
GEOGRAPHIC VARIATION IN THE WATER ECONOMY OF A LUNGLESS SALAMANDER

AMANDA WINTERS AND MATTHEW E. GIFFORD*

Department of Biology, University of Arkansas at Little Rock, Little Rock Arkansas 72204, USA
*Corresponding author, email: megifford@ualr.edu

Abstract.—Evaporative water loss is a critically important constraint on the activities and natural history of all amphibians, particularly for lungless salamanders that rely almost exclusively on cutaneous respiration. Although environmental and ecological factors often co-vary with rates of evaporative water loss across species, relatively few studies have examined variation in the water economy among populations within species. In this study we examined whether rates of evaporative water loss and rates of rehydration vary among populations of the Northern Gray-cheeked Salamander, *Plethodon montanus*, distributed along a gradient in elevation. Populations exhibited clinal variation in evaporative water-loss rate along the elevation gradient with salamanders from a high-elevation population (1890 m) losing water at a significantly greater rate than salamanders from a low-elevation population (975 m). Rehydration rates did not differ among populations, and variation in rehydration was a consequence of the amount of water lost through dehydration. Variation in evaporative water loss is associated with a prevailing elevational gradient of precipitation. It is unclear whether this variation is a consequence of local genetic adaptation or developmental acclimation. The specific mechanisms driving interpopulation variation in evaporative water loss are unknown, and we discuss several non-mutually exclusive possibilities. This study reveals that variation in evaporative water loss in plethodontid salamanders occurs at the level of populations and can have important implications for spatial variation in ecology and ultimately range limits. Studies such as this one can provide critically important information for developing mechanistic species-distribution models and for predicting species responses to climate change.

Key Words.—amphibians; evaporative water loss; Northern Gray-cheeked Salamander; *Plethodon montanus*; rehydration; southern Appalachian Mountains

INTRODUCTION

Most amphibian skin offers approximately similar resistance to evaporation as a free water surface (Littleford et al. 1947; Thorson 1955; Spotila and Berman 1976; Feder and Londos 1984). In lungless salamanders, permeability is necessary for the skin to function as an effective transfer surface for gases (Lillywhite and Licht 1975; Lillywhite and Maderson 1988). This requirement restricts the activities and habitat associations of lungless salamanders (Feder 1983). Feder and Londos (1984) found that the amount of water a salamander loses before it abandons foraging (the water time limit) is inversely correlated with the vapor pressure gradient of the atmosphere. In addition, they found that salamanders abandoned foraging at modest dehydration deficits, indicative of evolutionary pressures for water-conserving behaviors.

Habitat use in amphibians is diverse; therefore, inter- and intra-specific differences in dehydration and rehydration rates due to integument morphology and their relationships to the ecologies of these organisms have long been of interest to researchers. Roth (1973) studied vascularization in the ventral pelvic region of nine anuran species and was able to correlate the degree of pelvic vascularization to

habitat type. Christensen (1974) discovered that *Bufo bufo*, the most xeric of the species he studied, has in the pelvic region specialized structures in the skin surface (tube-like grooves acting as capillaries, in addition to extensive vascular perfusion) that contribute to its relatively high rehydration rate. Camp et al. (2007) found that lungless salamanders (*Desmognathus ocoee*) collected from intermittent streams exhibited water loss phenotypes more characteristic of terrestrial species (i.e., higher tolerance of desiccation and a greater capacity to delay reaching tolerance limits) compared to salamanders collected from more permanent streams. In another study, salamanders (*Salamandra salamandra*) from semi-arid environments were able to maintain their body mass on soil with lower moisture content than those from the moist habitats (Degani 1982). Finally, Grover (2000) examined rehydration rates of several plethodontid species in the context of their distributions along moisture gradients and found that average rates of rehydration were positively correlated with their median distance to a water source.

Mechanisms for limiting water loss or increasing water gain could potentially free up spatial or temporal niches and behaviors that were otherwise unavailable to species. These mechanisms can extend an organism's water-

time limit, thus potentially leading to increased opportunity for energy gain. Besides energetic advantages, other benefits of increased activity time include territory defense and increased opportunities for mating. In addition to altering the rate of evaporative water loss, changing the rate of rehydration could extend overall activity by decreasing the amount of time spent in a refuge for rehydration. However, there is evidence that the relationship between dehydration and rehydration is reciprocal (Thorson 1955; Spotila 1972). A third strategy to extending foraging time is to withstand greater dehydration deficits.

Although environmental factors or ecology appear to drive differences in water loss among species, there are relatively few studies that examine geographic variation in water economy within a species. In this study, we test whether dehydration and rehydration rates vary among populations of a lungless salamander distributed along an elevational gradient in the southern Appalachian Mountains.

MATERIALS AND METHODS

Collection and animal maintenance.—We collected individuals of *Plethodon montanus* by hand from four sites along an elevational gradient of approximately 900 meters in the Black Mountains of Yancey County, North Carolina. We collected 8–10 similarly sized salamanders per sampling site ($N = 35$ experiment-wide). Sampling sites were 975, 1,280, 1,676, and 1,890 meters above sea level (asl).

Plethodon montanus is a relatively small, terrestrial, lungless salamander (mean mass of animals used in this study = 2.91 ± 0.13 [SE] g) with a fairly broad distribution at high elevations (generally above 900 m asl) in the southern Appalachian mountains of eastern Tennessee, western North Carolina, and western Virginia. After collection we transported salamanders to the laboratory in a cooler and subsequently housed them in plastic containers (670 ml; GladWare®, Glad Products, Oakland, California, USA). We placed all containers in a temperature-controlled incubator set at 15° C with a 14:10 (L:D) photoperiod. Moist paper towels lining each enclosure insured that animals stayed hydrated, and once a week we cleaned containers and provided each salamander with 100 to 150 *Drosophila hydei*. All salamanders

maintained body mass during experimental trials.

Dehydration and rehydration trials.—Each experimental chamber consisted of two GladWare® containers and a lid. The bottom container held a salt solution that maintained a constant vapor pressure gradient within the chamber (i.e., constant relative humidity). We adhered foam tape to the outside of the upper container so that it was nestled inside the lower container. We cut out the plastic bottom of the upper container and replaced it with screening in order to expose the salamander to the effects of the solution. Approximately 1 cm was between the solution and the screen. We placed a partially translucent GladWare® lid on the uppermost container in order to block air flow. We made small pouches by melting together with a soldering iron both sides of a folded piece of fiberglass screening. We placed a salamander inside a pouch and closed the open side with a binder clip once the salamander was contained.

All trials occurred between 0900 and 1700 in a dark room maintained at ~22° C. Experimental chambers for dehydration trials maintained an environment with approximately 76% relative humidity (generated by a saturated NaCl solution [Winston and Bates 1960]). After expressing bladder contents, we measured each salamander's mass, both with and without the bag, prior to the beginning of the trial. We chose two salamanders, at random with respect to population, and placed them side-by-side in each chamber each in their own pouch. After five hours, we expressed the bladder contents of each salamander and recorded its mass (five hours is time to lose a minimum of 10% of body mass; determined by preliminary tests). Rehydration trials commenced immediately after dehydration trials. For rehydration trials, we placed salamanders individually in petri dishes filled with 1 cm of water. Each hour we removed a salamander from the water, carefully blotted it dry, and reweighed it. We expressed rates of dehydration and rehydration as RSA (respiratory surface area) hour⁻¹ in mg cm⁻² hr⁻¹. We followed Spotila (1972) and used respiratory surface area (RSA) rather than surface area because salamanders can lose water through the skin, mouth, and buccal cavity. We calculated RSA with the formula presented in Whitford and Hutchinson (1967): $S = 9.62W^{0.614}$, where S = RSA in cm² and W is mass (g). We measured all

masses with an analytical balance to the nearest 0.0001 g.

We analyzed dehydration data using ANCOVA with initial mass as a covariate and population as a factor. For rehydration data, we used mixed effects models with population and time interval (i.e., measurements at each hour, 1–5) as fixed effects and individual identity as a random effect. The latter analysis was implemented in the nlme package (Pinheiro et al. 2013) and parameters of the model were estimated using restricted maximum likelihood. We examined pairwise differences in dehydration between populations using Tukey-Kramer post hoc tests. We conducted all statistical tests in the R computing environment (R version 2.15.1; R Core Team 2012). Statistical significance was assessed at $\alpha = 0.05$.

RESULTS

Prior to analysis we \log_{10} -transformed body mass to normalize the data. Salamanders from each site did not differ significantly in \log_{10} -body mass (pop1 [975 m] = $2.99 \pm [SE] 0.27$ g, pop2 [1,280 m] = 2.69 ± 0.16 g, pop3 [1,678 m] = 3.24 ± 0.39 g, pop4 [1,890 m] = 2.72 ± 0.16 g; ANOVA: $F = 0.89$, $df = 3, 31$, $P = 0.46$). We did not detect a significant influence of \log_{10} -body mass on the rate of evaporative water loss (EWL; Table 1); however, \log_{10} -body mass was significantly correlated with total water loss over the experimental period ($r^2 = 0.134$, $F = 5.107$, $df = 1, 31$, $P = 0.031$) across the entire sample. In addition, the scaling of EWL did not differ among populations as indicated by a non-significant interaction term (Table 1). The EWL differed significantly among populations along the elevational gradient (Table 1; Fig. 1). In

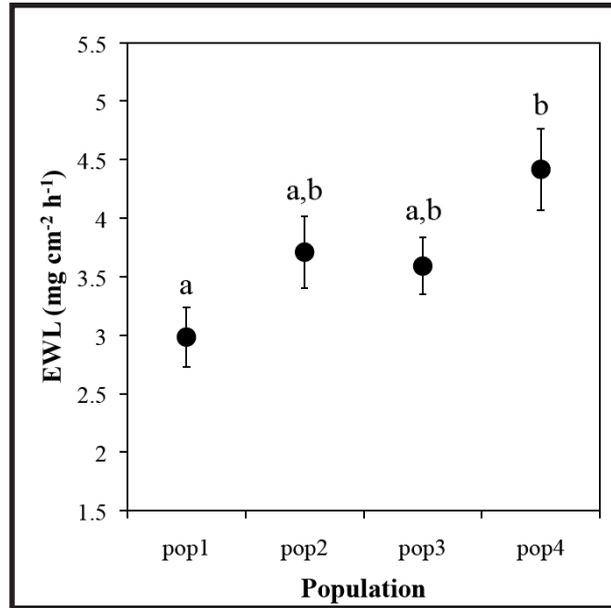


FIGURE 1. Variation in evaporative water loss rate (EWL, $\text{mg cm}^{-2} \text{h}^{-1}$, mean \pm SE) among populations of *Plethodon montanus* distributed along an elevation gradient. The X-axis lists populations from lowest to highest elevation (pop1 = 975 m, pop2 = 1,280 m, pop3 = 1,678 m, pop4 = 1,890 m). Symbols sharing letters are not significantly different from one another according to a Tukey-HSD posthoc test ($\alpha < 0.05$).

particular, salamanders from our lowest elevation site had EWL rates that were significantly lower than salamanders from our highest elevation site (Tukey HSD, $P = 0.014$; Fig. 1). No other populations differed significantly from one another. Across all populations rehydration rates varied over time, but the pattern of change in rehydration rate over time did not differ among populations (Table 1). Specifically rehydration rates were highest during the first hour and declined steadily over the following 4 h for all populations (Fig. 2).

TABLE 1. Analysis of Covariance results testing for differences in rates of dehydration (EWL, evaporative water loss in $\text{mg cm}^{-2} \text{hr}^{-1}$) and mixed effects ANCOVA results testing for differences in rates rehydration ($\text{mg cm}^{-2} \text{hr}^{-1}$) between populations of salamanders along an elevational gradient. For the latter analysis “Time” was used as a random effect (i.e., repeated measure). \log_{10} -transformed body mass was used as a covariate in the comparisons of EWL.

Comparison	df	F	P
EWL			
Mass	1, 31	1.770	0.195
Population	3, 31	3.379	0.033
Population \times Mass	3, 31	0.518	0.674
Rehydration			
Time	1, 124	89.971	< 0.0001
Population	3, 31	3.438	0.029
Time \times Population	12, 124	1.012	0.441

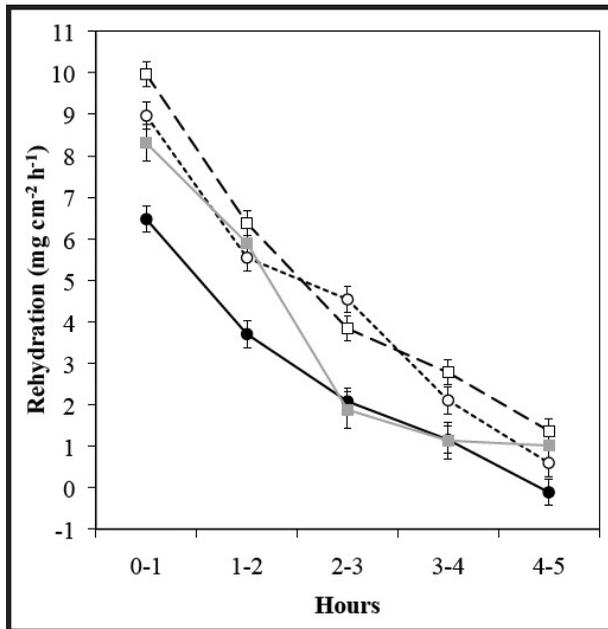


FIGURE 2. The rate of rehydration ($\text{mg cm}^{-2} \text{h}^{-1}$, mean \pm SE) varied over time and differed significantly among populations. Among-population variation in rehydration rate largely paralleled among-population variation in evaporative water loss rate (EWL, $\text{mg cm}^{-2} \text{h}^{-1}$). Symbols are coded by population (filled circles, pop1 [975 m]; open circles, pop2 [1,280 m], gray boxes, pop3 [1,678 m], open boxes, pop4 [1,890 m]).

However, salamanders from each population differed significantly in average rate of rehydration as indicated by the statistically significant intercept term (Table 1; Fig. 2). Among-population patterns of rehydration rates largely paralleled EWL rates. Therefore, differences among populations in rehydration are mostly a consequence of the amount of water lost through evaporation. Salamanders from each population rehydrated to within 3.0% of their initial mass by the end of the experiment (i.e., 5 h; pop1 = $1.6 \pm 0.3\%$, pop2 = $2.0 \pm 0.3\%$, pop3 = $2.9 \pm 0.9\%$, pop4 = $2.7 \pm 0.7\%$).

DISCUSSION

The water economy of plethodontid salamanders plays an important role in regulating temporal and spatial activity patterns. In addition to temperature, habitat moisture and intrinsic rates of dehydration and rehydration determine the times and locations of surface activity. Restriction of surface activity (away from a moist retreat), because of high rates of evaporative water loss, can be extremely costly

to plethodontids by limiting opportunities for foraging, growth, and reproduction (Feder 1983). In this study we examined rates of dehydration and rehydration under common conditions in *P. montanus* from four populations distributed along an elevation gradient. We found that salamanders from our lowest elevation site (975 m) had lower EWL rates than salamanders from our highest site (1,890 m). Rehydration rates varied significantly through time and also differed significantly among populations, although the latter is likely a consequence of variation among populations in water lost through dehydration.

Variation in EWL rate is predicted to influence the amount of time a salamander can remain outside a moist retreat prior to returning to rehydrate (water time limit). Feder and Londos (1984) demonstrated in *Desmognathus ochrophaeus* that the water time limit was significantly and positively correlated with the time necessary for a salamander to lose 1% of its body mass and hence is inversely correlated with EWL. Thus, under common conditions, *P. montanus* from our low-elevation site should be able to sustain surface activity for a longer period of time (i.e., exhibit a longer water time limit) than salamanders from our high-elevation site. Assuming that *P. montanus* will remain active and forage on the surface until it loses 10% of its body mass, we can calculate the potential foraging time (PFT) given our observed EWL measurements for each population using the following equation:

$$PFT = (0.1 \times M) / EWL$$

where M is body mass (g) and EWL is water loss rate (g h^{-1} ; Feder 1983). Using this calculation, a single foraging bout for *P. montanus* from our low elevation population could last 2.2 hours longer than for salamanders from our high elevation site (Fig. 3). Low elevation habitats around our sampling sites in the southern Appalachian Mountains tend to receive lower annual precipitation than higher elevation habitats ($r = 0.865$, $P = 0.058$, $N = 5$), and thus, the latter might provide a more equable hydric environment for *Plethodon*. Consistent with this idea, in the southern Appalachians *Plethodon* abundance increases with increasing elevation (Gifford and Kozak 2012; John C. Maerz, pers. comm.). A reduction in EWL in salamanders from lower elevations could allow these animals

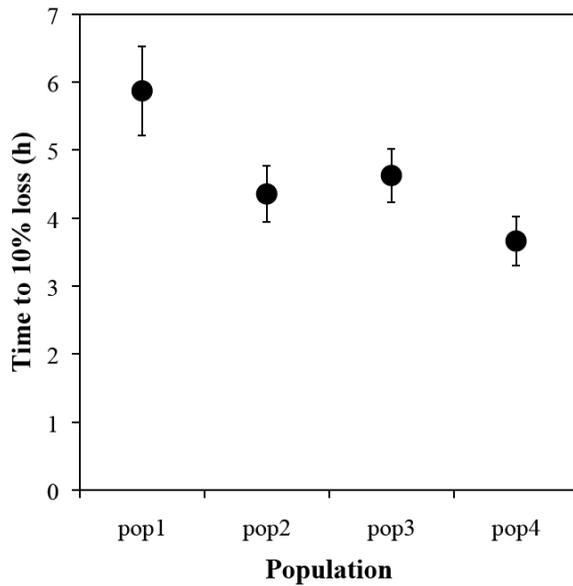


FIGURE 3. Predicted duration of activity for salamanders from each population, under experimental conditions, based on estimated rates of evaporative water loss (EWL, mean \pm SE). See text for details about this calculation. The X-axis lists populations from lowest to highest elevation (pop1 = 975 m, pop2 = 1,280 m, pop3 = 1,678 m, pop4 = 1,890 m).

to maintain comparable activity levels at generally lower relative humidity than those from higher elevations. It is unclear whether variation in EWL is a consequence of genetic differentiation among populations or a plastic response to local environmental conditions. Salamanders in this study were acclimated to laboratory conditions of moderate temperature (15° C) and high humidity for two months prior to experimentation providing some evidence that the observed responses are not a product of short-term, reversible acclimation. Unfortunately, we were unable to discriminate between genetic and developmental effects in this study given the focus on adult animals.

The specific mechanisms driving the observed clinal pattern of EWL are unclear, but we discuss a few candidates below. This is not an exhaustive review of potential mechanisms. Our goal is to suggest potentially fruitful avenues for future research. One possibility is that the skin of salamanders from high and low-elevation populations differs in structural characteristics, specifically the thickness of the stratum corneum. Generally speaking, the permeability of a semi-permeable membrane is correlated

with its thickness (i.e., length of the diffusion pathway). Therefore, our low elevation population could potentially reduce EWL by producing a thicker epidermis. Skin thickness is associated with habitat in some frogs and appears to be an important mechanism influencing water balance (Toledo and Jared 1993). A second potential mechanism influencing geographic patterns of EWL is deposition of lipids in the skin or secretion of lipids onto the skin surface. Although skin lipids have been studied in some amphibians in the context of water economy (Sadowski-Fugitt et al. 2012), we are unaware of any studies that have examined lipids in the skin or skin secretions of plethodontids in this context. Because lipids appear to play an important role in limiting EWL in a large variety of organisms, they provide a promising mechanism to explore in plethodontids. Plethodontids rely almost exclusively on cutaneous respiration, which necessitates a very thin epidermis. Therefore, most lipids in the skin might be concentrated in the dermal layer. Amey and Grigg (1995) found that among Australian hylid frogs, more drought-tolerant species have a lipid layer in the upper level of the epidermis, whereas frogs with higher cutaneous water loss rates had a lipid layer in the dermis. Therefore, variation in lipid content of skin secretions might play an additional (or primary) role in reducing water loss in lower elevation populations. Because the skin of plethodontids is generally assumed to supply negligible resistance to evaporative water loss, behavior is thought to be the primary means of limiting water loss under ecological conditions (Feder 1983; Feder and Londos 1984). It has been clearly demonstrated that microhabitat choice and posturing can significantly affect EWL (Pough et al. 1983). Our experimental design (confining salamanders to small pouches made of screen) should have limited behavioral and postural differences during experiments.

Our data demonstrate that salamanders from populations distributed along an environmental gradient show significant (and ecologically relevant) clinal variation in evaporative water loss rates; therefore, morphological and physiological mechanisms likely play an important role in driving these patterns. It is currently unclear which mechanism(s) are responsible for this variation and whether variation in water loss among populations is a consequence of developmental plasticity or local

genetic adaptation to prevailing conditions along the mountain slope. Models predicting responses of ectotherms to climate change often do not incorporate geographic variation in physiological parameters (but see Buckley 2008). This is most likely because of a dearth of physiological data at the population level. Studies such as ours are important because geographic variation in physiology can potentially allow us to make more realistic predictions about the fate of species and populations as climate changes.

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MATTHEW E. GIFFORD is an Assistant Professor at the University of Arkansas at Little Rock in Little Rock, Arkansas where he teaches Zoology, Comparative Physiology, and Herpetology. His primary research interests include the ecological and evolutionary physiology of vertebrate ectotherms (particularly salamanders, snakes, and lizards). (Photographed by Kenneth Kozak).