

FACTORS AFFECTING ABUNDANCE, PHYSIOLOGY, AND
FINE-SCALE GENETIC DIFFERENTIATION OF THE
WESTERN SLIMY SALAMANDER (*PLETHODON ALBAGULA*)

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OF THE WESTERN SLIMY SALAMANDER (*PLETHODON ALBAGULA*)

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ABSTRACT

Inferring process from pattern can be a challenging undertaking when dealing with ecological complexity. The distribution and abundance of organisms on the landscape is often interpreted through the lens of competition, movement, or physiology, as well as interactions with the abiotic environment. Further, movement, distribution, and abundance often coincide with favorable abiotic environments such as temperature, moisture, or nutrients. At its core, landscape genetics seeks to identify the spatial processes shaping the observed patterns of genetic diversity across the landscape, but most landscape genetic studies are predominantly exploratory and lack well-established hypotheses. To increase understanding of process-driven patterns in landscape genetics, I studied the western slimy salamander (*Plethodon albagula*) in east-central Missouri with three specific questions: (1) Where are salamanders on the landscape, and what environmental factors influence local abundance? (2) Is there a physiological constraint underlying the observed patterns of distribution and abundance? (3) How is spatial genetic structure shaped by abundance and physiology across the landscape? I utilized a combination of abundance modeling, spatial quantification of water loss using plaster of Paris models, and landscape genetics analyses to assess the factors contributing to genetic differentiation across a 1300 ha landscape.

Plethodontid salamanders are highly sensitive to water loss, in part due to their lack of lungs and cutaneous respiration. I found that abundance of salamanders was best predicted by canopy cover, topographic position (ridge, slope, ravine), and the interaction between wetness and solar exposure. The spatial relationships of these factors are such that abundance is predicted to be highest in forested ravines with lower solar exposure. Plaster models deployed across the landscape served as surrogates for live salamanders to quantify rates of water loss. I found that rates of water loss across the landscape were inversely related to predicted abundance, suggesting that water loss is likely a physiologically-limiting process underlying the distribution of salamanders. Finally, I determined that genetic distances were significantly correlated with ecological surfaces, and that the independent landscape features underlying these processes were poor predictors of genetic differentiation. My results highlight the importance of understanding basic ecological and physiological factors as mechanisms for interpreting spatial genetic patterns.

CHAPTER 1

Introduction

Ecology can be defined as ‘the scientific study of the interactions that determine the distribution and abundance of organisms’ (Krebs 1972). An understanding of distribution and abundance provides a necessary foundation for further scientific inquiry, and the ‘interactions’ affecting many organisms are with their local environment. Environmental gradients are instrumental in shaping the distribution and local abundance of species because at the most fundamental level, an organism’s performance is constrained by the environment it inhabits. In topographically complex landscapes, environmental gradients can occur over small spatial scales (Oliver *et al.* 2010), creating microclimates of temperature and moisture (Chen *et al.* 1999; Suggitt *et al.* 2011). The spatial distributions of both plants and animals are often closely tied to microclimatic conditions (Chen *et al.* 1999), emphasizing the importance of understanding the environment as a proximate cause of patterns in species’ distribution and abundance. Further, local abundance and population dynamics can often be related to an organism’s physiology, which can affect foraging, movement, and reproduction (Huey 1991).

Salamanders of the genus *Plethodon* have specialized physiological requirements, are highly philopatric, and have limited dispersal ability. The physical and behavioral constraints of such dispersal-limited taxa make them much more reliant on their immediate surroundings and limit active selection of favorable microclimates. Despite a long history of study, there are still significant gaps in our understanding of the basic ecology of plethodontid salamanders. Where are they on the landscape? What environmental and landscape features affect their distribution? What constrains distribution and abundance? How do salamanders move across the landscape? These are a few of the many basic questions that are largely unknown for terrestrial salamanders. With forested landscape continually being altered, degraded, or lost, and with the realities of the effects of climate change becoming more apparent, my dissertation attempts to answer some of these fundamental questions. In doing so, I hope to provide the necessary ecological information, as well as the field and analytical tools to make informed and effective management decisions for salamanders.

The objectives of my dissertation research are threefold: (1) to describe the fine-scale patterns of abundance in relation to environmental gradients (chapter 2); (2) to identify and describe a physiological mechanism constraining the distribution of abundance (chapter 3); (3) to determine the effects of the environment, abundance, and physiology on fine-scale genetic differentiation (chapter 4). I address each these objectives through studies of the western slimy salamander (*Plethodon albagula*) in Missouri. In chapter 2, I combine fine-scale spatial data (3-m resolution) with intensive field surveys to identify the environmental gradients affecting salamander abundance. Further, I identify differences in population dynamics that vary spatially with abiotic environmental gradients. In chapter 3, I explore water loss as a physiological limiting factor underlying the observed patterns of abundance. Using plaster models of salamanders, I measured rates of water loss across the landscape and determined the environmental and climatic factors affecting water loss. Finally, in chapter 4, I conducted a fine-scale landscape genetics study to determine how environmental and ecological factors affect gene flow.

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CHAPTER 2

Fine-scale habitat associations of a terrestrial salamander: the role of environmental gradients and implications for population dynamics

Abstract

Environmental gradients are instrumental in shaping the distribution and local abundance of species because at the most fundamental level, an organism's performance is constrained by the environment it inhabits. In topographically complex landscapes, slope, aspect, and vegetative cover interact to affect solar exposure, creating temperature-moisture gradients and unique microclimates. The significance of the interaction of abiotic gradients and biotic factors such as competition, movement, or physiology has long been recognized, but the scale at which these factors vary on the landscape has generally precluded their inclusion in spatial abundance models. We used fine-scale spatial data relating to surface-soil moisture, temperature, and canopy cover to describe the spatial distribution of abundance of a terrestrial salamander, *Plethodon albagula*, across the landscape. Abundance was greatest in dense-canopy ravine habitats with high moisture and low solar exposure, resulting in a patchy distribution of abundance. We hypothesize that these patterns reflect the physiological constraints of Plethodontid salamanders. Furthermore, demographic cohorts were not uniformly distributed among occupied plots on the landscape. The probability of gravid female occurrence was nearly uniform among occupied plots, but juveniles were much more likely to occur on plots with lower surface temperatures. The disconnect between reproductive effort and recruitment suggests that survival differs across the landscape and that local population dynamics vary spatially. Our study demonstrates a connection between abundance, fine-scale environmental gradients, and population dynamics, providing a foundation for future research concerning movement, population connectivity, and physiology.

Introduction

Gradients of biotic and abiotic factors are inherent to heterogeneous landscapes (Oliver *et al.* 2010).

Abiotic factors such as temperature, water, sunlight, pH, and nutrient concentrations, and biotic factors such as competition, prey availability, and predators, interact to determine species distributions and local population abundance (Brown 1984). Temperature is a critical abiotic factor related to species distribution at a regional scale, but temperature also can vary across small spatial scales. Climate and elevation generally dictate the temperature of a region (Barry 1992; Fridley 2009), but topographic and vegetative characteristics of the landscape affect temperature locally (Scherrer & Körner 2011; Suggitt *et al.* 2011). Topography and slope influence the amount of solar exposure and hence surface temperatures (Fridley 2009; Lookingbill & Urban 2003), which in turn can have significant effects on soil moisture (Lookingbill & Urban 2004). The land cover overlaying the physical landscape (e.g. forest, grassland) further influences the amount of solar exposure to create microclimates with unique temperature-moisture characteristics (Chen *et al.* 1999; Suggitt *et al.* 2011).

The spatial distributions of both plants and animals are often closely tied to microclimatic conditions (Chen *et al.* 1999), emphasizing the importance of understanding the environment as a proximate cause of patterns in species distribution and abundance. Gradients created by slope and aspect play a significant role in structuring plant communities (Bennie *et al.* 2008; Hutchinson *et al.* 1999; Whittaker 1956) and in determining the distribution and abundance of animals ranging from carabid beetles (Antvogel & Bonn 2001) and butterflies (Weiss *et al.* 1988), to amphibians (Heatwole 1962) and birds (Lloyd & Palmer 1998).

Acquisition and assimilation of energy from the environment is critical for survival and reproduction, and the distribution of temperature, water, and food resources can shape the life history, abundance, and distribution of species (Andrewartha & Birch 1954). In choosing optimal thermal environments, an organism seeks to maximize its energy consumption while minimizing metabolic expenditures (Brown *et al.* 2004), and in some species, rates of evaporative water loss are closely tied to temperature (Tracy 1976). Different taxa perceive and relate to their environment at different scales, with many terrestrial animals operating on a scale of meters or less. Vagile animals such as insects, birds, and reptiles often exhibit differential success in relation to microclimate, and actively select favorable microclimatic conditions (Adolph 1990; Wachob 1996; Weiss *et al.* 1988). Although plants cannot actively select their local

environment, seedling recruitment can nonetheless be affected by environmental gradients (Graae *et al.* 2011), thus shaping spatial patterns of distribution and abundance (Albrecht & McCarthy 2009). Many animals are much more plant-like in their habitat associations. While extremely dependent upon suitable temperature and moisture microclimates, terrestrial gastropods have limited dispersal abilities (Baur & Baur 1995; Kappes 2005). Similarly, terrestrial salamanders of the family Plethodontidae are generally highly philopatric (Kleeberger & Werner 1982), exhibiting minimal dispersal (Liebgold *et al.* 2011). The physical and behavioral constraints of dispersal-limited taxa make such species much more reliant on their immediate surroundings, limiting active selection of a favorable microclimate.

Plethodontid salamanders are unique among terrestrial vertebrates in that they are lungless; respiration occurs predominantly across the skin surface. Water balance is critical for survival in plethodontid salamanders, and has been shown to be an important determinant of surface activity (Heatwole 1962; Jørgensen 1997). Temperature and moisture conditions must be suitable for an adequate duration of time to allow salamanders to successfully forage and meet their energy requirements (Fraser 1976). From this energy budget perspective (Hall *et al.* 1992), a species' distribution will be limited to areas with a positive energy budget, and local abundance may positively correlate with energy budget surplus (Gifford & Kozak 2012).

One of the primary goals of ecology is to understand how biotic and abiotic factors influence species' distribution and abundance (Andrewartha & Birch 1954). These foundational relationships between an organism and its environment are often the basis of subsequent ecological inquiry into local population dynamics (Pattison & Mack 2009), dispersal (Harrison 1994), and evolutionary potential (Pabijan *et al.* 2012). More than ever, research concerning species habitat relationships is concerned with potential responses to, or outcomes of anthropogenic land use and climate change (Elith & Leathwick 2009). One of the major limitations to accurately describing distribution or abundance is that neither can be observed perfectly (MacKenzie 2005; Pellet & Schmidt 2005; Royle *et al.* 2007). Failure to account for observation error will result in an underrepresentation of true distribution or abundance (Royle & Dorazio 2008) and bias estimates of covariate relationships (Tyre *et al.* 2003). Observation error can be accounted for using models that allow for the simultaneous estimation of species abundance/occupancy and detection probability (MacKenzie *et al.* 2002; Royle 2004b; Tyre *et al.* 2003).

We used binomial mixture models and a metapopulation sampling design (Royle 2004a; Royle 2004b) to account for variable detection and obtain unbiased abundance estimates of a terrestrial plethodontid salamander, *Plethodon albagula*. Our objectives were to (1) determine the fine-scale environmental gradients that correlate with abundance, (2) describe the distribution of abundance across the landscape, and (3) determine the effects that environmental gradients have on population dynamics. We hypothesized that abundance in *P. albagula* would be positively associated with metrics relating to high moisture and cool temperatures and, correspondingly, would not be uniformly distributed across the landscape. Instead, we predicted that abundance would be topography-dependent with the greatest abundance in ravine habitats and lowest abundance in ridge habitats, which correspond to cool-moist and hot-dry microclimates, respectively (Bennie *et al.* 2008). We also hypothesized that reproductive success would be greatest in areas of high abundance, indicating favorable microclimates for survival, growth, and reproduction.

Materials and Methods

Ethics statement

This research was done in compliance with all laws and regulations of the state of Missouri and the USA, and was conducted under Missouri Wildlife Collector's Permit #15203 animal care protocol #7403 approved by the University of Missouri Animal Care and Use Committee.

Study site

Our study took place in east-central Missouri within the River Hills Ecoregion (Chapman *et al.* 2002). This physiographic region borders the Missouri River, and is characterized by forested ridges and valleys with slopes that are frequently covered by exposed rock or rock outcrops. Seasonal temperatures range from -6.8–31.2°C, and average annual rainfall is 94.2 cm. Our field site was located at Daniel Boone Conservation Area (DBCA; 38.78° N, 91.39° W; 157–280 m a.s.l.), which encompasses 1424.5 ha of mature (80–100 yrs old) second-growth forest consisting of oak (*Quercus* spp.) and hickory (*Carya* spp.) dominated overstory with varying amounts of sugar maple (*Acer saccharum*) and red cedar (*Juniperus virginiana*) in the understory (Semlitsch *et al.* 2008).

Study species

Plethodon albagula (western slimy salamander) is a large plethodontid salamander that lives in forested habitats throughout the Ozark and Ouchitae mountains of Missouri, Arkansas, eastern Oklahoma, and northeastern Texas, USA. Within these forested habitats, salamanders are most frequently associated with moist, sheltered ravine and valley habitats. Like other plethodontid salamanders, *P. albagula* spend much of the year in subterranean refugia, but are surface active in the spring and autumn months when temperature and moisture conditions are favorable (Petranka 1998). Foraging, dispersal, and courtship activities are generally nocturnal, and individuals retreat underground or seek refuge under rocks and logs during the day. Females lay 10–20 eggs under rotting logs, rocks, or in subterranean refugia (Trauth *et al.* 2004). Age at sexual maturity is unknown for *P. albagula*, but ranges from 3–5 yrs in other large plethodontid salamanders (Kéry & Royle 2009; Semlitsch 1980). Dispersal and home ranges are also largely unknown. Plethodontid salamanders are generally philopatric (Highton 1989; Trauth *et al.* 2004), and limited data on closely related species in the *P. glutinosus* complex suggests that home ranges of adult and juvenile salamanders are small ($< 4.0 \text{ m}^2$) (Cushman *et al.* 2013), and do not differ significantly between sexes or age classes. Although sympatric with other terrestrial salamanders in parts of its range, *P. albagula* is the only plethodontid species present in our study region (Daniel & Edmond 2012).

Field surveys

We surveyed for *P. albagula* using area-constrained daytime observations of 135 survey plots. Plots were 3 m x 3 m, and were a minimum of 75 m apart. Due to logistical constraints, plots were not randomly selected across the landscape, but were instead arranged in an offset grid or in linear transects (Fig. 1). The location of each plot was marked in the field using a hand held GPS (Garmin 62sc) with multiple locations being taken until the estimated precision was ≤ 3 m. Each plot was surveyed seven times from 8 April to 28 May 2011 from 0600–1700 CST, with at least six days between surveys. During each survey, all moveable cover objects, including rocks, logs, and bark, were carefully lifted and all salamanders were captured by hand. We measured snout-vent length (SVL) and total length of each captured salamander and determined sex based on SVL and presence of a mental gland (males) (Trauth *et al.* 2004). If salamanders were of adult size and not visibly male (≥ 55 mm SVL, no mental gland), we candled the salamander to determine if eggs were present (Gillette & Peterson 2001). Mass was recorded to 0.01 g using a portable digital balance (Durascale, My Weigh). Cover objects were returned to their original position and salamanders were

released following data collection. Leaf litter was not surveyed for salamanders because preliminary surveys deemed this largely ineffectual and too destructive of the plot habitat.

Several plot-level covariates were measured in the field. Surface soil temperature under each searched cover object was measured at a distance of 1 m during each survey using an infrared thermometer (Raytek MT4), and all measures from a plot were averaged for each survey. We also quantified the amount of available searchable cover as the surface area of logs, rocks, bark, as well as the total surface area of all these cover objects. Lastly, we measured leaf litter depth as the average of five measurements within each plot.

Spatial covariates

Spatial covariates were calculated in ArcGIS 9.3 (ESRI, Redlands, CA, USA). Using 1/9 arc second Nation Elevation Dataset (3 m resolution; <http://seamless.usgs.gov/products/9arc.php>), we derived the following spatial layers: northness (cosine of aspect; value range from 1 = north to -1 = south), eastness (sin of aspect; value range from 1 = east, -1 = west (Deng *et al.* 2007), slope, topographic position index (TPI), topographic wetness index (TWI), potential relative radiation (PRR), surface curvature, distance to stream, and maximum surface temperature. TPI was calculated as the slope position relative to the surrounding 90 m, with negative values indicating areas that are lower than the surrounding landscape (ravines) and positive values indicating areas that are higher than the surrounding landscape (ridges) (Jenness 2006). TWI was calculated accounting for solar insolation (azimuth = 178.3, altitude = 65.3) (Theobald 2007). PRR was calculated following the approach of Pierce *et al.* (2005) wherein we estimated the relative amount of solar exposure on the landscape for every hour of the first and 15th day of the months of April–October. The estimated solar exposure values for all hours and days were then summed together. Maximum surface temperature was calculated from a network of 61 ThermoChron iButton data loggers (Maxim) using a hierarchical mixed-effects model as described by Fridley (2009). For each of these derived layers, we then calculated a local average of each pixel by averaging the surrounding 9 m x 9 m area. In doing so, we smoothed each landscape surface as well as generalized the local landscape to help account for potential spatial error in plot location that can result from GPS imprecision. We also estimated canopy cover at our site using the normalized difference vegetation index (NDVI), which was calculated from Landsat 7 satellite imagery (Tucker 1979). We obtained cloud free images of our study area for 15 June, 20 July, 9

August 2011 (<http://glovis.usgs.gov/>); a mean NDVI was calculated by averaging these together. Because the resolution of the NDVI layer was 30 m, we resampled it to 3 m to match the resolution of our other spatial layers.

Temporal covariates

Because salamander surface activity can be highly sensitive to climatological variation, we collected data on millimeters of precipitation in the 24 hrs and 5 days preceding each survey, average temperature 24 hrs preceding each survey, the number of days since a soaking rainfall event of ≥ 5 mm, and the number of days since any rainfall. These data were collected and averaged from three weather stations ≤ 20 km from our study site (<http://www.wunderground.com>). The Julian date of each survey was also included as a temporal covariate.

Statistical analyses

We analyzed the repeated observations of *P. albagula* counts at plots using binomial mixture models (i.e. N-mixture model) (Royle 2004b). One of the major assumptions of binomial mixture models is that populations are closed to immigration, emigration, births, and deaths during the period of sampling. Plethodontid salamanders do exhibit vertical migration from the surface to underground refugia (Petranka 1998), but as long as this temporary emigration is random, then closed-population models such as the binomial mixture model should yield unbiased abundance estimates (Bailey *et al.* 2004a). By confining our sampling to the relatively short spring active season, we feel that the closure assumptions are largely met in our system and that vertical migration (i.e. temporary emigration), which will affect detection, is dependent upon temporal climate variation. We have attempted to account for this temporal variation in detection by fitting climate covariates to our detection model. Binomial mixture models are superior to logistic or Poisson regression when modeling distribution or abundance because of their hierarchical nature. Such models allow for the estimation of species abundance as a function of site-level covariates, while accounting for imperfect species detection (Royle *et al.* 2007). By correcting for variable detection of salamanders among sites and surveys, we reduce the bias in our abundance estimate to more accurately describe abundance across the landscape as it relates to environmental gradients (Kéry & Schmidt 2008; Royle 2004b). Binomial mixture models are a form of a hierarchical generalized linear mixed model (Kéry

2010), which can be solved through maximum likelihood estimation or Bayesian methods (Royle & Dorazio 2008).

We analyzed our models in a Bayesian hierarchical framework using the WinBUGS software package (v. 1.4.3) (Spiegelhalter *et al.* 2003), executed through R (v. 2.15) (Ihaka & Gentleman 1996) using the R library R2WinBUGS (Sturtz *et al.* 2005). Prior to modeling, all covariates were standardized by subtracting the arithmetic mean and dividing the standard deviation. We used uninformative normally distributed priors with a mean of zero and a variance of 10^6 for all model parameters. Posterior summaries were based on 500,000 Markov chain Monte Carlo iterations thinned at a rate of 50 following a burn-in of 250,000 iterations. From each model we calculated the mean and 95% credible interval (CRI) for all model parameters, as well as the latent abundance parameter at each site. Model convergence was assessed using the Gelman-Rubin statistic (Rhat) (Gelman *et al.* 2004). Posterior predictive assessment of model fit was done using Bayesian p-value as well as a Chi-square discrepancy measure (Gelman *et al.* 1996; Kéry 2010).

We constructed our models in a five step process. (1) We fit a full model with all potential explanatory covariates in both the abundance and detection hierarchies (see Electronic Table S1), and then assessed model fit. Poisson, zero-inflated Poisson, and random effects models were all fit to our data (Kéry & Schaub 2012). For the random effects parameterization, a normally distributed random effects term was included in our detection model to account for unexplained variation in our plot-survey detection probability (Kéry *et al.* 2009; Kéry & Royle 2010). (2) Using the best-fit model from step 1, we fit all covariates that could potentially affect detection of salamanders while holding abundance among sites constant. Parameter estimates for each covariate were assessed, and those that did not include zero in their 95% credible interval were retained. (3) Step 2 was then repeated with the retained covariates to confirm that the magnitude and sign of the parameter estimates did not change. (4) Using the detection model from step 3, we fit all potentially meaningful covariates to the abundance model. Parameter estimates for each covariate were assessed, and those that did not include zero in their 95% credible interval were retained. (5) Finally, the full model with all retained covariates was run and the sign and magnitude of the parameter estimates were assessed. To evaluate the predictive power of our model we conducted a leave-one-out cross-validation test. We iteratively omitted the observation data for a single site, and then re-ran the model

to obtain posterior predictions for abundance at that site. These predictions were then compared to the predictions made with the full model.

Our primary model described abundance of all *P. albagula* across the landscape in relation to environmental gradients, but given this distribution, we also wanted to know if the probabilities of gravid female and juvenile salamander occurrence were the same at occupied sites. We chose these demographic groups because they represent reproductive effort (gravid females) and successful recruitment (juveniles). To address this question, we constructed multistate models using a conditional binomial parameterization in program PRESENCE v3.1 (MacKenzie *et al.* 2009; Pellet & Schmidt 2005). Multistate models were fit separately for gravid females and for juvenile salamanders. Three states were present in each of these models: (1) no salamanders present; (2) salamanders present, but target salamander absent; (3) target salamander present, where target salamander is either gravid female or juvenile salamander, for each respective model. To parameterize the multistate model, we fit the same covariates to the detection and occupancy parameters in the multistate model as were found to be significant covariates of detection and abundance in the binomial mixture model (Table 1). The conditional state probability parameter (R), was also fit with the five significant abundance covariates.

Results

Over 7 survey periods we observed 487 salamanders at 88 of 135 surveyed plots. We found that a binomial mixture model with a random-effects parameterization of the detection process fit our data best (Bayesian p-value = 0.508; Chi-square discrepancy = 0.999). The average detection rate of salamanders across sites and observations was 0.164 (0.120–0.215 CRI), and was affected by survey date, time since the last soaking rainfall event, plot temperature, and the area of bark available to search (Table 1). After correcting for imperfect detection, we found that salamander abundance was best predicted by indices related to cooler temperatures and higher moisture. Predicted abundance was positively associated with higher canopy cover, ravine habitats (negative TPI), and areas on the landscape with low solar exposure and high

topographic wetness (Table 1, Fig. 2A–C). The estimated abundance at each 9 m² survey plot ranged from 0.0178–7.869 (mean = 1.968 ± 0.687 SD).

Spatially, these relationships with environmental gradients resulted in *P. albagula* abundance being patchily distributed across the landscape (Fig. 3). Small areas of high abundance are seen in ravines, but these high abundance areas are frequently isolated from each other by hotter, drier ridges with very low estimated abundance. Our model had moderately precise predictive ability (Fig. 4). Specifically, the cross-validation estimates of plot abundance were on average 1.18 (± 0.09 SD) different than the abundance estimates from the model run with the full observation data. Only five estimates of abundance exceeded the 95% credible intervals of the cross-validation test (error rate = 3.73%; Fig. 4). At sites with low predicted abundance, cross-validation over-predicted, and sites with high predicted abundance, cross-validation under-predicted. These results, in part, likely stem from the low detection rate and the model's reliance on repeated observations to estimate the latent abundance parameter.

We constructed separate multistate models for gravid females and juveniles to estimate the probability that they occur at a plot, conditional on the plot being occupied by salamanders. We found that gravid females and juveniles did not differ substantially in their respective detection probabilities (0.460 ± 0.05 SE; 0.403 ± 0.53 SE). In contrast, the conditional occurrence probabilities differed between the two groups. On average, females had a higher and less variable probability of occurring at an occupied plot (0.747 ± 0.006 SE) compared to juveniles (0.645 ± 0.013). The probability of juvenile occurrence showed a significant and positive relationship with estimated abundance ($F_{1,134} = 480.50$, $R^2 = 0.783$, $P < 0.001$; Fig 5A), but the relationship between gravid female occurrence and estimated abundance was much weaker ($F_{1,134} = 13.58$, $R^2 = 0.086$, $P = 0.0003$; Fig 5A). Maximum surface temperature was not a significant predictor in the abundance model, but abundance estimates decreased significantly as surface temperature increased ($F_{1,134} = 83.58$, $R^2 = 0.381$, $P < 0.001$; Fig. 6). Because maximum temperature was not significant in the full abundance model, yet showed a strong relationship with predicted abundance, we used it as a univariate metric to assess how gravid female and juvenile occurrence probabilities differed across the landscape. The probability of juvenile salamander occurrence significantly declined as temperature increased ($F_{1,134} = 190.21$, $R^2 = 0.587$, $P < 0.001$; Fig 5B), but there was no relationship between gravid female occurrence and maximum temperature ($F_{1,134} = 1.46$, $R^2 = 0.003$, $P = 0.229$; Fig 5B). There were

also highly significant interactions between the juvenile and gravid female probabilities of occurrence in relation to both abundance and maximum temperature ($P < 0.001$; Fig. 5A–B), suggesting a disconnect between microclimate influence on reproductive effort and successful recruitment.

Discussion

The distribution and abundance of species along environmental gradients often results from the interplay of biotic interactions such as predation and competition with the abiotic environment (Menge & Sutherland 1987). The complexity of biotic interactions often makes it difficult to identify the optimal range of environmental conditions that allow a species to maximize its fitness, and studies of plethodontid salamanders have shown that species interactions are pivotal in shaping distributions along environmental gradients (Gifford & Kozak 2012; Hairston 1987). Our study of *P. albagula* living in allopatry circumvents issues of biotic interactions with other salamanders, allowing us to clearly and directly assess distribution and abundance relationships with environmental gradients, and to make inferences concerning the population dynamics underlying the distribution of abundance across the landscape.

As we hypothesized, *P. albagula* abundance was significantly associated with environmental gradients relating to moisture and temperature. All plethodontid salamanders in the eastern United States are closely associated with mature forests (Petranka 1998), and abundance of plethodontid salamanders in topographically complex habitats is often greatest in ravine or cove forests (Petranka *et al.* 1993). In this study we found that abundance was substantially greater in areas with dense canopy cover (Fig. 2B), but within this closed canopy habitat, abundance was predicted to be more than seven times greater in ravine habitats (negative TPI) than on ridges (Fig. 2A). Canopy closure and topographic position are both critical factors influencing surface soil moisture (Scherrer & Körner 2011; Suggitt *et al.* 2011). Additionally, there was a significant interactive effect of topographic wetness and solar exposure wherein areas with high moisture and low solar exposure were predicted to support the most salamanders (Fig. 2C). Moisture is well understood to be critical to surface activity and foraging (Grover 1998; Keen 1979; Keen 1984), but the distribution of moist microhabitats across a topographically complex landscape and the effect on the spatial distribution of abundance has not been previously assessed in plethodontid salamanders.

The combination of environmental covariates and their relationship with salamander abundance across the landscape provides strong evidence that physiology is the underlying mechanism constraining abundance. Nonetheless, we cannot discount the potential effects that varying temperature and humidity has on prey availability, which could act as an indirect mechanism affecting salamander abundance through resource limitation (Indermaur *et al.* 2009). Temperature and moisture both play critical roles in the physiology and ecology of terrestrial plethodontid salamanders (Feder 1983; Spotila 1972). To facilitate cutaneous respiration, the skin must remain moist and permeable (Feder & Burggren 1985), concurrently increasing the susceptibility to water loss (Spotila & Berman 1976). Further, the diffusion of gases across the skin surface, and assimilation efficiencies of nutrients, increases at cooler temperatures (Bobka *et al.* 1981; Feder 1983). Although the links among physiology, temperature-moisture gradients, and population growth are apparent (Feder 1983), their implications for the spatial distribution of abundance and population dynamics are largely unknown. Using a mechanistic niche model, (Gifford & Kozak 2012) projected energy budgets across a montane landscape, demonstrating that temperature and water loss were significant factors relating to surface activity and subsequent energy intake. Our results provide confirmation of this critical linkage between physiology and species abundance.

By projecting our abundance model across the study landscape (Fig. 3), we determined that the spatial pattern of abundance was not uniform. While a large proportion of the landscape is predicted to have one or more salamanders, areas of high abundance (> 4) are patchily distributed among sheltered ravines. Hot, dry ridges as well as valleys with flat, floodplain-like characteristics are predicted to be largely uninhabited. Despite the negative effects that temperature can have on water loss, foraging activity, and metabolic rates, our spatial model of maximum surface temperature was not a significant predictor of abundance. We speculate that this lack of predictability occurred because the temperature surface was built from a linear model incorporating many of the same spatial attributes used to model abundance (e.g., NDVI, TWI, TPI, PRR, and distance to stream) (Fridley 2009). As such, the maximum temperature surface did not account for substantial variation in abundance not already accounted for by the independently modeled parameters. Nonetheless, maximum temperature was strongly correlated with overall predicted abundance (Fig. 6).

Our final question in this study concerned the effects of environmental gradients on population dynamics. Salamander abundance is clearly not uniformly distributed across the landscape (Fig. 3), and we further demonstrated additional spatial variation in distribution of life stages through our multistate modeling. Specifically, the mean probability of a gravid female occupying a plot suitable for *P. albagula* was 0.747. There was a very weak relationship with predicted abundance (Fig. 5A) and no relationship with maximum temperature (Fig. 5B). In contrast, the probability of juvenile *P. albagula* occupancy significantly increased in relation to both predicted abundance and maximum temperature (Fig. 5A–B). These interacting relationships highlight a disconnect between reproductive effort (gravid females) and realized recruitment (juveniles) in relation to microclimate. Adult females appear to be uniformly distributed among occupied plots that range in maximum temperature from 23–33°C, but only in cooler plots (< 27°C) do juveniles have an equivalent or greater probability of occurring. Foraging time for salamanders is highly dependent on local temperature and moisture conditions, with water loss dictating foraging duration (Spotila 1972). Smaller, juvenile salamanders that have significantly greater surface area relative to their mass will experience greater rates of water loss, further curtailing their foraging time relative to adults (Feder 1983), and restricting them to cool, moist areas.

Critical to the accuracy of our abundance estimates was the correction for imperfect detection. Plethodontid salamanders exhibit highly variable surface activity in both space and time (Bailey *et al.* 2004b), and this was true in our study. The issue of detection has contributed to the difficulty in estimating local abundance of plethodontid salamanders as well as determining relationships of abundance with local or regional landscape features (Dodd & Dorazio 2004; Hyde & Simons 2001). In our study, we had a low average detection rate (0.164; 0.120–0.215 CRI) that depended upon rainfall and temperature. After accounting for unexplained observation error in our detection model, our abundance model produced biologically realistic estimates of abundance that varied meaningfully with environmental gradients. Large woodland salamanders such as *P. albagula* have been estimated to occur at densities ranging from 0.418–0.844 m⁻² (adults and juveniles combined) (Semlitsch 1980); the mean density in our study was 0.219 m⁻² with a maximum of 0.874 m⁻². The cross-validation test of our model highlights the affect that low detection can have on abundance estimation, and the importance of correcting for these biases through

repeated observations (Fig. 4). Nonetheless, the cross-validation demonstrates that our model captures the essence of the system.

Plethodon albagula, especially juveniles, are more frequently encountered and more abundant in cooler, moister microclimates on the landscape. These findings are corroborated with our predicted abundance model, the demographic differences observed in the multistate modeling, and with plethodontid physiology. Our study did not determine whether areas of low predicted abundance, which are more likely to be occupied by gravid females than juveniles (Fig. 5A), exist as viable populations, harbor animals dispersing between high abundance patches, or represent sink populations being supported by high-abundance ravine populations. Studies on plethodontid salamanders have identified significant genetic differentiation over small spatial scales similar to what we studied (Cabe *et al.* 2007; Marsh *et al.* 2008), thus suggesting that dispersal is generally very limited (Liebgold *et al.* 2011). No studies have yet incorporated natural habitat variation as a factor affecting salamander movement and spatial structuring of populations. A clearer understanding of *P. albagula* movement ecology is required to gain further insight into the mechanisms underlying patterns of salamander abundance across the landscape. Although we describe a general phenomenon concerning the abundance-habitat relationships of terrestrial salamanders, few studies have sought to rigorously describe the abundance-habitat relationships of terrestrial salamanders. Despite this trend, we feel that abundance-habitat relationships provide the necessary lens for understanding other critical processes such as population dynamics, local adaptation, movement and dispersal, ecophysiology, climate change, and conservation biology. Our study elucidates these foundational relationships and demonstrates a strong connection between fine-scale environmental gradients, species abundance, and population dynamics.

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Table 1. Parameter estimates for detection and abundance models in hierarchical binomial mixture model. See methods for description of parameters. Detection model parameter estimates are on the logit scale and Abundance model parameter estimates are on the log scale.

Model	Parameter	Beta	95% Credible Interval	
			Lower	Upper
Detection				
	Intercept	-1.64	-1.994	-1.293
	Date	0.904	0.694	1.139
	Bark	0.263	0.095	0.431
	Soak.Rain	-0.491	-0.724	-0.276
	Soak.Rain ²	0.463	0.265	0.675
	Temp	0.144	-0.031	0.323
	Temp ²	-0.220	-0.379	-0.070
Abundance				
	Intercept	0.445	0.227	0.671
	NDVI	0.422	0.225	0.623
	TPI	-0.371	-0.551	-0.181
	TWI	-0.326	-0.532	-0.129
	PRR	-0.241	-0.448	-0.034
	TWI*PRR	-0.283	-0.460	-0.106

Figure 1. Hillshade relief map of Daniel Boone Conservation Area, MO, U.S.A. Points on the map indicate the 135 sample plot locations that are separated by at least 75 m. The counts represent the sum of all salamanders observed across the seven survey periods with no correction for imperfect detection or accounting for duplicate observations.

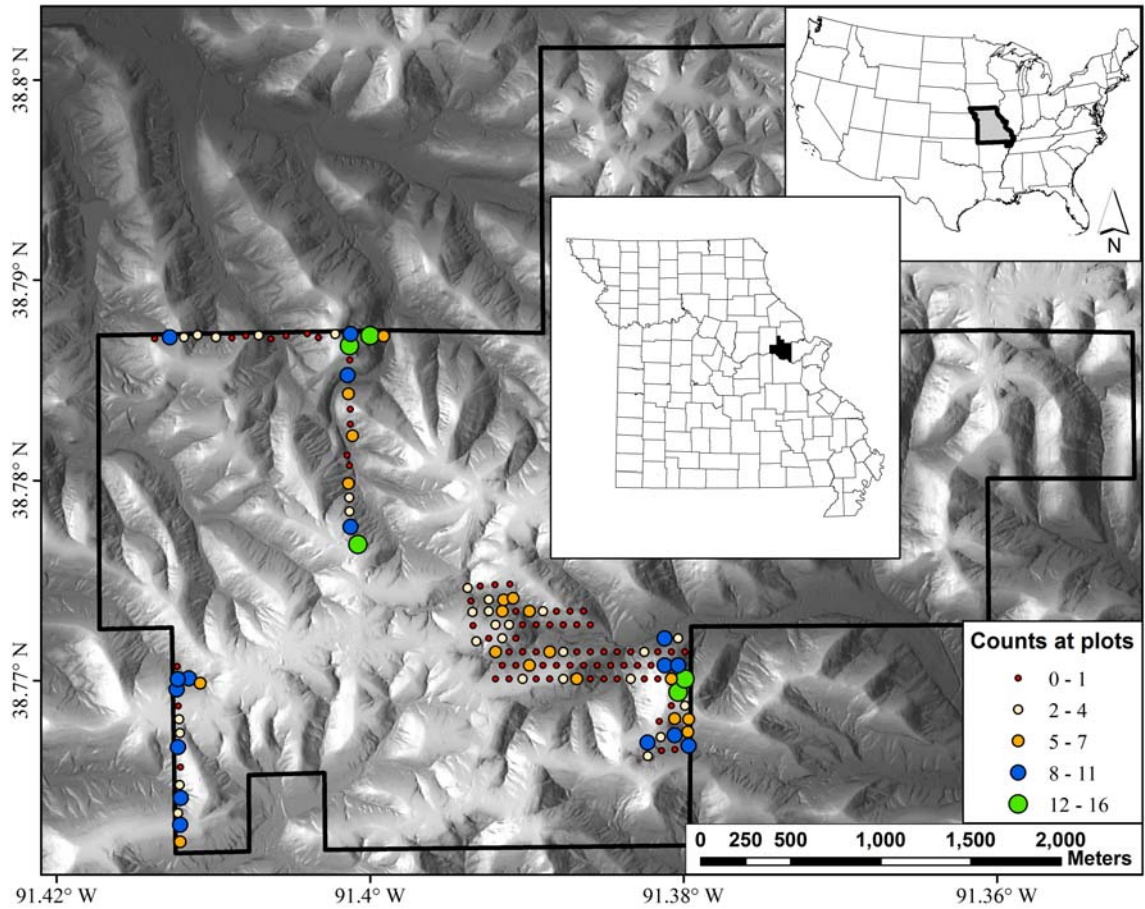


Figure 2. Estimated abundance relationships with (A) topographic position index; (B) canopy cover; and (C) interaction between topographic wetness and potential relative radiation. All covariates are scaled to a mean of zero. Low values of TPI indicate more ravine-like habitat and high values ridge-like habitat. Lower values of TWI, PRR, and NDVI indicate lower wetness, relative radiation, and canopy cover, respectively. Dashed lines around mean estimates of (A) and (B) represent 95% prediction intervals.

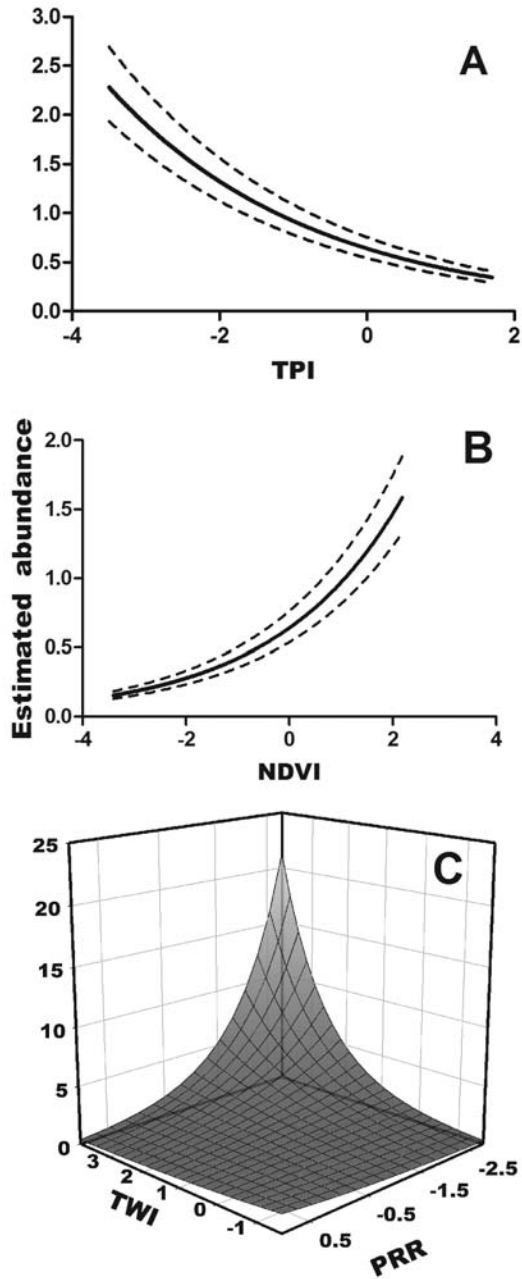


Figure 3. Spatial estimation of abundance across the landscape at Daniel Boone Conservation Area. Each pixel represents 9 m².

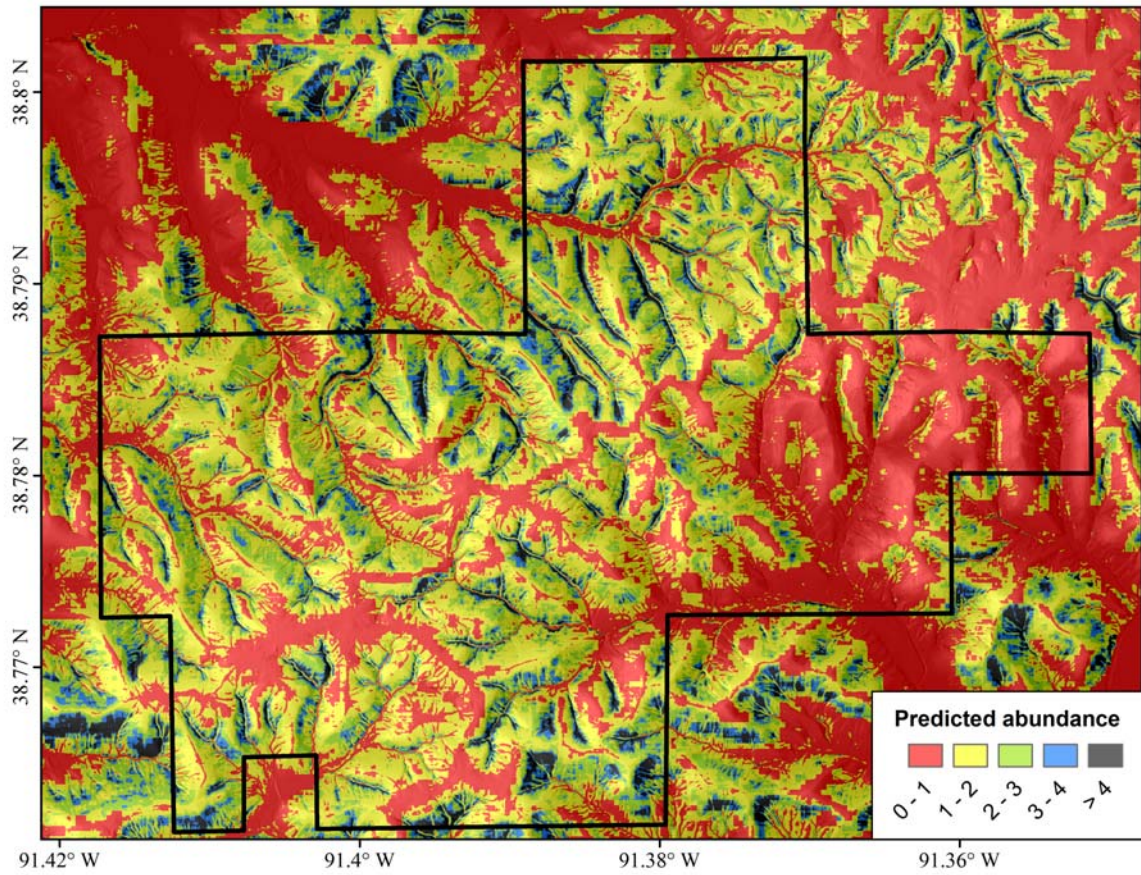


Figure 4. Results of the leave-one-out cross-validation of binomial-mixture model. The solid line indicates the abundance estimate for each plot from the full model, while the gray dashed line indicates the abundance estimate for each plot when observation data were omitted for that site. The gray shading indicates the 95% Bayesian credible interval around the leave-one-out plot estimate. Surveyed plots are arranged from lowest to highest predicted abundance along the x-axis.

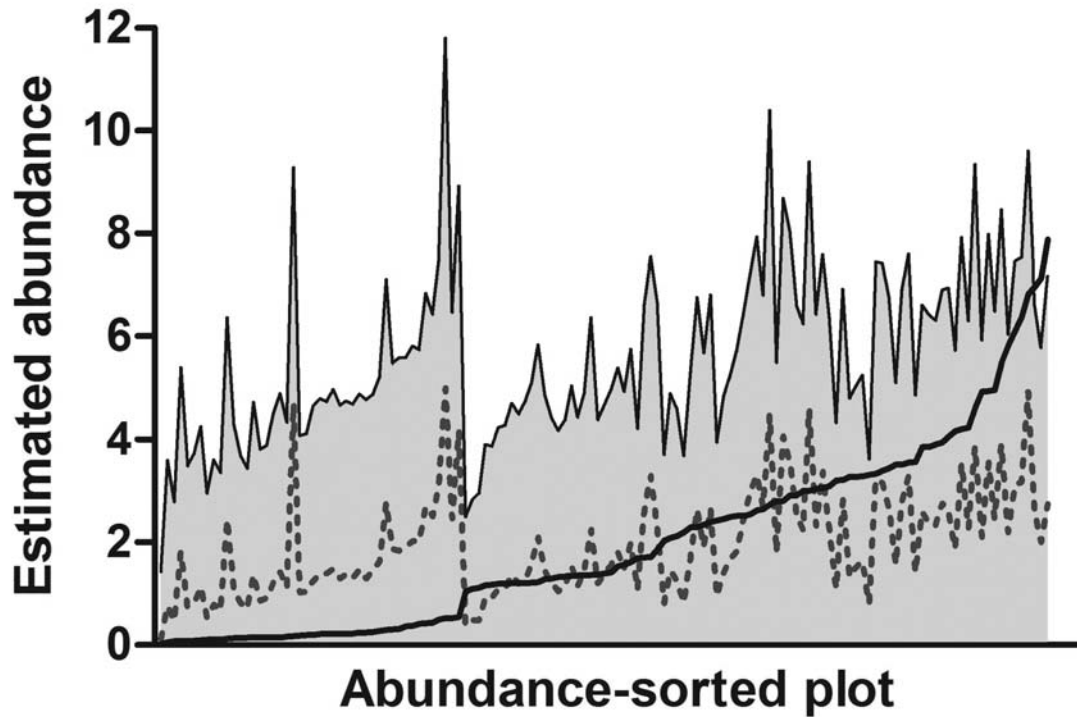


Figure 5. Probability that gravid female and juvenile *P. albagula* are present at occupied plots in relation to (A) the estimated abundance of the plot and (B) the maximum estimated temperature of the plot. Solid lines represent mean estimate, and dashed lines are the 95% confidence intervals.

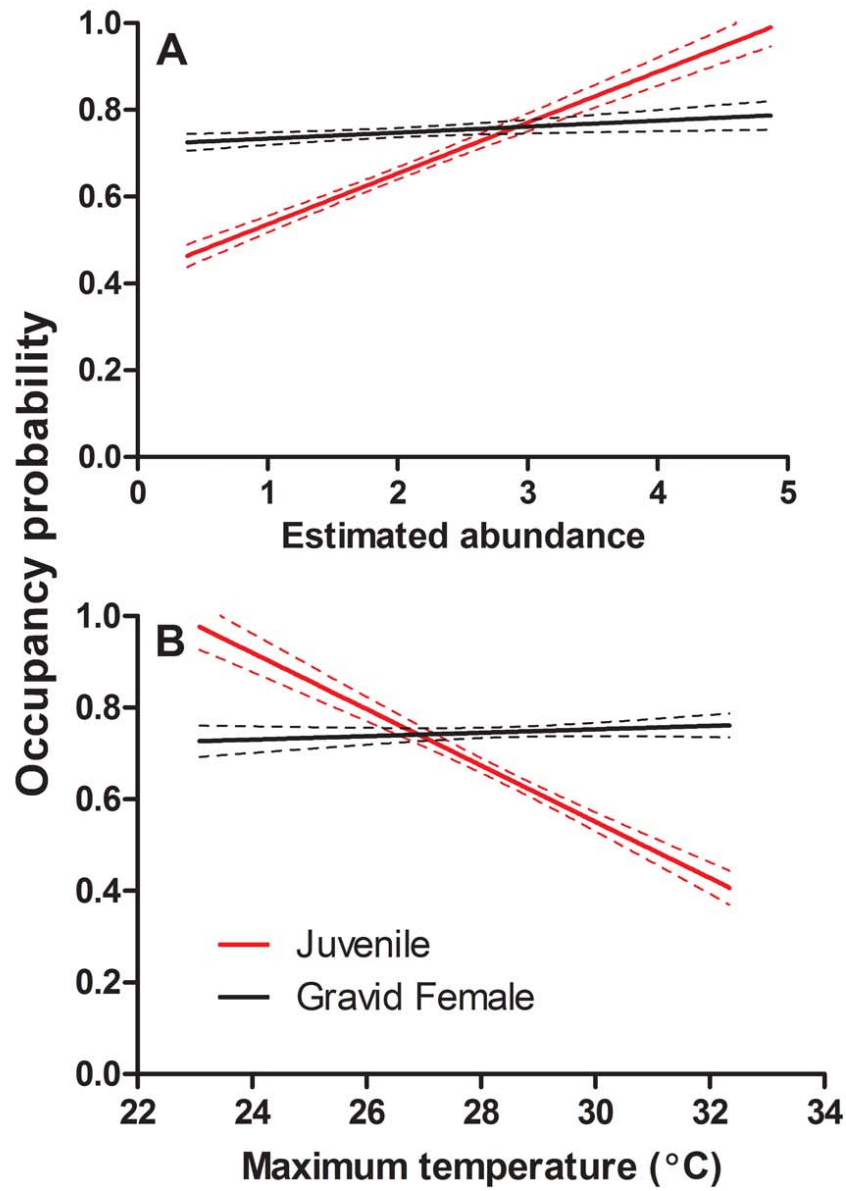
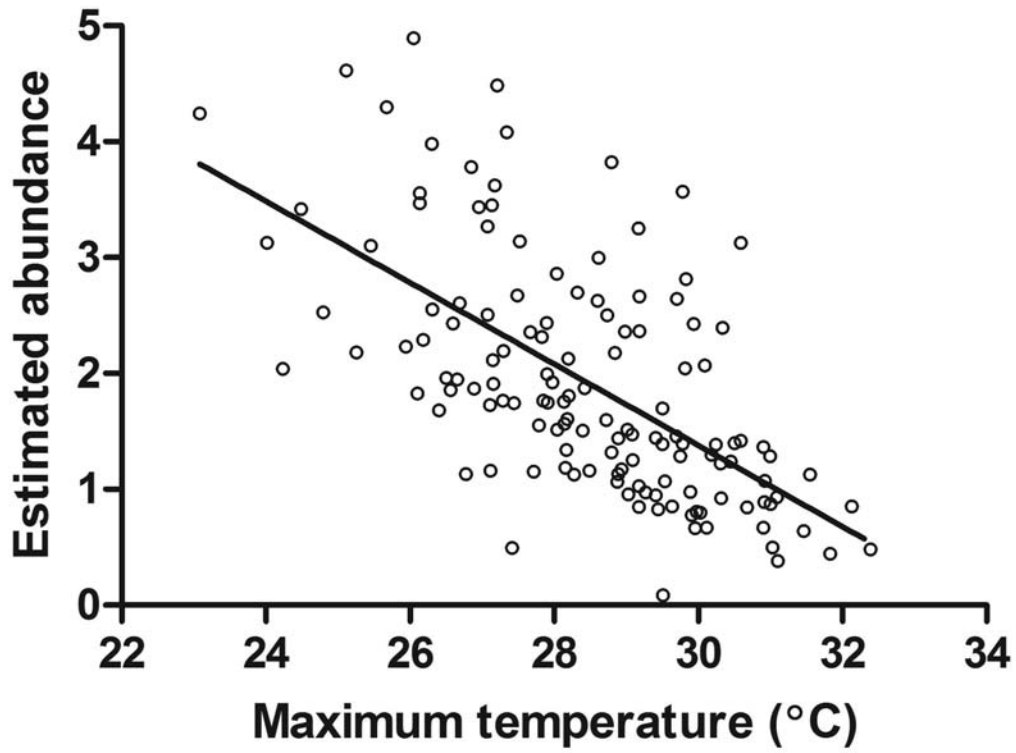


Figure 6. Relationship between estimated abundance and maximum surface temperature ($R^2 = 0.381$, $P < 0.001$).



CHAPTER 3

Spatial variation in water loss predicts terrestrial salamander distribution and population dynamics

Abstract

Many patterns observed in ecology, such as life history variation, habitat use, and distribution have physiological underpinnings. For many ectothermic organisms temperature relations shape these patterns, but for terrestrial amphibians, water balance may supersede temperature as the most critical physiological limiting factor. Terrestrial plethodontid salamanders lack lungs and have cutaneous respiration. This unique physiology results in little resistance to water loss, restricting them to moist microhabitats and may significantly affect surface activities such as foraging, dispersal, and courtship. In my study, I used Plaster of Paris models mimicking the rate of water loss of adult and juvenile *Plethodon albagula* (western slimy salamander) were used to measure water loss under ecologically-relevant field conditions. I used mixed effects models were used to estimate the effects of environmental gradients and local climate on rates of water loss, and fitted models were extrapolated across the focal landscape and converted to maps depicting surface activity times. Rates of water loss were significantly affected by topography, solar exposure, canopy cover, maximum air temperature and time since rain. Spatially, surface activity times were highest in forested ravine habitats and lowest on ridges. Surface activity time of adult models in the summer exhibited the highest correlation with predicted salamander abundance ($r=0.68$). I found that the mean size of salamanders measured in the field increased as predicted surface activity time decreased. Further, the probability of a juvenile salamander occupying an area with limited surface activity time was three times lower than an area of high surface activity. These findings suggesting that survival, recruitment, or both are demographic processes that are affected by water loss and the ability of salamanders to be surface active. Results from my study extend my understanding of plethodontid ecology, reinforcing the limitations imposed by their unique physiology, and highlighting the importance of water loss to spatial population dynamics.

Introduction

An organism's physiology is dynamically related to its environment; physiology dictates the habitats that are occupied and behaviour within these habitats, while the environment can affect physiological performance and subsequently, ecological performance (Huey 1991). In concert with the environment, physiology can affect an organism's performance at the local scale (Brewster *et al.* 2013), life history at a regional scale (Kearney 2012), and dictates limits on distribution (Buckley *et al.* 2010; Gifford & Kozak 2012; Kearney & Porter 2009). Further, potential responses to habitat or climate change can be modelled mechanistically by incorporating physiology (Kearney *et al.* 2008; Keith *et al.* 2008; Sinervo *et al.* 2010). The role of physiology is especially evident in ectothermic organisms, with the preponderance of emphasis being placed on thermal aspects of behaviour, physiology, and life history evolution (Angilletta 2009; Angilletta *et al.* 2004).

Although not independent of temperature and metabolic processes, water balance is another critical physiological characteristic that weighs heavily on the behaviour, distribution, and ecology of terrestrial taxa, especially amphibians (Wells 2007). Amphibian skin provides little to no resistance to water loss (Spight 1968; Spotila & Berman 1976), even when the atmosphere is near saturation (Adolph 1932). All terrestrial amphibians must manage their hydric relations, but it is particularly critical for woodland salamanders of the genus *Plethodon*. These salamanders are unique among terrestrial vertebrates in that they are lungless and respire cutaneously by diffusion (Whitford & Hutchison 1967). As a consequence, plethodontid skin must remain moist and permeable to facilitate gas exchange, but these requirements impose physiological and ecological limitations. Because of its permeability, the skin of plethodontid salamanders loses water at a rate that is nearly identical to a free water surface of equivalent surface area (Spotila & Berman 1976). Uninhibited water loss impinges upon salamander activity, potentially limiting foraging, dispersal, and reproductive efforts. Surface activity and foraging of salamanders is greatest under moist conditions (Grover 1998; Keen 1979; Keen 1984), and the duration of time spent foraging is directly tied to water balance (Feder & Londos 1984). To minimize water loss, salamanders are predominantly nocturnal, and are generally associated with cool, moist microhabitats (Heatwole 1962; Peterman & Semlitsch 2013; Spotila 1972).

From a physiological perspective, four measurements are needed to predict the duration of salamander surface activity: salamander mass (used to calculate surface area; Whitford & Hutchison 1967), air temperature, relative humidity, and wind speed (Feder 1983). These factors can be used to

predict that surface activity will be greatest for large salamanders when humidity is high, temperatures are cool, and there is no wind. Ecologically, this means that adults may have an advantage over juveniles in being able to sustain prolonged surface activity due to their smaller surface area to volume ratio, and microclimate variation produced by landscape features such as topography may profoundly affect surface activity times by modulating temperature, wind, and humidity. Limited surface activity may limit foraging time, and consequently affect individual growth and reproduction. Dispersal may also be curtailed, reducing gene flow among local populations.

Despite the intuitive effects that hydric constraints impose on terrestrial plethodontid salamander activity time, habitat use, and population dynamics, direct tests of these processes have been limited. Within a controlled laboratory setting, Feder and Londos (1984) found that a stream salamander (*Desmognathus ochrophaeus*, Cope) would abandon foraging in dry air twice as quickly as in moist air (3.8% vs. 7.5% loss of body mass, respectively). Grover (1998) experimentally demonstrated that surface activity of salamanders, especially juveniles, increased with increased soil moisture. Peterman and Semlitsch (2013) found that terrestrial salamander abundance was greatest in dense-canopy ravines with low solar exposure and high moisture, and found evidence of differential reproductive success related to these landscape features. Effects on population dynamics have indirectly been observed through variation in egg production. Grover and Wilbur (2002) found that salamanders in high moisture conditions produced more eggs, and both Milanovich *et al.* (2006) and Maiorana (1977) found that annual fecundity is influenced by precipitation. These findings suggest that wetter conditions may accommodate increased surface activity and foraging, increasing the energy available to be allocated to reproduction.

By incorporating physiology with spatial and temporal climate variation, mechanistic biophysical models are capable of accurately predicting the distribution (Kearney & Porter 2009), biotic interactions (Buckley & Roughgarden 2005; Gifford & Kozak 2012), and life history variation (Kearney 2012) of species. To encompass spatial heterogeneity, most of these studies cover broad geographical or elevational ranges. However, environmental gradients can occur over significantly smaller spatial scales in topographically complex landscapes (Bennie *et al.* 2008; Chen *et al.* 1999). Further, fine-scale variation in microclimate can affect species occurrence, population dynamics, and resilience to changing climatic conditions, especially in species with low vagility (Antvogel & Bonn 2001; Peterman & Semlitsch 2013; Scherrer & Körner 2011; Weiss *et al.* 1988). Although the

importance of fine-scale microclimatic variation is well-understood (Huey 1991), most analyses of physiological processes have not been spatially explicit.

We model physiological landscapes describing fine-scale spatial variation in water loss rates for a terrestrial plethodontid salamander, *Plethodon albagula* (western slimy salamander, Grobman). Our overarching hypothesis in this study is that water balance is a limiting factor for terrestrial salamanders, and that spatial variation in water loss rates result in differential patterns of surface activity and abundance that influence population dynamics across the landscape. Our objectives in calculating rates of water loss were to (1) determine the landscape and environmental factors influencing spatial variation in water loss in a topographically complex landscape, (2) determine whether realized salamander distribution on the landscape is a function of physiological limitations imposed by water loss and reduced activity time, and (3) assess the effects of water loss on spatial population dynamics. We hypothesized that rates of water loss would be dependent upon both topographical landscape features as well abiotic climatic conditions. Specifically, we predicted that topographic complexity would result in heterogeneous water loss rates across the landscape and that ravine habitat with low solar exposure would have the lowest rates of water loss. Temporally, we predicted that abiotic factors such as time since rain, air temperature, and relative humidity would significantly affect daily and seasonal patterns of water loss. Because Peterman and Semlitsch (2013) found salamander abundance to be greatest in sheltered ravine habitats and lowest on dry ridges, we hypothesized that spatial patterns of water loss would corroborate these patterns with ravines exhibiting low rates of water loss and ridges high rates of water loss. Further, as a mechanism limiting population growth, we hypothesized that evidence of successful reproduction would be greatest in ravines with lower rates of water loss.

Materials and methods

STUDY SPECIES

Plethodon albagula are a large plethodontid salamander of the *P. glutinosus* species complex (Highton 1989) that live in forested habitats throughout the Ozark and Ouachita mountains of Missouri, Arkansas, eastern Oklahoma, and northeastern Texas, USA. Within these forested habitats, salamander abundance is greatest in moist, forested ravines (Peterman & Semlitsch 2013). Surface activity varies seasonally, with peak activity occurring in spring and to a lesser extent during autumn (Milanovich *et*

al. 2011); terrestrial plethodontid salamanders generally seek subterranean refuge during hot, dry summer conditions (Taub 1961).

PLASTER MODELS

We assayed water loss across the landscape by using cylindrical plaster of Paris models as analogues for live salamanders. Plaster models were made following methods described by Peterman *et al.* (2013), and had surface areas equivalent to adult- and juvenile-sized salamanders that were 7.25 g and 2.25 g, respectively. Previous research has shown these models to lose water linearly and at rates equivalent to similarly sized salamanders (Peterman *et al.* 2013). Models were cured in a drying oven for 24 h at 70°C, and then weighed to the nearest 0.01 g on a portable digital balance (Durascale, My Weigh, Vancouver, BC).

Models were deployed at Daniel Boone Conservation Area (DBCA; Fig. 1) along 250-m long transects, spaced at approximately 50-m intervals (n=108 locations). Locations of model deployment were marked in the field using a handheld GPS (Garmin 62sc) with multiple locations being taken until the estimated precision was ≤ 3 m. Models were deployed in both spring (8 April–8 May 2012) and summer (15 August–28 August 2012). At each location, adult- and juvenile-sized models were deployed under the leaf litter, and another pair was deployed on top of the leaf litter. All models were housed within cylindrical cages made of 3 mm hardware cloth to prevent models from coming in direct contact with leaf litter or soil (Peterman *et al.* 2013). Prior to deployment, all models were soaked in water for at least four hours; models were deployed within one hour of sunset, and retrieved within one hour of sunrise. Each model was weighed with the portable digital balance upon deployment and retrieval.

SPATIAL AND TEMPORAL COVARIATES

Spatial covariates used in this analysis were calculated in ArcGIS 9.3 (ESRI, Redlands, CA, USA) and are described in detail in (Peterman & Semlitsch 2013). In this study we assessed the effects of topographic position (TPI), topographic wetness index (TWI), potential relative radiation (PRR), and distance from stream. These variables have a resolution of 3 m, and were calculated from 1/9 arc second National Elevation Dataset (<http://seamless.usgs.gov/products/9arc.php>). Canopy cover was also estimated at DBCA using the normalized difference vegetation index (NDVI), which was

calculated from cloud-free Landsat 7 satellite images of our study area taken on 15 June, 20 July, 9 August 2011 (<http://glovis.usgs.gov/>). A mean NDVI was calculated by averaging these days together. The resolution of the NDVI layer was 30 m, so it was resampled to a resolution of 3 m. Because the majority of our spring trials were conducted prior to full leaf-out, NDVI was not included in the spring models. For this analysis, we used time since rain, maximum overnight humidity, and maximum temperature of the previous day as temporal climatological covariates. These data were collected from the Big Spring weather station (<http://www.wunderground.com>), which is located 8 km west of DBCA. For extrapolating our model to the entire DBCA landscape, we determined averages for these measures in spring (1 April–31 May) and summer (1 June–31 August) for 2005–2012.

STATISTICAL ANALYSES

For each model we calculated the percentage of water lost per hour ($percent\ loss = [deployed\ mass - retrieved\ mass] / [deployed\ mass - dry\ mass] / time\ deployed$), which became our dependent variable. For this analysis, we did not have *a priori* hypotheses concerning the factors that would affect water loss, but rather, we were interested in fitting the best model possible to explain the spatial and temporal patterns of water loss in our plaster models. As such, we did not conduct extensive model selection on parameters to include or exclude from each model, but instead fit a small number of meaningful parameters to each model. Our modeling work flow proceeded as follows. We first divided our data by model size and season (size-season) to create four independent data sets (juvenile-spring, juvenile-summer, adult-spring, and adult-summer). We then assessed the correlation of each of our independent variables with each other, as well as their correlation with the dependent variable. If two variables had a Pearson's correlation $r \geq 0.70$, we excluded the variable that had the lowest correlation with the dependent variable. Lastly, to limit complexity we did not include interactions of independent variables, or variables that had $r < 0.10$ correlation with the dependent variable. To account for heterogeneous variance in our data, we fit different variance structures to our data using 'nlme' (Pinheiro *et al.* 2013) in R (R Core Team 2013); model selection was based on AIC (Akaike 1974). Using the model with the best-fit variance structure, we then tested different random effects parameterizations to account for the nested nature of our data (i.e. models within location, locations within transect, transects within date). The percent variance explained by our top model for each size-season combination was assessed using the marginal and conditional R^2 measures of Nakagawa and

Schielzeth (2013) and calculated with ‘MuMIn’ (Barton 2013). The marginal R^2 describes the percent variation explained in the fixed effects model alone, while the conditional R^2 is the total variation explained in the mixed effects model.

The fixed effects parameter estimates for the top size-season models were then used to predict water loss rates across the DBCA landscape. Model position (under leaves or on the surface) was a factor in each model, so for each size-season combination, we calculated a surface and a leaf water loss estimate. For the remainder of this paper we consider salamander surface activity to be evenly divided between these two states (i.e. 50% surface, 50% under leaves). Therefore, to calculate a single size-season water loss rate, we averaged the model predictions from surface and leaf models. Because the main objective in this study is to demonstrate water loss as a limiting factor for terrestrial salamanders, we converted water loss rates to surface activity times (SAT). There is no empirical data describing the threshold of water loss when terrestrial plethodontid salamanders cease surface activity and seek refuge, and only one study has experimentally assessed this in a stream-associated salamander (Feder & Londos 1984). Previous studies have used 10% of body mass lost as the point at which salamanders stop foraging (Feder 1983; Gifford & Kozak 2012). For our study, we used 10% of total water lost as the threshold; SAT was calculated as the time (hrs) to 10% water loss. It should be noted that the proportion of a salamander’s body mass comprised of water decreases as mass increases: *Percent Water* = $(-0.0168 * \text{live salamander wet mass (g)} + 0.8747)$ (Peterman *et al.* 2013). Ten percent mass loss for juvenile and adult salamanders of sizes equivalent to our models would result in 11.9% and 13.3% loss of water, respectively. Assuming that the physiological tolerances and behavioral responses are equivalent among size classes within a species, our use of percent water loss to estimate SAT is therefore preferable.

We generated 5,000 random points separated by a minimum of 50 m to correlate our size-season SAT predictions with each other, as well as with predicted abundance. Predicted salamander abundance used in our analysis was obtained from a Bayesian binomial mixture model described by Peterman and Semlitsch (2013). We also assessed spatial patterns of correlation between SAT and abundance within ArcGIS using a moving window correlation (Dilts 2010) with a window size of 51 m (17 x 17 pixels).

Peterman and Semlitsch (2013) used multistate models to identify a potential disconnect between reproductive effort (presence of gravid females) and realized recruitment (presence of

juveniles). We generalize that analysis for our study to assess how the probability of juvenile and adult occurrence on the landscape is related to SAT. We constructed multistate models using a conditional binomial parameterization in program PRESENCE v3.1 (MacKenzie *et al.* 2009). Models were fit separately for adult and juvenile salamanders, with three states being present in each model: (1) no salamanders present; (2) salamanders present, but focal size class absent; (3) focal size class present, where the focal size class is either adult (snout-vent length [SVL] ≥ 55 mm; Milanovich *et al.* 2006) or juvenile (SVL < 55 mm), respectively. Details on data used for this analysis and model parameterization are in Peterman and Semlitsch (2013) and Supplement S2. From these models we estimated the conditional probability of occurrence, which is the probability of a focal demographic group occurring, given that a site is occupied by salamanders.

Results

Correlations among independent variables revealed that TPI and distance from stream were highly correlated ($r = 0.74$), but TPI had a greater correlation with rate of water loss in the spring data sets, and distance to stream had a greater correlation in summer data sets. We also found TWI and maximum overnight humidity to have low correlation with water loss across all size-season combinations ($r \leq 0.07$), so these variables were not included in mixed effects models. To account for heterogeneity within our data, an exponential variance structure was fit to both the juvenile and adult spring data, a combined identity-exponential variance structure was fit to the juvenile summer data, and an identity variance structure was fit to the adult summer data (Table 1). Random-effects fit to each model had both slopes and intercepts varying by covariates (Table 1). The average interval between rainfall events, as determined from the seven years of climate data, is 1.5 days (± 1.98 SD) and 2.2 days (± 2.85 SD) and the average daily maximum temperature is 22.5°C (± 6.22) and 31.2°C (± 4.00 SD) for spring and summer seasons, respectively.

Our final mixed effects models explained the majority of the variance in our data ($R^2_{\text{GLMM}(m)} = 82.90\% - 98.69\%$, $R^2_{\text{GLMM}(c)} = 96.70\% - 99.95\%$; Table 1). Notably, simple linear regression models that do not account for heterogeneity in variance or the nestedness of our sampling design described 67.15%–81.60% of variation in our data (Table 1). Plaster model position was significant for both plaster model sizes in both seasons, with models on the surface losing 1.26%–2.64% more water per hour than adjacent models placed under leaves (Table 1). In the spring, water loss in juvenile plaster

models increased significantly with topographic position (TPI) meaning that water loss was greater in ridge-like habitat and lesser in ravine-like habitats. In contrast, topographic position had no effect on adult plaster models. Distance from stream had a significant effect on both juvenile and adult plaster model water loss in the summer with water loss rates increasing with distance from streams. Solar exposure (PRR) had no effect in the spring, but significantly increased rates of water loss in the summer (Table 1). The number of days since rainfall also significantly increased the rate of water loss in all size-season models. As anticipated, water loss increased with maximum temperature in the spring for both juvenile and adult plaster models. Surprisingly, temperature had no effect on adult plaster model water loss in the summer, and had a negative effect on juvenile water loss (Table 1). Lastly, canopy cover as measured by NDVI was found to have no effect on juvenile water loss, but had a significant effect on adult water loss; as canopy cover increased, adult model water loss decreased.

Spatially, there is extensive congruence among each size-season SAT map (Fig. 2), and correlations among these ranged from 0.62–0.95 (Table 2). The highest SAT are concentrated within ravine habitats, which are separated by ridges with lower SAT. Mean SAT on the landscape ranged from 1.94 hrs for juveniles in the summer, to 9.90 hrs for adults in the spring (Table 3). Paired t-tests revealed that juvenile SAT is significantly less than adult SAT in spring and summer, and that all SAT are significantly less in the summer (all tests $P < 0.0001$). In general, the estimated SAT is 3 times longer in spring than summer, and is about 1.5 times longer for adults than juveniles, regardless of season (Table 3, Fig. 2). Correlations of predicted salamander abundance with SAT were also high ($r = 0.35$ – 0.68 ; Table 4). Adult summer SAT had the highest correlation with predicted abundance ($r = 0.68$; Fig. 3), largely because of the significance of canopy cover in mitigating water loss (Table 1). Spatial similarities between predicted salamander abundance and adult summer SAT are evident (Fig. 4a–b); the correlation between abundance and SAT is generally highest in areas of low predicted abundance and low SAT (Fig. 4c).

Because adult summer SAT had the highest correlation with abundance, we explored in more detail its relations with abundance, salamander size distribution, and probability of occurrence. We do note, however, that the other size-season models also had significant correlations with predicted abundance ($r = 0.35$ – 0.44 ; Table 4). A linear model using adult summer SAT to predict abundance explained 45% of the variance ($F_{1, 4998} = 4,082$; $P < 0.0001$; $R^2 = 0.446$; Table 4), with predicted abundance significantly increasing as SAT increases (Fig. 4a). Further, we found that the mean SVL of

salamanders observed at 88 plots ($n = 407$ unique salamanders measured; Peterman & Semlitsch 2013) significantly increased as SAT decreased ($F_{1, 86} = 8.38$; $P=0.005$; $R^2=0.089$; Fig. 4b), suggesting that, on average, larger salamanders are found in areas with limited SAT. This finding could potentially be biased due to unequal detection of adult and juvenile salamanders. In further support of this pattern, we found that the probability of juvenile salamander occupancy at a suitable site, after correcting for imperfect detection, significantly increased as SAT increased ($F_{1, 133} = 199.20$; $P<0.0001$; $R^2=0.598$; Table 4, Fig. 4c). In contrast, the probability of adult occupancy at a suitable site changed very little with regard to adult summer SAT (Fig. 4c). Although there was a significant and positive relationship of adult occupancy probability with SAT ($F_{1, 133} = 39.54$; $P<0.0001$; $R^2=0.225$; Fig. 4c), there was little variation in adult occupancy probability across the range of SAT (adult occupancy probability = 0.78–0.97). Juvenile occupancy probability at the same sites ranged from (0.28–0.94; Fig. 4c).

Discussion

Our study assessed patterns of water loss as a process that varies spatially and temporally as a function of fine-scale environmental gradients and temporal climatic conditions. We found that spatial estimates of SAT derived from rates of water loss were significantly correlated with estimates of salamander abundance as well as population demographic characteristics. Importantly, our SAT estimates were independently derived from plaster models deployed under field conditions, and were in no way contingent upon actual salamander distributions. Results from our study extend our understanding of plethodontid ecology and reinforce the limitations imposed by their unique physiology. Previous research has only speculated the importance of hydric relations and surface activity as mechanisms underlying local distribution and population dynamics by extrapolating results from controlled laboratory experiments or indirectly through field observations. The level of corroborative agreement between our independently-derived abundance and activity time estimates provides compelling evidence that water loss is a limiting factor affecting not only the spatial distribution of salamanders on the landscape, but population dynamics as well

Water balance can be particularly critical for smaller organisms, and we found that juvenile-sized plaster models lost water at 1.5–3 times greater rate than adult-sized plaster models. Such differences significantly curtail surface activity, and could lead to differential survival across the landscape. In support of this, we found that the mean SVL of salamanders was smaller in plots with

lower rates of water loss and high SAT (Fig. 4b). Further, we found that the probability of encountering a juvenile salamander in areas of high SAT was significantly greater than areas low SAT, while the probability of encountering an adult was high and nearly constant across the range a range of SAT (Fig. 4c). These patterns suggest that reproductive rates may be greater in high SAT regions of the landscape, or that survival of juvenile salamanders is higher in high SAT areas. Either or both of these processes would contribute to the increased abundance of salamanders in high SAT regions (Fig. 4a). Differentiating these processes as mechanisms underlying the spatial variation in size distribution is likely only possible through long-term, detailed studies of local demographic processes.

In corroboration with seasonal patterns of surface activity of salamanders in the field (Milanovich *et al.* 2006), estimated SAT differed significantly among spring and summer seasons (Table 3; Fig. 2). Although SAT was 3 times greater in the spring, there is still pronounced spatial heterogeneity in SAT due to the influence of topographic position in affecting water loss. The mixed effects models describing the spatial patterns of water loss for adult- and juvenile-sized plaster models in the spring were nearly identical (Table 1). In the summer, juvenile models had no relationship with canopy cover, while adult models lost significantly less water as canopy cover increased. We speculate that the rate of water loss was so rapid in the high surface area juvenile models that canopy cover did little to attenuate losses. Although Peterman *et al.* (2013) found water loss rates of plaster models to be linear over an 8-hr laboratory test with up to 35% water loss, we note the possibility that rates of water loss could become non-linear as dehydration deficits approaches 100% (summer dehydration deficit for juvenile models: mean=60.3%, max=98.5%; adult models: mean= 39.4%, max=82.1%). Such non-linearity could contribute to the observed differences in parameter estimates for adult and juvenile models.

If reproductive success differs across the landscape, then *P. albagula* may best be described as existing as a spatially structured population (Harrison 1991; Thomas & Kunin 1999). Specifically, reproductive rates and success may be greatest within forested ravines with high SAT, and be negligible or non-existent where SAT is low. As such, the presence of adult salamanders in low SAT areas of the landscape would predominantly depend upon salamanders dispersing from high SAT regions, implying fine-scale source-sink dynamics (Pulliam 1988). Little is known concerning dispersal in plethodontid salamanders, but as adults they are generally considered to be highly philopatric with small home ranges (Kleeberger & Werner 1982; Ousterhout & Liebgold 2010). *Plethodon cinereus*

(Green), a smaller species of woodland salamander, have been found to have significant genetic differentiation over small spatial scales within continuously forested habitat (200 m; Cabe *et al.* 2007) and to have male-biased dispersal (Liebgold *et al.* 2011). Marsh *et al.* (2004) also found the majority of dispersing *P. cinereus* to be young adults. From a water loss perspective, smaller individuals with higher surface areas will incur the greatest cost of dispersing, so the finding of Marsh *et al.* (2004) that young adults are the dispersing size class may indicate a trade-off between maximizing the benefits of dispersing (e.g., reduction of kin competition, metapopulation processes, inbreeding avoidance; Hamilton & May 1977; Olivieri *et al.* 1995; Waser *et al.* 1986) while minimizing costs by not dispersing as very small, desiccant-prone juveniles. Explicit testing of how spatial variation in activity time affects population genetic structure may provide greater insight into how physiology shapes population processes.

Water relations temporally and seasonally shape activity times, locally dictate habitat use, and regionally delineate distributions. Nonetheless, water loss is not a physiological process working in isolation. Metabolic rates of ectotherms are temperature dependent, increasing with environmental temperature. Because evaporative water loss also increases with temperature, plethodontid salamanders are doubly challenged under hot, dry conditions. As metabolic demands increase with temperature there is a greater need for energy intake, but surface activity will likely be curtailed at higher temperatures due to increased rates of water loss. The relationship of energy expenditure and intake, as a function of temperature and foraging time (limited by water loss), was incorporated into a mechanistic energy budget model and used to accurately predict the elevational distribution of a montane woodland salamander (Gifford & Kozak 2012). Although temperature variation exists across our landscape and correlates with predicted abundance (Peterman & Semlitsch 2013), the independent (or interactive) role that spatial variation in temperature has on salamander metabolic rate and subsequently on abundance and population dynamics is unclear. Mechanistic modelling approaches, as used by Gifford and Kozak (2012), may be able to provide insight into these questions.

Although we observed significant correlation between SAT and predicted salamander abundance, correlations were not perfect. Included in the spatial abundance model of Peterman and Semlitsch (2013) was topographic wetness and an interaction between topographic wetness and solar exposure. These terms were not included in our mixed effects models to limit model complexity and because there was minimal correlation with measured rates of water loss. Exclusion of these factors

could explain some of the SAT-abundance discrepancies, although our mixed effects models were able to explain the majority of the variation in our data, leaving little unexplained variance to be accounted for by other factors.

Plaster models effectively mimicked water loss rates of living salamanders (Peterman *et al.* 2013), but we nonetheless made several simplifying assumptions. First, evaporative water loss in wet-skinned amphibians is determined by the moisture content of the air and the difference in the water vapour density at the surface of the animal (Spotila *et al.* 1992), but atmospheric moisture can vary over small spatial scales and as a function of topography and vegetation (Campbell & Norman 1998). While we attempted to account for humidity variation by using synoptic meteorological measurements, relative humidity did not correlate with water loss and was omitted from our mixed effect models. Fine-scale estimation of variation of relative humidity is likely necessary to more accurately estimate evaporative water loss in salamanders, but TPI and distance from stream in our study likely correlate strongly with fine-scale humidity variation (Holden & Jolly 2011). Second, under wind-free conditions, a boundary layer will form around a stationary object (Tracy 1976), which reduces the rate of evaporative water loss. Our estimates of water loss from plaster models are therefore likely conservative as foraging or dispersal movements of surface active salamanders would disrupt the boundary layer and increase rates of water loss. Third, a critical aspect of terrestrial salamander water balance is their ability to rehydrate by absorbing water across their skin (Spotila 1972), but we sought to avoid contact of our models with the leaf litter and soil to minimize the potentially confounding effects of these factors on evaporative water loss.

Our study is the first to estimate spatially-explicit rates of water loss for a terrestrial amphibian under relevant ecological field conditions. Previous research has carefully detailed the physiological relationships of amphibians with their environment (reviewed by Feder 1983; Shoemaker *et al.* 1992; Spotila *et al.* 1992; Wells 2007), but only superficial attempts have been made to relate physiology with patterns observed in nature (Spotila 1972). While water loss is unlikely to be the only limiting factor for terrestrial salamanders, our results provide strong support that it is critical. Future work in this system should explore how temperature, metabolic rate, and spatial energy budgets (Gifford & Kozak 2012) relate to patterns of abundance and population processes. Additionally, spatial genetic processes of terrestrial salamanders are largely unknown, but understanding how fine-scale environmental gradients relate to population genetics may provide critical insight into how physiology

affects local population dynamics and dispersal. Lastly, our findings that abundance and spatial demographic patterns are closely related to SAT has implications for the future persistence terrestrial salamanders. Across seasons, we found that maximum temperature and time since rain were critical predictors of water loss. Climate change scenarios forecast more extreme temperatures and increased variability in the interval and amount of rainfall (Field *et al.* 2012), and changes in these climatological parameters may profoundly affect terrestrial salamanders (Milanovich *et al.* 2010; Walls 2009).

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Table 1. Parameter estimates, standard errors (SE), and parameter significance from mixed effects model analyses of water loss rate for adult- and juvenile-sized plaster of Paris models.

Fixed effects parameters	Spring water loss models						Summer water loss models					
	Juvenile			Adult			Juvenile			Adult		
	Estimate	SE	<i>P</i>	Estimate	SE	<i>P</i>	Estimate	SE	<i>P</i>	Estimate	SE	<i>P</i>
Intercept	-3.968	0.931	<0.0001	-1.794	0.402	<0.0001	16.075	6.874	0.022	6.360	4.903	0.201
Position (surface)	2.264	0.352	<0.0001	1.155	0.184	<0.0001	1.721	0.669	0.012	1.256	0.109	<0.0001
TPI ^a	0.060	0.017	0.0006	0.004	0.007	0.546	-	-	-	-	-	-
Dist from stream ^a	-	-	-	-	-	-	0.007	0.002	0.005	0.007	0.002	0.006
PRR	0.000053	0.000060	0.378	0.000034	0.000027	0.206	0.000334	0.000110	0.003	0.000254	0.000107	0.022
Max. Temp	0.142	0.024	0.028	0.059	0.010	0.028	-0.537	0.170	0.002	0.063	0.101	0.534
Time since rain	0.415	0.060	0.021	0.205	0.024	0.013	0.256	0.020	<0.0001	0.167	0.013	<0.0001
NDVI ^b	-	-	-	-	-	-	0.348	6.922	0.960	-15.830	6.464	0.019
$R^2_{GLMM(m)}$	89.79%			93.68%			98.69%			82.90%		
$R^2_{GLMM(c)}$	99.82%			98.69%			99.95%			96.70%		
Linear model R^2	70.93%			67.15%			73.74%			81.60%		
Variance structure	Exponential (Max. Temp*position)			Exponential (Max. Temp*position)			Combined Identity(position) Exponential (Max. Temp*position)			Identity (position)		
Random effects	~1 + position date/transect/locale			~1 + position date/transect/locale			~1 + position date/transect/locale			~1 + position locale		

^a These parameters were correlated with each other; the parameter with the highest correlation with water loss rate was retained

^b Spring models were deployed pre leaf-out, so canopy cover was not used as a predictor of water loss

Table 2. Table of Pearson's r correlation coefficients for surface activity times estimated from adult- and juvenile-sized models in spring and summer. All correlations are significant at $P < 0.0001$

Model	Juvenile spring	Adult spring	Juvenile summer	Adult summer
Juvenile spring	-			
Adult spring	0.95	-		
Juvenile summer	0.77	0.85	-	
Adult summer	0.56	0.62	0.69	-

Table 3. Summary of estimated surface activity times (SAT*) for juvenile- and adult-sized models in spring and summer seasons. Values are summaries of 5,000 randomly selected points across the landscape.

Model	Surface active time (hrs)		
	Mean	SD	Range
Juvenile spring	6.25	0.62	5.00–9.48
Adult spring	9.90	0.49	8.96–12.57
Juvenile summer	1.94	0.15	1.59–2.65
Adult summer	3.67	0.73	1.15–8.97

* $SAT = 10 / \text{percent water lost}^{-hr}$

Table 4. Summary of Pearson’s correlation and linear model (LM) fit for juvenile- and adult-sized plaster model activity times in spring and summer in relation to estimated abundance, probability of juvenile salamander occupancy, and probability of adult salamander occupancy. Bolded and italicized values were significant at $P < 0.001$, and otherwise not significant.

Model	Predicted abundance		Probability of juvenile occupancy		Probability of adult occupancy	
	Pearson's r	LM R^2	Pearson's r	LM R^2	Pearson's r	LM R^2
Juvenile spring	<i>0.43</i>	<i>18.86%</i>	<i>0.44</i>	<i>19.15%</i>	0.08	1.00%
Adult spring	<i>0.44</i>	<i>19.23%</i>	<i>0.49</i>	<i>24.11%</i>	-0.02	<1.00%
Juvenile summer	<i>0.35</i>	<i>12.36%</i>	<i>0.50</i>	<i>25.31%</i>	0.14	<1.00%
Adult summer	<i>0.68</i>	<i>44.62%</i>	<i>0.78</i>	<i>59.84%</i>	<i>0.48</i>	<i>22.47%</i>

Figure 1. Locations of plaster model deployment at Daniel Boone Conservation Area, Missouri, USA.

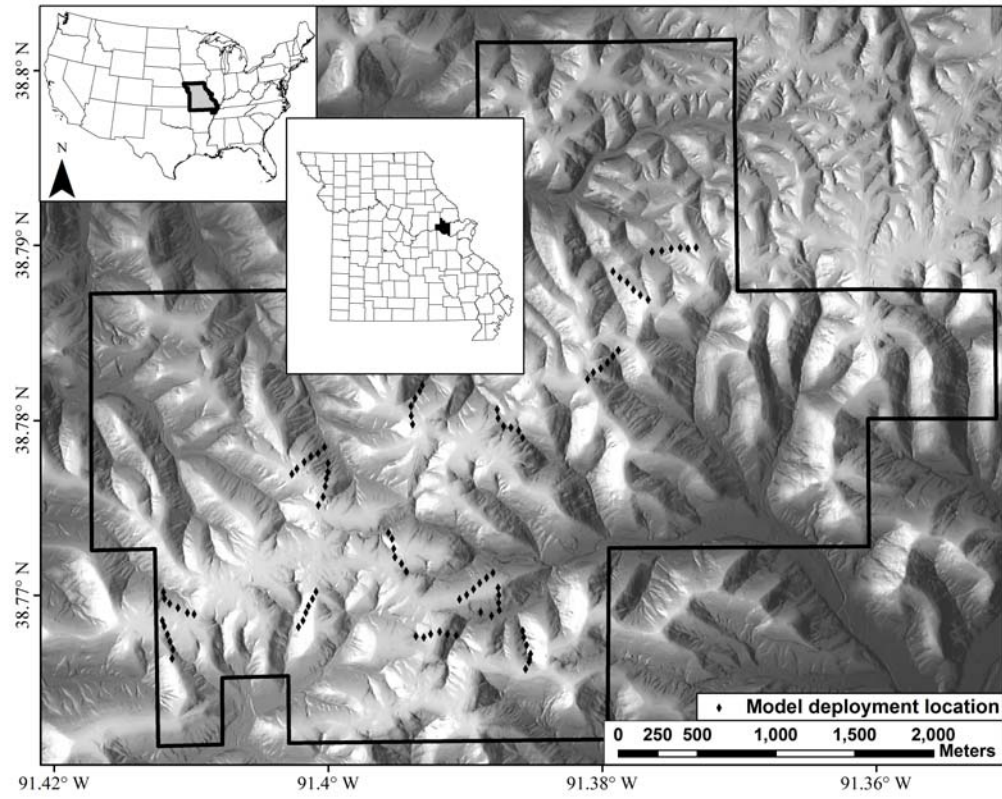


Figure 2. Maps of surface activity times estimated for adult- and juvenile-sized models in spring and summer. Note that the range and scale of activity differs for each map.

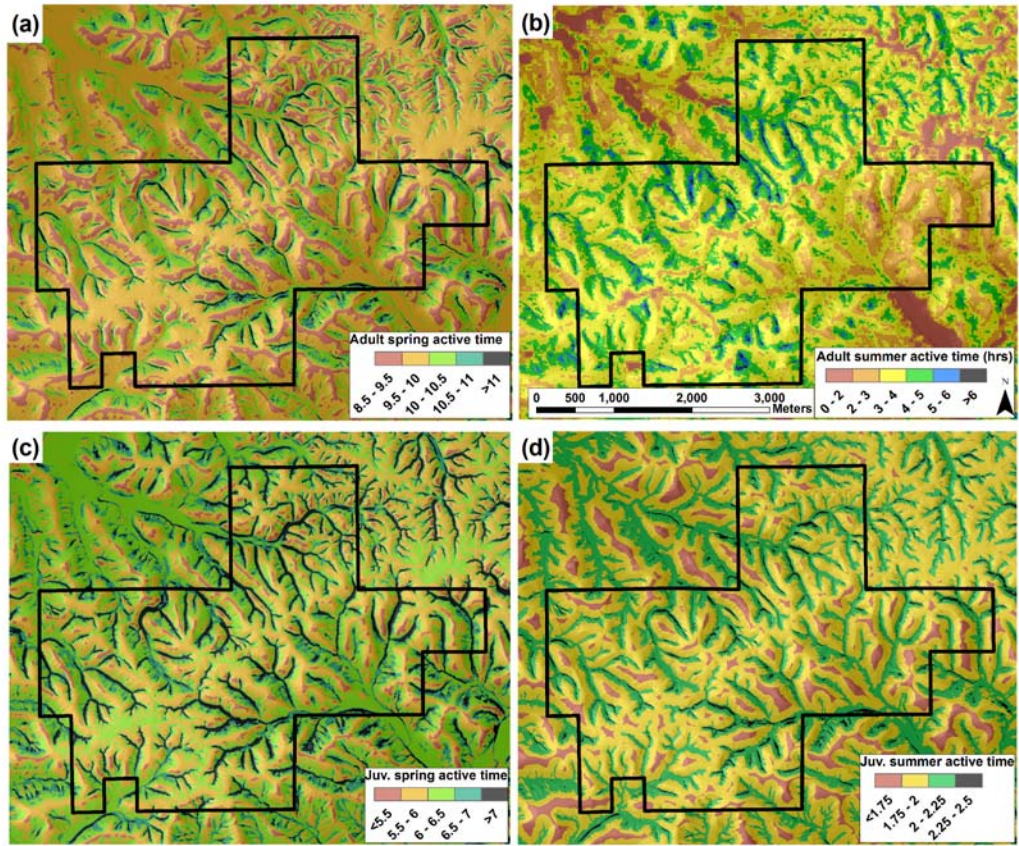


Figure 3. Maps of (a) predicted salamander abundance, (b) summer surface activity time estimated from adult-sized plaster models, and (c) spatial Pearson's r correlation values.

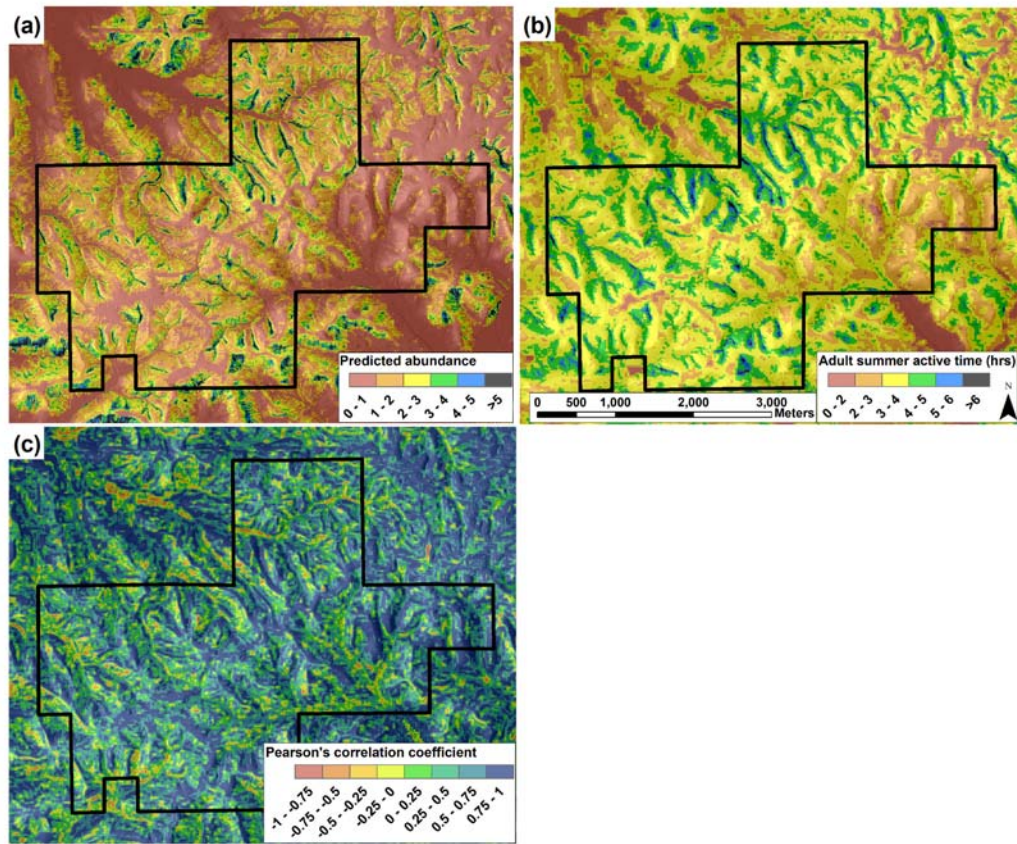
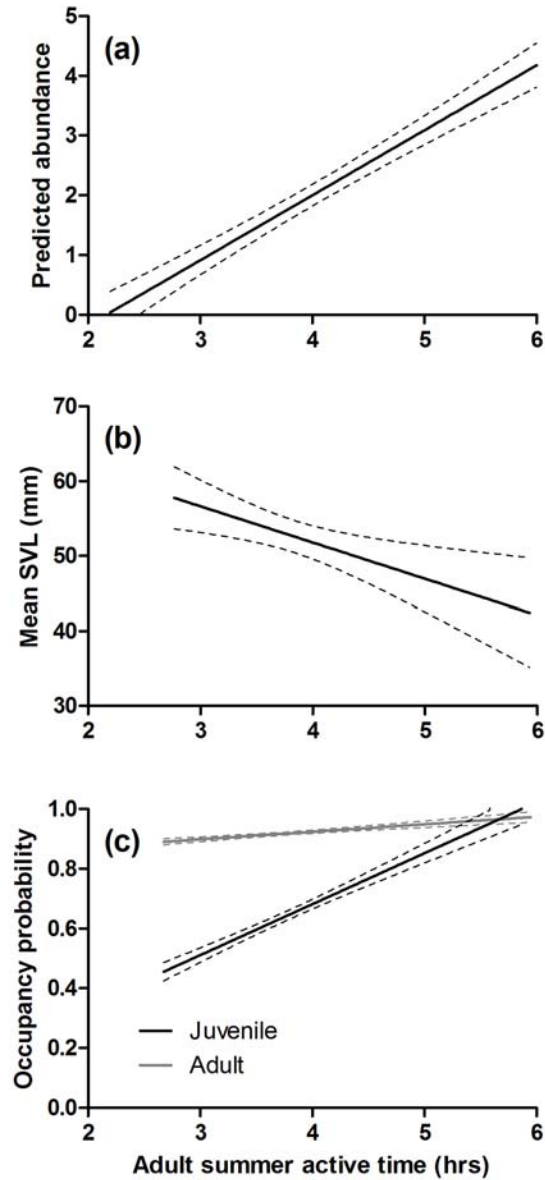


Figure 4. Relationship of adult summer active time with (a) predicted abundance from 5,000 random points on the landscape ($P < 0.0001$, $R^2 = 0.446$); (b) mean SVL from salamanders captured at 135 survey plots ($P = 0.005$; $R^2 = 0.089$); and (c) probability of occurrence of adult ($P < 0.0001$; $R^2 = 0.225$) and juvenile ($P < 0.0001$; $R^2 = 0.598$) salamanders at 135 plots.



CHAPTER 4

Multivariate ecological resistance surfaces predict fine scale genetic differentiation in a terrestrial woodland salamander

Abstract

Landscape genetics has seen tremendous advances since its introduction, but parameterization and optimization of resistance surfaces still poses significant challenges. Despite increased availability and resolution of spatial data, studies have largely failed to integrate these in spatially-explicit, ecologically-relevant ways. In my study, I build upon previous research to determine the landscape and ecological factors affecting gene flow in the western slimy salamander (*Plethodon albagula*). Ecological surfaces representing abundance, rate of water loss, and maximum surface temperature were made through combinations of canopy cover, topographic wetness, topographic position, solar exposure, and distance from ravine. Utilizing a novel resistance optimization method, we determined the transformation of each surface that maximized the partial Mantel correlation. We then used model selection to determine the resistance surfaces with the greatest support based on AICc, and found clear support for the ecologically-based, multivariate resistance surfaces representing rate of water loss experienced by adult salamanders in the summer and maximum surface temperature. All resistance surfaces affected gene flow contrary our predictions, but these patterns can be explained in light of compensatory movement behaviours through suboptimal habitat. My results demonstrate that multivariate representations of ecologic processes can provide novel insight and can better explain genetic differentiation than combinations of univariate surfaces.

Introduction

Since its formal introduction, landscape genetics has sought to combine landscape ecology, population genetics, and spatial statistics (Manel & Holderegger 2013; Manel *et al.* 2003). Significant advances have been made in analytical methods and frameworks (e.g., Cushman *et al.* 2006; Dyer *et al.* 2010; Murphy *et al.* 2008; Van Strien *et al.* 2012), simulation environments (Landguth & Cushman 2009; Rebaudo *et al.* 2013), and methods to optimize resistance surfaces (Graves *et al.* 2013; Shirk *et al.* 2010; Wang *et al.* 2009). Despite these advances, there is still a paucity of studies utilizing ecologically-based, empirically-derived resistance surfaces to test landscape genetics hypotheses (Spear *et al.* 2010). While methodological and technological advances are necessary, there will likely never be a panacea by which we can sample individuals or populations in space to determine (and correctly interpret) the ecological underpinnings of landscape features correlated with gene flow. Only through on-the-ground ecological research and experimentation can we begin to understand the multivariate effects of the landscape on ecological processes. The development of resistance surfaces that represent these ecological processes (e.g., dispersal, foraging, reproduction, survival, etc.), offers novel insights into functional connectivity and a deeper understanding of specifically how the landscape affects gene flow (Michels *et al.* 2001; Watts *et al.* 2004). Moving towards greater integration of ecological data to test hypotheses of landscape resistance is particularly pertinent given the evidence that expert opinion rarely performs satisfactorily (Charney 2012; Shirk *et al.* 2010) and that resistance surfaces are sensitive to incorrect parameterizations (Beier *et al.* 2009; Rayfield *et al.* 2010).

To better incorporate ecology into resistance modelling, researchers must not only identify landscape features important to their study organism but also understand how those features are related to ecological processes. Methods to parameterize ecological resistance surfaces have been reviewed by Spear *et al.* (2010) and can include telemetry or tracking data (e.g., Driezen *et al.* 2007), presence-absence data (e.g., Wang *et al.* 2008), or movement studies (e.g., Stevens *et al.* 2006b). The majority of these studies seek to identify habitat features that are conducive to species occurrence or to determine the relative effect that habitat features have on movement. The resultant resistance surfaces are generally univariate and assign resistance values to land cover types. Only studies utilizing habitat suitability models (e.g., Wang *et al.* 2008) or resource selection functions (e.g., Chetkiewicz & Boyce 2009) have incorporated multiple

landscape features to create novel, ecologically-relevant multivariate surfaces. Given the increasing availability and resolution of spatial data, there is great potential for researchers to develop multivariate surfaces that meaningfully relate to the ecology of their study organism. It can be relatively straightforward to derive surfaces such as soil moisture (Lookingbill & Urban 2004), solar exposure (Lookingbill & Urban 2003; McCune & Keon 2002), and surface temperature (Fridley 2009; Lookingbill & Urban 2003) at relevant spatial scales. Any of these surfaces may serve as good proxies for ecological processes, although explicit modelling of ecological processes in space will likely lead to the most profound insights.

As in all landscape studies, the spatial extent and resolution of the landscape must match the biology of the study organism. Small, terrestrial animals such as gastropods and amphibians are often closely associated with temperature and moisture microclimates (Baur & Baur 1995; Peterman & Semlitsch 2013), which may lead to differential survival, movement, and abundance across the landscape. A close dependence on microclimate may make these taxa particularly relevant for studies seeking to assess the effects of fine scale ecological processes on gene flow. Unique among terrestrial vertebrates, plethodontid salamanders are lungless and respire cutaneously (Whitford & Hutchison 1967). As a result, their skin must remain moist and permeable, requirements that impose physiological and ecological constraints. *Plethodon* is the most speciose genus of amphibian in North America, consisting of 55 species (Collins & Taggart 2009). Species diversity has been attributed to niche conservatism (Kozak 2006; Kozak & Wiens 2010b; Shepard & Burbrink 2008, 2009) wherein species are closely tied to the climatic niches in which they originally evolved. In montane or topographically complex regions, species and populations are confined to a limited geographic or elevational range, and are isolated by unsuitable lowland climates. The inability to move or adapt to a changing climate may have profound implications for the long term persistence of many plethodontid species (Milanovich *et al.* 2010). Further, studies have shown that plethodontid salamanders are sensitive to land uses such as logging (Hocking *et al.* 2013; Reichenbach & Sattler 2007) and urbanization (Gibbs 1998; Marsh *et al.* 2008; Noël *et al.* 2007). Potentially exacerbating the effects of land use and climate change is the fact that plethodontid salamander are generally perceived to be highly philopatric (Kleeberger & Werner 1982) and to exhibit minimal dispersal (Liebgold *et al.* 2011). Data from species in the *P. glutinosus* complex indicate that home ranges of adult and juvenile salamanders are <4.0 m² (Marvin 1998), and do not differ significantly between sexes or age classes. Significant genetic

differentiation in *P. cinereus* has been found at distances of only 200 m within continuous forest habitat (Cabe *et al.* 2007), and natural features such as low-order streams have been found to increase genetic differentiation among populations (Marsh *et al.* 2007). These life history characteristics suggest that genetic differentiation is likely to occur over fine spatial scales. Despite the multitude of threats facing woodland salamanders, population and landscape studies are largely absent from the literature. There is a pressing need to gain insight into processes affecting gene flow among local populations, a scale that is relevant for species management and conservation.

We conducted the first fine scale landscape genetic assessment of a terrestrial salamander, *Plethodon albagula* (western slimy salamander), with specific emphasis on building upon empirical ecological data acquired through field surveys and experimentation. *Plethodon albagula* are large plethodontid salamanders of the *P. glutinosus* species complex (Highton 1989) that live in forested habitats throughout the Ozark and Ouachita Mountains of Missouri, Arkansas, eastern Oklahoma, and northeastern Texas, USA. In Missouri, Peterman and Semlitsch (2013) found that abundance of *P. albagula* is greatest in ravines with dense canopy cover, high moisture, and low solar exposure. As a potential mechanism shaping patterns of abundance on the landscape, Peterman (2013) estimated rates of water loss across the landscape finding that water loss was most affected by topographic position, solar exposure, canopy cover, maximum daily temperature, and time since rain. The resulting water loss landscape was highly correlated ($r=0.68$) with predicted abundance on the landscape (Peterman 2013). There is also evidence that population dynamics vary spatially. Peterman and Semlitsch (2013) found that the probability of encountering a juvenile salamander increased significantly as predicted abundance increased, while the probability of encountering a gravid female was nearly equivalent across the landscape. Similarly, the probability of encountering juvenile salamanders was greatest in areas with low rates of water loss, but adult salamanders were predicted to occupy all areas with high probability (Peterman 2013). These findings were interpreted as a disconnect between reproductive effort and realized recruitment, with successful reproduction being limited to cool, moist ravine habitats (Peterman 2013; Peterman & Semlitsch 2013).

Although a convincing case can be made for water loss as a physiological mechanism shaping the spatial distribution of abundance, functional connectivity across the landscape remains unknown. Using our observations of the landscape features affecting abundance and water loss, as well as the multivariate

spatial estimates of these two ecological factors, we designed this study to determine the factors affecting gene flow of *P. albagula* across the landscape. We hypothesized that (i) fine-scale population genetic differentiation would be evident for *P. albagula*; (ii) gene flow would be best predicted by ecologically-derived resistance surfaces representing local population dynamics (abundance; Peterman & Semlitsch 2013), relative surface activity time (rate of water loss and maximum temperature; Feder & Londos 1984; Peterman 2013) and metabolic rate (maximum temperature; Feder 1976); and (iii) gene flow would be greatest through landscape and ecological features conducive to salamander reproduction and survival (i.e. ravines, high moisture, low solar exposure, high canopy, low maximum temperature, high predicted abundance, low rates of water loss).

Methods

Study site and species

Our study took place in east-central Missouri within the River Hills Ecoregion (Chapman *et al.* 2002) at Daniel Boone Conservation Area (DBCA; 38.78° N, 91.39° W; 157–280 m a.s.l.; Fig. 1a). This physiographic region and conservation area border the Missouri River, and are characterized by forested ridges and valleys with slopes that are frequently covered by exposed rock or rock outcrops. DBCA encompasses 1424.5 ha of mature (80–100 yrs old) second-growth forest (Semlitsch *et al.* 2008).

Population sampling

Tissue was collected from 1024 *P. albagula* at each of 21 sample locations by taking 0.5 cm of tail tissue, which was stored in 95% EtOH at -20°C until DNA extraction. All salamanders were collected within a 25 m² area. When possible, adult salamanders were preferentially chosen over juvenile or hatchling salamanders in an attempt to minimize the number of sibling pairs. The centre of each sample location was marked with a handheld GPS (Garmin 60sc) with repeated measurements until the estimated precision was ≤ 3 m. The minimum and maximum distances between sample locations were 75 m and 3 978 m, respectively (mean=1 725 m \pm 947 m).

Population genetic analyses

DNA was extracted using the Wizard SV 96 Genomic DNA Purification System (Promega, Madison, WI, USA) according to the manufacturer's protocols. Twenty-four tetra- and penta-nucleotide microsatellite loci were amplified using PCR; primers were fluorescently-labelled and arranged into two multiplex reactions as described in Spatola *et al.* (2013). Amplification products were sized on an ABI 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA, USA) using Liz 600 size standard at the University of Missouri DNA Core Facility, and results were scored using GENEMARKER (v.1.97; Softgenetics, State College, PA, USA). Before proceeding with analyses we tested for, and removed, full siblings from our data set using COLONY (Jones & Wang 2010). Following this, we had 343 individuals in our data set (mean=16.33/site; Table 1).

Genepop 4.2 (Raymond & Rousset 1995; Rousset 2008) was used to test if loci conformed to expected heterozygosity values under Hardy-Weinberg equilibrium (HWE) and to test for linkage disequilibrium among pairs of loci. Both tests were conducted using 250 batches with 2 500 iterations following a burn-in of 2 500. We tested for presence of null alleles using Micro-Checker (Van Oosterhout *et al.* 2004). Rarefied allelic richness and private alleles were calculated using HP-RARE (Kalinowski 2005). Observed and expected heterozygosity, spatial autocorrelation of genetic distances, as well as F_{ST} and chord distances (D_C) were calculated using GenoDive (Meirmans & Van Tienderen 2004). For the spatial autocorrelation analysis, we made 30 equiproportional distance classes, and assessed autocorrelation based on 9 999 permutations with Mantel r as the test statistics. We chose to assess D_C as an alternative metric to F_{ST} because mutation is insignificant relative to drift in D_C , and Goldberg and Waits (2010) suggested that use of this metric may be optimal for assessing fine-scale patterns. Pairwise differentiation among sample locations was assessed based on 9 999 permutations in GenoDive.

Landscape resistance surfaces

Eleven continuous landscape resistance surfaces, which were hypothesized to affect survival or movement of *P. albagula*, were created in ArcGIS 9.3 (ESRI, Redlands, CA, USA)(Fig. 1b–i). Topographic wetness index (TWI; Fig. 1b), topographic position index (TPI; Fig. 1c), distance from ravine (Fig. 1d), and potential relative radiation (PRR; Fig. 1e) were all derived from 1/9 arc second Nation Elevation Dataset (~3 m resolution; <http://seamless.usgs.gov/products/9arc.php>), while normalized difference vegetation

index (NDVI= canopy cover; Fig. 1f) was calculated from Landsat 7 satellite imagery and had an original resolution of 30 m. All of these resistance surfaces were previously assessed for their influence on abundance (Peterman & Semlitsch 2013) and rate of water loss (Peterman 2013). Details of surface development can be found in Peterman and Semlitsch (2013). Additionally, maximum temperature on the landscape surface (maxt; Fig. 1g) was estimated using a hierarchical mixed-effects model as described by Fridley (2009) from 61 Thermochron iButton data loggers (Maxim) deployed across our landscape (Peterman & Semlitsch 2013). The maximum temperature surface is a composite surface that incorporates all of the previously described resistance surfaces to predict surface soil temperature at a 3-m resolution. In addition to the maximum temperature composite surface, we also included predicted salamander abundance (Fig. 1h), which was estimated from a hierarchical binomial mixture model that included TPI, TWI, PRR, and NDVI as independent variables (Peterman & Semlitsch 2013). Finally, we included four different water loss surfaces representing predicted rates of water loss for adult and juvenile salamanders in both spring and summer (Fig. 1i). These surfaces were generated from linear mixed effects models that included different combinations TPI, PRR, NDVI, and distance from ravine as independent variables (Peterman 2013). We predicted that abundance, NDVI, and TWI would promote gene flow (i.e. higher values would have lower resistance), while all other surfaces would limit gene flow (i.e. higher values would have increased resistance).

A major challenge to the modelling of landscape resistance is the parameterization of resistance surfaces, with expert opinion or limited empirical data generally guiding the parameterization process (Spear *et al.* 2010). At best, some studies assess different resistance parameterizations through limited testing of haphazardly chosen resistance values. Although we have *a priori* predictions concerning the relationships of resistance surfaces to gene flow, we employ a novel optimization procedure to more extensively search parameter space to determine the optimal relationship between resistance values and genetic distance. We do so by making no *a priori* assumptions concerning the relationships of resistance surfaces with genetic distance. and used two equations to modify resistance surfaces:

$$R_{exp} = y^{data} \quad (1)$$

$$R_{log} = y \cdot y^{(1-data)} \quad (2)$$

In these equations, *data* is the original resistance surface and *y* is a base value that is varied to change the shape of the resistance relationship (Fig. 2). We refer to equations 1 and 2 as R_{exp} and R_{log} because of the shape of the transformations that they generate when $y > 1$ (Fig. 2a). We varied the value of *y* from 0.02–0.98 by 0.02, and from 1–10 by 0.2. When $0 < y < 1$, equation 1 results in extremely small decimal values and equation 2 results in negative values. To make these surfaces useable for resistance modelling and to standardize all resistance surfaces, we rescaled each transformed surface to a range of 1–5 using the equation:

$$(range\ MAX - range\ MIN) / (data\ MAX - data\ MIN) * (data - data\ MIN) + range\ MIN \quad (3)$$

Where *range MAX* and *MIN* represent the desired maximum and minimum values for rescaling the data, and *data MAX* and *MIN* represent the maximum and minimum values present in the original data. This rescaling preserves the relative relationship between values of the data. Following rescaling, data transformations when $0 < y < 1$ result in the curves shown in Fig 2b. When $y = 1$, $R_{exp} = 1$ and $R_{log} = 0$; we changed the zero-values of R_{log} to 1, and these two homogeneous surfaces served as tests of isolation-by-distance. We also included positive and negative linear relationships of each resistance surface. In total, 192 potential resistance surfaces were tested for each original resistance surface. Resistance distance between sample locations was then measured for each resistance surface using CIRCUITSCAPE (v.3.5.8; McRae 2006). This approach assesses flow of electrical current across a grid of resistors (or conductors), which are represented by landscape surfaces. The flow of electrical current across the landscape is analogous to individuals dispersing via a random walk, and assesses all possible pathways between any two points. By incorporating multiple dispersal pathways, circuit-based methods may better simulate gene flow that occurs over multiple generations (McRae 2006). For this analysis we assessed connectivity based on average resistances using an eight neighbour connection scheme. To make the Circuitscape analyses tractable, all resistance surfaces were resampled to a resolution of 10 m. An assumption of circuit-based resistance methods is that each pixel of the resistance surface represents a population (McRae *et al.* 2008). While this assumption is likely frequently violated in most applications of circuit theory, it is plausible in our study system. Individuals of *P. albagula* are distributed across our focal landscape, although abundance varies spatially. Given the scale and continuity of our landscape and the biological relevance of circuit theory to our system, we only used Circuitscape to assess resistance distance. Genetic distance was

significantly correlated with geographic distance, so we used partial Mantel tests (Smouse *et al.* 1986) to partial out the effects of geographic distance and test the remaining correlation between Circuitscape resistance distance with both F_{ST} and D_C . Partial Mantel tests were run using *ecodist* (Goslee & Urban 2007) in R 2.15.2 (R Core Team 2013) using 9 999 permutations, and the best-fit transformation of each resistance surface was determined as the surface with the highest partial Mantel correlation coefficient. If the best-fit transformation was on the boundary of the values assessed for y (i.e. 0.02 or 10), we extended the range of y until a peak in the partial Mantel correlation statistic was found.

Statistical modelling

We tested for the relative support for each of the 11 optimized resistance surfaces using two methods. First, we used *nlme* (Pinheiro *et al.* 2013) in R to fit linear mixed effects models using generalized least squares regression (GLS). To correct for non-independence between pairwise genetic and resistance distance measurements, we first fit three covariance structures to our data: compound symmetry [CS], first-order autoregressive [AR(1)], and first-order autoregressive moving-average [ARMA(1,1)] (Meeuwig *et al.* 2010; Pavlacky *et al.* 2009; Yang 2004). The best-fit covariance structure was determined by fitting models using restricted maximum likelihood and comparing AIC values; the ARMA(1,1) covariance structure minimized AIC in all of our models. Using geographic distance and the optimized resistance distance for each resistance surface as independent variables and genetic distance as the dependent variable, we then fit GLS models using maximum likelihood. Relative support for each was assessed using AICc calculated based on 21 observations using *AICcmodavg* (Mazerolle 2012) in R. Although this approach could allow us to fit more complex multivariate models to assess multiple resistance factors at once (Meeuwig *et al.* 2010; Pavlacky *et al.* 2009), this was not feasible with our data as all optimized resistance distances were highly correlated ($r \geq 0.61$, $\text{mean} = 0.84$). We also used the approach detailed by Richardson (2012) to conduct regression analyses on residuals to partial out the effects of geographic distance. Specifically, we (1) regressed geographic distance on genetic distance and saved the residuals; (2) regressed geographic distance on resistance distance and saved the residuals; and (3) constructed a linear model using the residuals of the previous models. From this final regression of residuals we calculated the AICc value for each model. As noted by Richardson (2012), the Pearson's correlation coefficient between the residuals of

the first two regressions is equivalent to the partial Mantel correlation coefficient. Finally, we built a composite resistance surface by combining the surfaces with the greatest support, as determined by AICc values. Because each resistance surface is unlikely to have the same influence (i.e. equal weight), we created all combinations of the top resistance surfaces by changing the relative weight of each by 5% increments, such that the weights always summed to one (e.g. Resist1_0.5+Resist2_0.95 gives 5% of the weight to resistance surface 1 and 95% of the weight to resistance surface 2).

Results

Population genetic analyses

Three microsatellite loci were monomorphic (PLAL_791, PG_RIH, and PG_3XI) and PLAL_EIXNY had an extremely high error rate and evidence of null alleles. The remaining 20 loci had 2–27 alleles (mean = 8.20 ± 5.76) across all samples. All loci and populations conformed to HWE expectations, and there was no evidence for linkage between pairs of loci. Observed heterozygosity at each sample location ranged from 0.42–0.53 (mean=0.49; Table 1). Significant spatial autocorrelation of pairwise genetic distances was evident at distances <300 m ($p \leq 0.041$; Table S1). Pairwise estimates of F_{ST} ranged from 0–0.0529 (mean = 0.0156 ± 0.0114 ; Table S2), and mean distance between significant pairwise comparisons was 1 920 m (± 825 m).

Comparison of genetic and landscape distances

A single optimal transformation was identified for each resistance surface, and the optimized resistance surface was nearly the same for both F_{ST} and D_C in most cases (Table 2–3, Table S3). Notable exceptions were PRR and NDVI, both of which had relatively low correlations with F_{ST} and D_C relative to the other surfaces analyzed. For all surfaces except NDVI, the optimized model was within the originally searched parameter space of γ (0.02–10). Unexpectedly, all of the optimized resistance surfaces had the opposite effect on gene flow from what we predicted. Higher values of abundance, TWI, and NDVI corresponded with higher resistance, while high values of all the other surfaces corresponded with low resistance. Most of the resistance surfaces were optimized using equation 1, with only PRR and TWI being optimized with equation 2 (Table 2, Table 3). The partial Mantel correlation statistic for each optimized surface ranged

from 0.178–0.488 (mean = 0.333) using F_{ST} as the response, and 0.123–0.491 (mean = 0.355) using D_C as the response (Table S3). We first assessed biotic models (i.e. abundance and water loss) separately from abiotic models (all other resistance surfaces; Table 2a-b). There was unequivocal support for the rate of water loss experienced by adult salamanders in the summer (Table 2a) and maximum surface soil temperature (Table 2b) when fitting models to F_{ST} . Results were nearly the same with models fit to D_C , but distance to ravine had moderate support in the linear mixed effects model (Table 3b). Using the optimized adult rate of water loss and maximum surface temperature resistance surfaces, we assessed all weighted combinations. For F_{ST} , we found the optimal composite resistance surface to be 60% adult summer water loss and 40% maximum temperature (Fig. 3), and this composite layer has moderately better support than the summer water loss alone (Table 2c, 2f). For D_C , the best supported composite model was 100% adult summer water loss and 0% maximum surface temperature (i.e. adult summer water loss was the best resistance surface; Table 3c). In terms of model assessment, both linear mixed effects models fitted with ARMA(1,1) covariance (Table 2a–c, Table 3a–c) and regression of residuals partialling out distance (Table 2d–f, Table 3d–f) resulted in nearly congruent results. In all cases, support for the top two models remained the same between methods when comparing all models, but relative ranks of less supported models did differ between the methods (Table 2-3).

Although there is a weak but significant correlation of geographic distance with genetic distance ($r = 0.237/0.229$, $p = 0.016/0.023$ for F_{ST}/D_C ; Table S2), distance does not appear to have significant influence at the spatial scale of our study. In our linear mixed effects models, distance was a marginally significant parameter in models predicting F_{ST} , and distance was not a significant parameter predicting D_C (Table 4). In all these models distance had a negative effect. Further, if we assess our Mantel analyses in a causal modelling framework (Cushman *et al.* 2006), we only find support for isolation by resistance (Table S3, S4).

Discussion

In support of our first two hypotheses we found unequivocal evidence that landscape resistance created by a composite of landscape features representing ecological processes is significantly affecting gene flow of *P. albagula* at DBCA. The rate of water loss experienced by adult salamanders on the landscape was the

best biotic resistance surface and single best overall resistance surface regardless of genetic distance metric, and maximum surface temperature was the best abiotic resistance surface. Unexpectedly, both of these resistance surfaces, and all other resistance surfaces assessed, affected gene flow contrary to our predictions. This result refutes our third hypothesis in which we predicted gene flow to be greatest when landscape and ecological features conducive to salamander reproduction and survival. Peterman and Semlitsch (2013) found that the probability of encountering a gravid female was significantly higher in hotter, drier regions of the landscape and that the probability of encountering juveniles was significantly lower in these same areas. Further, the abundance of salamanders was predicted to be significantly greater in cool, moist regions of the landscape. These patterns were originally interpreted as a disconnect between reproductive effort and recruitment, wherein offspring from gravid females inhabiting hot and dry regions of landscape would most likely perish and that while these females represented reproductive sinks (Peterman & Semlitsch 2013), offspring from females in cool, moist areas would survive. This pattern was reinforced by Peterman (2013), who found that desiccation of salamanders was greatest on dry ridges and that predicted abundance increased as desiccation rate decreased. In contrast, results from this study clearly demonstrate that low-abundance, desiccation-prone regions of the landscape are low resistance habitats that do not inhibit the movement of individuals and genes across the landscape.

Although surprising, these counterintuitive resistance effects can be explained in light of movement behaviour. Plethodontid salamanders are surface active almost exclusively at night, and activity is greatest during or immediately following rain (Petranka 1998). Under these conditions, water loss may not be a limiting factor for dispersing salamanders. However, if salamanders fail to completely traverse inhospitable habitat in a single night, they must settle, potentially increasing their risk of mortality. To minimize the need to settle, salamanders may exhibit different dispersal behaviours depending upon local landscape features. Movement ability and behaviour can be significantly affected by landscape context (Goodwin & Fahrig 2002), and Semlitsch *et al.* (2012) found that the rate of movement and straightness of movement paths increased when salamanders (*P. metcalfi*) were placed on artificial substrates. Similar compensatory movement behaviours have been experimentally observed in another terrestrial salamander, *Ensatina escholtzii*, which increased velocity and were less likely to settle when traversing bare soil (Rosenberg *et al.* 1998). The experimental findings of Semlitsch *et al.* (2012) and (Rosenberg *et al.* 1998)

suggest that the motivation and subsequent pattern of dispersal for salamanders can vary depending upon the surrounding landscape. Individuals inhabiting cool, moist habitats may make more exploratory movements and move only as far as necessary to establish a territory, while individuals in hot, dry habitats may make more rapid and directed movements when environmental conditions permit. Moving directly and rapidly through inhospitable habitats may be a general evolutionary dispersal strategy that reduces mortality risk, and exploratory movements within suitable habitat may best maximize fitness (Knowlton & Graham 2010). Despite the patterns of dispersal observed in this study, we emphasize that our study focused on a small region of continuously forested habitat, with only minimal influence of anthropogenic alteration (gravel road, Fig. 1a). Land uses such as logging, agriculture, or urbanization would undoubtedly increase rates of water loss and surface temperatures on the landscape, and the scale of such land uses may profoundly alter dispersal and connectivity and result in different patterns of resistance than measured in our study. Previous research at DBCA has found that abundance and apparent recruitment of *P. albagula* is significantly reduced in clear-cut logged habitats for up to seven years (Hocking *et al.* 2013), and Connette and Semlitsch (*in press*) found that populations of *P. shermani* in North Carolina, USA may take 100 yrs to fully recover following logging. These larger-scale anthropogenic disturbances may have profound impacts on local genetic diversity and affect the level genetic differentiation.

Other genetic-based studies have revealed unexpected movement patterns of animals. Keller and Holderegger (2013) found that short-distance movements of damselflies were predominantly confined to stream corridors, but long-distance dispersal was best described by straight line paths across agricultural matrix. A study of *Ambystoma californiense*, a species associated with grassland habitat, found that dispersal costs were greatest through grassland habitat (Wang *et al.* 2009). Stevens *et al.* (2006b) used behavioural experiments with natterjack toads (*Epidalea calamita*) to parameterize alternate resistance models that reflected relative resistance values of habitat type (Stevens *et al.* 2004) and habitat boundary permeability (Stevens *et al.* 2006a). They determined that habitat permeability was significantly correlated with genetic distance, while speed of movement through habitats was not.

Despite gene flow being affected contrary to our predictions when assessed at scales of 75 m–4 000 m, there is compelling research suggesting that our hypotheses concerning the role of habitat and physiology on gene flow may be important for plethodontid salamanders. Water balance is a limiting factor

that affects foraging time (Feder & Londos 1984), which may ultimately affect egg production and fecundity (Maiorana 1977; Milanovich *et al.* 2006). A spatially-explicit, mechanistic energy budget model, wherein food intake was limited by surface activity time and surface activity by water loss, was used by Gifford and Kozak (2012) to accurately predict the distribution of *P. jordani* in the Great Smoky Mountains, USA. Shepard and Burbrink (2011) found that gene flow, at the scale of kilometres, within distinct lineages of *P. caddoensis* located in the Ouachita Mountains, USA, was best explained by stream distance and geology. Collectively, these results suggest that the ecological resistance surfaces tested in our study may affect salamanders as we initially hypothesized, but that these processes may occur at different spatial scales than we assessed.

Parameterization and optimization of resistance surfaces is an ever-present challenge in landscape genetics studies and has received considerable attention (reviewed by Spear *et al.* 2010). Although optimization of univariate resistance surfaces is relatively tractable, biological realism may be better captured by using multivariate resistance surfaces, which can become exceedingly complex to parameterize (Spear *et al.* 2010). When parameter space is relatively limited, it may be possible to assess all combinations of resistance values (Wang *et al.* 2009). Other approaches have attempted to iteratively optimize, combine, and re-optimize resistance surfaces by parameters that alter the shape and magnitude of resistance (Dudaniec *et al.* 2013; Shirk *et al.* 2010), but do so over a limited parameter space. A major limitation to most parameterization/optimization methods utilized to date is that the direction of the effect must be predetermined. Although there seems to be growing consensus that expert opinion often poorly describes the ecological processes being modelled (Charney 2012; Shirk *et al.* 2010), researchers are still injecting bias into their analyses by not completely searching parameter space. Our optimization framework, although not exhaustive, allows researchers to explore relationships between resistance surfaces and genetic distances that might otherwise be ignored. We emphasize that the absolute values used in resistance modelling are not critical, but rather the relative values (Rayfield *et al.* 2010). In our study, we used the partial Mantel correlation statistic to determine the optimized transformation for each resistance surface. A recent simulation and optimization study by Graves *et al.* (2013) found that the Mantel correlation surface can be very flat over large regions of multivariate parameter space, which can pose significant challenges to optimization and may bring into question the accuracy of optimized models.

Nonetheless, our method of optimizing a single surface led to a single and clear solution in all cases. To determine the optimized resistance surface that best explained the variation in spatial genetic differentiation, we employed model selection using AICc, following analysis with linear mixed effects models and regression on residuals, partialling out the effects of distance. Frameworks for analysing landscape genetics data are many, and there likely is no single best analytical tool (Spear *et al.* 2010). Our analysis using two different analytical methods with two different genetic distance measures yielded largely congruent results for all combinations of methods. As such, we are confident that we have correctly identified water loss and maximum surface temperature, both empirically-derived multivariate resistance surfaces, as primary drivers of genetic differentiation in *P. albagula* at the scale we assessed.

Recent studies have shown that sampling design can affect inferences in landscape genetic studies (Oyler-McCance *et al.* 2012; Schwartz & McKelvey 2009). Although *P. albagula* at DBCA are widely distributed across the landscape, abundance is greatest in ravine habitats (Peterman & Semlitsch 2013). A major challenge to studying a species such as *P. albagula* using individual-based methods is the number and density of animals on the landscape (estimated to be as high as 0.874 salamanders m⁻² at DBCA; Peterman & Semlitsch 2013). Most simulation studies testing individual-based methods have found that sampling 10% or more of the simulated population increases the likelihood of identifying the data generating process. For our study, we focused our sampling efforts on a select number of high-abundance localities that encompassed the variation in landscape and ecological processes of interest, and that spanned the range of relevant spatial scales.

The scale at which ecological processes occur is a cornerstone of landscape ecology and , extension, landscape genetics. Plethodontid salamanders have long been the focus of studies with an emphasis on broad temporal and spatial scales (e.g., Devitt *et al.* 2013; Kozak & Wiens 2010a; Shepard & Burbrink 2008), but fine scale studies at the population level are lacking. Our study is the first attempt to fill this void in a widespread and speciose group of amphibians by studying a fine, but ecologically relevant, spatial scale. Future research on plethodontid salamanders should consider sampling designs to utilize individual-based methods to avoid the need to delineate populations from continuously distributed animals. Additionally, researchers should continue to assess the generality of the patterns observed in our study, as well as assess gene flow at different spatial scales, as advocated by Storfer *et al.* (2010).

To our knowledge, our study is the first to develop spatially-explicit resistance surfaces representing ecological processes to test their effects on spatial genetic structure. Previous studies have converted correlative habitat suitability models to resistance surfaces (Richards-Zawacki 2009; Wang 2012; Wang *et al.* 2008). Although these methods may integrate multiple habitat features into a single resistance surface, they are generally used as ‘black box’ methods (Yackulic *et al.* 2013) and lack ecological grounding (but see Laiolo & Tella 2006; Wang *et al.* 2008). We have gone beyond correlation of landscape features that influence connectivity and specifically tested hypotheses concerning how ecological processes affect population connectivity. By conducting relevant field research and experimentation, we were able to derive composite multivariate surfaces representing the spatial distribution of abundance and rate of water loss, as well as maximum surface temperatures. These ecological resistance surfaces are the multivariate realization that naïve studies of landscape genetics hope to achieve. But because these surfaces have relevant ecological meaning, we can go beyond simply stating that certain combinations of landscape features affect dispersal, and begin to formulate more specific hypotheses about how and why the observed patterns of spatial genetic structure have emerged. While methods for optimizing and weighting multivariate resistance surfaces continue to be sought (e.g., Graves *et al.* 2013), we also advocate that greater emphasis be placed on developing ecologically relevant resistance surfaces. To date, landscape genetics has been a predominantly exploratory field (Manel & Holderegger 2013), seeking correlations with habitat and landscape features, with plausible ecological explanations being built around these results. While novel insight into cryptic or hard to study species will always be a strength of landscape genetics, it should not preclude the inclusion of empirical, field-derived ecological data.

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Table 1. Population genetic summary statistics for 21 sample sites in the Daniel Boone Conservation Area, MO, USA. N is the number of samples after removal of full siblings, H_E is expected heterozygosity, H_O is observed heterozygosity, and A_R and A_P are the mean rarefied allelic richness and frequency of private alleles.

Population	Northing	Easting	N	HE	HO	AR	AP
103p	4292372.86	640720.62	15	0.50	0.47	3.88	0
146p	4292226.41	640701.14	21	0.49	0.42	3.93	0.15
148p	4292152.06	640774.62	14	0.49	0.51	3.85	0.04
149p	4292134.41	640701.42	20	0.49	0.50	3.67	0.09
151p	4292093.46	640550.17	14	0.52	0.53	3.93	0.03
158p	4292894.26	639806.07	19	0.51	0.51	3.95	0.05
2R	4293194.32	638944.23	12	0.50	0.48	3.73	0.07
300p	4293291.61	638904.60	24	0.48	0.50	3.73	0.04
301p	4294348.96	639012.25	22	0.49	0.47	3.79	0.06
315p	4291542.88	637959.14	20	0.52	0.51	3.84	0.05
330p	4291785.98	637962.56	11	0.47	0.46	3.69	0
333p	4292449.62	638006.56	12	0.49	0.48	3.68	0.03
343p	4292672.86	640645.62	15	0.45	0.45	3.55	0.04
37R	4292597.86	639708.12	18	0.52	0.46	3.61	0.02
40R	4292522.85	640720.62	20	0.56	0.53	4.08	0.08
41R	4294131.57	638880.38	19	0.51	0.51	3.7	0.02
44p	4293694.71	640463.13	10	0.51	0.48	3.95	0.02
46p	4292049.02	640282.59	11	0.46	0.47	3.68	0.02
74p	4292022.60	640657.48	16	0.48	0.46	3.84	0.05
7R	4294549.24	640359.22	13	0.50	0.49	3.64	0.11
8R	4294768.24	640286.79	17	0.51	0.50	3.71	0.06
Average			16.33	0.50	0.49	3.78	0.05

Table 2. Model selection results for linear mixed effects models (a–c) and linear regression models controlling for the effects of distance (d–f) fit to F_{ST} . Biotic resistance models are presented in *a* and *d*, abiotic resistance models are presented in *b* and *e*, and all resistance models are in *c* and *f*. Each optimized resistance surface is represented by the original surface name, followed by the value of γ and the optimization equation that maximized the partial Mantel r . Model a.sum.r0.6_maxt0.4 is a composite model from the top biotic and abiotic models, with decimal values representing relative weights of each surface in the composite.

(a)						(d)					
Biotic model*	K	AICc	ΔAICc	AICcWt	LL	Biotic model*	K	AICc	ΔAICc	AICcWt	LL
a.sum.r_0.06exp + Distance	6	-1319.19	0	1	668.6	a.sum.r_0.06exp	3	-1326.90	0	1	667.15
abund_1.4exp + Distance	6	-1299.96	19.23	0	658.98	abund_1.4exp	3	-1306.34	20.56	0	656.88
j.sp.r_0.42exp + Distance	6	-1294.23	24.96	0	656.12	j.sp.r_0.42exp	3	-1300.29	26.60	0	653.85
a.sp.r_0.48exp + Distance	6	-1292.77	26.42	0	655.38	a.sp.r_0.48exp	3	-1299.45	27.45	0	653.43
j.sum.r_0.46exp + Distance	6	-1292.15	27.04	0	655.07	j.sum.r_0.46exp	3	-1296.94	29.95	0	652.18
Distance	5	-1276.22	42.97	0	645.11						
Null	4	-1261.56	57.63	0	636.03						

(b)						(e)					
Abiotic model	K	AICc	ΔAICc	AICcWt	LL	Abiotic model	K	AICc	ΔAICc	AICcWt	LL
maxt_0.1exp + Distance	6	-1314.08	0	1	666.04	maxt_0.1exp	3	-1320.08	0	1	663.74
rav.dist_0.22exp + Distance	6	-1294.56	19.52	0	656.28	rav.dist_0.22exp	3	-1293.59	26.48	0	650.5
tpi_0.7exp + Distance	6	-1284.55	29.53	0	651.28	pr_r_0.8log	3	-1287.37	32.71	0	647.39
pr_r_0.8log	6	-1281.94	32.14	0	649.97	tpi_0.7exp	3	-1285.46	34.62	0	646.44
ndvi_35exp + Distance	6	-1277.95	36.14	0	647.97	ndvi_35exp	3	-1277.66	42.41	0	642.54
Distance	5	-1276.22	37.87	0	645.11	twi_1.6log	3	-1276.72	43.35	0	642.07
twi_1.6log + Distance	6	-1275.85	38.23	0	646.93						
Null	4	-1261.56	52.52	0	636.03						

(c)						(f)					
Model	K	AICc	Δ AICc	AICcWt	LL	Model	K	AICc	Δ AICc	AICcWt	LL
a.sum.r0.6_maxt0.4 + Distance	6	-1320.56	0	0.65	669.28	a.sum.r0.6_maxt0.4	3	-1327.95	0	0.62	667.68
a.sum.r_0.06exp + Distance	6	-1319.19	1.37	0.33	668.6	a.sum.r_0.06exp	3	-1326.90	1.06	0.37	667.15
maxt_0.1exp + Distance	6	-1314.08	6.48	0.03	666.04	maxt_0.1exp	3	-1320.08	7.88	0.01	663.74
abund_1.4exp + Distance	6	-1299.96	20.60	0	658.98	abund_1.4exp	3	-1306.34	21.61	0	656.88
rav.dist_0.22exp + Distance	6	-1294.56	26.00	0	656.28	j.sp.r_0.42exp	3	-1300.29	27.66	0	653.85
j.sp.r_0.42exp + Distance	6	-1294.23	26.33	0	656.12	a.sp.r_0.48exp	3	-1299.45	28.50	0	653.43
a.sp.r_0.48exp + Distance	6	-1292.77	27.79	0	655.38	j.sum.r_0.46exp	3	-1296.94	31.01	0	652.18
j.sum.r_0.46exp + Distance	6	-1292.15	28.41	0	655.07	rav.dist_0.22exp	3	-1293.59	34.36	0	650.5
tpi_0.7exp + Distance	6	-1284.55	36.01	0	651.28	pr_r_0.8log	3	-1287.37	40.58	0	647.39
pr_r_0.8log + Distance	6	-1281.94	38.62	0	649.97	tpi_0.7exp	3	-1285.46	42.49	0	646.44
ndvi_35exp + Distance	6	-1277.95	42.61	0	647.97	ndvi_35exp	3	-1277.66	50.29	0	642.54
Distance	5	-1276.22	44.34	0	645.11	twi_1.6log	3	-1276.72	51.23	0	642.07
twi_1.6log	6	-1275.85	44.70	0	646.93						
Null	4	-1261.56	59	0	636.03						

*a.sum.r, a.sp.r., j.sum.r, and j.sp.r represent rate of water loss in adults during summer and spring, and rate of water loss in juveniles during summer and spring, respectively

Table 3. Model selection results for linear mixed effects models (*a-c*) and linear regression models controlling for the effects of distance (*d-f*) fit to chord distance (D_C). Biotic resistance models are presented in *a* and *d*, abiotic resistance models are presented in *b* and *e*, and all resistance models are in *c* and *f*. Each optimized resistance surface is represented by the original surface name, followed by the value of y and the optimization equation that maximized the partial Mantel r .

(a)

Biotic model**	K	AICc	ΔAICc	AICcWt	LL
a.sum.r_0.06exp	6	-785.78	0	1	401.89
j.sum.r_0.46exp	6	-769.80	15.98	0	393.9
j.sp.r_0.48exp	6	-766.87	18.91	0	392.43
a.sp.r_0.48exp	6	-764.99	20.79	0	391.49
abund_1.8exp	6	-759.67	26.11	0	388.84
Distance	5	-746.71	39.07	0	380.36
Null	4	-730.70	55.08	0	370.6

(b)

Abiotic model	K	AICc	ΔAICc	AICcWt	LL
maxt_0.08exp	6	-775.23	0	0.88	396.62
rav.dist_0.32exp	6	-771.30	3.93	0.12	394.65
prr_0.56exp	6	-752.06	23.17	0	385.03
Distance	5	-746.71	28.52	0	380.36
ndvi_130exp	6	-746.58	28.65	0	382.29
Null	4	-730.70	44.53	0	370.6

(c)

(d)

Biotic model**	K	AICc	ΔAICc	AICcWt	LL
a.sum.r_0.06exp	3	-792.5	0	1	399.96
j.sum.r_0.46exp	3	-775.47	17.03	0	391.44
a.sp.r_0.48exp	3	-771.20	21.30	0	389.31
j.sp.r_0.48exp	3	-771.03	21.47	0	389.22
abund_1.8exp	3	-763.13	29.38	0	385.27

(e)

Abiotic model	K	AICc	ΔAICc	AICcWt	LL
maxt_0.08exp	3	-777.04	0	0.99	392.22
rav.dist_0.32exp	3	-768.17	8.87	0.01	387.79
prr_0.56exp	3	-754.21	22.82	0	380.81
tpi_0.7exp	3	-753.72	23.32	0	380.56
twi_1.4log	3	-740.90	36.14	0	374.15
ndvi_130exp	3	-737.90	39.14	0	372.66

(f)

Model	K	AICc	Δ AICc	AICcWt	LL	Model	K	AICc	Δ AICc	AICcWt	LL
a.sum.r_0.06exp*	6	-785.78	0	0.99	401.89	a.sum.r_0.06exp	3	-792.5	0	1	399.96
maxt_0.08exp	6	-775.23	10.55	0.01	396.62	maxt_0.08exp	3	-777.04	15.47	0	392.22
rav.dist_0.32exp	6	-771.30	14.48	0	394.65	j.sum.r_0.46exp	3	-775.47	17.03	0	391.44
j.sum.r_0.46exp	6	-769.80	15.98	0	393.9	a.sp.r_0.48exp	3	-771.20	21.30	0	389.31
j.sp.r_0.48exp	6	-766.87	18.91	0	392.43	j.sp.r_0.48exp	3	-771.03	21.47	0	389.22
a.sp.r_0.48exp	6	-764.99	20.79	0	391.49	rav.dist_0.32exp	3	-768.17	24.33	0	387.79
abund_1.8exp	6	-759.67	26.11	0	388.84	abund_1.8exp	3	-763.13	29.38	0	385.27
tpi_0.7exp	6	-758.13	27.65	0	388.07	pr_r_0.56exp	3	-754.21	38.29	0	380.81
pr_r_0.56exp	6	-752.06	33.71	0	385.03	tpi_0.7exp	3	-753.72	38.78	0	380.56
Distance	5	-746.71	39.07	0	380.36	twi_1.4log	3	-740.90	51.60	0	374.15
ndvi_130exp	6	-746.58	39.20	0	382.29	ndvi_130exp	3	-737.90	54.60	0	372.66
twi_1.4log	6	-745.61	40.17	0	381.8						
Null	4	-730.70	55.08	0	370.6						

* The best-supported composite resistance model was a.sum.r1.0_maxt0.0

**a.sum.r, a.sp.r., j.sum.r, and j.sp.r represent rate of water loss in adult salamanders during summer and spring, and rate of water loss in juvenile salamanders during summer and spring, respectively.

Table 4. Parameter estimates from the best-supported linear mixed effects models.

Genetic Distance	Model	Parameter	β	S.E.	<i>P</i>
<i>F_{ST}</i>	a.sum.r_0.06exp + Distance	Intercept	-0.0137	0.0034	0.0001
		a.sum.r_0.06exp	0.0172	0.0023	<0.001
		Distance	-0.000002	0.000001	0.0841
<i>F_{ST}</i>	maxt_0.1exp + Distance	Intercept	-0.0200	0.0044	<0.001
		maxt_0.1exp	0.0262	0.0038	<0.001
		Distance	-0.000003	0.000001	0.0242
<i>F_{ST}</i>	A.sum0.6_MxT0.4 + Distance	Intercept	-0.0200	0.0044	<0.001
		A.sum0.6_MxT0.4	0.0262	0.0038	<0.001
		Distance	-0.000003	0.000001	0.0242
<i>D_C</i>	a.sum.r_0.06exp + Distance	Intercept	0.2006	0.0121	<0.001
		a.sum.r_0.06exp	0.0607	0.0083	<0.001
		Distance	-0.000006	0.000004	0.1625
<i>D_C</i>	maxt_0.08exp	Intercept	0.1864	0.0163	<0.001
		maxt_0.08exp	0.0878	0.0146	<0.001
		Distance	-0.000006	0.000005	0.2152

Figure 1. Map of the Daniel Boone Conservation Area in Warren County, MO, USA. The 21 sample locations, hillshade relief, conservation area boundaries (thin black line), and focal landscape (blue box) are depicted in panel a. (b) topographic wetness (TWI), (c) topographic position index (TPI), (d) distance from ravine, (e) potential relative radiation (PRR), (f) canopy cover (NDVI), (g) maximum surface temperature, (h) predicted abundance, and (i) estimated rate of water loss for adult *P. albagula* in the summer, respectively. Blue represents low, green intermediate, and red high values in each respective surface.

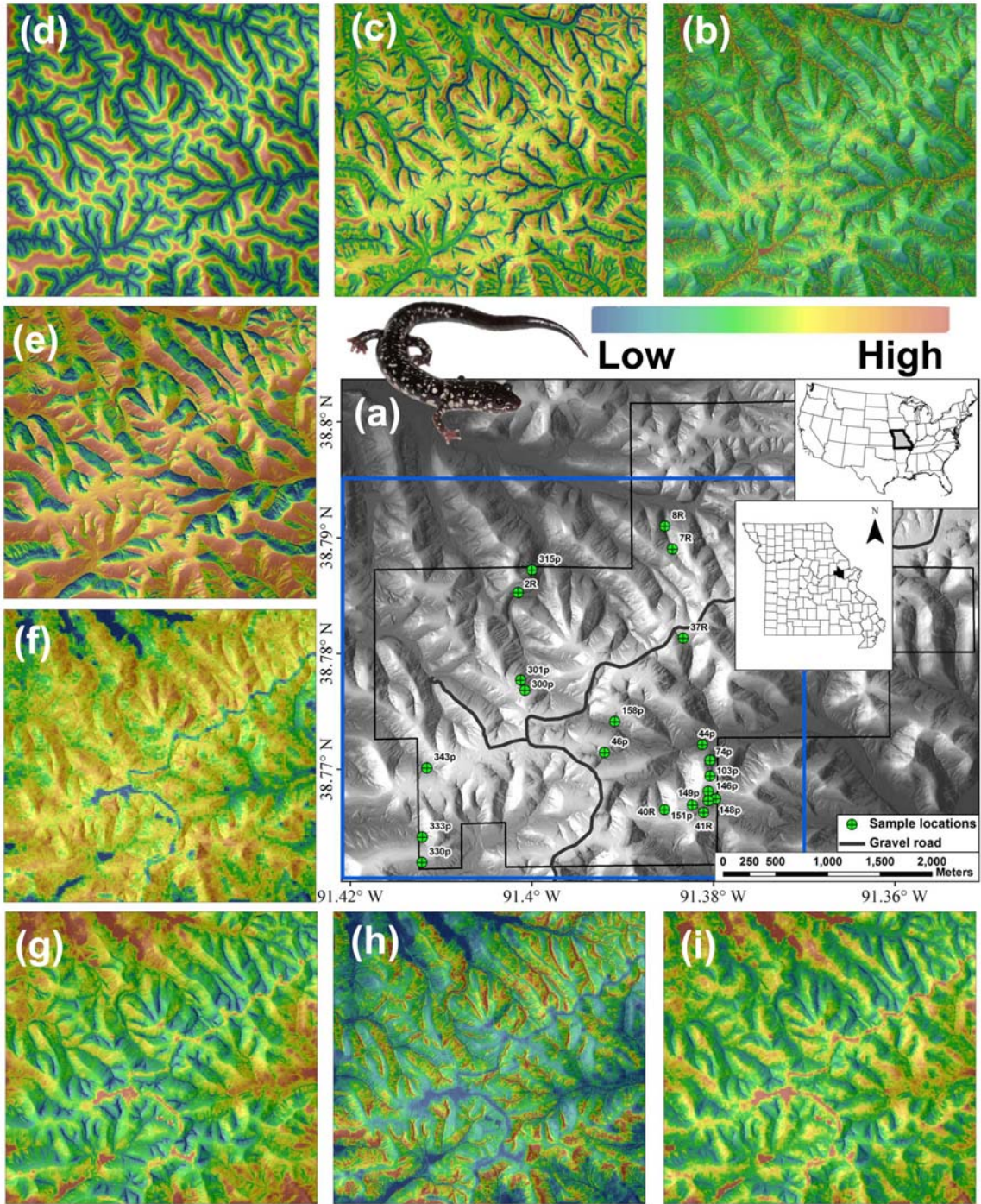


Figure 2. Results of data transformations used to optimize resistance surfaces. The solid black line represents eq. 1 and the solid gray line represents eq. 2 as described in the methods. When $y > 1$, surfaces will be transformed as in (a); when $0 < y < 1$, surfaces will be transformed as in (b). The curves drawn represent the maximum (a) and minimum (b) values of y (10 and 0.02, respectively). As y approaches 1, the shape of the transformation will become more linear, as indicated by the arrows.

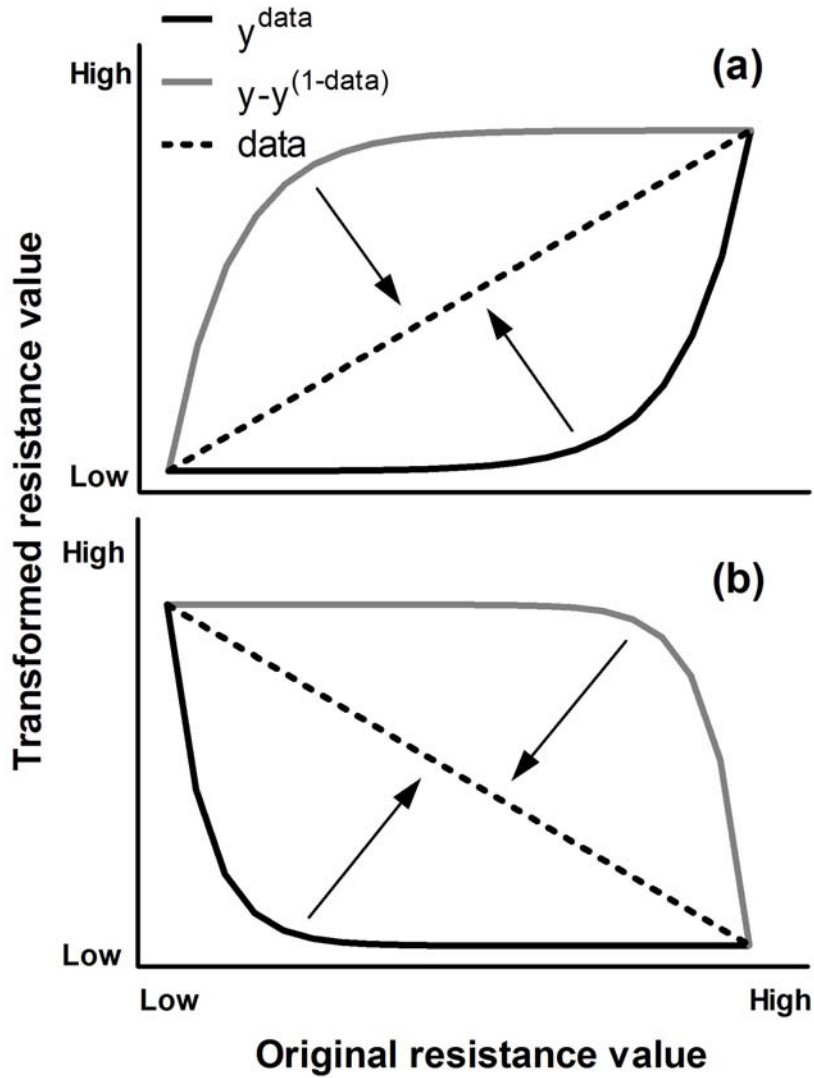
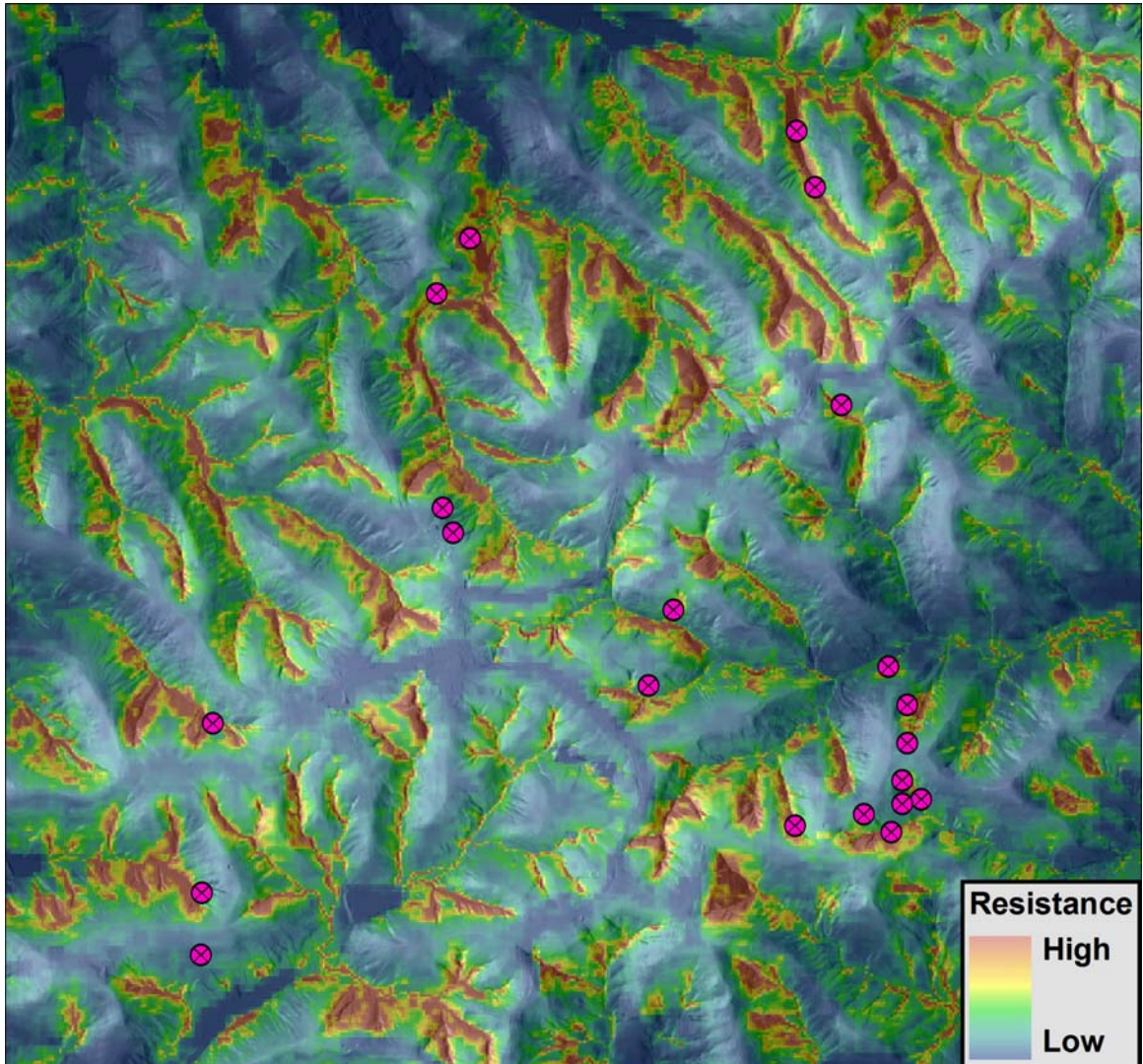


Figure 3. Resistance across the landscape for the best-supported resistance surface surface optimized on F_{ST} : a.sum0.6_maxt0.4. This surface is a combination of a.sum.r_0.06exp (60% of the weight) and maxt_0.1exp (40% of the weight).



CHAPTER 5

Summary

Understanding species' distribution and abundance forms the cornerstone of ecological research. This dissertation reverts to these basic principles to gain greater insight into the environmental factors affecting population dynamics, the physiological constraints imposed by the landscape, and the ultimate effect on movement and gene flow across the landscape. What follows is a summary of each dissertation chapter's main findings.

Chapter 2 - Fine-scale habitat associations of a terrestrial salamander: the role of environmental gradients and implications for population dynamics

- Salamander abundance is not uniformly distributed across the landscape
- Abundance is predicted to be greatest in ravines with dense canopy, low solar exposure, and high moisture
- Given that a site is suitable for salamanders, juvenile salamanders are more likely to occur in areas of high abundance and low temperature, but gravid females are likely to occur with equal and high probability, regardless of abundance or temperature

Chapter 3 - Spatial variation in water loss predicts terrestrial salamander distribution and population dynamics

- Rates of water loss were significantly lower in adults than in juveniles, and significantly lower in spring than in summer
- Rates of water loss were lowest in ravines with dense canopy cover and low solar exposure
- Water loss increased as air temperature and time since rain increased
- The predicted spatial variation in water loss was significantly correlated with predicted abundance

Chapter 4 - Multivariate ecological resistance surfaces predict fine scale genetic differentiation in a terrestrial woodland salamander

- Multivariate resistance surfaces derived from empirical field research were better predictors of genetic differentiation than single factor resistance surfaces
- The rate of water loss experienced by adult salamanders in the summer and maximum surface temperature were the best supported resistance models explaining gene flow
- Contrary to my predictions, gene flow among ravines was greatest through high water loss, high temperature regions of the landscape
- Empirically-derived ecological data provided novel insight into factors affecting gene flow, and when possible, should be incorporated into future landscape genetic studies

VITA

William Earl Peterman was born 21 November 1982 in Fremont, Michigan. He grew up in Ionia, Michigan and graduated from Ionia High School in 2001 and continued his education at Butler University in Indianapolis, Indiana. Bill graduated with high honors from Butler in 2005 with a B.S. in Biological Sciences. He began research at the University of Missouri (Columbia) in May 2005, studying stream salamanders in the southern Appalachian Mountains. Bill was married to Kimberly D. Huck in September 2007, and then graduated with a M.A. degree from the Division of Biological Sciences with an emphasis in Ecology and Evolution in May 2008. Bill began his PhD at the University of Missouri in 2009. His dissertation focused on *Plethodon albagula*, but Bill participated in numerous side projects studying wood frogs as well as ringed, spotted, marbled, and Jefferson's salamanders. On 6 December 2012, Grant Eugene Peterman joined the Peterman family, and in July 2013 Bill completed his dissertation. Bill will continue his research, first at Missouri, studying source-sink population dynamics of ringed salamanders. In 2014, Bill will begin a post doc with the Illinois Natural History Survey at the University of Illinois where he will be studying factors affecting statewide amphibian species richness and connectivity.