

Dimension 1 accounted for 49.2% of the variation, and we subsequently used the Dimension 1 scores for each individual in the growth and sCJS modeling discussed below. Hybrid scores ranged from -2.20 to 2.49 with positive values indicating more hybrid characteristics and negative values indicating more ‘pure’ *P. shermani* characteristics (Appendix B: Figure B.2). The scoring method accounts only for the observed phenotypic traits of hybrids, neglecting genetic variability or gene combinations, and should thus be considered a coarse metric of hybridization.

### Count surveys

We established 87-paired (total plots = 174) count plots, each 3 x 3 m in size situated across elevation (700 – 1550 m) and stream-distance (0 – 250 m) gradients. Each plot was visually surveyed for surface active salamanders between 2130 and 0230 EST. We estimated the age (juvenile or adult) of each counted individual by visual estimation of snout-vent-length (SVL), whereby adults were > 45 mm. Plots were surveyed 4 times in

2017 and once in 2018 and 2019. Specific details of the count data can be found in Gade and Peterman (2019).

### Growth Model

We estimated the effect of elevation, stream distance, hybridization score, and precipitation on individual growth during the active season using Fabens formulation of the von Bertalanffy curve growth model (Fabens 1965). We used the average SVL of an individual if it was captured multiple times over a single year and we only included animals captured over at least two years. Since the sCMR plots were arranged at high and low elevation, we included elevation as a binary variable and stream distance as a continuous variable. Precipitation data was obtained from the Wayah Bald Mountain WINE weather station from the North Carolina Climate Retrieval and Observation Network of the Southeast Database (<https://climate.ncsu.edu/cronos>). The WINE station is the nearest weather station to our sites, located at the at one of our high elevation sCMR sites. We calculated the cumulative precipitation for the active season 01 May to 31 August for each survey year (Appendix B: Figure B.3).

The growth function was defined for individual  $i$  at time  $t$  as:

$$SVL0_{i,t} = SVL0_{i,t-1} + \left\{ L - SVL0_{i,t-1} \times \left[ 1 - \exp\left(-K_{i,t} \times \frac{I}{365}\right) \right] \right\}$$

where asymptotic size,  $L$ , was estimated as a function of categorical elevation ( $ELE$ ; low, high) as:

$$L = L_0 + L_1 \times ELE_i.$$

$SVL0_{i,t}$  is size at first capture and follows a Uniform distribution with a minimum of 10 and maximum of 80,  $L_0$  was estimated from a Normally distributed prior with a mean of

60 and precision of 0.01 while  $L_1$  was estimated from a Normally distributed prior with a mean of 0 and precision of 0.01.  $K$  represents the individual growth rate, and  $I$  is the annual scaling interval between captures. We considered  $K$  as a function of categorical elevation (high/low), and continuous distance to stream ( $STR$ ), precipitation ( $PREC$ ), and hybrid status ( $HYB$ ) such that:

$$K_{i,t} = \beta_0^{[K]} + \beta_1^{[K]} \times ELE_i + \beta_2^{[K]} \times STR_i + \beta_3^{[K]} \times ELE_i \times STR_i + \beta_4^{[K]} \times PREC_t + \beta_5^{[K]} \times HYB_s.$$

We used vague normal priors for all growth rate covariates with a mean of 0 and precision of 0.01. We fit the model in JAGS (Plummer 2003) using *jagsUI* (Kellner 2017). Following a burn in of 1,000 and adaptation phase of 5,000, 5 MCMC chains were run for 12,000 iterations, thinned at a rate of 5 for a total posterior sample of 18,220.

We then used the growth rates to estimate time to maturity at both high and low elevations. For high elevations, we estimated time to maturity based on the size of the smallest gravid female *P. shermani* as estimated by Connette (2014), 49.4 mm, because we did not capture any gravid females during our surveys. For low elevations, we estimated the size of maturity for *P. shermani* hybrids based on the nearest reported estimate of size at maturity of a closely related species to *P. teyahalee*, *P. glutinosis*, in Giles County, VA (Highton 1962), which was 57 mm. We then averaged the mature sizes of *P. glutinosis* and *P. shermani* (53 mm) because hybrid individuals often exhibit traits intermediate of their parent species (Seehausen 2004). We used the average hatchling

size at high elevation (19.15 mm) and low elevation (22.09 mm) as the starting size to generate two growth curves (Connette, 2014).

#### Spatial Cormack-Jolly-Seber Model

We used a spatial Cormack-Jolly-Seber (sCJS) model (Schaub and Royle 2014) to examine the spatial variation in survival and dispersal. A sCJS model differs from traditional CJS models by incorporating locations of each individual relative to the study area; in our study, spatial locations refer to the 1-m<sup>2</sup> grid cell each salamander was captured. The model assumes that death, birth, immigration, and emigration could occur between, but not within years and equal recapture probability across all individuals and years. The true latent status of an individual  $i$  at time  $t$ ,  $z_{i,t}$  (1 for alive and 0 for dead), was modeled with a Bernoulli distribution given the status in the previous time step and survival probability such that:

$$z_{i,t}|z_{i,t-1} \sim \text{Bernoulli}(z_{i,t-1} \times \phi_{i,t}),$$

in which survival probability ( $\phi_{i,t}$ ) was modeled as a function of categorical elevation (high/low), continuous stream distance, and site-level hybrid score such that:

$$\text{logit}(\phi_{i,t}) = \beta_0^{[\phi]} + \beta_1^{[\phi]} \times ELE_i + \beta_2^{[\phi]} \times STR_i + \beta_3^{[\phi]} \times ELE_i \times STR_i + \beta_4^{[\phi]} \times HYB_s.$$

Survival estimates were scaled to annual survival by including a term indicating the length of time between capture periods.

The change in location (G) for individual  $i$  between the time  $t$  ( $G_{i,t}$ ) and time  $t+1$  ( $G_{i,t+1}$ ) is modeled using a random walk (Turchin and Thoeny 1993) such that:

$$G_{i,t+1} \sim \text{Normal}(G_{i,t}, \chi_i^2).$$

in which the variance  $\chi_i^2$  is a function of covariates such that:

$$\log(\chi_i^2) = \beta_0^{[\chi]} + \beta_1^{[\chi]} \times ELE_i + \beta_2^{[\chi]} \times STR_i + \beta_3^{[\chi]} \times ELE_i \times STR_i + \beta_4^{[\chi]} \times HYB_s.$$

In this way the metric of dispersal is a measure of activity center variance between survey periods (Munoz et al. 2016; Schaub and Royle 2014).

The observation process of the model which is conditional on survival and presence in the study area, indicated by a step function of whether individual  $i$  is inside ( $r_{i,t} = 1$ ) or outside ( $r_{i,t} = 0$ ) of the study area at time  $t$ . The observation process is then expressed as:

$$y_{i,t} | z_{i,t}, r_{i,t} \sim \text{Bernoulli}(z_{i,t} \times r_{i,t} \times p_{i,t}).$$

where  $p_{i,t}$  is the recapture probability of individual  $i$  at time  $t$ . We modeled recapture probability as a function of air temperature (ATEMP), relative humidity (HUMID), and surface soil temperature (STEMP) such that:

$$\text{logit}(p_{i,t}) = \beta_0^{[p]} + \beta_1^{[p]} \times ATEMP_t + \beta_2^{[p]} \times HUMID_t + \beta_3^{[p]} \times STEMP_t + \varepsilon_{i,t}^{[p]}.$$

We used vague normal priors for all covariates with a mean of 0 and precision of 0.01.

We fit the model in JAGS (Plummer 2003) using *jagsUI* (Kellner 2017). Following a burn in of 75,000 and adaptation phase of 60,000, 10 MCMC chains were run for 165,000 iterations, thinned at a rate of 5 for a total posterior sample of 305,000.

### Spatially Explicit Integrated Population Model

We used a spatially explicit integrated population model (SEIPM) to account for spatiotemporal variation in initial population size, immigration, emigration, survival, and per capita reproduction rate in relation to habitat and density covariates (Zhao 2020). This model jointly analyzes count and CMR data by combining a spatially explicit dynamic N-

mixture model (Zhao et al. 2017) with a multistate capture-recapture model (Arnason 1973). The particular benefit of the SEIPM in this study is that it allowed us to leverage multiple data sets that spanned different spatial, temporal, and information coverage for more robust demographic estimation. Unlike the sCJS model, SEIPM allowed for estimations along continuous stream and elevation gradients because of the sampling strategy of the count plots.

For the purposes of the SEIPM, we split the landscape into 100 x 100 m grids and combined the counts of all plots within a grid to reduce the total number of plots from 174 to 50 and reduce overall variation. The model with reduced number of plots resulted in improved mixing of MCMC chains and more efficient sampling of posterior distributions, in comparison to the same model with the original number of plots. Six of the twelve capture-recapture plots overlapped spatial locations with count plots (Fig 1). We included an offset term ( $\log[area]$ ) to account for variable search area within each grid dependent on the number of combined count plots. We did not include hybrid score in this analysis since we did not photograph salamanders from the count plots.

The SEIPM model is a hierarchical Bayesian model containing a process model which describes how local population sizes vary spatially and temporally and an observation model which describes how count and CMR data are obtained. The process model assumes that the local population size in the first year at habitat patch  $s$ , denoted  $N_{s,1}$ , follows a Poisson distribution such that  $N_{s,1} \sim \text{Poisson}(\lambda_s^{[0]})$ , in which  $\lambda_s^{[0]}$  is a function of continuous elevation and stream distance such that:

$$\log(\lambda_s^{[0]}) = \beta_0^{[0]} + \beta_1^{[0]} \times ELE_s + \beta_2^{[0]} \times STR_s + \beta_3^{[0]} \times ELE_s \times STR_s + \varepsilon_s^{[0]} + \log(area),$$

in which the residuals  $\varepsilon_s^{[0]}$  follow a Normal distribution with mean 0 and standard deviation  $\sigma^{[0]}$ . For subsequent years, the model assumes that variation in local population sizes are a consequence of demographic processes including survival, reproduction, immigration, and emigration, and thus have  $N_{s,t} = R_{s,t} + S_{s,t} - E_{s,t} + I_{s,t}$ , where  $R_{s,t}$  is the number of individuals that are reproduced in habitat patch  $s$  and year  $t$ ,  $S_{s,t}$  is the number of individuals in habitat patch  $s$  that survived from year  $t-1$  to  $t$ ,  $E_{s,t}$  is the number of individuals that emigrated from habitat patch  $s$  in year  $t$ , and  $I_{s,t}$  is the number of individuals that immigrated into habitat patch  $s$  in year  $t$ . We further assumed that  $R_{s,t}$  follows a Poisson distribution such that  $R_{s,t} \sim \text{Poisson}(N_{s,t-1} \times \gamma_{s,t-1})$ , in which the per capita reproduction rate  $\gamma_{s,t-1}$  is a function of local population size, elevation and stream distance such that:

$$\log(\gamma_{s,t}) = \beta_0^{[R]} + \beta_1^{[R]} \times (N_{s,t} - \lambda_s^{[0]}) / \lambda_s^{[0]} + \beta_2^{[R]} \times ELE_s + \beta_3^{[R]} \times STR_s + \beta_4^{[R]} \times ELE_s \times STR_s + \varepsilon_{s,t}^{[R]} + \log(area),$$

in which the residuals  $\varepsilon_{s,t}^{[R]}$  follows a Normal distribution with mean 0 and standard deviation  $\sigma^{[R]}$ .

We assumed that  $S_{s,t}$  follows a Binomial distribution such that  $S_{s,t} \sim \text{Binomial}(N_{s,t-1}, \omega_{s,t-1})$ , in which the survival probability  $\omega_{s,t-1}$  is a function of local population size, elevation and stream distance:

$$\log(\omega_{s,t}) = \beta_0^{[S]} + \beta_1^{[S]} \times (N_{s,t} - \lambda_s^{[0]}) / \lambda_s^{[0]} + \beta_2^{[S]} \times ELE_s + \beta_3^{[S]} \times STR_s + \beta_4^{[S]} \times ELE_s \times STR_s + \varepsilon_{s,t}^{[S]} + \log(area),$$

in which the residuals  $\varepsilon_{s,t}^{[S]}$  follows a Normal distribution with mean 0 and standard deviation  $\sigma^{[S]}$ .

We further assumed that  $E_{s,t}$  followed a Binomial distribution such that:

$$E_{s,t} \sim \text{Binomial}(S_{s,t}, \kappa),$$

in which  $\kappa$  is the probability of emigration given that an individual survived. Immigrant individuals are assumed to be emigrants from other patches, and the number of immigrants was calculated as

$$I_{s,t} = \sum_{j=1}^n M_{j,s,t},$$

where  $M_{j,s,t}$  is the number of individuals that moved from grid  $j$  to grid  $s$  at year  $t$  and follows a Multinomial distribution such that  $M_{j,s,t} \sim \text{Multinomial}(E_{j,t}, w_{j,s})$ . The movement rate between grid cells  $j$  and  $s$  is a function of the distance between the centroids of these two grids ( $d_{j,s}$ ):

$$w_{j,s} = \exp(-\theta \times d_{j,s} + \varepsilon_{j,s}^{[M]}) / \sum_{s=1}^n \exp(-\theta \times d_{j,s} + \varepsilon_{j,s}^{[M]})$$

where  $\theta$  is the decay parameter and the error term  $\varepsilon_{j,s}^{[M]}$  follows a Normal distribution with mean 0 and standard deviation  $\sigma^{[M]}$ .

The observation model assumes that the age-structured counts are generated for adults and juveniles separately without individual identification. Counts for adults at site  $s$  and year  $t$  during survey  $k$  informs local populations  $N_{s,t}$  such that

$Y_{s,t,k}^{[a]} \sim \text{Binomial}(N_{s,t}, p_t^{[obs]})$ , and counts for juveniles informs  $R_{s,t}$  such that  
 $Y_{s,t,k}^{[j]} \sim \text{Binomial}(R_{s,t}, p_t^{[obs]})$ , in which the detection probability  $p_t^{[obs]}$  is a random effect  
such that  $\text{logit}(p_t^{[obs]})$  follows a Normal distribution with mean  $\mu^{[obs]}$  and standard  
deviation  $\sigma^{[obs]}$ . The capture-recapture process also assumes a capture probability  $p_t^{[cap]}$   
which again is a random effect such that  $\text{logit}(p_t^{[cap]})$  follows a Normal distribution  
with mean  $\mu^{[cap]}$  and standard deviation  $\sigma^{[cap]}$ . The likelihood of the individual  
encounter history is based on  $\omega_{s,t}$ ,  $\kappa$ ,  $w_{j,s}$ , and  $p_t^{[cap]}$ .

We used vague normal priors for all covariates with a mean of 0 and precision of  
0.01. We fit the model in JAGS (Plummer 2003) using *jagsUI* (Kellner 2017). Following  
a burn in of 2,500 and adaptation phase of 5,000, 15 MCMC chains were run for 35,000  
iterations, thinned at a rate of 15 for a total posterior sample of 30,495. Fully annotated  
code for each of the above-described models can be found at  
<https://github.com/meaghanregina/Plethodon-IPM-SCJS>. For all estimates and covariates  
we considered this meaningful support for the direction of the effect if >85% of the  
posterior density was to one side of zero.

## Results

Across 15 surveys over 4 years, we captured a total of 2,310 salamanders representing six  
species (*Desmognathus occoeae*, *D. monticola*, *D. quadramaculatus*, *D. wrighti*, *Eurycea*  
*wilderae*, and *Plethodon shermani*), among which 532 (23.0%) were recaptured. We  
captured 1,712 individuals of the target species, *P. shermani*, recapturing 447 animals at

least once (range: 1–6; 26.1% recapture rate). The average SVL of all *P. shermani* captured was 53.66 mm (range: 20.83 – 77.70 mm). The average SVL for adult females across all sites was 59.31 mm (range: 37.15 – 77.70) and adult males was 57.98 (range: 40.24 – 74.45). Out of 753 animals captured and photographed across the 6 low elevation sites, 730 (97.50%) exhibited some level of visible hybrid traits. No high elevation animals exhibited hybrid traits.

*Growth Model* — Salamanders from high elevations had higher growth rates (*K*) (mean: 0.561, 95% CRI: 0.477, 0.652) than those from low elevation (mean: 0.457, 95% CRI: 0.387, 0.536) (Table 1). There was a negative effect of the distance from stream on growth at high elevations (mean: -0.034, 95% CRI: -0.066, -0.004) but the effect of stream distance was positive at low elevation (mean = 0.020, 95% CRI: -0.011, 0.055). There was also a strong positive effect of precipitation at both high (mean = 0.209, 95% CRI: 0.102, 0.320) and low elevations (mean = 0.063 95% CRI: -0.022, 0.149) on growth. At low elevations, animals with more hybrid characteristics had reduced growth (mean = -0.015, 95% CRI: -0.048, 0.016), Salamanders at high elevation reached a lower asymptotic size (66.01 mm, 95% CRI: 64.28, 67.85) than those at low elevations (73.52 mm, 95% CRI: 71.65, 75.50) (Table 3.1). The mean estimated age of maturity at high elevation is 1.81 years (95% CRI: 1.77, 1.84) and for low elevation hybrids is 2.02 years (95% CRI: 1.98, 2.12) (Figure 3.2).

*sCJS* —Across our survey period, the average annual survival of salamanders was 0.595 (95% CRI: 0.508, 0.694) at high elevation and 0.942 (95% CRI: 0.744, 1.00) at low elevations. At high elevation, there was no effect of stream distance on survival (mean =

0.056, 95% CRI: -0.498, 5.16) and stream distance had a marginally negative effect on survival at low elevations (mean = -1.046, 95% CRI: -9.773, 3.15). Animals with more hybrid characteristics had increased survival probability (mean = 0.221, 95% CI: -0.128, 0.575, Figure 3.3) and decreased movement probability (mean: -0.578, 95% CRI: -0.763, -0.383; Fig 3.3). Salamanders from high elevation had a mean movement of 1.822 m (95% CRI: 0.058, 6.504) while those at low elevation had a mean movement of 3.323 m (95% CRI: 0.107, 12.001) (Fig 3.4A). There was greater variance in movement at low elevations (10.341; 95% CRI: 6.966, 14.831) than high elevations (3.113, 95% CRI: 2.579, 3.794) (Fig 3.4B). The distance from stream had a marginally negative effect on movement at high elevation (-0.082, 95% CRI: -0.233, 0.062) but a substantial positive effect at low elevation (0.320, 95% CRI: -0.038, 0.681) (Fig 3.4A). The average individual recapture probability across all sites was 0.157 and recapture was positively influenced by relative humidity and surface soil temperature and negatively influenced by air temperature (Figure 3.5).

*SEIPM*—Our models show that initial population size increased with elevation (mean = 0.529, 95% CRI: 0.305, 0.749) and decreased with the distance from stream (mean = -0.118, 95% CRI: -0.362, 0.128) without an interaction between elevation and stream distance (mean = 0.018, 95% CRI: -0.255, 0.277). The average survival across all sites was 0.432 (95% CRI: 0.315, 0.513) and was not density dependent (mean = 0.175, 95% CRI: -1.877, 1.903). There was a significant negative interaction between elevation and stream distance on survival, meaning that survival was highest at low elevation near streams and declined with the distance from stream at low elevations (Figure 3.6). Per

capita reproduction averaged 0.249 (95% CRI: 0.153, 0.421) across the landscape and was marginally negatively influenced by population density (mean = -0.657, 95% CRI: -1.840, 0.054). There was also a significant positive interaction between elevation and stream distance on per capita reproduction rate whereby reproduction increases with elevation and at low elevation, reproduction decreases with stream distance (Figure 3.7). Emigration probabilities were low (mean = 0.014, 95% CRI: 0.001, 0.073) with a low mean dispersal parameter (mean = 0.015, 95% CRI: 0.001, 0.177) indicating a low probability that an individual will move from their 100-m grid cell, with an average number of individuals moved per year at each patch across the landscape 0.079 (95% CRI: 0.001, 0.100). The average detection probability for animals across all count plots was 0.270 and average recapture probability across all sMRC sites was 0.338.

## Discussion

Estimating demographic variation in populations across space provides a window into how landscape heterogeneity drives ecological processes (Gurevitch et al. 2016). Using elevation and stream distance as proxies for temperature and moisture variation, we reveal nuanced spatial patterns in population demography across temperature, moisture and hybridization gradients, as well as how demographic rates at a fine scale have consequences for species performance at broader landscape scales. Previously, Gade and Peterman (2019) showed that salamander abundance is linked to cool and moist microhabitats which vary across a montane landscape. Our present study shows that spatial heterogeneity of microhabitats drives demographic rates such that growth,

movement, and survival are highest at low elevations and both growth and movement increase with stream distance at low elevation. We also estimated survival and per capita reproductive rate to decline with stream distance at low elevations, but reproduction rates to increase with elevation.

High elevation salamanders grow faster and reach maturity approximately two and half months earlier than low elevations animals, but low elevation animals reach a larger size. Larger asymptotic sizes at low elevation is likely attributed to the larger hatchling sizes of hybrid animals. Hairston (1983) similarly noted that *P. jordani* (a closely related species to *P. shermani*) and *P. teyahalee* populations had similar size distributions at all ages, but *P. teyahalee* reached a larger maximum size. Interestingly, our estimates for age at maturity across elevations were lower than previous estimates. Hairston (1982) estimated that *P. teyahalee* reaches maturity at approximately 5-years of age and Connette (2014) estimated female *P. shermani* reach maturity at 2.53 years. Such differences in estimates may be a result of the environmental conditions both preceding and during surveys. While growth rates have been shown to increase with precipitation (Connette et al. 2015; Caruso and Rissler 2018), large adult *P. shermani* appear to be disproportionately active under drier conditions (Connette et al., 2015). During the survey period of Connette et al. (2015), the average cumulative annual rainfall was 535.23 mm and the four years preceding their sampling time frame were extreme drought years, whereas the average cumulative rainfall during our survey period was 553.01 mm and the four years preceding our sampling time frame were exceptionally wet (Appendix B: Figure B.2). It is possible that the overall drier annual conditions before and during

Connette (2014) lead to a systematic bias towards capturing larger individuals that grow at a slower rate relative to animals captured during our wetter study period.

Our results also suggest that precipitation has a much larger effect on growth at high elevations than low elevations (Table 3.1). Relative to *P. shermani*, *P. teyahalee* are larger bodied and subsequently have higher resistance to water loss (Riddell and Sears 2015). With greater resistance to water loss, larger hybrid animals may have less reliance on precipitation for growth (Table 3.1). While growth rates in our study were primarily affected by precipitation, stream distance also impacted growth rates across elevations, albeit in opposite directions and with nominal effect (Table 3.1). Given our model estimates and previous work (Connette et al. 2015; Caruso and Rissler 2018; Caruso et al. 2020), precipitation influences growth rates more strongly than spatial variation in moisture or temperature. In the Southern Appalachian region, precipitation is expected to increase in variability in the future (Kunkel et al. 2020), which could have significant demographic consequences. For example, annual growth rates may increase in variability over time leading to changes in time to maturity, reduced fecundity, and changes in population size structures.

Terrestrial plethodontid salamanders are known to have small home ranges and limited dispersal capacity of less than 3 m (Cabe et al. 2007; Muñoz et al. 2016; Caruso and Rissler 2018). While trends broadly show low dispersal capacity in *Plethodon*, our results suggest that high elevation *P. shermani* move less and with less variation than low elevation animals, and movement decreases with stream distance at high elevations and increases with stream distance at low elevations (Figure 3.4). Movement rates may be

tied to temperature considering low elevations are warmer than high elevations, especially far from streams (Gade and Peterman 2019). Temperature increases surface activity probability in *Plethodon* (Gade et al. 2020), other salamander families (Johnson et al. 2010), and other ectotherms like lizards and fish (Xiang et al. 1996, Petty et al. 2012) and so long as the temperature lies within the thermal window of the animal. Additionally, large-bodied individuals, such as those at low elevations, have higher metabolic rates that are further increased with warmer environmental conditions (Gifford et al. 2013). As such, more surface activity dedicated to nutrient acquisition may be required to offset the metabolic costs associated with low elevation habitats and further feedback into the lower reproduction rates estimated at low elevations (Figure 3.7). Additionally, movement may be tied to resources availability. For example, arthropod abundance increases with elevation at mid-latitudes (Supriya et al. 2019) and low elevations in the Smoky Mountain National Park harbor a greater diversity of low quality *P. shermani* prey items including ants (Jaeger 1980; Lessard et al. 2011; Lewis et al. 2014). Thus, low elevation animals must move more to obtain necessary resources. Finally, increased movement at low elevation could be related to decreased population density (Gade and Peterman 2019b). Our plot-level estimates of movement from the sCJS suggest little movement within a plot, and our IPM provides further evidence that there is very little dispersal and emigration across the broader landscape.

By using multiple modeling frameworks, we obtained both fine- and broad-scale understanding of demographic parameters including survival rates. The sCJS model reflects individual, plot-level survival estimates derived only from mark-recapture data

on 10x10-m plots, whereas the SEIPM survival estimates are reflective of local population averages of the entire landscape (i.e. all habitat patches) and are derived from both annual mark-recapture and count data. While trends in sCJS and SEIPM survival rates similarly showed that survival decreased with elevation and that stream distance has differential effects across elevation, the absolute value of survival probabilities varied. The sCJS estimated altogether higher survival probability than the SEIPM, but the SEIPM allowed for a continuous and more nuanced understanding of the interaction between elevation and stream distance across the landscape. The reduced survival estimates from the SEIPM may therefore be attributable to the greater variation and heterogeneity of the landscape-scale count data. Additionally, count data can be quite noisy and variable. Terrestrial salamanders are cryptic and highly sensitive to environmental conditions to become surface active and detectable (O'Donnell et al. 2015; Gade et al. 2020; Hocking et al. 2020). Aside from our first year of data collection, we visited count plots only once, providing a snapshot of the population. We are thus putting a heavy burden on our ability to account for individual-level detection, but our estimated detection rates suggest this should not be an issue. It is only through our integration of independent data sources in the SEIPM that we could estimate demographic rates across continuous elevation and stream distance gradients, which was not achievable with the sCJS model without more extensive, and likely intractable, mark-recapture sampling across the landscape.

Our SEIPM estimated that annual *P. shermani* survival was highest at low elevations. Caruso and Rissler (2018) described a similar survival trend in a closely

related species, *P. montanus*, such that animals from low elevation had higher survival than those at high elevation and that survival was positively associated with temperature. Temperature appears to be linked with higher energy assimilation and survival rates, but only to an optimal temperature, which when surpassed, rates decline rapidly (Caruso & Rissler, 2018; Clay & Gifford, 2018a). In our system, we see further evidence for this relationship in our stream distance gradient whereby survival probability dropped to 0.19 in animals found at low elevations and far from streams (Figure 3.6). Temperature increases and humidity decreases with stream distance at low elevations (Gade & Peterman, 2019), creating high vapor pressure deficits that exacerbate water loss and provide unsuitable conditions for terrestrial salamanders (Riddell et al. 2017; Peterman and Gade 2017). The hot and dry conditions far from streams provide a combination of abiotic stressors that salamanders may not be able to survive. For example, Chapter 4 of this dissertation found that salamanders can compensate their hormonal stress response to one environmental stressor (e.g. temperature), but are overwhelmed by multiple abiotic stressors (e.g. temperature and lack of moisture). Thus, multiple environmental stressors appear to significantly impact the overall fitness of animals. While our SEIPM suggests a strong positive effect of stream distance on survival at high elevations (Figure 3.6), this trend is due to increased uncertainty in model estimates (Appendix B: Figure B.4 and B.5), as these regions were not well sampled due to logistical constraints. Gade and Peterman (2019) suggest similar model limitations with regions at high elevation and far from streams showing the greatest uncertainty in abundance estimates.

Per capita reproduction increases with elevation (Figure 3.7) and may be a contributing factor to the positive association between elevation and abundance patterns observed in Gade and Peterman (2019). In a related *Plethodon* spp., population growth rates were lowest at low elevations, aligning with our reduced reproductive rate estimates (Caruso et al. 2020). Higher reproductive rates at high elevations may also serve as a compensatory mechanism for the lower estimated survival probability at high elevations (Muths et al. 2011; Vilellas et al. 2015; Buckley et al. 2021). Negative covariance between vital rates can occur to maintain demographic performance over environmental gradients. Such demographic compensation between reproduction and survival may contribute to local adaptation to environmental conditions (Angilletta et al. 2003) and we observed other vital rates that may contribute to energy tradeoffs and local adaptation across temperature and moisture gradients. For example, since low elevation animals experience warmer temperatures and higher metabolic demands (Clay and Gifford 2018b), individuals must remain surface active to obtain the necessary resources for growth potentially at the cost to reproductive output. Conversely, at high elevations animals move less while still maintaining equal growth rates allowing for greater investment into reproduction. Further, lungless salamanders must constantly regulate and invest energy into water loss resistance, which is achieved through capillary bed regeneration or regression (Riddell et al. 2019). With low elevation animals moving more and experiencing higher desiccation probability, there is likely a significant amount of energy diverted from reproduction and invested into water loss resistance for survival purposes. Alternatively, at high elevations where conditions are broadly cooler and

wetter, there may be less energy budgeted to water loss regulation, allowing energy investment into reproduction. We also observed both reproduction and survival rates declining with stream distance at low elevations (Figure 3.6, 3.7) suggesting the hot and dry conditions in these habitats surpass any ability of salamanders to maintain viable populations. The complex spatial patterns of vital demographic rates provide valuable insight into compensations between life-history and energy allocation across a landscape with multiple abiotic gradients.

Our study focuses on the spatial variation in demographic rates across abiotic gradients that represent the range of optimal to extreme environmental conditions experienced by individuals. This spatial approach offers an understanding into the role of abiotic gradients on demography but neglects the temporal aspect of demographic rates. Due to the somewhat limited timescale of our study (4 years) especially relative to the lifespan of *Plethodon* (~10 years; Staub, 2016), we do not capture enough temporal variation to make robust estimates. Demographic rates are influenced at different time scales. For example, survival is often affected by short term exposure to extreme environmental conditions whereas fecundity and reproduction tend to be an integrated response to longer-term environmental stochasticity (Levins 1968; Gilchrist 1995; Buckley et al. 2021). While our present study, to some extent, substitutes space-for-time to understand how temperature and moisture gradients influence population vital rates at individual plot-levels as well as across the entire landscape, we are limited in our inference of how stochasticity in weather events (i.e., droughts, heat waves) affect vital

rates in the long term. Continued monitoring of these populations will be necessary to disentangle temporal effects on demography.

Hybridization has the potential to allow species to survive in rapidly changing environments by promoting phenotypic and genotypic novelty more quickly than typical evolutionary mechanisms (Arnold 1997; Rieseberg et al. 2003). In our study, hybrid individuals, as quantified by purely phenotypic traits, had a higher survival probability than non-hybrid, “pure” *P. shermani* (Figure 3.3). Hybridization between populations adapted to diminishing abiotic environments and those preadapted to emerging environments offers a unique avenue for species to survive climate change (Chunco 2014). The warmer temperatures expected with climate change in the future may particularly benefit hybrid *P. shermani* because *P. teyahalee* are preadapted to occupy warmer and drier microhabitats (Hairston et al., 1992). In fact, Gifford and Kozak (2012) found that temperatures are limiting the low elevation range of *P. jordani* while competition limits the upper elevational range of *P. teyahalee*. Thus, *P. shermani* hybrids may have physiological traits that predispose them for survival in warmer, drier conditions. However, *P. teyahalee* tend to occupy warmer but moist microhabitats that minimize water loss (Farallo et al. 2020), which may potentially balance the costs of higher temperatures. Our survival estimates decline with stream distance at low elevation, where temperatures are high and humidity is low, a microhabitat that appears unsuitable even for hybrid individuals. Thus, hybridization may only be beneficial if moist microhabitats are maintained in the future.

The larger body size of *P. shermani* hybrids may further benefit their future persistence because large-bodied salamanders have higher resistance to water loss (Riddell & Sears, 2015). Interestingly, we saw that hybrids moved less than “pure” *P. shermani*, despite lower elevation salamanders moving more than those at high elevations, especially at further distances from streams (Figure 3.3A). However, we do see greater variance in movement rates at low elevations (Figure 3.4B), which may be attributed to the contributions from variable hybrid individual movement probability. Hybrid dysfunction in movement has been observed in burst-speed performance of *Ambystoma* larvae (Fitzpatrick 2008) but other work has found adult *Ambystoma* hybrids move greater distances and faster, especially under warmer temperatures (Johnson et al. 2010). It is possible that since hybrids are larger in body size, they occupy and defend higher-quality cover objects that provide better resources, and thus require animals to move less (Krzysik 1979; Keen 1982; Mathis 1990). However, more research is needed to further untangle the complex movement patterns in hybrid *Plethodon*. Contemporary hybridization may facilitate Plethodontid diversification through increased speciation and decreased extinction rates (Patton et al. 2020), and may be a creative strategy for rapid adaptation to novel stressors expected with climate change. It will be critical to continue monitoring hybrid populations for greater resolution of the role hybridization may play in this and other *Plethodon* species.

Evaluating the spatial variation in multiple demographic rates not only provides understanding of how such rates respond to environmental heterogeneity but also helps to guide our understanding of the ongoing effects of climate change. Mountains are

excellent natural laboratories to assess climate change (Tito et al. 2020) and in our system, it appears that regions at low elevations and close to streams may be representative of how high elevation habitats could be altered with climate change. In the context of our study, this assumption means that in the future, salamanders may experience increased survival with reduced growth rates, but reproductive rates could decline. However, if extreme warming and drying occurs, we may see higher elevation habitat changes into those more akin to low elevations far from streams, where we estimated declines in both survival probability and reproductive rates. If such habitat change occurs, there is a potential for salamander population collapse in the future.

Our study demonstrates that demographic estimates can vary by the scale at which they are assessed. Robustly sampled fine-scale estimates provide valuable information but may not necessarily represent landscape-scale patterns. Our study shows that data sources measured at different scales can be combined to estimate nuanced spatial variation in critical demographic rates across a heterogeneous landscape and highlights the need for future studies to evaluate vital rates and life history across relevant environmental gradients. Estimation of landscape-scale demographic rates has previously been limited by time, costs, and logistics. SEIPMs provide a rigorous and coherent framework for maximally leveraging disparate data sources and a promising tool for future spatial demographic studies.

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Table 3.1. Parameter estimates for the growth model of *P. shermani*. Parenthetical high and low refer to elevation-specific intercept estimates. L is the asymptotic size, K is growth rate, and f indicates percent of the estimates that lies to one side of zero. Stream distance, hybrid score, and precipitation are continuous covariates of K with elevation-specific estimates.

Parameter	Mean	2.5% CRI	97.5% CRI	f
L (High)	66.01	64.38	67.85	1.00
L (Low)	73.51	71.67	75.50	1.00
K (High)	0.56	0.48	0.65	1.00
K (Low)	0.46	0.39	0.54	1.00
Stream (High)	-0.03	-0.07	0.00	0.99
Stream (Low)	0.02	-0.01	0.05	0.89
Hybrid (High)	0.00	-0.36	0.41	0.54
Hybrid (Low)	-0.02	-0.05	0.01	0.84
Precipitation (High)	0.21	0.10	0.32	1.00
Precipitation (Low)	0.06	-0.02	0.15	0.93

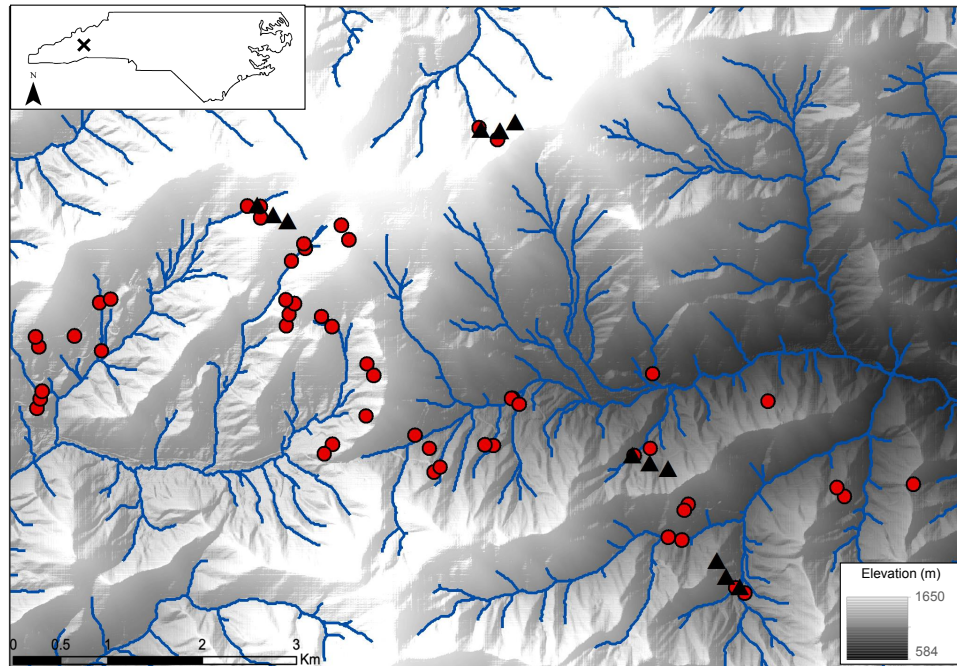


Figure 3.1. Locations of the salamander count plots (red circles) and mark-recapture plots (black triangles) on Wayah Mountain in the Nantahala National Forest in western North Carolina.

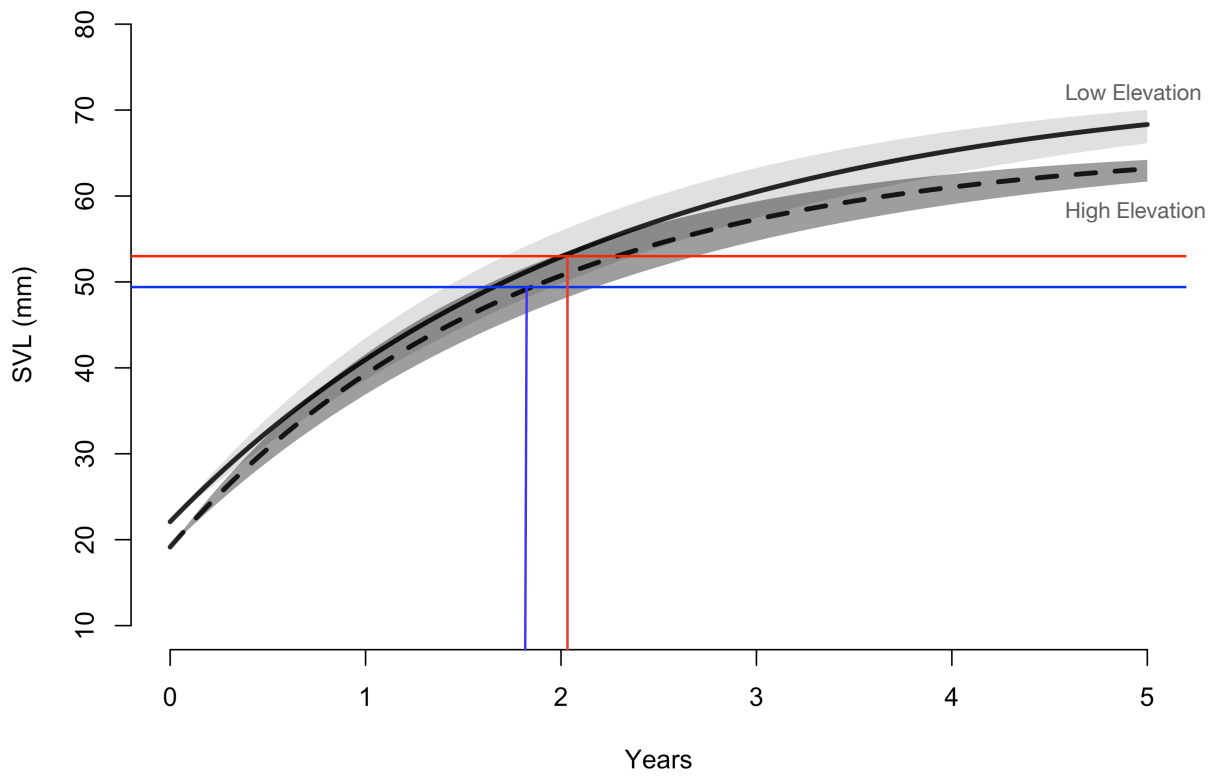


Figure 3.2. Growth projection for high elevation (dotted line) and low elevation (solid line) *P. shermani* based on the growth model. The horizontal lines represent minimum size (in snout vent length, SVL, in mm) at maturity for high elevation ‘pure’ *P. shermani* (blue) and low elevation hybrid *P. shermani*.

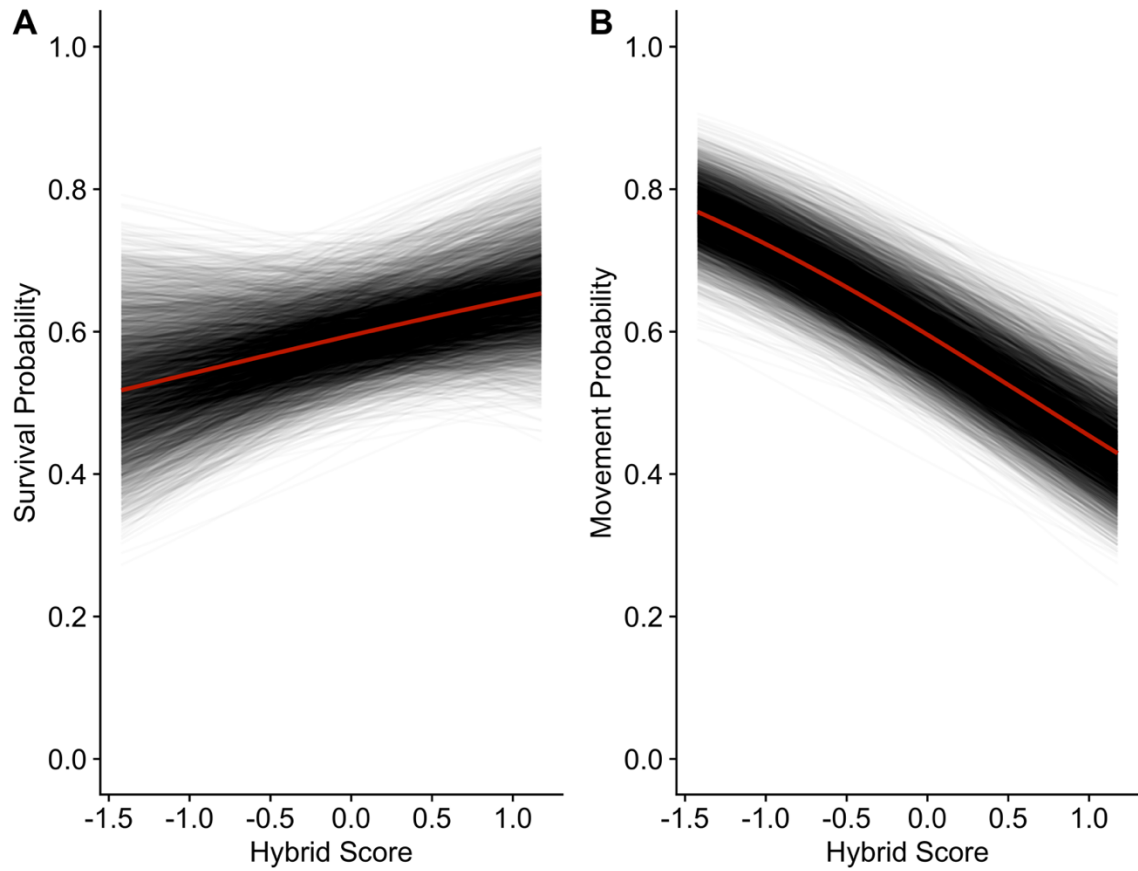


Figure 3.3 The effect of hybrid score on (A) survival probability and (B) movement probability of *P. shermani* from 5000 random draws of the posterior of the sCJS model. Positive hybrid scores indicate more characteristics of *P. teyahalee* and thus represent more hybridization.

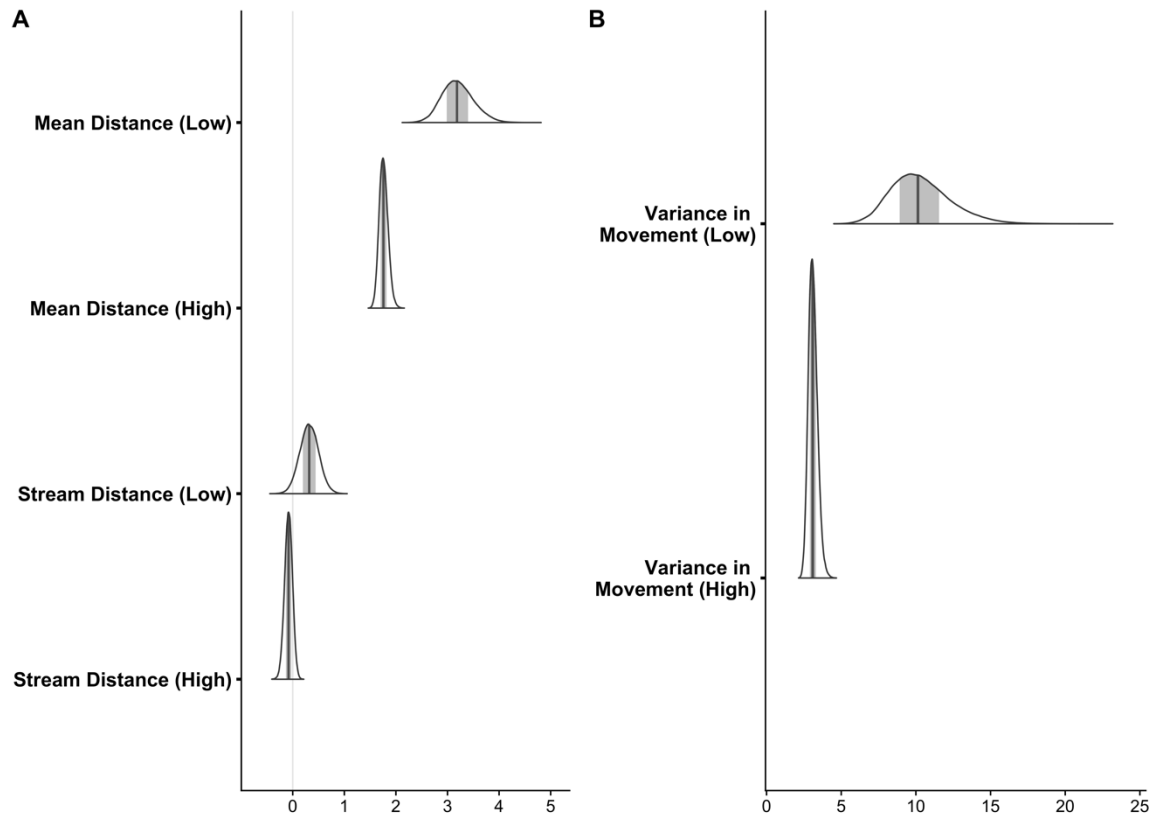


Figure 3.4. Posterior mean densities for the (A) mean movement distance and stream distance covariates of *P. shermani* and (B) the variance in movement estimated in the sCJS. The parenthetical High and Low refer to the two elevation intercepts estimated. The shaded region indicates 50% of the posterior.

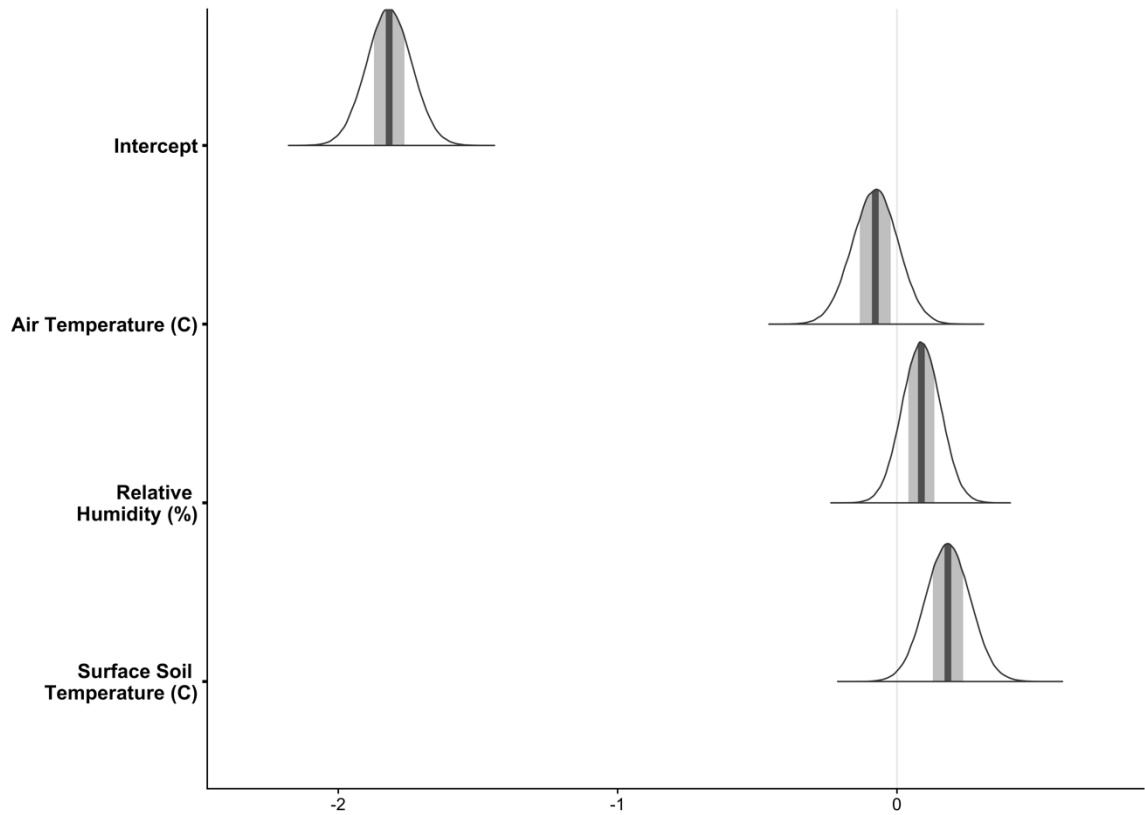


Figure 3.5. Posterior mean density estimates for the detection covariates of *P. shermani* estimated in the sCJS. The shaded region includes 50% of the posterior.

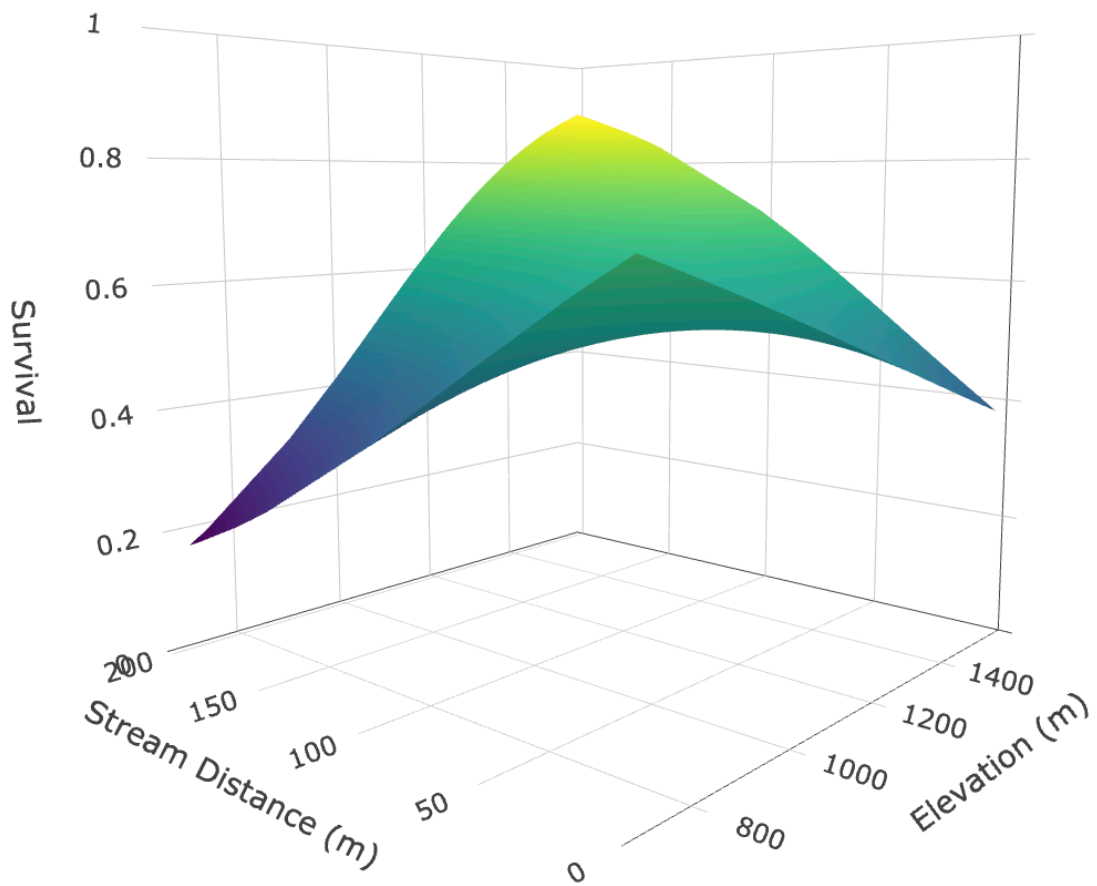


Figure 3.6. Interaction between elevation and stream distance on the survival probability of *P. shermani* from the SEIPM. Survival probability is highest at low elevation and decreases with stream distance at lower elevations.

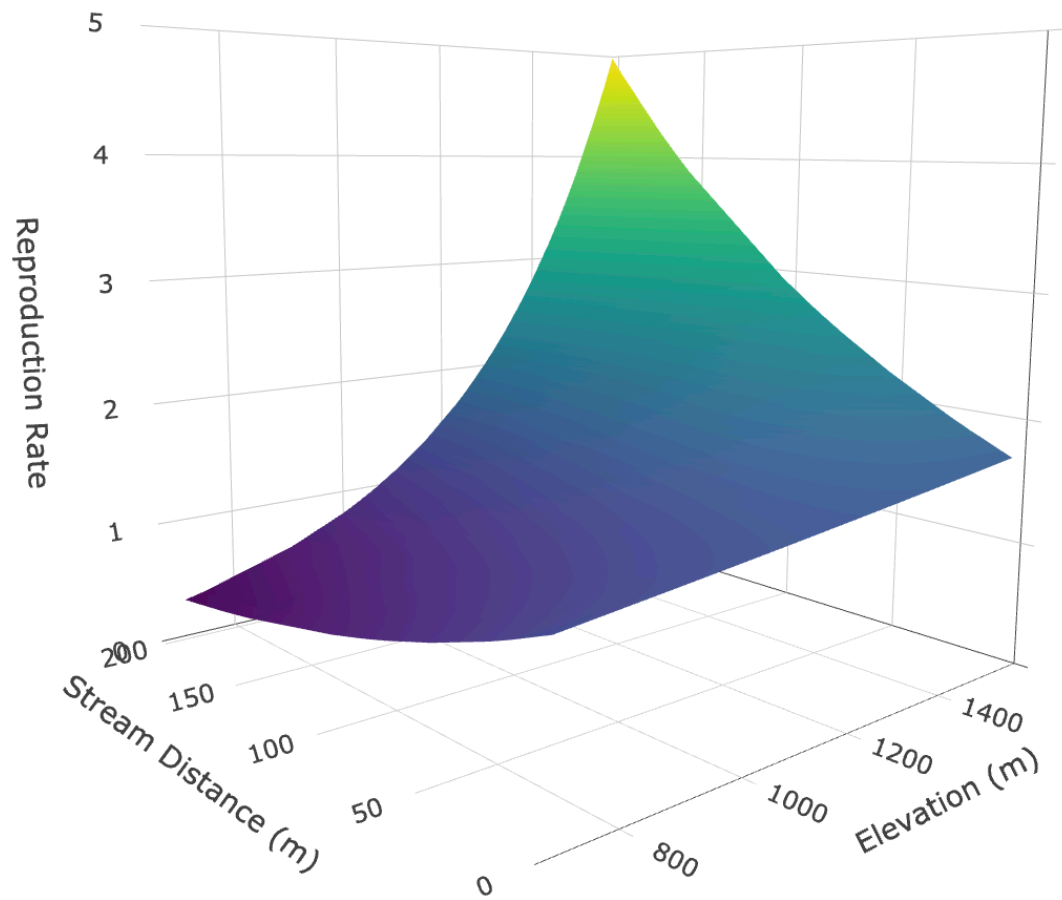


Figure 3.7. Interaction between elevation and stream distance on per capita reproduction rate of *P. shermani* from the SEIPM. Per capita reproduction increases with elevation and at low elevations, decreases with stream distance.

## Chapter 4. Dermal Corticosterone variation across the landscape and in the face of climate change in a montane amphibian

### Abstract

In response to the current and increasing threat of climate change, many organisms exhibit stress responses that can negatively impact their fitness and survival. Evaluating current stress levels as well as predicting stress responses to future conditions is critical to informing actionable conservation management strategies. However, the predicted shift towards hotter and drier conditions is likely to differentially impact animals across the landscape because of fine-scale microhabitat differences. It is unclear how organisms sensitive to environmental perturbations like amphibians will physiologically respond to climate change across a landscape. We had two goals: (1) measure baseline dermal CORT (dCORT) levels of a montane salamander (*Plethodon shermani*) across temperature and moisture gradients *in situ* and (2) measure dCORT levels when experimentally exposed to climatically stressful conditions. We found that high elevation salamanders had higher baseline dCORT levels than those at low elevations, but there was no difference in dCORT across moisture gradients at any elevation. When experimentally exposed to dry conditions (50% humidity), salamanders showed less dCORT, had no difference in dCORT levels after exposure to hot conditions (25°C), and showed additively higher amounts of dCORT when exposed to the combination of hot and dry conditions. Yet, salamanders from all landscape origins responded similarly to

each treatment. Our results suggest dCORT likely indicates a stress response such that salamanders may be able to tolerate the effects of a single stressor but become overwhelmed by multiple stressors. Further, the dCORT metric appears to be associated with glucocorticoid responses and may serve an important role in physiological regulation across salamander's elevational range. By integrating both field and experimental approaches with a novel methodological technique, we highlight the importance of evaluating multiple possible stressors of wildlife to best predict potential resilience in the face of climate change.

## Introduction

Wildlife are increasingly exposed to numerous environmental stressors such as habitat modification, pollution, and changing climate. With these growing threats, it is imperative to mechanistically understand how wildlife will respond so effective conservation can be implemented. Anthropogenic climate change may be among the most pervasive threats to wildlife, especially for ectothermic organisms whose performance and survival are directly reliant on environmental conditions. However, climate change effects are spatially complex and vary by latitudinal position and/or topographic exposure (Sears et al. 2016; Nadeau et al. 2017). Thus, individuals of the same population that occupy a spatially heterogeneous environment likely experience and respond to climate change differently (Dobrowski 2011). Understanding fine-scale responses to environmental spatial heterogeneity is vital to accurately assessing organismal responses to climate change.

Organisms have a suite of physiological mechanisms for coping with environmental heterogeneity. Physiological processes such as metabolism, oxygen consumption,

ingestion rates, and stress are often temperature dependent, whereby organisms evolve maximum physiological performance within an optimal thermal window, and performance quickly declines outside the bounds of the window (Huey and Stevenson 1979). Although the effects of temperature on physiology are important to understanding species responses to climate change, moisture may supersede or interact with temperature as a driving force regulating physiological processes (Carey and Alexander 2003; Peterman and Semlitsch 2014). In fact, the explicit incorporation of moisture conditions is often neglected, resulting in oversimplification of organismal responses to environmental change (but see Studds and Marra 2007, Studds et al. 2008). For example, compared to the effects of temperature alone, amphibians were at a 60% higher risk of declines when changes in both temperature and precipitation were modeled (McCain and Colwell 2011). Incorporating climatic complexity into future physiological response predictions will bolster our understanding of how organisms will respond to future climatic conditions.

Stress is a key climate-sensitive physiological regulatory process because hormone levels interact with other vital processes such as reproduction, behavior, growth, and immune function (Ricklefs and Wikelski 2002). Stress is often quantified using circulating glucocorticoids (GC), which activate the hypothalamic-pituitary-interrenal axis (HPI) in amphibians and reptiles (Sapolsky et al. 2000). HPI activation mobilizes energy stores to cope with external stressors and reallocates energy away from other vital processes (Sapolsky et al. 2000, Wingfield and Romero 2000). GC's have also been shown to mediate blood flow and regulate water and salt balance, which is particularly important for desiccation-vulnerable species such as amphibians (McCormick and

Bradshaw 2006). These effects of GC release have the benefit of avoiding negative impacts of acute stressors, but a prolonged response to chronic stressors can result in elevated baseline levels with severe impacts (Wingfield and Romero 2000). Thus, quantifying GC secretion may provide a measurement of animal health and condition and provide a window into how stress levels vary across environments (Wingfield and Romero 2000; Ellis et al. 2012; but see: Dickens and Romero 2013).

In this study, we use both mensurative and manipulative approaches to assess responses to natural climatic gradients and chronic climatic stressors in plethodontid salamanders. Terrestrial plethodontid salamanders are a group of amphibians particularly sensitive to the predicted warmer and drier summers in the future because of their lungless anatomy and subsequent strict reliance on cool and moist conditions (Sutton et al. 2015; USGCRP 2017). Abundance and diversity of plethodontid salamanders are greatest in the Southern Appalachian Mountains of the United States, where the topography is complex and climatic conditions, such as temperature and moisture, vary over small spatial scales. For example, low elevations tend to be warmer and drier than high elevations, and areas close to streams are cooler and moister than those far from streams (e.g., riparian zones vs. ridgelines). Terrestrial salamander distribution and abundance follows these environmental patterns such that at low elevations, salamanders are most abundant in stream-side riparian habitat, whereas at higher elevations salamanders are more uniformly distributed across the landscape (Gade & Peterman, 2019). While we know where salamanders exist across a montane landscape, we lack complete knowledge of the health and condition of salamanders living in these different habitat types.

Stress GC's have been studied across taxa including mammals (e.g. Reeder and Kramer 2005), birds (e.g. Newberry et al. 2018), reptiles (e.g. Weigand 2019), and amphibians (e.g. Charbonnier et al. 2018) as a way to understand the mechanisms underlying population responses. Stress physiology has been studied in plethodontid salamanders (Wack et al. 2012, 2013; Woodley 2017), but primarily in the context of reproduction and behavior, with the notable exception of Novarro *et al.* (2018) who assessed the latitudinal variation in stress in *Plethodon cinereus*. Novarro *et al.* (2018) found that corticosterone (CORT), the primary GC in amphibians (Moore and Jessop 2003), excretion had a positive relationship with temperature across all latitudes, with salamanders from the southernmost (warmest) sites showing the highest overall levels of CORT, providing evidence of regional variation and local adaptation in stress responses. However, *P. cinereus* is unique among plethodontids in its broad latitudinal range — the majority of plethodontid species have restricted ranges in the eastern mountains of the United States (Kozak and Wiens 2010). Montane biodiversity is predicted to be at higher risk to climate change because of their geographic isolation, limited range size, and narrow physiological tolerances (La Sorte and Jetz 2010; McCain and Colwell 2011), emphasizing the need to determine climate change effects on montane species. Further, Novarro et al. (2018) did not investigate the role of hydric state on stress. Hydric state is of clear importance to salamanders and may help to buffer the effects of thermal stress. Thus, explicit incorporation of both temperature and moisture is needed for a more comprehensive understanding of salamander responses to climate change.

Salamanders are notoriously secretive, only emerging when climatic conditions are optimal (Feder 1983), thereby detecting population declines in a timely manner is

challenging. Monitoring stress in individuals within and between populations may serve as a relevant surrogate to intensive population monitoring. However, previous work assessing stress (CORT) and other hormonal physiology in terrestrial salamanders have primarily used fecal samples (Crane et al. 2012), blood samples (Homan et al. 2003), and water-borne assays (Gabor et al., 2016; Novarro et al., 2018). These sampling techniques are either strictly lab-based, time-intensive, and/or lethal to the animal, reducing sample sizes and preventing repeated and *in situ* measurements. Recently, a novel, non-invasive method was described (Santymire et. al. 2018) and further validated (Scheun et al. 2019) using skin swabs to measure dermal CORT (hereafter: dCORT) levels as a potential metric for stress. This methodology provides a promising tool toward obtaining rapid, non-lethal measures of stress. Here, we apply the dermal swab technique to quantify variation in dCORT levels across a montane landscape in a salamander population as well as quantify salamander dCORT responses to abiotic conditions expected with climate change. We predicted that animals from warmer and drier conditions (low elevations, especially far from streams) will have greater dCORT than those from cooler and wetter habitats (low elevation/close to stream and high elevations at both close and far distances from streams) due to chronically experiencing more abiotically stressful conditions. We also predicted that in response to experimentally hot and dry conditions, low elevation salamanders will exhibit less change in dCORT because they are locally adapted to hot and dry conditions.

## Methods

### Field Methods

*Handling Test*– In summer of 2018, we conducted a pilot study to validate dCORT as a viable matrix to assess GC levels by evaluating the response of dCORT to handling stress in the red legged salamander, *Plethodon shermani*. We hand-captured ten salamanders, 5 from high elevation and 5 from low elevation, and swabbed each individual within three minutes (see below for more details). Once the “pre-stressor” swab was obtained, we securely closed and gently shook a plastic container (12.6 x 10.5 x 9.5 cm ) for 5 minutes to induce a stressor. Following the five-minute stress test, we obtained a “post-stressor” swab and released the animal at the exact capture location.

*Field Study*– In May – August of 2019, we sampled salamanders across four habitat types that capture a temperature and moisture gradient experienced by salamanders in this region: High Elevation/Close to streams (HC), High elevation/Far from streams (HF), Low elevation/Close to streams (LC), and Low elevation/Far from streams (LF). High elevation sites were those greater than 1200 m asl and low elevation were those below 1000 m. Close to stream sites were within 5 m of a stream and far from stream sites were at least 175 m from a stream. High elevation sites are typically cooler and wetter than low elevation and stream-sides provide a moist and cool climate refugia relative to ridgelines or exposed slopes, especially at low elevations (Gade and Peterman, 2019; Riddell and Sears, 2015). Our study was conducted across Wayah Mountain (35.158 N, -83.574W) in the Nantahala National Forest in western North Carolina, USA. We collected salamanders from three replicate sites for each habitat type for a total of 12 sites. Five to eight adult (>45mm snout-vent length) *Plethodon shermani* were collected and swabbed at each site. Upon capture, salamanders were placed in a plastic container

and swabbed within 3-minutes of initial capture. Obtaining a sample within three minutes has been shown to reflect the baseline, unstressed state of the animal prior to the stress of capture (Romero and Reed 2005). Each salamander was swabbed 10 times along the dorsum using a 3-cm sterile cotton-tipped swab. We avoided swabbing the anterior end of the dorsum and the tail because defensive mucous is secreted in these areas which may interfere with hormone extraction (Largen and Woodley 2008). Swabs were individually placed in a labeled 2.0 ml tube containing 1ml of 90% ethanol and stored at 5°C until processing. Once salamanders were swabbed, they were individually placed in a sealable plastic bag filled with moist leaf litter and transported to Highlands Biological Station (HBS) approximately 60 km away. We collected 120 animals (30 from each Origin) in May and the remaining 120 animals (30 from each Origin) in July due to housing space limitation at HBS (See “Experimental Protocol” below for more details).

#### Experimental Protocol

Once at HBS, salamanders were sexed, weighed (g), the snout-vent length (mm) measured, and placed into individual plastic containers (36 x 25 x 13 cm) lined with two moist paper towels and one crumpled paper towel to serve as a retreat. The containers were then placed in a temperature and humidity controlled environmental chamber set to 17°C and 95% humidity with a 10L:14D light cycle to mimic summer light conditions. The housing containers had a mesh lid to circulate air from the environmental chamber to container. The acclimation temperature was selected because it is the average temperature experienced by salamanders across elevations at the study site (Gade and Peterman, 2019) . Salamanders acclimated to lab conditions for two weeks and were fed ~30 fruit

flies per week, similar to the husbandry protocol used by Novarro et al. (2018). We allowed 24-hours to pass between feeding and the start of the experiment to account for potential digestive effort confounding the results. To ensure consistent levels of moisture inside containers, 50mL of water was added at the start of the acclimation period and 20mL was added at the start of each subsequent week.

Following the two-week acclimation period, salamanders were swabbed using the same protocol described above to obtain an “experimental baseline” measure.

Salamanders were then randomly assigned to one of four treatments. Each treatment had 15 individuals from each habitat type (hereafter, “origin”), for a total of 60 salamanders per treatment. The four treatments were: (1) Increase temperature (hereafter, Hot), (2) Decrease humidity (hereafter, Dry), (3) Increase temperature + decrease humidity (hereafter, Hot+Dry), and (4) Control. HBS only has two environmental chambers, thus the experiment was conducted in two phases. Phase I tested treatments Hot and Dry and Phase II tested treatments Hot+Dry and Control. Phase I salamanders were collected and acclimated at the same time, and at the conclusion of Phase I, Phase II salamanders were collected and acclimated.

Following the collection of the experimental baseline swab, the experiment began. Depending on the treatment, the temperature was increased by 2°C per day for six days until temperatures reached 25°C, a physiologically challenging temperature for salamanders reasonably expected under climate change predictions (IPCC 2014, Clay and Gifford 2017; Novarro et al. 2018). Humidity was lowered by 12.5% each day for six days until conditions reached 50%. For the Hot Treatment, the humidity was maintained at 95% while temperature was adjusted, and in the Dry Treatment, temperature was

maintained at 17°C while humidity was adjusted (Table 4.1). In the Control treatment, salamanders were kept at their acclimation conditions for the duration of the experiment (6 days). Salamanders were kept at their experimental conditions for 24-h, after which, we took another dermal swab using the same methods described above. All swabs were taken between 1200 – 1400h to minimize any daily variations in dCORT (Dauphin-Villemant and Xavier 1987; Dupoué et al. 2018). Salamanders were weighed after the final swab to determine mass changes following the experiment. Hydrochon iButton data loggers were placed in each chamber to record humidity and temperature at 30-minute increments to ensure experimental conditions were met.

#### Hormone Extraction Procedure

We evaluated corticosterone concentrations from the dermal swabs using methods adapted from Santymire et. al. (2018). The methodology was modified and validated using assay parallelism from the handling stress pilot data described above. To confirm assay parallelism, we compared the slopes of serial dilutions of assay kit standards to serial dilutions from swabs taken from two individuals. Using ANCOVA, we found no difference between standards and unknowns in the slope of percent of the assay conjugate bound to plate antibody (relative binding) compared to log transformed CORT concentration (group\*concentration interaction:  $F_{1,6} = 0.64$ ,  $P = 0.45$ ; Appendix C: Figure C.1).

To process swabs, we vortexed each tube for 3 minutes and pipetted the ethanol from the tube into a drying tube. Swabs were tapped on the side of the drying tube to expel as much ethanol as possible. The drying tubes containing samples were submerged

in a 50°C shaking water bath and dried under forced nitrogen air until all ethanol was evaporated (~45 minutes). Once dried, samples were reconstituted using 1mL saline buffer solution (supplied from assay kits; see below) and vortexed for 1 minute. Samples were then run in duplicate on a commercial enzyme immunoassay (EIA) from Neogen Corporations (product no. 402810, Lexington, KY). Samples were measured against a standard curve on each plate following the Neogen protocols and materials. Samples from every treatment were randomly analyzed across 17 assays from September 19 – November 25, 2019. Inter-assay variation based on kit standards was 15.27% and inter-assay variation based on the coefficient of variation between the duplicate unknown samples was on average 4.81%.

### Statistical Analyses

We use a two-sample t-test to determine differences in total dCORT between pre- and post-handling stress test individuals as well as between males and females of all captured salamanders. We analyzed t-tests in a Bayesian framework using the *BayesianFirstAid* package (Bååth 2013). To assess differences in dCORT between salamanders from different origins *in-situ*, we used a mixed-effect model with total dCORT as the response variable, origin as a fixed effect, and site as a random effect because we collected salamanders from three sites per origin. All dCORT values were divided by the individual's SVL to account for variable swabbing lengths between individuals.

To determine the effect of each experimental treatment on dCORT levels between salamanders from the four habitat origins, we used a mixed-effect model with  $\Delta$ dCORT of each individual ( $\Delta$ dCORT = dCORT<sub>final</sub> – dCORT<sub>initial</sub>) as the response and the

interaction of origin and experimental treatment as fixed effects, with a random effect of individual nested in site to account for repeated measures of individuals. We analyzed all mixed-effects models in a Bayesian framework with the *brms* package (Bürkner 2018) in R v 3.6.1. We used the lognormal family to account for non-normality of data. Because some  $\Delta$ dCORT values were negative, a constant was added to all  $\Delta$ dCORT measures. All models were run across 4 chains for 3,000 iterations with a 1,500-iteration warmup and a thinning rate of 1. Model convergence was based on R-hat values ( $<1.1$ ) and visual inspection of MCMC chain mixing. We used the *emmeans* package for Tukey post-hoc contrast analyses (Lenth, 2019).

## Results

The handling-stress study showed a significant increase in dCORT levels following a 5-minute handling stress test ( $\text{mean}_{\text{pre}} = 26.83$  pg/swab,  $\text{mean}_{\text{post}} = 36.88$  pg/swab; CRI: -1.90, 0.13), with a probability of 97.6% of difference of means being less than zero.

There was no difference in dCORT between male and female salamanders, with the difference of the means ( $\text{mean}_{\text{female}} = 7.30$  CRI: 6.5, 8.0;  $\text{mean}_{\text{male}} = 7.00$ , CRI: 6.00, 8.11) having a 66.10% probability being greater than zero. Thus we did not assess sex differences in subsequent analyses. dCORT levels *in situ* differed between origins such that both high elevation origins, HC ( $\beta = 0.99$ , CRI: 0.82, 1.82) and HF ( $\beta = -0.01$ , CRI: -0.26, 0.24), had higher mean dCORT excretion than both LC ( $\beta = -0.23$ , CRI: -0.48, 0.02) and LF ( $\beta = -0.25$ , CRI: -0.51, -0.01) (Figure 4.1). After salamanders were acclimated to lab conditions, they had significantly lower dCORT when compared to their *in-situ*

measures ( $\beta = -0.14$ , CRI: -0.24, -0.03), but dCORT between salamanders of different origins did not differ post-acclimation.

Throughout the experimental procedure, conditions within the chambers were consistent with the given treatment and acclimation conditions followed our protocols for incrementally changing temperature or humidity throughout the acclimation and experimental procedure (Appendix C: Figure C.2). There was no change in dCORT between pre-and post-exposure in the control treatment ( $\text{mean}_{\text{pre}} = 7.40$ , CRI: 5.91, 8.82;  $\text{mean}_{\text{post}} = 8.60$ , CRI: 6.52, 11.01), with the difference of means having a 17% probability of being greater than zero, providing confidence that the control group is a suitable comparison for experimental groups. Relative to the control treatment,  $\Delta\text{dCORT}$  was not different in the Hot Treatment ( $\Delta\text{dCORT} = 2.33 \pm (\text{SE})0.41$ ;  $\beta = 0.01$ , CRI: -0.43, 0.43), had an 83% probability of being lower than Control animals in the Dry Treatment ( $\Delta\text{dCORT} = 1.08 \pm 0.68$ ;  $\beta = -0.22$ , CRI: -0.70, 0.20), and a 91% probability of being higher than Control animals in the Hot+Dry Treatment ( $\Delta\text{dCORT} = 8.43 \pm 1.38$ ;  $\beta = 0.30$ , CRI: -0.14, 0.73) (Figure 4.2). There was no difference in  $\Delta\text{dCORT}$  between origins within the Hot or Hot+Dry Treatment, but in the Dry Treatment, salamanders from HF had lower  $\Delta\text{dCORT}$  than both LF ( $\beta = -0.29$ , CRI: -0.73, 0.13) and LC ( $\beta = -0.45$ , CRI: -0.85, 0.01) (Appendix C: Figure C.3 and C.4).

## Discussion

As the effects of climate change continue to mount, evaluating an organism's physiological responses to changes in the environment will reveal their potential resilience and ultimate survival in the future. We found that dCORT varies across the

landscape as a function of elevation. Salamanders at high elevations, regardless of proximity to stream-sides, had higher dCORT than their low elevation counterparts. Low elevations are often warmer and drier than high elevations (Barry, 2008; Gade and Peterman, 2019; Riddell and Sears, 2015), and thus salamanders at low elevation are more chronically exposed to stressful climatic conditions. As such, individuals may lower dCORT to downregulate maintenance costs and save energy and water (Preest and Cree 2008; Dupoué et al. 2018). At low elevations, salamanders have increased metabolic rates as a result of the higher experienced temperatures (Catenazzi 2016), and may require metabolic regulation to maintain energy balances. Elevated plasma CORT appears to elevate oxygen consumption and metabolic rates in male *P. shermani* (Wack et al. 2012), and thus dCORT downregulation may be particularly beneficial for low elevation populations.

Reduction of circulating CORT has been associated with increasing vascular blood flow and subsequent increased desiccation resistance (McCormick and Bradshaw 2006). In our experimental Dry treatment, we saw that individuals reduced dCORT levels relative to the Control treatment, potentially further indicating that dCORT may be associated with regulating water loss. Plethodontid salamanders from low elevation have been shown to exhibit higher desiccation resistance relative to high elevation counterparts (Riddell and Sears, 2015), and a reduction in water loss rates correspond to simultaneous reductions in metabolic rates (Riddell et al., 2018). Similar patterns have been shown in wild populations of cane toads (*Rhinella marina*), which have the lowest evaporative water loss when CORT levels are low (Jessop et al. 2013). Given both *in situ* and experimental results, it may be possible that the dCORT metric is associated with GC

regulation and may serve an important role in physiological regulation across their elevational range. However, it is alternatively possible that dCORT is lower in dry conditions both *in situ* and experimentally because CORT evaporates off the skin surface more rapidly or reduced water loss results in reduced CORT excretion through the skin. Future work is required to test these hypotheses.

In addition to evaluating baseline dCORT levels across the landscape, we experimentally exposed salamanders to climatically stressful conditions to evaluate dCORT responses to climate change. We designed the experiment to assess not only dCORT levels in response to abiotic stressors but also to evaluate how animals from different origins respond. Our selection of ‘origins’ (LC, LF, HC, HF) are representative of the microhabitats terrestrial salamanders are found in this region, albeit at different abundances (Gade and Peterman 2019). Aside from one case (HF differing from LF and LC in the dry treatment), salamanders consistently responded to stressors despite their origin. Salamanders from all origins were acclimated to the same conditions (17°C and 95% humidity) and had similar dCORT levels following a 2-week acclimation period, further suggesting salamander’s ability for acclimation. Similarly, Novarro et al. (2018) found no difference in CORT release rates at higher temperatures between salamanders collected across latitudes, suggesting the salamanders may express adaptative plasticity. Plethodon salamanders appear capable of local adaptation and/or acclimation with resistance to water loss (Riddell and Sears, 2015), thus salamanders may acclimate to local conditions through multiple physiological modifications. However, a potential caveat to the experimental design in both the present study and Novarro et al. (2018) is that all salamanders were exposed, regardless of origin, to the same stressful conditions,

which salamanders may not realistically experience. For example, high elevations may never actually reach 25°C, while low elevations may significantly surpass 25°C in the future. Achieving precise biological realism in experimental studies is often impossible and tradeoffs are required to test certain hypotheses. Future studies should attempt to capture more nuanced responses to climate change.

Contrary to expectations, dCORT levels in our Hot treatment did not differ from the Control. Other studies from a range of taxa have shown that temperature influences circulating CORT release rate (Romero et al. 2000; Narayan and Hero 2014; Telemeco and Addis 2014; Jessop et al. 2016). It is possible that the temperature in the Hot treatment was not high enough to elicit a dCORT response. Spotila (1972) found that the critical thermal maximum (CTM) of a closely related species (*P. jordani*) was 33°C and a more recent study found the optimal preferred temperature of *P. montanus* was 22.6°C (Clay and Gifford 2017). We selected 25° C for two reasons: it is sub-lethal (below CTM) but still physiologically challenging and it is representative of a realistic change in temperature predicted with climate change over the next 100 years (IPCC 2018). Further, 25°C was used by Novarro et al. (2018), which ultimately elicited a stress response from another plethodontid species (*P. cinereus*). However, previous research has found that individuals acclimated at higher temperatures expressed higher critical thermal maxima (Spotila 1972). For example, *P. jordani* acclimated at 15°C had a CTM of 33°C, while those acclimated at 25°C, had a CTM of 36°C (Spotila 1972). We acclimated animals at 17°C, which was 2°C warmer than Novarro et al. (2018) and may have modified the thermal tolerance and CTM of individuals. Salamanders may adaptively respond to past

thermal regimes (Spotila 1972), which may have confounded their responses to thermal stress in our experiment.

Alternatively, we may have not observed changes in dCORT levels in the Hot treatment because we measured stress during the process of downregulation (Mell et al. 2016; Dupoué et al. 2018). Lizards appear to downregulate CORT in response to seasonal heat waves to minimize maintenance costs (Jessop et al. 2016; Dupoué et al. 2018). It is possible since we incrementally increased temperature over six days, salamanders initially increased dCORT in response to acute heat stress, but after a period of constant heat stress, began to compensate by downregulating CORT release rates. Although six days of heat stress likely does not constitute a chronic or long-term stressor, we maintained the temperature throughout day- and night- time, such that there was no reprieve from the heat, which could have been enough to induce a physiological modification in the form of dCORT downregulation, reflected in the lack of dCORT increase. Additionally, with future temperatures predicted to increase in variability over the next century, especially during summer months (Bathiany et al. 2018), stress responses will likely fluctuate between responding to chronic and acute stressors. It will, therefore, be important for future studies to evaluate physiological responses across multiple time points to capture variations in dCORT and temperature variation.

In line with our predictions, we saw an increase in dCORT relative to the control in the combined Hot + Dry treatment (Figure 4.2). Organisms appear capable of allostasis or regulating homeostasis and energetic balances in the face of change. However, when allostatic overload occurs, whereby the energy required to moderate change exceeds the overall energy budget, an extreme physiological response occurs (Bonier et al. 2009). In

our experiment, individuals appear to be able to maintain allostasis with one abiotic stressor, but in combination, were likely pushed into allostatic overload resulting in higher dCORT. Novarro (2018) found that *P. cinereus* exposed to dry conditions preferred a lower body temperature resulting reduced energy efficiency in laboratory conditions, suggesting a tradeoff between immediate water loss and long-term performance. We did not provide an option for a hydric-thermal tradeoff in our experiment, likely further exacerbating allostatic load. Focusing on a single stressor may be misleading or incomplete given the number of other factors organisms are responding to (McCain and Colwell 2011). The effects of multiple stressors offer greater realism to current and predicted future conditions with climate change (Todgham and Stillman 2013). For example, in streamside salamanders (*Ambystoma barbouri*), there was an interactive effect of temperature and moisture whereby at higher temperatures, moist conditions resulted in lower survival whereas dry conditions were more detrimental at lower temperatures (Rohr and Palmer 2013). Investigating multiple stressors will reveal nuanced responses that might be overlooked if only a single stressor is assessed.

Water balance and desiccation are among the most vital processes for terrestrial salamanders. Temperature exponentially increases the drying power of air because the requisite amount of water vapor to saturate the air increases with temperature (Leighly, 1937; Riddell et. al. 2019). By synergistically adjusting temperature and humidity in our experiment, we magnified evaporative loss rates, which may misrepresent a salamander's capacity to modify behaviors and express physiological plasticity to cope with stressors (Riddell and Sears 2020). With the expected increases in temperature in the future, water loss rates are likely to increase, and although salamanders appear capable of adjusting

their resistance to water loss (Riddell et. al. 2018), their ability to moderate such plasticity over a realistic time frame and temperature change rate is unknown. The ability of animals to respond and adapt to climate change at a relevant time scale will affect future persistence and survival. For example, many birds are tracking climate change through range shifts but at a slower rate than temperatures are increasing (Devictor et al. 2008) and migratory birds that are not adjusting breeding and migration timing are declining relative to species that are (Møller et al. 2008).

From a conservation perspective, proactive strategies for detecting population vulnerability or decline are often more successful and cost-effective as opposed to reactive approaches (Drechsler et al. 2011). Thus, developing tools that allow for rapid identification of population vulnerability are key to effective conservation. The dermal swab methodology offers a potential metric for the proactive evaluation of population health in amphibians. Santymire et. al. (2018) initially described and tested the dermal swab methodology across 15 amphibian species of varying life histories (terrestrial, semi-aquatic, and fully aquatic), and found the dermal secretions were a viable matrix to assess GC levels, albeit with small sample sizes per group. Scheun et al. (2019) validated the technique on African anurans and found additional support for dermal secretions from either the dorsum or ventral regions as suitable measures of physiological stress. Multiple levels of validation including biological and chemical, of a given methodology are required to ensure reliable results. Biological validation is necessary to show that there are changes in hormone levels in response to an external change, either an experimentally induced change, such as a synthetic adrenocorticotrophic hormone (ACTH) challenges (Scheun et al. 2019), or through exposure to a presumed stressor such as handling or

abiotic stressors. Although we cannot conclusively say dCORT is an equivalent measure to blood circulating CORT in *P. shermani*, we show that when *P. shermani* is exposed to a stressor, the dCORT metric responds in an expected direction. Plasma CORT levels have been shown to increase in response to capture and handling (Schubert et al. 2009, Woodley and Lacey 2010), and we show a similar pattern with dCORT in our handling experiment. The results of our experiment show measurable responses to known abiotic stressors, indicating dCORT are likely biologically relevant signals. Further, chemical validation determines whether the chosen hormone assay is reporting consistent and reliable values of the target hormone. Both Santymire and Manjerovic (2018) and Scheun et al. (2019) chemically validated their methods and we have demonstrated that commercial assay kits are effective at measuring CORT in dermal swabs (Appendix C: Figure C.1). Monitoring the population health of sensitive species like amphibians will be critical for management and conservation practices into the future. Although we acknowledge this method requires additional validation, both our biological and chemical validations suggests that the dermal swab methodology can be a useful, noninvasive, and real-time method for monitoring the health of amphibian populations.

Future predictions of climate change suggest that summer months will become hotter and drier, making plethodontid salamanders particularly at-risk to future persistence. To best manage and conserve sensitive species, we must evaluate responses to the current climate across the landscape and predict responses to future climates. Stress provides a useful lens by which to view responses to abiotic stressors. We show that terrestrial salamanders exhibit spatially heterogeneous dCORT levels and our experiment suggests salamanders may be capable of tolerating the effects of one stressor, but are

physiologically overwhelmed when exposed to multiple stressors. While our experiment does not reflect the full reality of environment exposures experienced by salamanders and the dermal swab methodology requires additional validation, our study is an important first step to understanding spatial variation and the future responses across a spatially heterogeneous landscape in response to climate change. Developing new, easily accessible metrics for measuring population health is critical for conservation and management, and dCORT is potential tool to be added to both manager and researcher's toolbox.

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Table 4.1 Final exposure temperature (°C) and humidity (%) conditions for each treatment of the stress experiment on *Plethodon shermani*.

Treatment	Temperature (°C)	Humidity (%)
1. Hot	25	95
2. Dry	17	45
3. Hot + Dry	25	45
4. Control	17	95

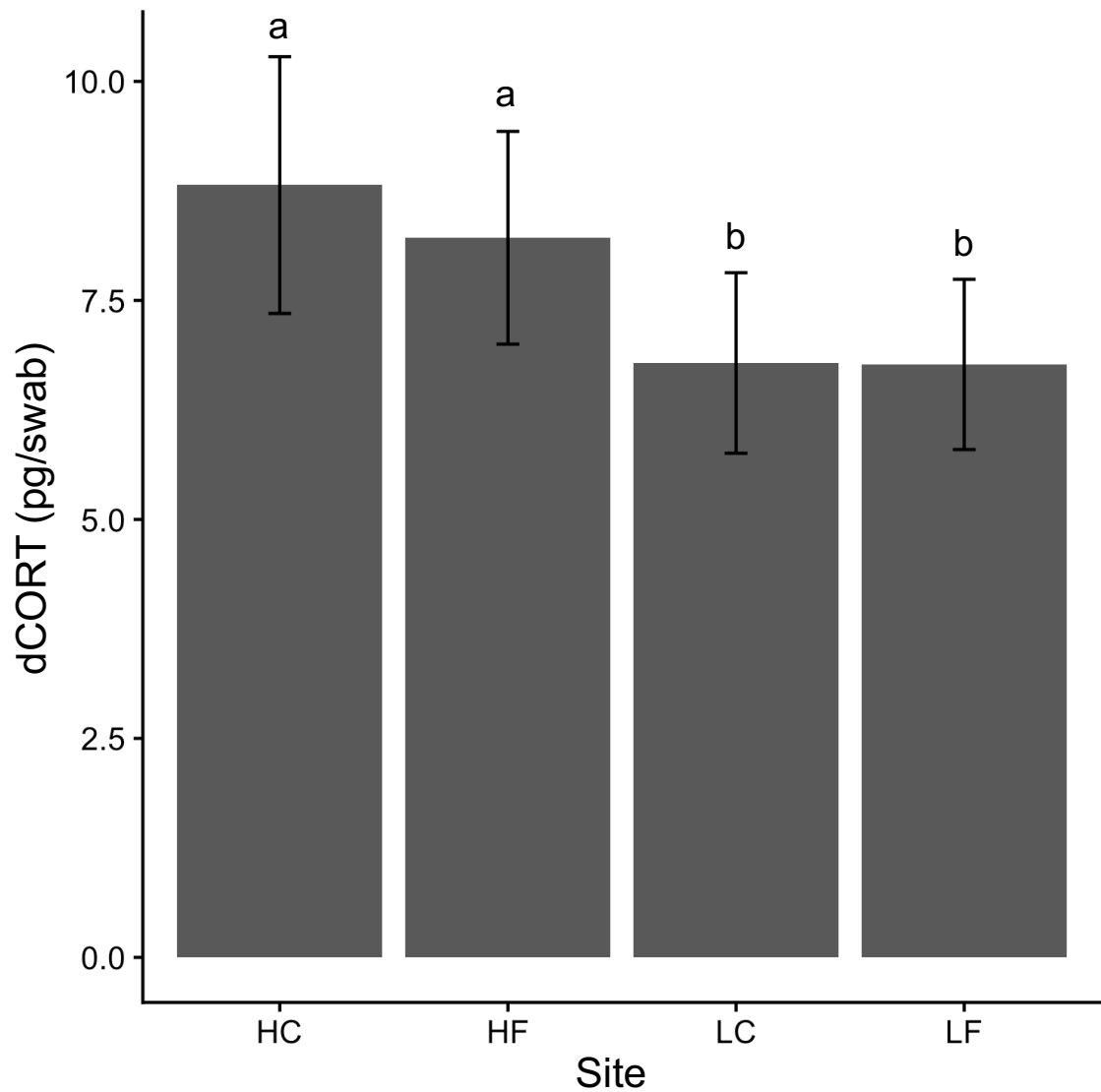


Figure 4.1 Mean ( $\pm$ SE) dCORT excretion (pg/swab) standardized by SVL in *Plethodon shermani* collected at four sites in-situ. The sites are High elevation/Close to stream (HC), High elevation/Far from stream (HF), Low elevation/Close to stream (LC) and Low elevation/Far from stream (LF). Letters above bars represent significant differences based on 80% CRI having the same sign as the mean estimate.

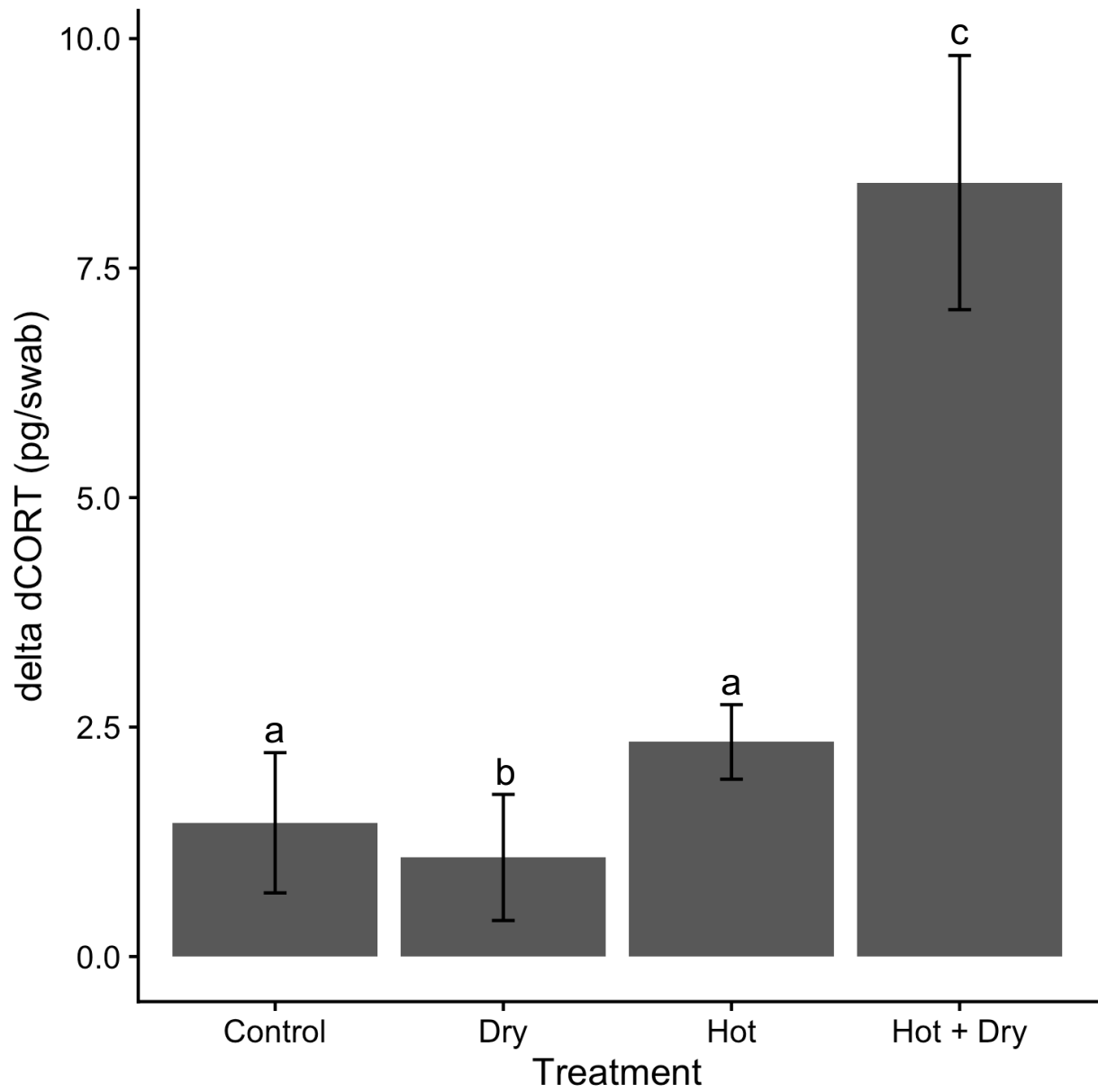


Figure 4.2 Mean ( $\pm$ SE) delta dCORT (post-exposure – pre-exposure) to for each experimental treatment (See Table 4.1 for final experimental conditions). We averaged dCORT across salamanders from all origin locations (HC, HF, LC, LF) since our models showed no difference in dCORT between origins.

## Chapter 5: Predicted alteration of surface activity as a consequence of climate change

### Abstract

Wildlife are faced with numerous threats to survival, none more pressing than that of climate change. Understanding how species will respond to changing climate behaviorally, physiologically, and demographically is a cornerstone of many contemporary ecological studies, especially for organisms, such as amphibians, whose persistence is closely tied to abiotic conditions. Activity is a useful parameter for understanding the effects of climate change because activity is directly linked to fitness as it dictates foraging times, energy budgets, and mating opportunities. However, activity can be challenging to directly measure, especially for secretive organisms like plethodontid salamanders which only become surface active when conditions are cool and moist due to their anatomical and physiological restrictions. We estimated abiotic predictors of surface activity for the seven species of the *Plethodon jordani* complex. Five independent datasets collected from 2004-2017 were used to determine the parameters driving salamander surface activity in the present-day, which were then used to predict potential activity changes over the next 80 years. Average active season temperature and vapor pressure deficit were the strongest predictors of salamander surface activity and, without physiological or behavioral modifications, salamanders were predicted to exhibit a higher probability of surface activity during peak active season under future climate conditions. Temperatures during the active season likely do not

exceed salamander thermal maxima to cause activity suppression and, until physiological limits are reached, future conditions may continue to increase activity. Our model is the first comprehensive field-based study to assess current and future surface activity probability. Our study provides insights into how a key behavior driving fitness may be affected by climate change.

## Introduction

Organisms are facing unprecedented challenges as a result of climate change, and predicting behavioral, demographic, and physiological responses dominates current research. Broadly, climate change is expected to increase temperatures and alter periodicity in precipitation (IPCC 2014). Ectothermic organisms, whose physiology and behavior are closely linked to abiotic conditions, are disproportionately impacted by the effects of climate change (Blaustein et al. 2010; Li et al. 2013). Contemporary climate change has already negatively affected many ectotherms (Alford and Richards 1999; Collins and Storfer 2003). For example, freshwater fish experience reduced reproductive output as a result of warmer water temperatures (Ficke et al. 2007; Holt and Jørgensen 2015), and several ectotherms have seen reductions in body size and depressed physiological performance correlated with warming temperatures (Reading 2007; Huey et al. 2009; Sheridan and Bickford 2011; Ohlberger 2013). To estimate wildlife responses to future climate change, we can incorporate the known behavioral and physiological responses of an organism at existing conditions into future climate predictions. Consideration of such biophysical processes has indicated that some organisms may exhibit resilience to change through physiological acclimation and behavioral modification (Kearney et al. 2009; Seebacher et al. 2015; Riddell et al. 2018). Linking

relevant species-specific parameters such as physiological traits, behavior, and dispersal ability to climate change predictions may reveal novel relationships otherwise overlooked. The inclusion of more biological realism into our predictions of climate sensitivity is necessary to estimate the likelihood of persistence and mitigate the impacts of climate change on species.

Determining the distribution and abundance of organisms in relation to environmental variables is a fundamental ecological goal and developing models to best estimate such patterns is essential for informing conservation and management strategies that account for climate change. Hierarchical binomial mixture modeling has become a staple in ecological research due to its ability to accommodate and partition underlying ecological patterns (i.e. abundance or occupancy) and observational processes that may bias sampling (Royle et al. 2007; Kery and Schaub 2012). Central to many observation processes is “detectability”, or the idea that we are unable to perfectly observe all individuals of the focal organism during every survey event. Often, failure to observe a species or individual at a given location does not necessarily indicate its absence but instead, may reflect factors such as observer proximity to the organism, timing (daily or seasonal), general species biology (calling frequency, phenology, etc.), or the environmental conditions driving surface availability.

In addition to addressing bias in estimating other state parameters (e.g., abundance, occurrence, survival), the ‘detection’ process can be biologically relevant for species such as plethodontid salamanders because of its direct link to surface activity, which affects energy intake and overall fitness. Plethodontid salamanders are lungless and rely on highly permeable and moist skin for gas exchange (Feder 1983).

Consequently, plethodontids generally require cool and moist conditions to avoid rapid water loss (Feder 1983). Individuals spend significant amounts of time under cover objects or below ground to reduce evaporative water loss (Jaeger 1980; O'Donnell and Semlitsch 2015), and when salamanders do become surface active, it is often for short periods during cool and moist times (McEntire and Maerz 2019). In such a system, the detection component of a binomial mixture model is better capturing surface availability (i.e. the likelihood salamanders will be surface active) instead of the observer's ability to detect an individual. Only when surface active do plethodontids forage, grow, and search for mates. Therefore, surface availability is a reasonable proxy of surface activity which represents energy intake and subsequently, fitness (Gifford and Kozak 2012; McEntire and Maerz 2019; Peterman and Gade 2017; Riddell and Sears 2015). Evaluating surface activity under predicted climatic change provides a mechanistical understanding of how future climate may affect plethodontid performance and population persistence.

Direct and reliable observations of surface activity in the field are challenging to measure. Previous work in other systems has modeled surface activity indirectly through known physiological limits and/or energy budgets at given abiotic conditions. For example, Buckley (2008) assumed eastern fence lizards (*Sceloporus undulatus*) became active once a minimum temperature was reached, a temperature that was measured in a laboratory. In plethodontids, activity time has been modeled as a function of resistance to water loss at a given temperature and vapor pressure deficit in the atmosphere (Riddell and Sears 2015). Instead of using indirect physiological models to infer activity, it may be possible to use surface availability estimates as a more direct surrogate for surface activity, especially because terrestrial plethodontids are only detectable when surface

active, and have inherently low detection levels consistent with their restricted surface activity (Bailey et al. 2004).

The Appalachian region is a global hotspot of salamander species richness and abundance, and members of the Plethodontidae family account for much of the diversity within this region. Given their high abundance and biomass, combined with their role as vital members of the terrestrial food web and contributions to forest nutrient cycling (Davic and Welsh 2004; Best and Welsh 2014; Hocking and Babbitt 2014), and their predicted decline as a consequence of climate change (Milanovich et al. 2010; Sutton et al. 2015), protection and conservation are warranted. Our goal was to determine common weather parameters driving salamander surface activity in the present-day at a broad scale to inform potential changes in surface activity probability in the future. We used the *Plethodon jordani* species complex as a focal group representative of other Appalachian terrestrial plethodontid species to estimate surface activity probabilities. Originally thought to all be subspecies of *P. jordani*, Highton and Peabody (2000) described seven distinct species using allozyme variation. Each species in the complex has geographically distinct color variation and are high elevation isolates across the Southern Appalachian Mountains (Figure 5.1). As high-elevation specialists, species of the complex share similar climatic niches and exhibit niche conservatism, providing a unique opportunity to apply data from a subset of species to all species in the complex (Wiens et al. 2010; Riddell et al. 2018; Farallo et al. 2020). Members of the *P. jordani* complex are considered “sky island” species whereby populations are confined to isolated, but adjacent, mountain peaks (McCormack et al. 2008; Gifford and Kozak 2012). Sky island species are often vulnerable to the effects of climate change because of their genetic

isolation, inability to disperse from unsuitable conditions, and narrow physiological tolerances (Kozak and Wiens 2010; Gifford and Kozak 2012; Wiens et al. 2019), underscoring the need for determining responses to climate change as a vital step towards conservation action.

## Methods

### Datasets Description

Using five independent data sets, we developed a binomial mixture model to estimate abundance while accounting for surface availability probability (Royle 2004). Each dataset was collected using area-constrained repeated count surveys to estimate abundance across five distinct areas of the Southern Appalachian Mountains (Figure 5.1). Surveys occurred between 2004–2017, with one dataset collected over multiple years and the four others consisting of a single season of observations. The multi-year dataset (dataset E) used a robust-design survey method (Pollock 1982) with three sampling occasions per season in Spring, Summer, and Fall of 2016 and 2017. Our count data includes three of the seven species in the *Plethodon jordani* complex: *P. shermani*, *P. metcalfei*, and *P. jordani*. The datasets were initially collected for other purposes and were adapted for the cross-species assessment in the present study. The specific details of data collection for each data set can be found in Appendix D: Table D.1. Sample plot areas ranged from 9 m<sup>2</sup> to 400 m<sup>2</sup> between datasets with 14 –195 individual sampling locations in each dataset (Table 5.1).

### Surface Activity and Abundance Covariates

Due to a lack of common survey-level environmental covariates measured across all datasets (see Appendix D: Table D.2), and to ensure consistency between datasets, we

downloaded daily weather covariates from Daymet ([daymet.ornl.gov](http://daymet.ornl.gov)) using the *Daymetr* package (Hufkens et al. 2018) in R (version 3.5.1, R Core Team 2013). The Daymet dataset interpolates local weather station observations to produce a 1 x 1 km gridded weather estimate. To validate that Daymet data was a reasonable surrogate for local, field-collected weather data, we downloaded temperature data from a series of weather stations at Coweeta Hydrologic Lab (CHL), where dataset E was collected, and compared it to the Daymet data at CHL from the years 2004–2017. Coweeta Hydrological Lab is within close proximity to all other datasets (Figure 5.1), serving as a proxy for the other datasets to validate the Daymet data used in our models.

From Daymet, we extracted daily precipitation (mm), maximum and minimum air temperature (°C) and water vapor pressure (Pa) to point locations of each sampling site in the five datasets (Figure 5.1). We averaged the minimum and maximum air temperature and used rolling window analyses to assess the 1, 2, and 3- day mean temperature, total number of days since a rainfall event (i.e. dry days), and 1, 2, and 3- day total precipitation. We then averaged the one day and two-day rolling windows for all weather variables to obtain a ‘corrected’ 1-day rolling window because all sampling occurred nocturnally, and thus overlapped two calendar days.

For the abundance sub-model, we used site-level topographic variables including elevation, slope, topographic position index (TPI), aspect, and stream distance. Variables were derived from a 9-m<sup>2</sup> resolution digital elevation model obtained from EarthExplorer ASTER Global DEM ([earthexplorer.usgs.gov](http://earthexplorer.usgs.gov)). TPI was calculated as the slope position relative to the surrounding 90 m and aspect was transformed into linear measures of eastness (sine of aspect) and northness (cosine of aspect). Streams were delineated using

a flow accumulation layer with a 450-pixel cell threshold, resulting in a drainage area greater than or equal to 0.405 hectares (Gade and Peterman 2019). Stream distance was then calculated using the Euclidean Distance tool in ArcGIS v.10.2.

### Model Description

We developed a binomial mixture model for each of the five individual datasets using the same abundance and surface availability covariates in each model. The general expected abundance ( $\lambda_{ij}$ ) and surface activity ( $p_{ijk}$ ) as well as the realized (latent) abundance ( $N_{ij}$ ) equations are shown below, with  $i$  indicating data set,  $j$  indicating survey plot, and  $k$  indicating sampling occasion. The bracketed ellipses indicate slope terms for the multiple covariates used in the model.

$$N_{ij} \sim \text{Poisson}(\lambda_{ij}) \quad 1$$

$$\text{Log}(\lambda_{i,j}) = \alpha 0_i + \alpha 1_i \times x_{1,i,j} + \alpha 2_i \times x_{2,i,j} + [...] + \log(\text{plot size}) + \varepsilon_j \quad 2$$

$$\text{logit}(p_{i,j,k}) = \beta 0_i + \beta 1_i \times x_{1,j,k} + \beta 2_i \times x_{2,j,k} + [...] \quad 3$$

$$y_{i,j,k} | N_{i,j} \sim \text{Binomial}(N_{i,j}, p_{i,j,k}) \quad 4$$

We assume that all salamander species are responding to environmental and habitat covariates similarly, so the dataset-level parameters defined above are modeled as coming from a common distribution. We included hyperparameters to represent an additional hierarchical level and define the global distributions that summarize the dataset level parameters.

$$\alpha'_a \sim \text{dnorm}(\mu_{\alpha i'}, \sigma_{\alpha i'}) \quad 5$$

$$\beta'_i \sim \text{dnorm}(\mu_{\beta i'}, \sigma_{\beta i'}) \quad 6$$

We used normally distributed, weakly informative priors with a mean of zero and precision of 0.1 for all priors, global and dataset-specific. To accommodate overdispersion in counts, a plot-specific random effect error term,  $\varepsilon_j$ , was included in each dataset. An offset term (*log* of the plot area) was also added to account for differences in survey plot sizes between datasets (Eqn. 2). We constructed a global model that included all abundance and activity covariates mentioned above and subsequently removed any covariate where <75% of the posterior distribution had the same sign as the mean parameter estimate to create our final model. Temperature (minimum/maximum/average and rolling windows) were correlated, thus we ran separate models including one temperature covariate at a time along with the other activity covariates. We analyzed our models in a Bayesian hierarchical framework using the JAGS (v.4.3.0; Plummer 2003) in R using the *jagsUI* (Kellner 2017) in R. All covariates were standardized prior to analysis by subtracting their mean and dividing by the standard deviation. Posterior summaries of the final model were based on 223,300 MCMC iterations on four chains thinned at a rate of 10 following an adaptation of 318,000 and burn-in of 210,000. Model convergence was assessed using the Gelman-Rubin statistic ( $R_{hat} < 1.1$ ) and visual inspection of MCMC chain mixing.

#### Future Surface Activity

Using the estimates from the binomial-mixture model described above which were informed by data from 3 species of the *P. jordani* complex, we estimated future surface activity for the seven species of the *P. jordani* complex. Species of the complex are closely related and are geographic replacements as they all inhabit high elevations and

share similar climate and physiological requirements (Weisrock and Larson 2006). We incorporated statistically downscaled daily future weather predictions from ten global circulation models (GCM) using the Multivariate Adaptive Constructed Analogs (MACA) dataset (Abatzoglou and Brown 2012) into the global surface availability model we developed above. The MACA method downscales GCMs from a coarse resolution (Appendix D: Table D.3) to a 4 km spatial resolution for the years 2005–2099 at Representative Concentration Pathway (RCP) 4.5 and 8.5. RCP 4.5 represents a stabilized scenario whereby moderate effort is needed to curb emissions and RCP 8.5 represents a scenario where no action is taken to mitigate emissions and greenhouse gas concentration levels increase substantially (Clarke et al. 2007; Riahi et al. 2007). Although 20 GCMs are available in the MACA dataset, we selected 10 representative models that: (1) incorporated the greatest variation in how the model was developed (using atmospheric chemistry, interactions with biogeochemical processes, etc.), (2) were developed at the finest spatial resolution, and (3) were either recommended by the MACA dataset developers and/or were evaluated as ‘highly credible’ from Rupp et al. (2013). Refer to Appendix D: Table D.3 for details on the selected GCMs.

We downloaded daily weather data from the MACA dataset to 1,012 unique point locations that spatially encompassed the ranges of the seven *P. jordani* complex species (Figure 1). Range maps of the species complex were obtained from the IUCN Redlist website ([www.iucnredlist.org](http://www.iucnredlist.org)). Points were uniformly placed 5 km apart to capture the variation in daily weather variables across the range of the *P. jordani* species complex within the distribution of each species. Data were downloaded from 15 May–15 August for the years 2020–2099 to represent the peak salamander active season. This time frame

was also selected as it is representative of the sampling dates of datasets A–D (Appendix D: Table D.1). We used minimum and maximum temperature, minimum and maximum relative humidity, and the total precipitation at both RCP 4.5 and 8.5. All data were downloaded using the *ClimateR* package in R (Johnson 2019). Similar to the aforementioned methods, we averaged the minimum and maximum temperature and relative humidity and used rolling window analyses to determine the number of dry days and the 1, 2, 3-day average temperature and total precipitation. We averaged one day and two-day rolling windows for temperature and precipitation to obtain a ‘corrected’ 1 day rolling average. Vapor Pressure (*vpa*) was derived as a function of the minimum daily temperature ( $T_{mn}$ ) as described in Peterman and Gade (2017) using the following equation:

$$\exp(vpa) = 6.467 + 0.0657 \times T_{mn} \quad 7$$

All future-climate covariates were rescaled using the mean and standard deviation of the current climate variables that were used to fit the original hierarchical model from the Daymet dataset.

We estimated future surface activity probability at each sample point for each day of the 2020 – 2099 active season using 5,000 random posterior samples generated based on our binomial mixture model for salamander abundance and activity probability. We used the following inverse logit equation to estimate activity:

$$p = \frac{1}{1 + \exp(-1 \times (\mu\beta_0 + \mu\beta_1' \times \chi_1 + \mu\beta_2' \times \chi + [\dots])} \quad 8$$

We made daily surface activity probability predictions at each of the 1,012 locations by averaging 5,000-posterior samples for every day at each site. The daily activity probability estimates at each location were subsequently averaged together by species and year. The final dataset included a yearly mean ( $\pm$ SD) activity probability for each of the seven species. To account for the physiological limitations of salamanders, we assumed surface activity was zero if the temperature on any day exceeded 25°C (Caruso & Rissler, 2018; Peterman & Gade, 2017). We also calculated the coefficient of variation by dividing the mean surface activity by the standard deviation for each species for each year. We then spatially projected the mean surface activity probability for the years 2020, 2050, and 2099 by averaging the activity probability estimates across the year at each point and rasterizing each point to a 5-km resolution.

We assessed the changes in surface activity probability for each species over time using mixed- effect models fit with the *brms* package (Bürkner 2017). In all models, surface activity probability was the response variable, year, RCP, and year \* RCP interaction were modeled as predictor variables, and GCM was the random effect. We used a Gaussian distribution with uninformative student-t priors (*brms* default), run for 3,000 iterations on 4 chains after a burn-in phase of 1,000 and thinned at a rate of 1. Model fit was assessed using the Gelman-Rubin statistic ( $R_{hat} < 1.1$ ). We also projected the change of each weather variable using a mixed-effect model with the weather covariate as the response; year, RCP, and year \* RCP interaction as predictor variables, and GCM as the random effect.

## Results

### Global Surface Activity Model

Across the five datasets, the average individual surface activity probabilities ranged from  $0.04 (\pm 0.19)$  –  $0.12 (\pm 0.05)$ . Global surface activity was positively related to 1-day precipitation, 3-day mean temperature, and water vapor pressure. Global surface activity was negatively related to the number of dry days, 3-day precipitation, and 1-day mean temperature (Figure 5.3A). Global abundance was related to elevation, stream distance, and the interaction of elevation and stream distance (Figure 5.3B; Appendix D: Table D.4). The abundance covariates had greater variation in magnitude and direction between datasets relative to activity covariates, which showed more consistency between datasets (Appendix D: Figure D.1). Further, the Coweeta weather station data was highly correlated with the Daymet data for the same region from 2004–2017 ( $r = 0.98$ ;  $p < 0.005$ ; Figure 5.2).

### Future Surface Activity Probability

Future projections of surface activity probabilities indicated that mean individual surface activity rates increased with time for all seven species of the *P. jordani* (Figure 5.4). All species had similar increases in activity probability over time, with *P. metcalfi*, *P. amplus*, and *P. meridianus* estimated to increase by 0.08% per year (CRI:  $\pm 0.001$ ) and *P. cheoah*, *P. jordani*, *P. montanus*, and *P. shermani* were estimated to increase by 0.05% per year (CRI:  $\pm 0.001$ ) (Figure 5.5). There was no difference in surface activity probability over time between the RCP 4.5 and 8.5 scenarios for all species (average  $\beta = 0.001$ , CRI:  $\pm 0.001$ ). Across all species, the coefficient of variation in surface activity probability increased over time (Appendix D: Figure D.3). *Plethodon metcalfi*, *P. amplus*, and *P. cheoah* had a similar increase in CV with  $\beta = 0.040$  (CRI: 0.040, 0.050).

*Plethodon jordani*, *P. meridianus*, *P. montanus*, and *P. shermani* had similar increases in CV over time with  $\beta = 0.05$  (CRI: 0.04, 0.05). The CV for all species did not differ between RCP 4.5 and RCP 8.5 ( $\beta = 0.0001$ , CRI:  $\pm 0.0001$ ). There was no significant interaction between year and RCP for any species. Predicted activity probabilities from models run with the 25°C physiological threshold forcing activity to zero and models without the threshold were highly correlated ( $r = 0.97$ ,  $p < 0.001$ ).

Over the next 80 years, the MACA dataset showed no significant trend in dry days over time ( $\beta = 0.09$ , CRI: -0.17, 0.34) or between RCPs ( $\beta = -323.19$ , CRI: -1117.98, 466.63); 1-day precipitation over time ( $\beta = 0.01$ , CRI: -0.01, 0.01) or between RCPs ( $\beta = 5.91$ , CRI: -6.20, 18.15); or 3-day precipitation over time ( $\beta = -0.001$ , CRI: -0.01, 0.01) or between RCPs ( $\beta = 14.48$ , CRI: -22.51, 52.33). There was a significant increase of VPA over time ( $\beta = 3.25$ , CRI: 2.95, 3.55) and RCP 4.5 was significantly lower than RCP 8.5 ( $\beta = -11446.01$ , CRI: -12407.23, -10478.78). Both 1-day and 3-day temperature increased over time ( $\beta = 0.03$ , CRI: 0.02, 0.03 and  $\beta = 0.03$ , CRI: 0.02, 0.03, respectively) and RCP 4.5 was significantly lower than RCP 8.5 ( $\beta = -81.3$ , CRI: -89.17, -73.40 and  $\beta = -81.73$ , CRI: -90.05, -73.29; Appendix D: Figure D.4).

## Discussion

The threats of climate change are ubiquitous and require immediate attention to understand how species will persist in the future. Our study assessed how surface activity, a fitness-related behavior of terrestrial salamanders, may change over time in the face of global climate change. We developed a model to describe the environmental covariates driving salamander abundance and surface activity probability at a broad scale

by integrating five independent datasets. Several previous studies have used hierarchical models to assess similar questions (e.g. Chandler & King, 2011; Connette and Semlitsch 2013; Dodd and Dorazio 2004; Gade and Peterman 2019; Kéry, Royle, and Schmid, 2005; Peterman and Semlitsch 2013; Studds et al. 2017), and have found myriad of factors driving abundance and more consistent factors predicting activity probability. For example, independent investigations have found temperature and precipitation to be reliable predictors of activity (Connette, Crawford, and Peterman 2015; Gade and Peterman 2019; Muñoz, Hesed, Grant, and Miller 2016). Even across our five datasets, activity covariates maintained more consistent parameter estimates (e.g. covariate “3-day temperature”, the parameter estimate ranged from 1.052 to 1.558; Appendix D: Figure D.1). Surface activity is more likely to be affected by abiotic conditions consistent with the physiological limitations of *Plethodon*. Abundance, alternatively, is affected by both abiotic and biotic factors and often, the site and survey-level nuances, parameters researchers choose to measure in the field, and *a priori* model decision criteria drive abundance predictors, likely resulting in the greater variance in abundance predictors. Between our five datasets, there were no consistent site-level covariates measured (Appendix D: Table D.2), and our parameter estimates for the abundance covariates significantly varied between each dataset (Appendix D: Figure D.1). Our approach to model and estimate common parameters for both abundance and activity is therefore novel and allows for inferences to be made at broad scales across similar species.

Using our global model parameter estimates, we projected surface activity into the future and found an overall increase in surface activity probability over time, largely coinciding with changes in temperature and VPA. Temperature and VPA had the largest

standardized effect sizes in our global model (Figure 5.3) and had significant increases over time (Appendix D: Figure D.4). Both temperature and VPA are key variables relating to plethodontid physiology. Plethodontids are lungless and become surface active during wet and cool nights (Feder and Londos 1984). However, we saw salamander activity increase with increasing temperatures and VPA, a seemingly contradictory result. Southern Appalachian plethodontid salamanders are typically most active during the middle of the summer (Connette, Crawford, and Peterman 2015) and our sampling window for future projections occurred during the peak activity season when yearly temperatures reach their maximum (Figure 5.2). Temperatures likely do not exceed salamander thermal maxima to cause activity suppression, even into the future, which is further supported by our high correlation between models that included a 25°C threshold of assumed zero activity and models without the threshold. Clay and Gifford (2017) showed the estimated performance breadth of one species of the *P. jordani* complex (*P. montanus*) ranged from ~17 – 27°C, with 30°C representing the critical thermal maxima of other *Plethodon* species (Spotila 1972). Increased temperatures have also been shown to increase survival probability in *P. montanus*, likely because temperatures never exceeded the species' thermal maxima (Caruso and Rissler 2018). Thus, for high-elevation species with peak activity at the warmest times of the year, future climates might be expected to increase activity until conditions approach and surpass physiological limits.

Globally, ectotherms' current activity times are restricted to the warm summer months, and trends suggest that in temperate regions, there will be an increase of potential surface activity with climate change (Buckley et al. 2012). Our observed

increased of activity probability over time is thus likely a warm-season effect. Increases in temperature affect nearly all physiological processes in ectotherms (Rome et al. 1992; Homyack et al. 2010). Warm temperatures increase the metabolic demands of salamanders, which may force more surface activity to maximize foraging opportunities. Connette and Semlitsch (2015) found that salamander surface activity increased in timber harvest plots relative to control plots, which could be a potential result of increased energy demands in the hotter harvest plots. Further, in a lab-based study, Novarro et al. (2018) found that *P. cinereus* had had greater ingestion rates at hotter temperatures, presumably in an attempt to counteract increased metabolic demands. Although Novarro et al. (2018) did not assess activity rates, their results suggest that salamanders would have to increase their surface activity to increase ingestion rates. Further, energy assimilation tends to decrease at higher temperatures, suggesting salamanders will need to increase feeding frequency to maintain energy balances (Clay and Gifford 2017; Fontaine et al. 2018; Huey and Kingsolver 2019). Increased metabolism could also have important population-level demographic consequences. For example, higher metabolism associated with increased temperature has been shown to decrease body sizes and growth rates in *Plethodon* (Caruso et. al. 2014; Homyack, Haas, and Hopkins, 2010; Muñoz et al. 2016). Reductions in body sizes could lead to delayed maturity or reduced fecundity, ultimately leading to population declines. Thus, although our model predicts greater opportunity for surface activity under future conditions, there may be physiological tradeoffs that collectively have negative consequences for salamander populations.

Salamander physiological tolerance and performance appears to be dependent on the climatic history of populations, which often exhibit local adaptation to the

experienced background climate (Spotila 1972; Riddell and Sears 2015; Clay and Gifford 2017; Novarro et al. 2018). For example, temperature differentially impacts growth across elevational gradients in *P. montanus* whereby salamanders inhabiting high elevations have increased growth as temperatures increase, while those at low elevation show the opposite growth trend (Caruso and Rissler 2018). The elevational variation in growth rate is likely a result of the conditions experienced at each elevation; higher elevation temperature increases are within the thermal tolerances of *P. montanus*, while low elevation increases may exceed such tolerances, resulting in decreased growth (Caruso and Rissler 2018). Our modeling framework assesses surface activity probability across the entire range (latitudinal and elevation) of the species complex, combining high and low elevation individuals. The average weather conditions across the entire range of the *P. jordani* species complex appear to not exceed the species' thermal maxima, thus explaining why our model predicts continued increases in surface activity probability over the next 80 years.

Concurrent with increased surface activity probability over time is an increase in variation in our activity probability (Appendix D: Figure D.3), which corresponds with increased GCM uncertainty. Global circulation models typically have high uncertainty, especially towards the end of the century (Rupp et al. 2013; Northrop and Chandler 2014), with precipitation predictions being more variable than temperature, particularly at the regional scale (Knutti et al. 2008; Northrop and Chandler 2014). We found precipitation to impact surface activity probability in our global model estimates (Figure 5.3), supporting previous findings (Caruso and Rissler 2018; Connette et al. 2015; Connette and Semlitsch 2013; Muñoz et al. 2016). Yet, there was no significant trend of

precipitation into the future likely as a result of the increased uncertainty in GCMs (Figure 5.5), thus resulting in no discernable effects on our future surface activity probability estimates. As expected, the RCP 8.5 scenario had greater estimates of changes in VPA and temperature relative to RCP 4.5 (Figure 5.5). These scenarios are representative of possible climate futures given emission levels and were specifically included to capture a range of variation in our model. There was no difference in surface activity probability between RCP scenarios, and all species responded with similar rates of increase in surface activity probability. This uniform response across the seven species is not surprising given that members of the *P. jordani* complex exhibit levels of niche conservatism. Mid to high elevations are the cradle of biodiversity for *Plethodon* likely because the climatic conditions experienced here have constrained species to specific climate (elevation) zones for millions of years, leading to greater species accumulation. Geographic isolation and limited dispersal through warmer low elevations has led to species diversification, and resulted in adaptation to the climatic conditions at these elevational regions (Kozak and Wiens 2010).

Our model predicts the probability of a salamander being surface active on a given night into the future, which is related to, but distinct from, the total time a salamander is active during a given night. This distinction is important because the duration of surface activity must be sufficient to fulfill the foraging requirements necessary for growth and survival. Further, mate finding and courtship occur on the forest floor (Feder 1983; Jaeger 1980; Petranksa 1998), therefore reproductive opportunities are also tied to surface activity. However, teasing apart the probability of activity and total activity time is challenging. Total season activity has been previously modeled to

increase with predicted rainfall (McEntire and Maerz 2019), and total activity time increases with dehydration resistance and water loss thresholds of salamanders, suggesting the importance of wet nights on activity time (Riddell et al. 2018). Our model cannot disentangle these effects. Additionally, discerning between the effects of environmental conditions on surface activity and annual and seasonal cycles is challenging. For instance, we have not observed salamanders at our sites becoming active under suitable conditions outside of their normal active season (*personal observations*). There may be a strong seasonal circadian rhythm to salamander activity that our model cannot detect. Photoperiod circadian rhythm can be a driver of activity initiation and metabolic activity in other urodeles, both of which can be modified by experimentally shifting photoperiod (Adler 1969; Hervant et al. 2000; Maerz et al. 2001). Bird migrations and many mammalian behaviors are strongly influenced by circadian and circannual rhythms (Gwinner 1996; Reppert and Weaver 2002; Albrecht and Eichele 2003). It could, therefore, be possible that the *P. jordani* species complex activity patterns have circadian patterns based on the summer season independent of the proximate environmental conditions.

Understanding surface activity of salamanders has been a long-standing challenge, and previous studies have used agent-based simulations (McEntire and Maerz 2019) and intensive lab-based physiological experiments (Riddell and Sears 2015) to understand and model surface activity and there are a variety of existing models that attempt to discern future climate change effects on species ranges or biophysical processes (e.g. correlative approaches or mechanistic biophysical models). Our modeling framework is unique in that we adapted an existing framework to directly understand a

targeted behavior: surface activity. Our model is the first comprehensive field-based study to assess surface activity probability at a regional scale and then project activity into the future under climate change scenarios. Our model does, however, have important limitations to consider. Our approach assumes that abundance is constant through time, which may not be wholly representative. There have been numerous studies suggesting declines in amphibians at global scales (Houlahan et al. 2000) and in North America (Grant et al. 2016). Using occupancy models, Grant et al. (2016) found that declines are driven by interacting stressors, including human modification, pesticides, and climate at a local scale. Milanovich et. al. (2010) suggested that suitable climate in the Appalachian highlands will significantly decrease, resulting in range contractions that would likely be driven by reductions in abundance over time. Our future surface activity model does not account for changes in abundance, nor does it account for the interactive and multiplicative stressors amphibians face that may contribute to declines (Blaustein et al. 2010). Our model also uses data from only three out of the seven species in the *P. jordanii* complex, and our sampling efforts are somewhat west skewed geographically (Figure 5.1). However, all species of the complex are constrained to similar elevations that experience similar environmental conditions. All of the species had similar projected activity probability in the future (Figure 5.5), suggesting our sampling bias does not strongly impact the results for all species in the complex. Finally, our model projects surface activity assuming that salamanders do not modify any behavior or physiology. Short-term lab studies suggest that salamanders have plastic physiological responses with metabolic and water loss suppression during the warmest parts of the year (Riddell et al. 2018). Salamanders may also modify the timing of surface activity to exploit more

optimal conditions, a pattern seen commonly in other taxa (Fielding et al. 1999; Tingley et al. 2012; Schuster et al. 2014; Buckley et al. 2015; Muñoz et al. 2016). However, the ultimate demographic consequences of sustained physiological adjustments are unknown. Assessing surface activity cost tradeoffs and phenological shifts in activity should be priorities for future studies.

Climate change will favor species with wide thermal tolerances, short generation times, and a range of genotypes among populations (i.e. high adaptive capacity; Ficke et al. 2007). Species of the *P. jordani* complex are unfortunately lacking in all three attributes. They have relatively narrow thermal performance windows (Clay and Gifford 2018), long generation times (Staub 2016), and small and geographically-isolated populations with limited gene flow (Highton and Peabody 2000), emphasizing their conservation concern. Our regional, multi-species model demonstrates that temperature, precipitation, and vapor pressure strongly influence surface activity probability, and without physiological or behavioral changes, these salamanders will exhibit higher levels of surface activity during their peak active season under future climate conditions. Our study provides necessary insight into how a key behavior driving fitness of plethodontid salamanders will be affected by climate change.

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Table 5.1 Summary of the individual datasets used to develop the global surface activity model.

<b>Dataset</b>	<b>Location</b>	<b>Sampling Years</b>	<b>Plot Area (m<sup>2</sup>)</b>	<b>Number of Plots</b>	<b>Elevational Range (m)</b>	<b>Type of Plot</b>	<b>Plethodon species</b>
A	35.04, -83.19	2004	400	14	718–1248	Transect	<i>P. metcalfi</i>
B	35.18, -83.59	2012	19.6	136	1228–1571	Point	<i>P. shermani</i> *
C	35.15, -83.55	2017	9	176	953–1582	Point	<i>P. shermani</i> *
D	35.61, -83.43	2012	100	195	412–2021	Transect	<i>P. jordani</i>
E	35.06, -83.44	2016–2017	25	72	739–1422	Point	<i>P. shermani</i> *

\* *P. shermani* is known to hybridize with *P. teyahalee* at intermediate elevations. Counts are inclusive of hybrids.

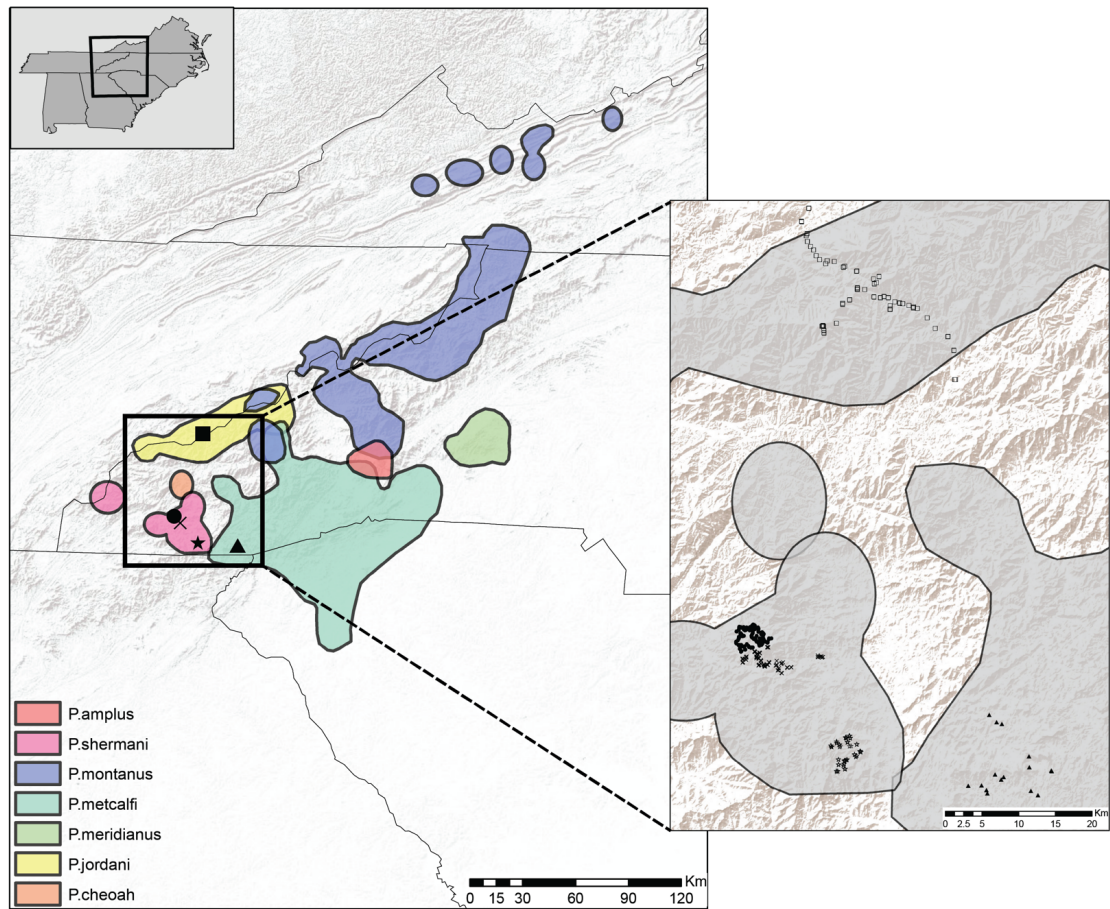


Figure 5.1 Map indicating the geographic ranges for each species of the *Plethodon jordani* complex (from iucnredlist.org) with the data sets used to develop the hierarchical model symbolized. Inset map shows every sampling point from each data set. Triangles are dataset A, circles are dataset B, X's are dataset C, squares are dataset D, and stars are dataset E (in reference to datasets outlined in Table 5.1).

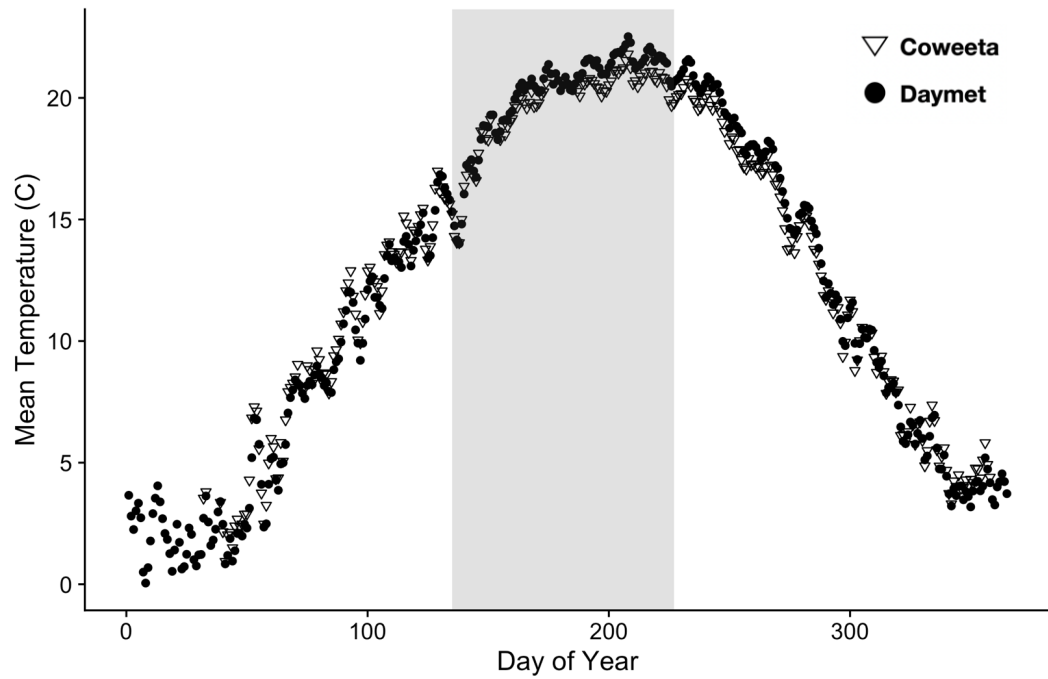


Figure 5.2 Mean temperature over time averaged from five weather stations at Coweeta Hydrological Lab (CHL; triangle) and downloaded from the Daymet database (circle) at the same spatial locations of CHL. Data is averaged from the years 2004 - 2017. The grey shaded region represents the ‘active season’ time frame we used for the future projections of surface activity.

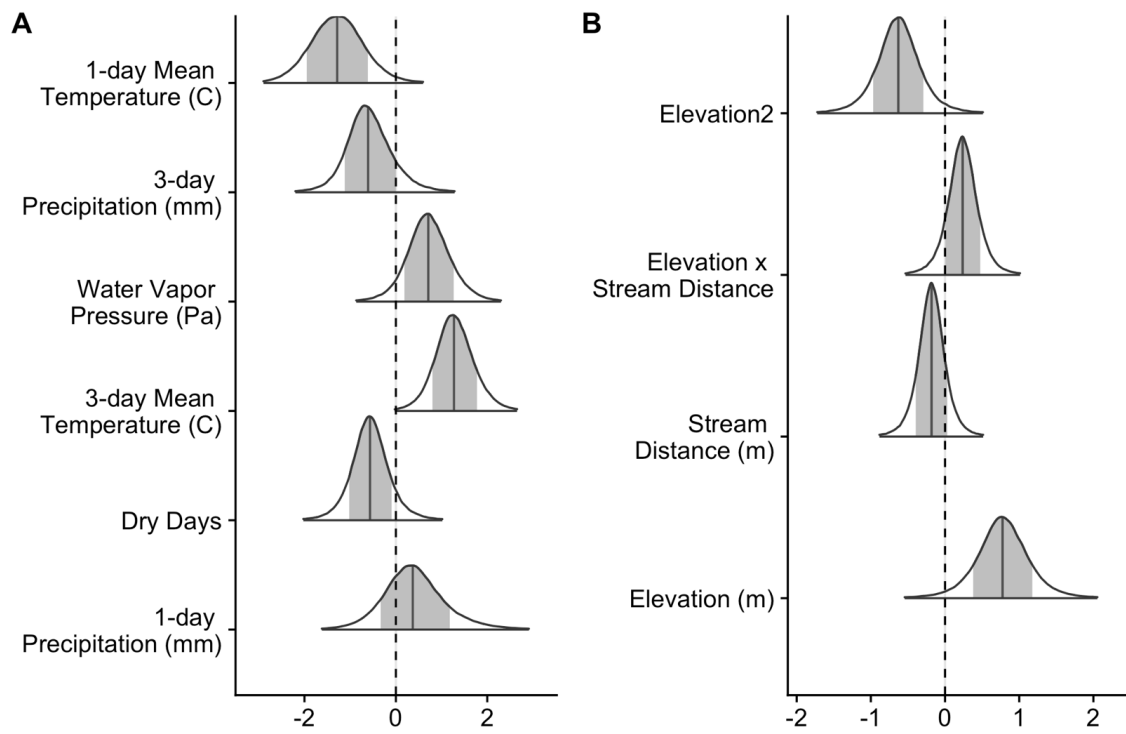


Figure 5.3 Mean estimates for (A) surface activity covariates and (B) abundance covariates from the global hierarchical model. Shaded region includes 75% of posterior region.

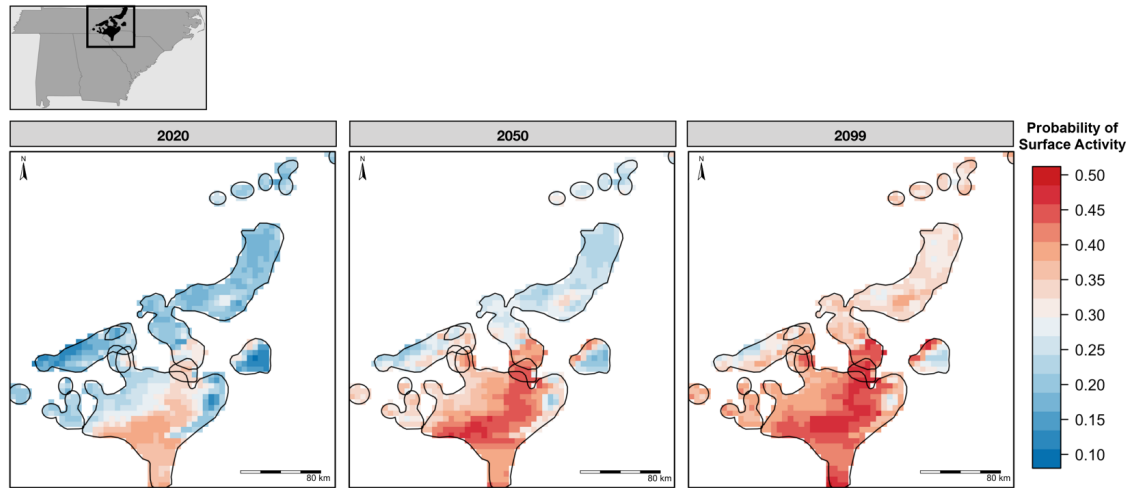


Figure 5.4 Average active season surface activity probability across the range of the seven species of the *Plethodon jordani* complex for the years 2020, 2050, and 2099.

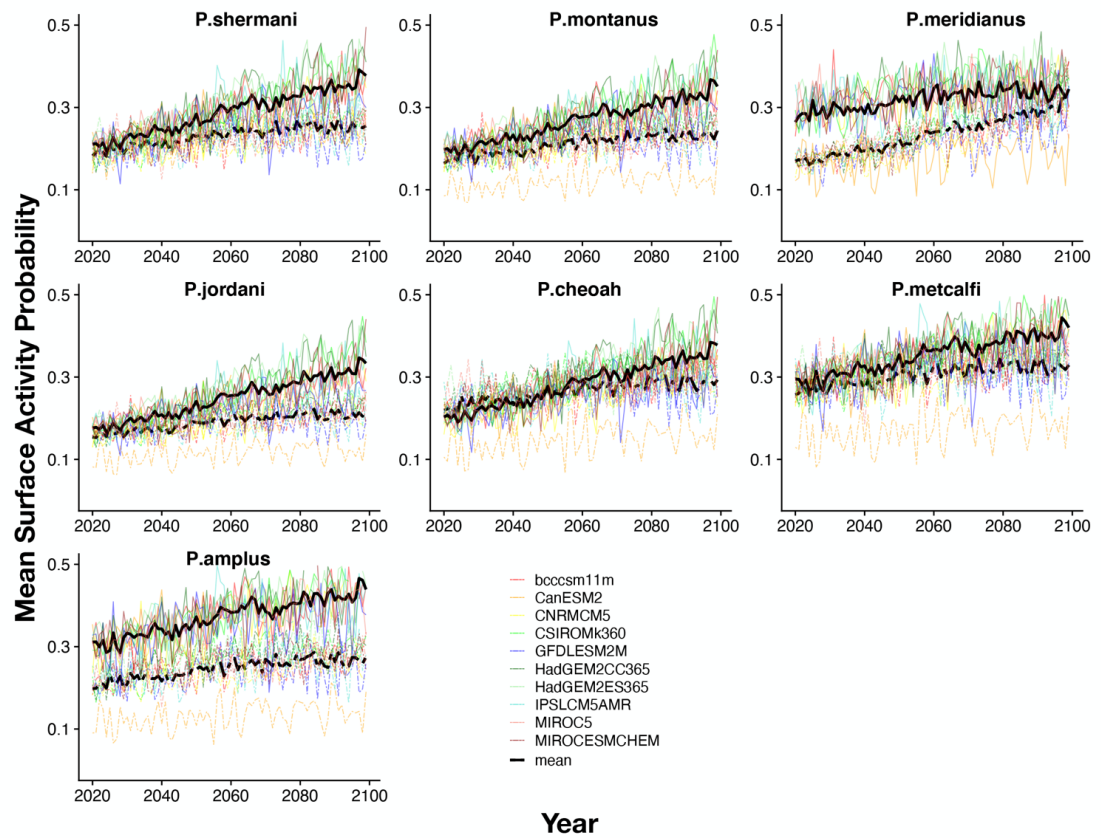


Figure 5.5 Mean surface activity probability for the seven species in the *Plethodon jordani* complex for the years 2020 – 2100. Colored lines represent individual GCMs, with the black line indicating the mean of the 10 GCMs. Dotted lines show RCP 4.5 and solid lines show RCP 8.5.

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## Appendix A: Model Description and Stand Age Histogram

Table A.1. Parameter estimates for the global detection and abundance hierarchical n-mixture model. N effective refers to the effective sample size from posterior samples and f refers to the proportion of overlap of zero. Bolded parameters indicate those with  $f < 0.75$ , which were subsequently removed for the reduced model

Model	Parameter	Beta	Lower CI	Upper CI	f	n effective
<b>Detection</b>	Intercept	-3.136	-4.694	-2.147	0.910	344
	Ground cover	0.047	-0.066	0.159	0.799	4,758
	<b>24-hour precipitation</b>	<b>0.021</b>	<b>-0.080</b>	<b>0.122</b>	<b>0.655</b>	<b>34,619</b>
	3-day precipitation	0.085	-0.082	0.249	0.843	36,742
	<b>Dry days</b>	<b>0.027</b>	<b>-0.153</b>	<b>0.209</b>	<b>0.614</b>	<b>182,000</b>
	24-hour temperature	0.084	-0.066	0.232	0.867	15,232
	Relative humidity	0.077	-0.016	0.171	0.948	11,465
	Surface soil temperature	-0.152	-0.333	0.028	0.950	4,824
	Belowground soil temperature	0.088	-0.035	0.215	0.917	15,010
	<b>Julian Date</b>	<b>-0.031</b>	<b>-0.192</b>	<b>0.132</b>	<b>0.651</b>	<b>10,417</b>
<b>Abundance</b>	Intercept	2.317	-2.606	4.513	0.910	1,267
	Elevation	0.890	-0.130	1.579	0.964	3,059
	Elevation2	-0.763	-1.403	0.418	0.928	1,383
	<b>Slope</b>	<b>0.001</b>	<b>0.088</b>	<b>0.175</b>	<b>0.504</b>	<b>22,771</b>
	Northness	-0.034	-0.124	0.055	0.772	4,886
	<b>Eastness</b>	<b>0.001</b>	<b>-0.091</b>	<b>0.092</b>	<b>0.505</b>	<b>16,104</b>
	Canopy cover	0.067	-0.028	0.161	0.918	7,189
	Leaf litter depth	0.063	-0.040	0.162	0.887	25,018
	<b>TPI</b>	<b>-0.044</b>	<b>-0.192</b>	<b>0.102</b>	<b>0.722</b>	<b>16,867</b>
	<b>Stand Age</b>	<b>-0.017</b>	<b>-0.206</b>	<b>0.173</b>	<b>0.572</b>	<b>12,156</b>
	Stream distance	-0.301	-0.575	0.025	0.966	2,958
	Elevation * Stream distance	0.296	-0.165	0.618	0.914	2,150

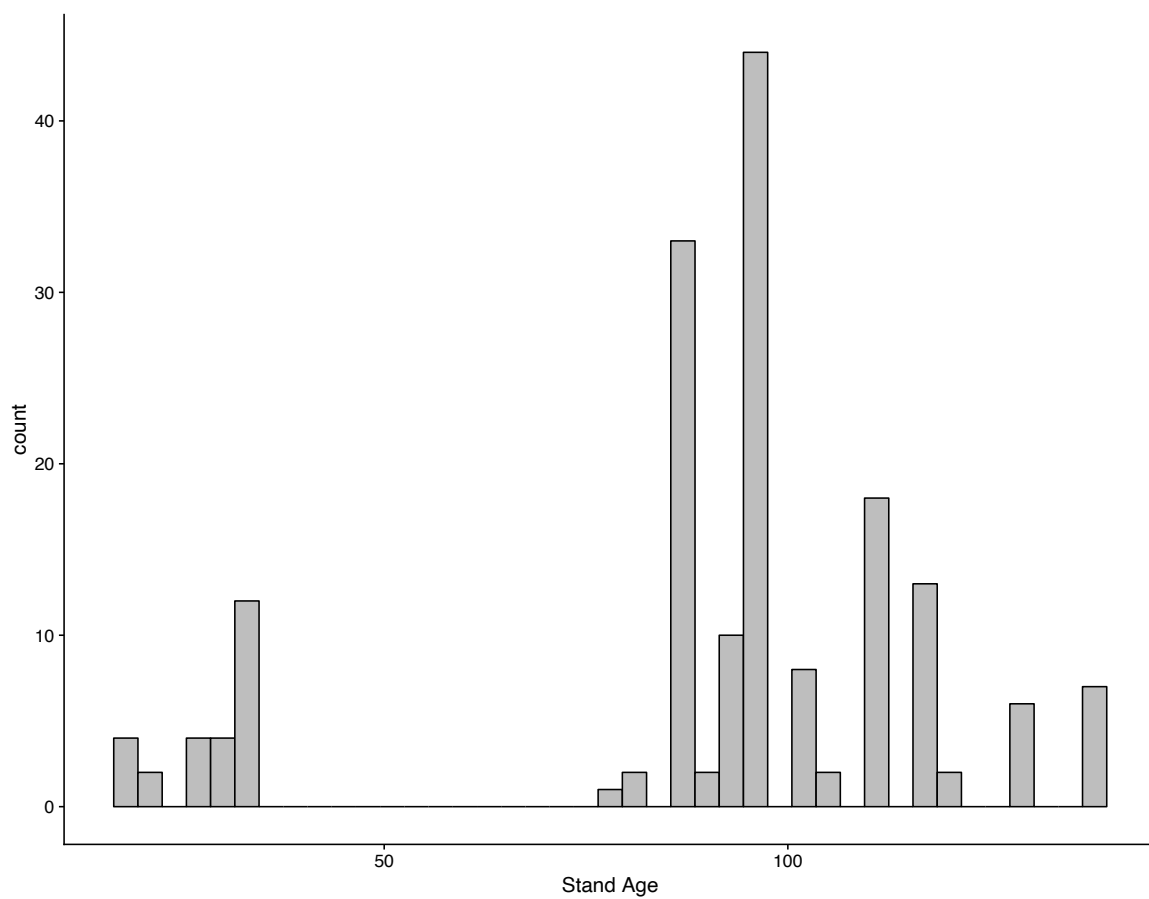


Figure A.1. Histogram of the range of stand ages where sampling plots were located across the study site.

## Appendix B: Demographic rate and hybridization

Table B.1: Topographic characteristics of the twelve Mark-Recapture plots used in the present study. The plot column indicates High or Low elevation, and the replicate close, medium, and far from stream plots.

<b>Plot</b>	<b>Elevation (m)</b>	<b>Stream Distance (m)</b>	<b>Slope (deg)</b>	<b>Stand Age (years)</b>	<b>Aspect</b>
High					
Close	1465	3	18	95	257
Close	1494	10	21	128	276
Medium	1534	117	24	95	299
Medium	1535	141	18	119	304
Far	1559	195	17	119	289
Far	1545	203	32	111	154
Low					
Close	968	3	19	92	69
Close	984	5	32	87	208
Medium	1001	101	34	137	39
Medium	1015	119	36	137	352
Far	1029	192	32	137	105
Far	1035	145	28	137	326

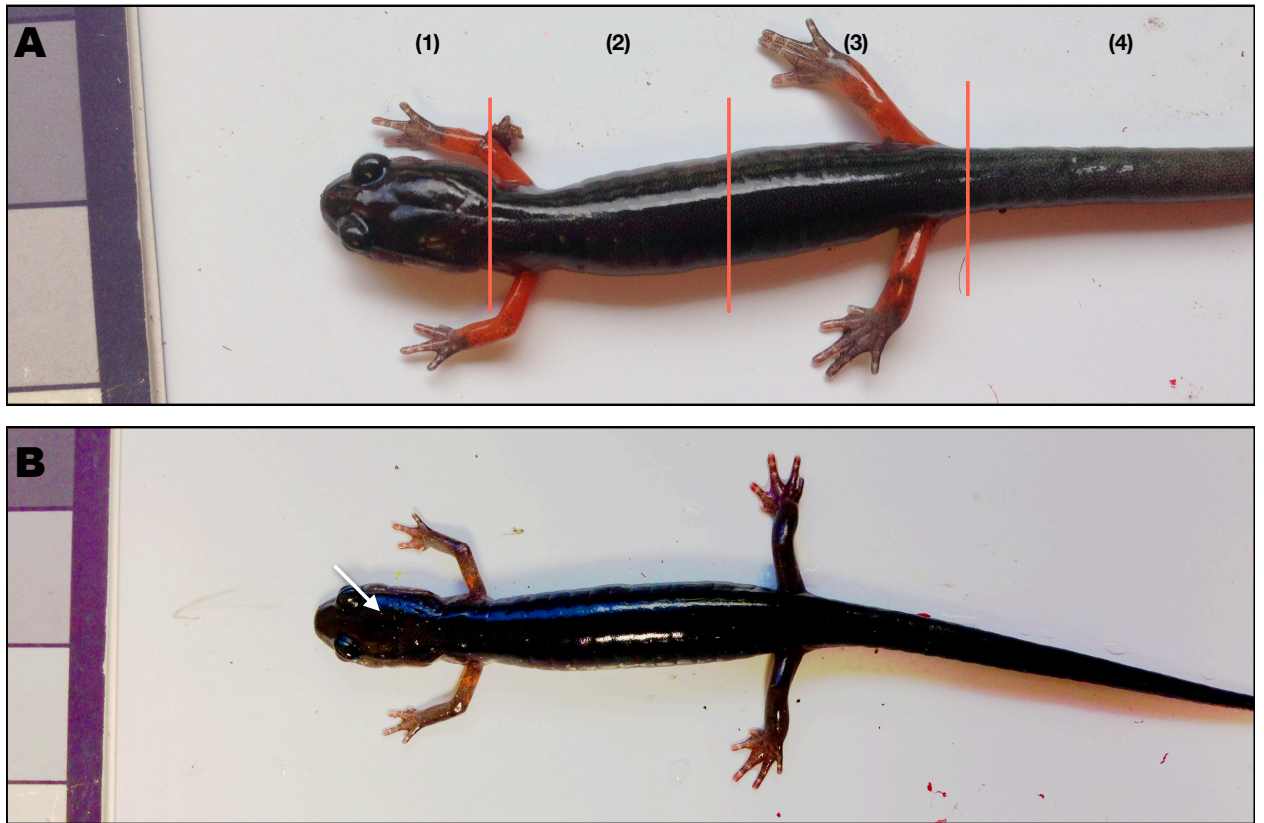


Figure B.1. Representative individuals of (A) high elevation "pure" *P. shermani* and (B) low elevation *P. shermani* x *P. teyahalee* hybrid. The red vertical lines in (A) show the 4-body region designation that the *P. teyahalee* characteristic white spots were counted to include in the hybrid score PCA. The white arrow in (B) shows examples of the white spots.

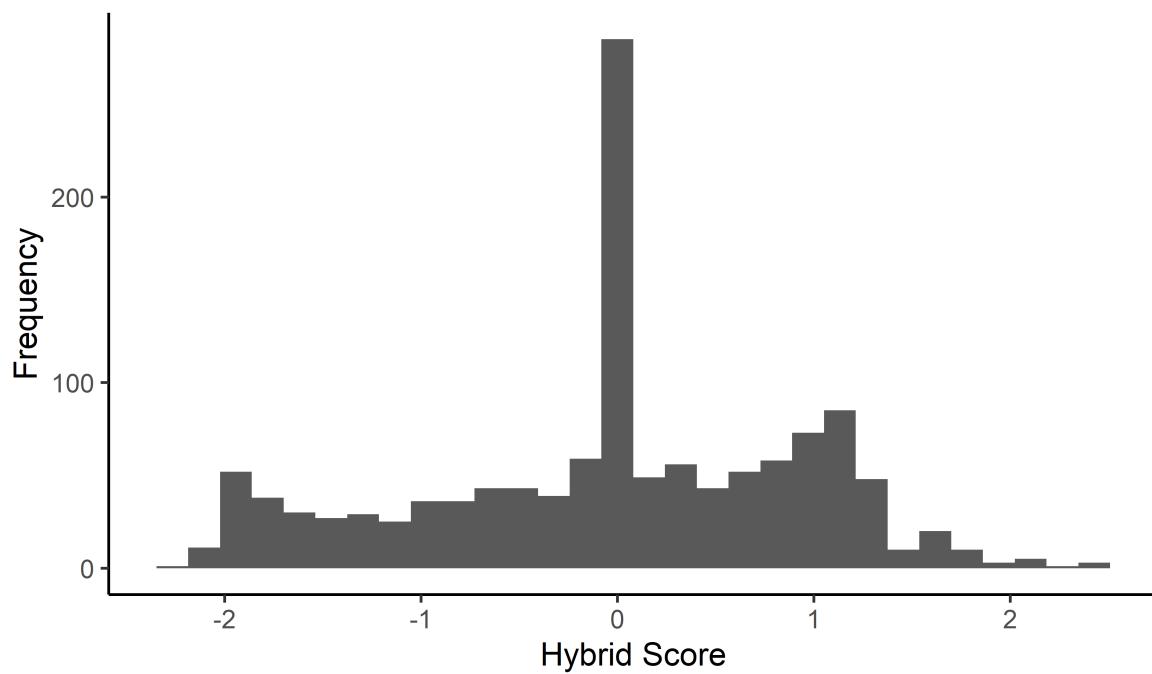


Figure B.2. Histogram of hybrid scores of the *P. shermani*. Positive values indicate more characteristics of *P. teyahalee* indicating more hybridization.

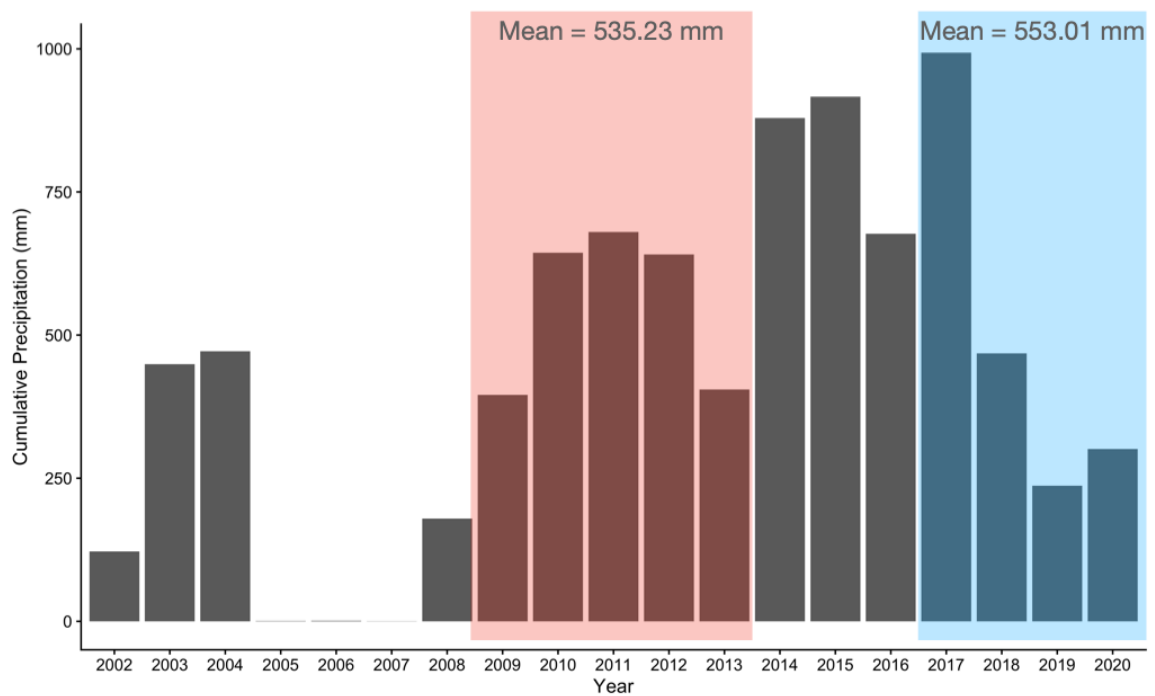


Figure B.3. Cumulative precipitation from 01 May to 31 August from 2002 – 2020 from the Wayah Bald Mountain WINE weather station (CRONOS). The red box shows the time scale of Connette (2014) and the blue box shows the time scale of the current study.

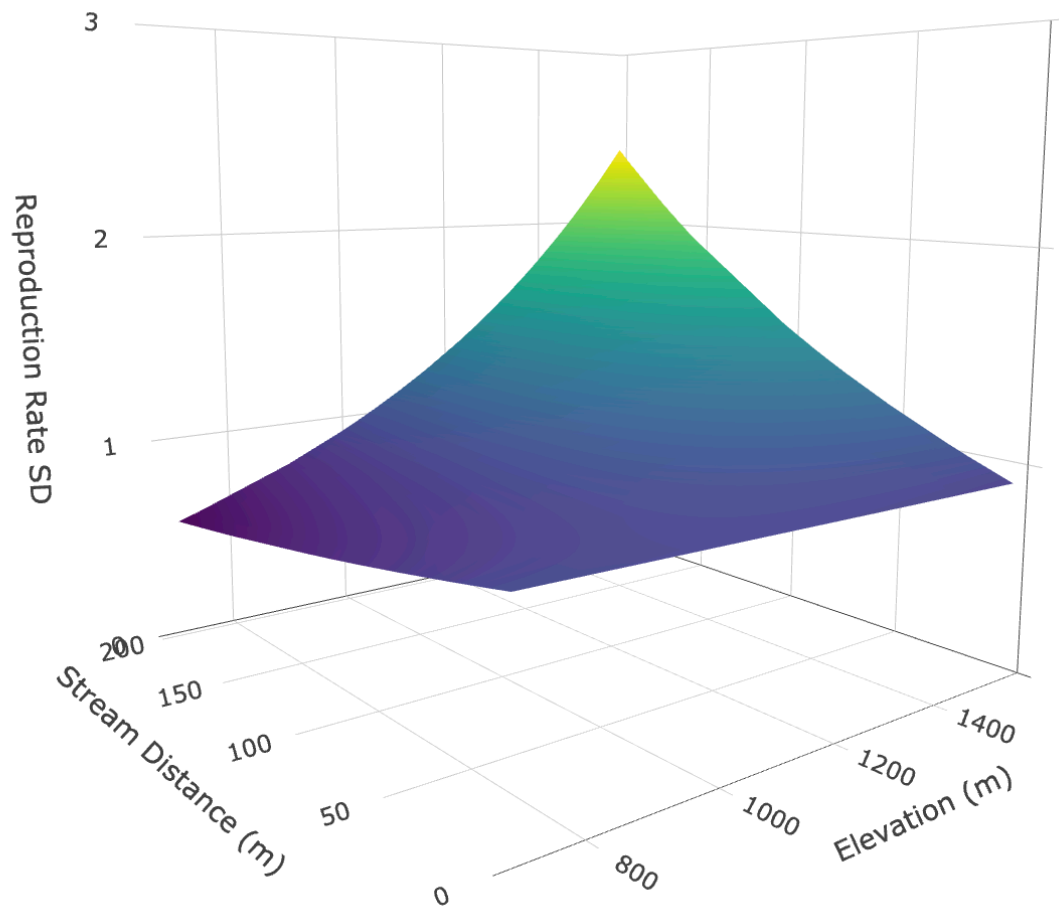


Figure B.4. The standard deviation in reproductive rate estimates in the interaction between elevation and stream distance in *P. shermani* from the SEIPM.

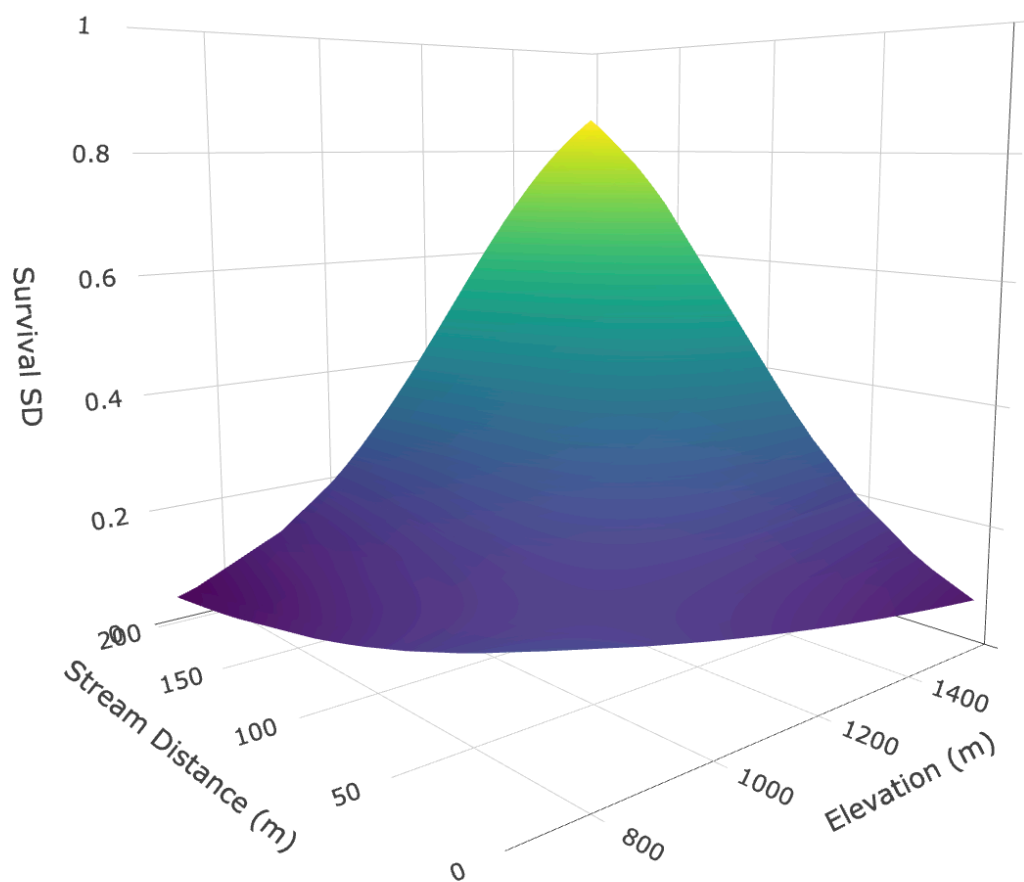


Figure B.5. The standard deviation in survival estimates in the interaction between elevation and stream distance in *P. shermani* from the SEIPM.

Appendix C: Experimental dCORT parallelism and chamber conditions

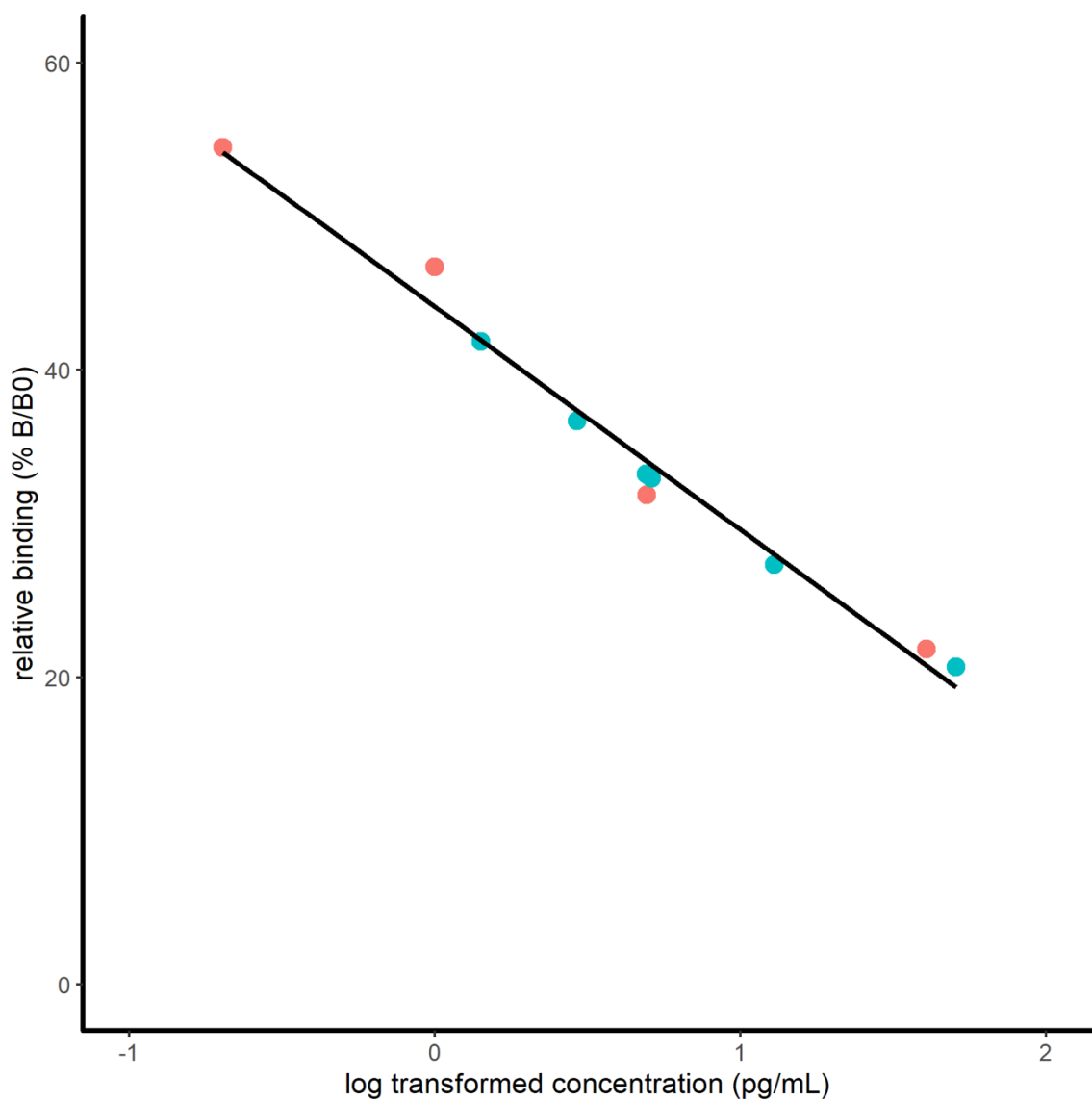


Figure C.1 Relative binding (%B/B0) in relation to log transformed CORT concentration (pg/mL) for serially diluted standards (red points) and salamander swab samples from two individuals (blue points).

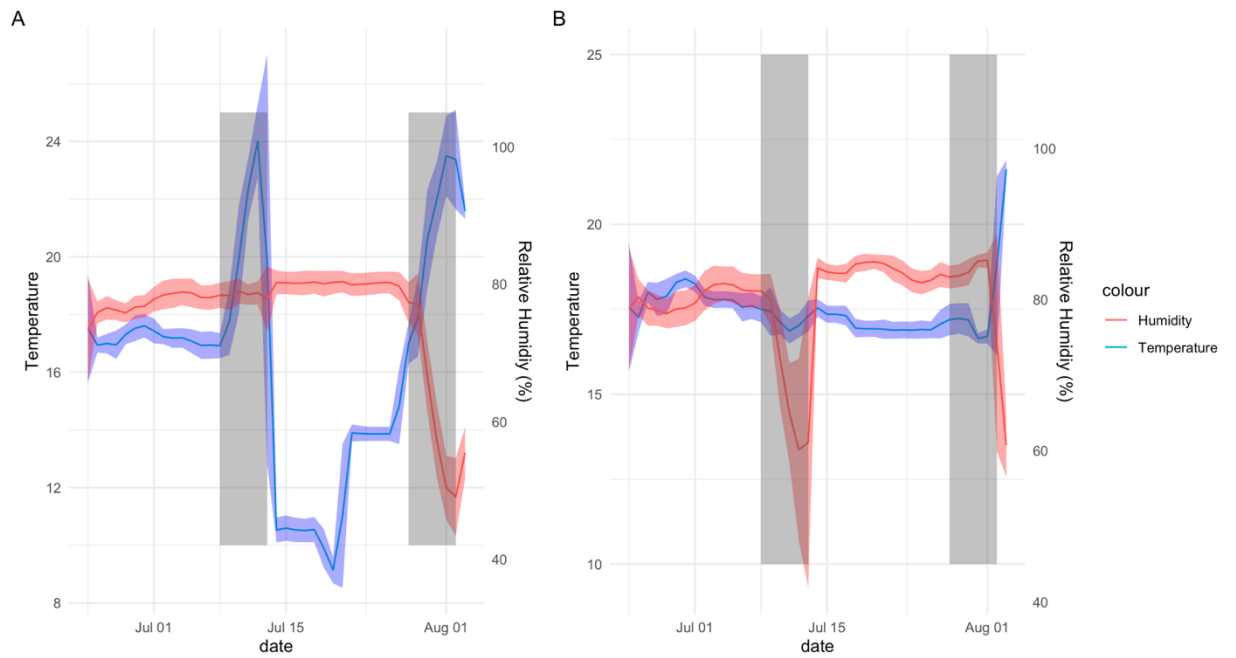


Figure C.2 Temperature and humidity conditions inside experimental chambers. Grey boxes show the time frame that Phase I and Phase II of the experiment occurred. (A) Treatment 1 (Hot) and Treatment 3 (Hot + Dry). (B) Treatment 2 (Dry) and Treatment 4 (Control).

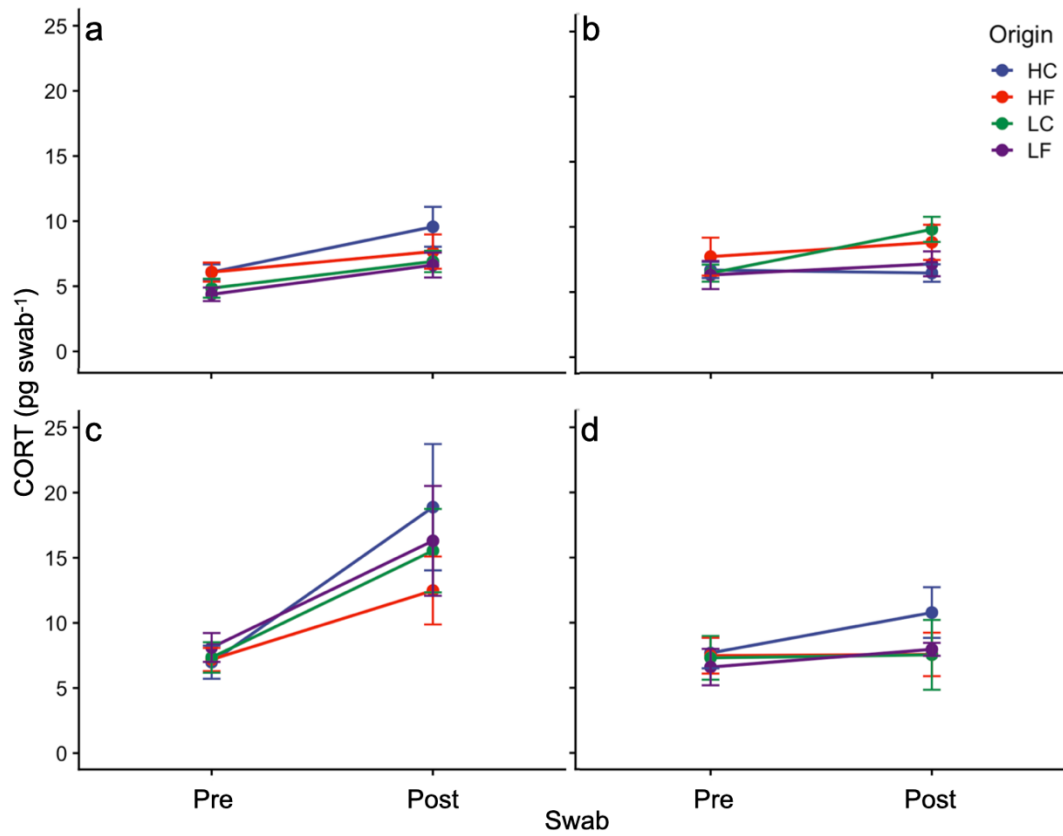


Figure C.3 Mean ( $\pm$  SE) dCORT excretion between pre-exposure and post exposure to (a) increased temperatures, (b) decreased humidity, (c) the combination of increased temperature and decreased humidity, and (d) control. Colored lines represent the average for individuals collected from a given origin.

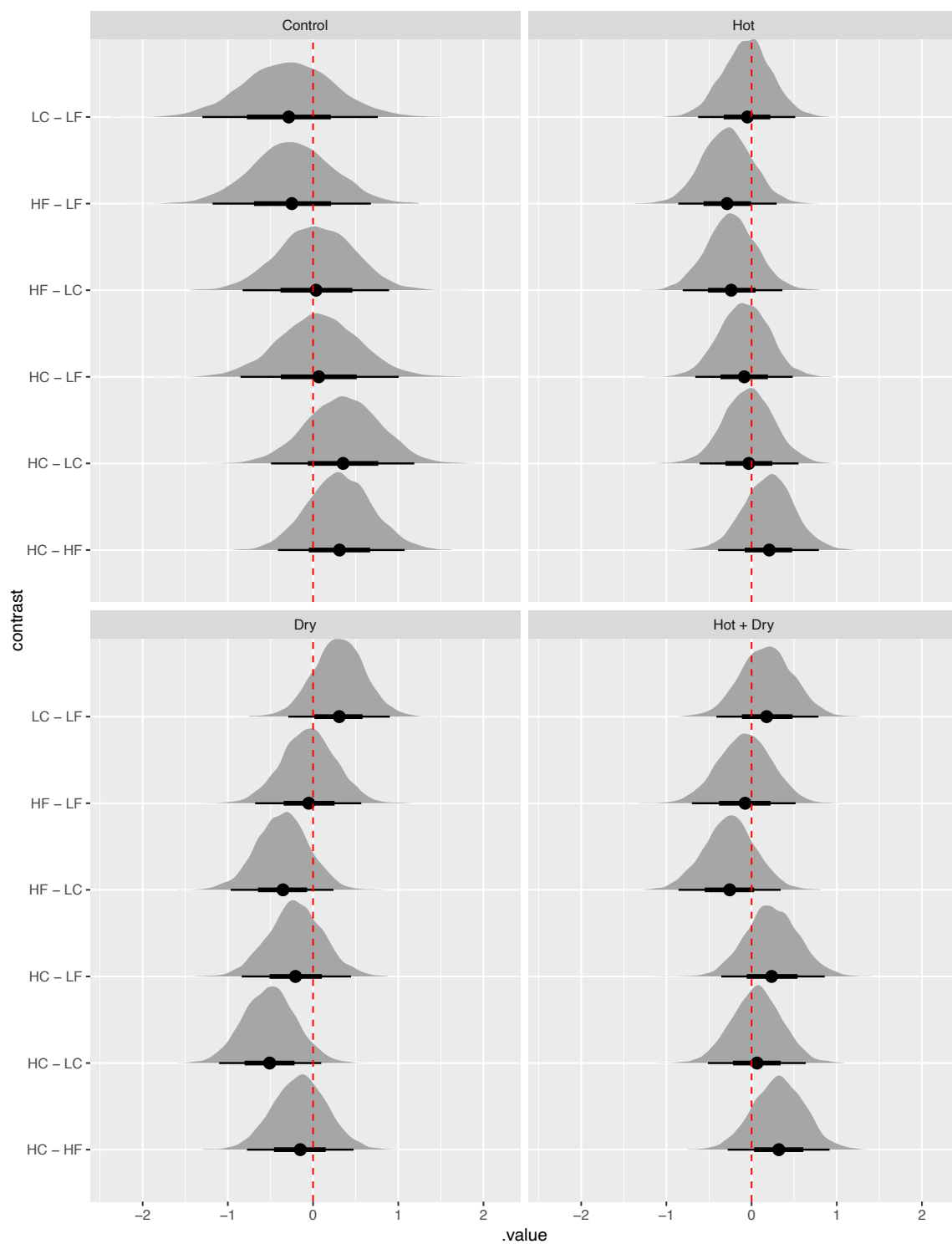


Figure C.4 Origin contrasts for each Treatment. Red dotted line represents zero, with thicker lines indicating 75% of the posterior and thin lines representing 95% of the posterior distribution

## Appendix D: Dataset and Global Circulation Details

Table D.1. Description of the sampling methodology for each dataset included in the global detection model.

### Dataset A: Collected by John A. Crawford (2004)

To define core terrestrial habitat use by an assemblage of stream salamanders, we sampled riparian forests adjacent to 14 headwater streams (streams were the unit of replication) in the southern Appalachian Mountains, Nantahala National Forest, Macon County, North Carolina (U.S.A.). All sites were located between 718 and 1248 m elevations, had not been subject to logging for at least 80 years, and were located at least 1 km apart.

We used a visual encounter search (VES) during the three night transects to capture surface active salamanders. Two researchers walked a straight line that was perpendicular from the stream edge (defined as the edge of the streambed) out to 100 m and recorded distance from the stream edge for each salamander encountered. While walking the perpendicular transect, each researcher searched 2.5 m to the right and left of the transect line. We identified all salamanders (including terrestrial *Plethodon* species) to species, weighed and measured for snout-vent length and total length, and determined sex. We determined age class (adult/juvenile) by comparing measured snout-vent lengths of each individual to published size classes for each species (Petranka 1998). We released all salamanders at the site of capture.

### Dataset B: Collected by Grant M. Connette (2012)

We conducted repeated-count surveys of salamanders at 68 timber stands to examine patterns of salamander abundance relative to historic timber harvest. Our surveys targeted 34 timber stands that had been harvested in the past 75 years (range 13–73 years) and 34 relatively late-successional stands (>82 years). Younger stands tended to be smaller (mean [SD] = 12.0 ha [17.5]) than older stands (26.0 ha [24.6]). Within each stand, we conducted counts at 2 independent survey points which were marked with a survey flag. One point was close to the center of the stand, and the second was between the center and the forest edge. Survey locations were accessed by foot, and the precise locations of survey points were determined in ArcGIS 9.3 (ESRI, Redlands, California) prior to visiting each site. Each survey point was 5 m in diameter and was surveyed on 5 separate dates between 25 June and 8 August 2012. During each survey, we performed a nighttime visual encounter survey during which a single observer exhaustively searched the survey area for salamanders active on the ground surface. Surveys were conducted between 21:20 and 05:59 eastern standard time and typically took 3–5 min. We recorded the time at which each survey was initiated and used an infrared thermometer to measure ground surface temperature. Daily rainfall totals were obtained from a weather station in

Rainbow Springs, North Carolina (<10 km from the study area). We also calculated the average leaf-litter depth for each survey point from 3 haphazard measurements taken within each survey area

Dataset C: Collected by Meaghan R. Gade (2017)

Eighty-seven locations were generated in ArcGIS v.10.2 in a stratified random design to incorporate the full range of available elevation and stream distances. Elevation ranged from 700 to 1600 m and distance from stream, ranging from 5m to 300 m was used as a proxy for moisture. We incorporated elevation and stream distance to independently assess temperature and moisture. Each of the 87 plots were at least 30-m from each other to insure independence of plots (Connette and Semlitsch 2013). Once each plot location was determined in the field, a second, paired plot was established 10 m in a random direction from the original plot, to maximize survey areas due to other logistical constraints, resulting a total of 174 survey plots. Each plot was 3 m x 3 m in size. Plots were surveyed on four separate occasions between 4 June and 2 August 2017, during peak activity time between 2130 and 0230 EST. Two observers walked through the center of each plot looking for surface active salamanders. Surveys took approximately 3–5 min and, in addition to the total number of observed salamanders was recorded.

Dataset D: Collected by Hocking, Crawford, Peterman, Milanovich (2012)

Surveys of salamanders were conducted as follows. We conducted nighttime visual encounter surveys (VES) along transects to locate surface-active salamanders. To conduct a survey, one of four observers (the authors) slowly walked a transect and recorded the number of each salamander species observed within 2 m of either side of the center transect line, resulting in a 100 m<sup>2</sup> transect survey plot (25 x 4 m). Salamanders were not disturbed except to handle for further identification. It required 10 – 20 minutes to conduct a VES on a transect depending on the terrain, density of vegetation, and number of animals that had to be handled for identification.

We surveyed each transect on 1–6 occasions (mean = 4.31) on 21 nights between 13 June – 20 July 2012. At least 2 transects were visited at every site on at least 5 occasions. Observers were randomly assigned to a transect on the first occasion and then rotated through the transects at each site on future occasions to avoid observer bias. A transect was skipped if the surveyor was unavailable on a given night. Survey sites were chosen each night using a stratified random sampling design along the elevational gradient. We randomly selected 3–5 sites from each of three elevations: low (412 – 1,000 m), medium (1,000 – 1,500 m), and high (1,500 – 2,025 m). We only surveyed transects at a site once during each of the six sampling occasions. Each night we also randomized our starting location then proceeded in the most convenient route to the other sites. This prevented sampling sites or specific elevations at the same time of night on each occasion. We conducted all surveys between 21:00 and 03:00 hours EDT.

Dataset E: Collected by John C. Maerz (2016 – 2017)

We distributed 72, 5 m X 5 m plots among 20 sites across the Coweeta Hydrologic Laboratory, Macon County, North Carolina. Sites were distributed evenly among north and south facing aspects and across an east to west gradient of declining mean annual precipitation. Starting in 2016, we conducted repeated counts over three consecutive nights in the spring, summer, and fall of each year following a robust design (Pollock 1982) resulting in 6 primary periods composed of 18 secondary periods. The spring sample was always conducted in May, the summer sample in July, and the fall sample in September and October. Sampling began approximately one hour after sunset and was completed between midnight and 4:00 AM depending on the number of salamanders captured and processed. Each plot was searched for a minimum of 15 person-minutes and 4 complete passes of each plot. Because capturing and bagging salamanders required time, the minimum number of passes insured sufficient sampling of each plot regardless of numbers captured. Salamanders were captured by hand and contained individually in plastic Ziploc bags until the search time ended. Only animals active on the surface were collected. Salamanders were released at the center of the plot before searchers moved to the next plot.

Table D.2. Comparison of all covariates originally collected at the survey- and site-level for the 5 datasets included in the global detection model. 1 represents ‘collected’ and 0 represents ‘not collected’ in original dataset.

	<b><u>Dataset</u></b>				
	<b>Crawford (A)</b>	<b>Connette (B)</b>	<b>Gade (C)</b>	<b>GSMNP (D)</b>	<b>Maerz (E)</b>
<b>Survey Level</b>					
Qualitative <sup>‡</sup>	1	0	0	1	1
Air Temperature (C)	1	0	1	1	1
Rel. Humidity (%)	1	0	1	1	1
Wind speed (m/s)	1	0	1	0	1
Baro. Pressure (mmHg)	1	0	0	0	0
Wet Bulb	1	0	0	0	0
Dew Point	1	0	0	0	0
Ground Temp. (C)	0	1	1	0	0
Belowground Soil Temperature(C)	1	1	1	1	1
Moon	0	0	1	0	0
Time	0	0	1	0	1
<b>Site Level</b>					
Leaf Litter Depth (mm)	1	1	1	1	0
Canopy Cover (%)	1	0	1	1	0
Ground Cover (%)	0	0	1	1	0
Stand Age (yrs)	0	1	1	0	0
<b>GIS-Derived Covariates</b>					
Elevation (m)	1	1	1	1	1
Slope (deg)	0	1	1	0	1
Aspect	0	1	1	0	1
TPI	0	1	1	1	0
Solar Exposure	0	1	0	1	0
TWI	0	0	1	1	0
Northing	0	1	0	0	0
Easting	0	1	0	0	0
Eastness	0	1	1	0	0
Northness	0	1	1	0	0
Stream Distance (m)	0	1	1	0	0

<b>Weather Station-Derived</b>					
Max Temperature (C)	1	0	1	0	0
Min Temperature (C)	1	0	1	0	0
24-h Precipitation (mm)	1	1	1	1	0
3-day Precipitation (mm)	0	1	1	0	0
7-day Precipitation (mm)	0	1	1	0	0
Sea Level Pressure (mm)	0	0	1	0	0
<b>Salamander Counts<sup>Ω</sup></b>					
PSHE	0	1	1	0	1
DMON	1	0	1	1	1
DOCO	1	1	1	1	1
DQUA	1	0	1	1	1
EGUT	1	0	0	0	0
EWIL	1	1	1	1	1
PMET	1	0	0	0	0
PTEY	1	0	1	1	1
DWRI	0	1	1	1	1
NOVI	0	1	0	1	1
GPOR	0	1	1	1	1
DEAE	0	1	0	0	1
PSER	0	0	0	1	0
PJOR	0	0	0	1	0
PGLU	0	0	0	1	0
DSAN	0	0	0	1	0
DIMI	0	0	0	1	0
DCON	0	0	0	1	0
PRUB	0	0	0	0	1
Snout Vent Length (mm)	1	0	0	0	1
Total Length (mm)	1	0	0	0	1
Mass (g)	1	0	0	0	1
Count	1	1	1	1	1
Life Stage	0	1	1	0	1

### Other

Sample Area (m <sup>2</sup> )	400	19.6	9	100	100
Number of Plots	14	136	176	195	72
Date of Surveys		June 25 -			
	May 17 - July 25	Aug 8	June 4 - July 30	June 13 - July 20	May 2 - Oct 2
Year	2004	2012	2017	2012	2016-2018

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<sup>‡</sup>Qualitative refers to any written description of the site during the survey (e.g. “wet leaves”, “leaf litter dry at bottom”, “recent bear activity”, etc.).

<sup>Ω</sup>PSHE = *Plethodon shermani*; DMON = *Desmognathus monticola*; DOCO = *Desmognathus occoe*; DQUA = *Desmognathus quadramaculatus*; EGUT = *Eurycea guttata*; EWIL = *Eurycea wilderae*; PMET = *Plethodon metcalfi*; PTEY = *Plethodon teyahalee*; DWRI = *Desmognathus wrighti*; NOVI = *Notophthalmus viridescens*; GPOR = *Gyrinophilus porphyriticus*; DEAE = *Desmognathus aeneus*; PSER = *Plethodon serratus*; PJOR = *Plethodon jordani*; PGLU = *Plethodon glutinosus*; DSAN = *Desmognathus santila*; DIMI = *Desmognathus imitator*; DCON = *Desmognathus conanti*; PRUB = *Pseudotriton ruber*

Table D.3. Comparison of the ten global circulation models used for the future projections.

<b>Model</b>	<b>Country Source</b>	<b>Rupp et al. 2013 Ranking (out of 30 models)</b>	<b>Resolution (degrees)</b>	<b>Type*</b>	<b>Vertical Layers of Atmosphere</b>
bcc-csm1-1-m	China	13	1.12 x 1.12		26
CanESM2	Canada	9	2.8 x 2.8	ESM	35
CNRM-CM5	France	5	1.4 x 1.4	ESM	31
CSIRO-Mk3-6-0	Australia	15	1.8 x 1.8		18
GFDL-ESM2M	USA	16	2.5 x 2	ESM	48
HadGEM2-ES365	UK	10	1.88 x 1.25	ESM	38
HadGEM2-CC365	UK	11	1.88 x 1.25	Carbon Cycle	60
IPSL-CM5A-MR	France		2.5 x 1.25		39
MIROC5	Japan	8	1.4 x 1.4		40
MIROC-ESM-CHEM	Japan	30	2.8 x 2.8	ESM/CHEM	80

\*ESM is Earth System  
Model

Table D.4. Parameter estimates for the global hierarchical model. Only global covariates are shown.

<b>Model</b>	<b>Parameter</b>	<b>Beta</b>	<b>Lower CI</b>	<b>Upper CI</b>	<b>f*</b>
Detection	Intercept	-2.357	-3.316	-1.441	0.999
	1-day Precipitation	0.410	-0.937	2.023	0.731
	Dry Days	-0.558	-1.467	0.410	0.909
	3-day Mean				
	Temperature	1.282	0.398	2.220	0.994
	Water Vapor				
	Pressure	0.718	-0.299	1.761	0.933
	3-day Precipitation	-0.575	-1.599	0.615	0.875
	1-day Mean				
	Temperature	-1.277	-2.452	-0.042	0.978
Abundance	Intercept	-0.857	-2.102	0.498	0.922
	Elevation	0.776	-0.031	1.580	0.972
	Stream Distance	-0.183	-0.615	0.244	0.843
	Elevation * Stream				
	Distance	0.238	-0.240	0.714	0.877
	Elevation <sup>2</sup>	-0.629	-1.311	0.066	0.967

\* f indicates the proportion of the posterior with the same sign as the mean estimate

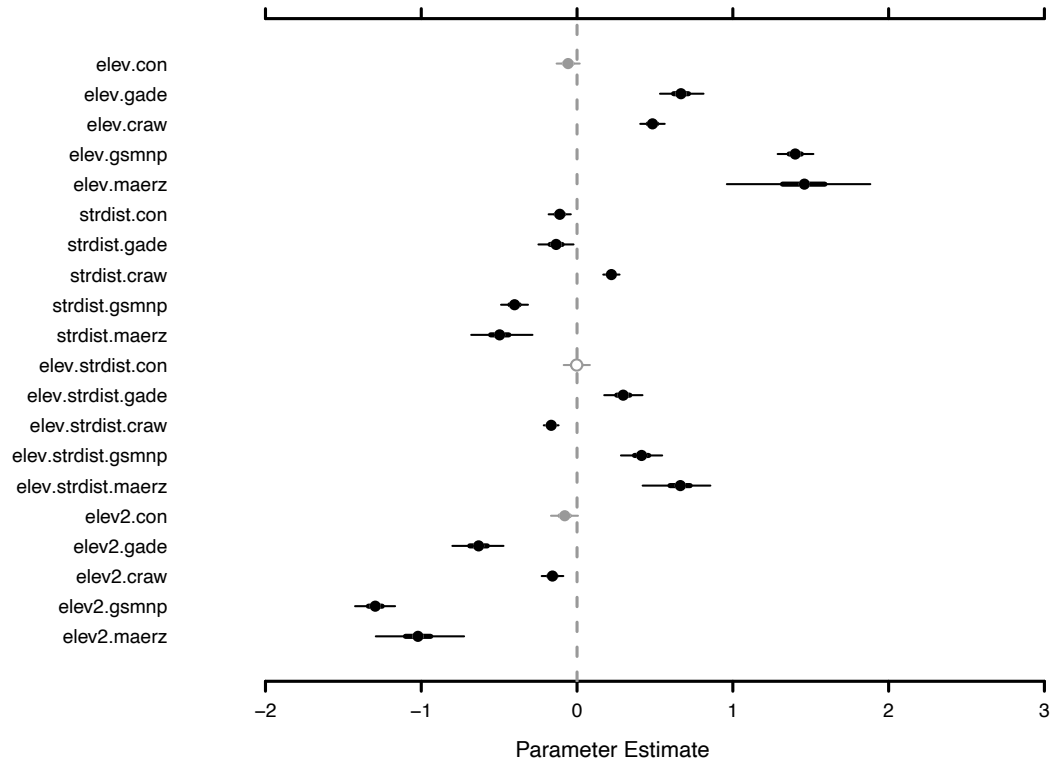


Figure D.1. Data-set specific parameter estimates for the abundance covariates in the global detection model. Covariates are arranged such that each parameter is listed for each dataset in the following order: con=Connette (B), gade=Gade (C), craw=Crawford (A), gsmnp=GSMNP (D), maerz=Maerz (E). Open circles indicate parameter where 50% credible intervals overlap zero, Parameters where 50% credible intervals do not overlap 0 AND 95 percent credible intervals do overlap 0 are indicated by 'closed' grey circles. Parameters where 95 percent credible intervals do not overlap 0 are indicated by 'closed' black circles (Youngflesh 2018).

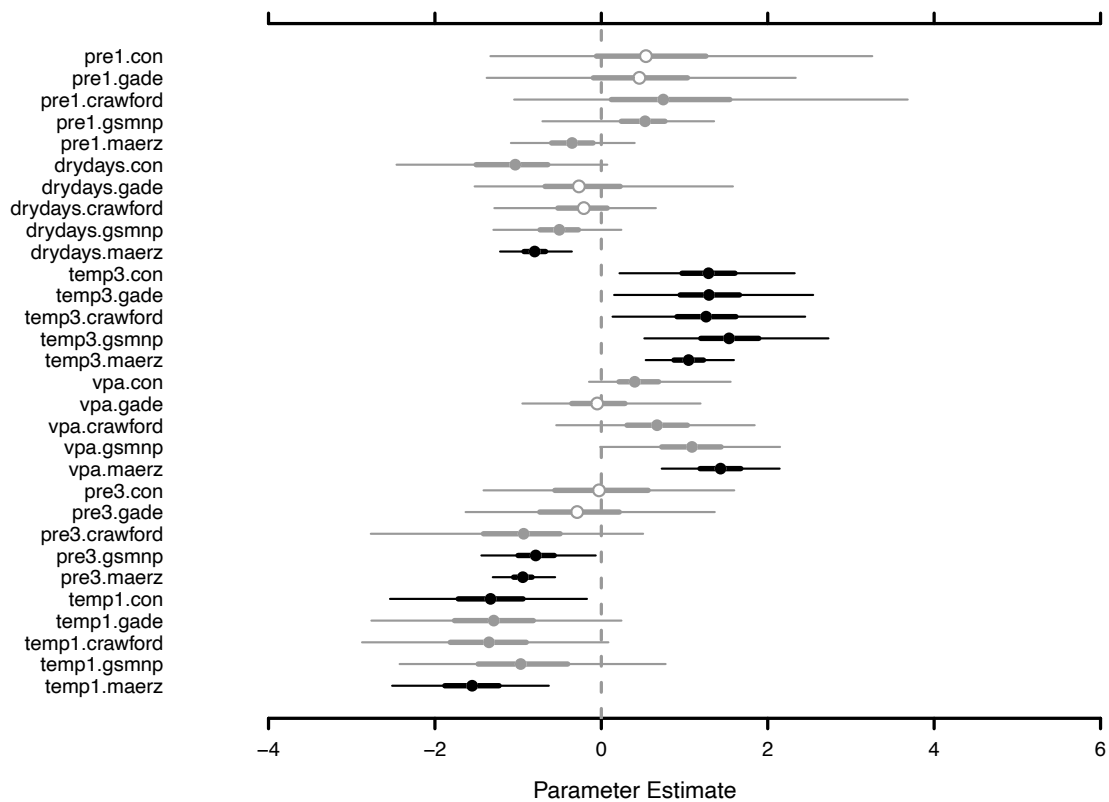


Figure D.2. Data-set specific parameter estimates for the detection covariates in the global detection model. Covariates are arranged such that each parameter is listed for each dataset in the following order: con=Connette (B), gade=Gade (C), craw=Crawford (A), gsmnp=GSMNP (D), maerz=Maerz (E). Open circles indicate parameter where 50% credible intervals overlap zero, Parameters where 50% credible intervals do not overlap 0 AND 95 percent credible intervals do overlap 0 are indicated by 'closed' grey circles. Parameters where 95 percent credible intervals do not overlap 0 are indicated by 'closed' black circles (Youngflesh 2018).

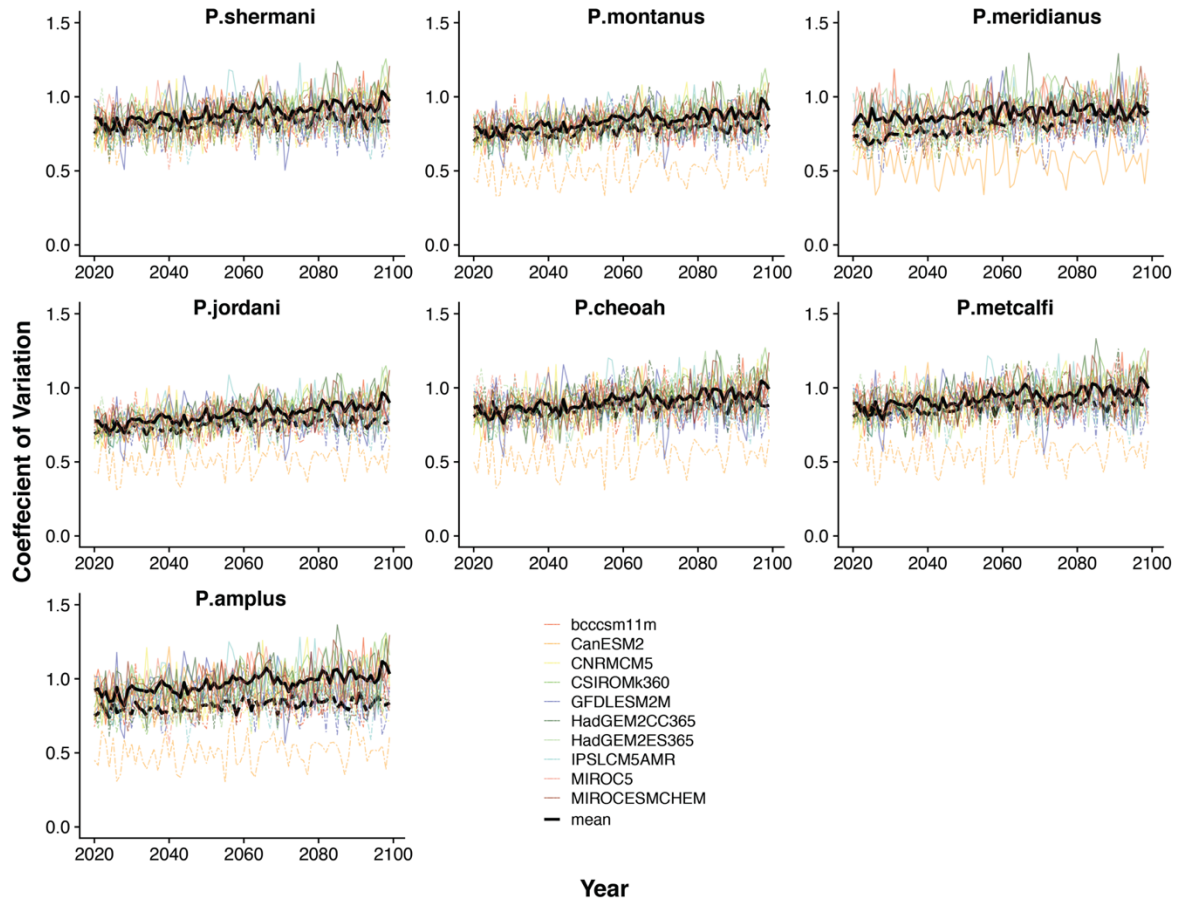


Figure D.3. Coefficient of variation for the seven species in the *Plethodon jordani* complex for the years 2020 – 2100. Colored lines represent the GCM, with the black line indicating the mean of the 10 GCMs. Dotted lines show RCP 4.5 and solid lines show RCP 8.5.

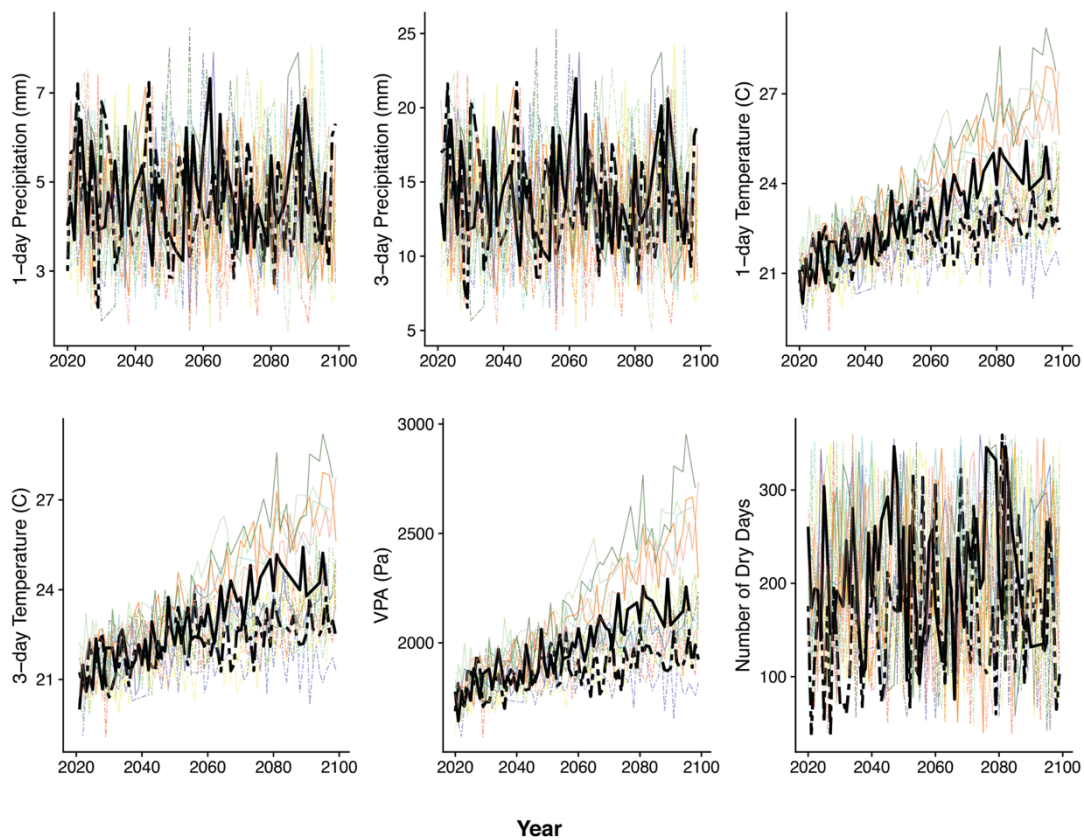


Figure D.4. Downscaled climate variables from the MACA dataset used in the detection analysis for year 2020 – 2100. Colored lines represent the GCM, with the black line indicating the mean of the 10 GCMs. Dotted lines show RCP 4.5 and solid lines showing RCP 8.5.