Population Level Differences in Thermal Sensitivity of Energy Assimilation in Terrestrial Salamanders

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Running Title: Population level differences of thermal sensitivity

Keywords: Thermal performance curve; hotter is better; thermal sensitivity; Plethodon

19 **Abstract**

20 Thermal adaptation predicts that thermal sensitivity of physiological traits should be optimized to
21 thermal conditions most frequently experienced. Furthermore, thermodynamic constraints predict
22 that species with higher thermal optima should have higher performance maxima and narrower
23 performance breadths. We tested these predictions by examining the thermal sensitivity of energy
24 assimilation between populations within two species of terrestrial-lungless salamanders,
25 *Plethodon albagula* and *P. montanus*. Within *P. albagula*, we examined populations that were
26 latitudinally separated by >450km. Within *P. montanus*, we examined populations that were
27 elevationally separated by >900m. Thermal sensitivity of energy assimilation varied substantially
28 between populations of *P. albagula* separated latitudinally, but did not vary between populations
29 of *P. montanus* separated elevationally. Specifically, in *P. albagula*, the lower latitude
30 population had a higher thermal optimum, higher maximal performance, and narrower
31 performance breadth compared to the higher latitude population. Furthermore, across all
32 individuals as thermal optima increased, performance maxima also increased, providing support
33 for the theory that “hotter is better”.

34

Introduction

Temperature constrains and affects the performance of almost all biological processes. Low temperatures produce slower reaction rates, thus reducing the performance of an organism. As temperature increases, so do the reaction rates of biological processes until performance is maximized, followed by a precipitous drop in performance (Huey and Stevenson 1979; Angilletta 2002; Bulte and Blouin-Demers 2006). Thermal sensitivity of performance is often depicted in the form of a reaction norm or thermal performance curve. From thermal performance curves, researchers often extract three useful performance metrics: the thermal optimum, maximum performance, and the performance breadth (Huey and Stevenson 1979). The thermal optimum is defined as the temperature at which performance is maximized. Performance breadth is the range of temperatures over which an organism can maintain or exceed some level of performance; in most studies a value of 80% of maximum performance is used. These metrics and their interrelatedness have produced many models and hypotheses (Huey and Kingsolver 1993; Angilletta et al. 2002).

Since biological processes are highly dependent on temperature, natural selection should favor individuals that maximize fitness at their most frequent body temperature, which should represent a compromise among many performance traits. (Angilletta et al. 2002; Martin and Huey 2008; Gvoždík 2015). This idea has produced a number of hypotheses concerning body temperature and thermal performance metrics. First, the thermal optimum should be positively correlated with the most frequently experienced body temperature of the organism, and second, the performance breadth should be positively correlated with the magnitude of variation in body temperature experienced (Huey and Stevenson 1979; Hertz et al. 1983; van Berkum 1986). Furthermore, the shape of the performance curve can influence the relationship between thermal

performance breadth and maximal performance due to specialist-generalist trade-offs, with specialization resulting in relatively higher performance with narrower breadths (Huey and Hertz 1984). The average body temperature an organism experiences is often the result of a complex interplay between the environment and the costs and benefits of thermoregulation (Huey and Slatkin 1976). Many organisms are relatively inefficient at adjusting their body temperatures and maintain temperatures that are very similar to their environment (Huey 1982). In these “thermoconformers”, one would predict that thermal optima would be highly correlated with average operative temperatures, and thermal performance breadth should be correlated with the magnitude of variation in environmental temperatures (Huey and Hertz 1984; Gilchrist 1995).

Given the thermodynamic constraints of biological processes, organisms that have thermal optima that are relatively warmer should exhibit a higher level of performance at the optimum temperature compared to those with cooler thermal optima. This idea has led to the theory that “hotter is better” or “warmer is better” (Bennet 1987; Huey and Kingsolver 1989), which suggests that cool adapted species or populations from relatively high latitudes or elevations will perform poorer than those adapted to warmer temperatures at their respective thermal optima (Gillooly et al. 2001). However, organisms may be able to counteract thermodynamic constraints through molecular adaptations, thereby allowing similar maximal performance regardless of their optimal temperature (Hochachka and Somero 2002; Clarke 2003).

Tests of thermodynamic constraints on the evolution of thermal performance have typically involved species or higher taxa (Angilletta et al. 2010). Such an approach has generally confirmed that warmer thermal optima are correlated with higher maximal performances (Bauwens et al. 1995; Heilmayer et al. 2004; Frazier et al. 2006), but see (Gvoždík and Van

Damme 2008). However, since such comparisons involve species with long evolutionary histories, it is unknown if the observed differences result from thermodynamic or evolutionary constraints. Intraspecific studies of genotypes or populations with differing thermal optima should be compared to explicitly test if thermodynamic constraints are limiting. Previous tests of thermodynamic constraints have produced inconsistent results with some taxa being more evolutionarily conservative with respect to “hotter is better” (Herron 1996; Izem and Kingsolver 2005), than others (Wilson 2001; Knies et al. 2009).

We determined geographic variation in thermal sensitivity of energy assimilation in two species of lungless terrestrial salamanders, *Plethodon albagula* and *P. montanus*. In both species, small body size, limited movement, and nocturnal nature limit their capacity to thermoregulate, and they are often considered thermoconformers (Olalla-Tarraga 2010). Thus, we expected thermal metrics to be highly correlated to their thermal environments. We chose these two species because of the large environmental gradients that occur within their range; *P. albagula* occurs over a large latitudinal range (>450km) and *P. montanus* occurs over a large elevational range (>900m). We chose energy assimilation as a response variable because it is a manifestation of many physiological and behavioral processes and should be more sensitive to temperature than any single constituent process (Angilletta 2001). Furthermore, because of its importance in constraining life history traits, assimilated energy should be under strong selection pressure (Dunham et al. 1989; Blackenhorn et al. 1995). We predicted that populations occurring in cooler climates would have cooler thermal optima, while populations from warmer climates would have warmer thermal optima. We also predicted that populations from environments that were more thermally variable would have larger performance breadths compared to populations from more stable environments.

104

105 **Methods**

106 Collection sites were chosen in a manner that would maximize the disparity between mean
107 temperatures. For *P. albagula*, the southern site was in the Ouachita National Forest within the
108 Interior Highlands of Arkansas (SOUTH; N34.4656, W093.6319, elevation 280m, mean
109 temperature 15.0 °C) and the northern site was in the Daniel Boone Conservation Area within
110 the Ozark Highlands of Missouri (NORTH; N38.7589, W91.4065, elevation 270m, mean
111 temperature 12.1 °C), a latitudinal separation greater than 450km. For *P. montanus*, both
112 populations were collected from the north-facing slope of Mt. Mitchell in North Carolina. The
113 elevational range between the high elevation site (HIGH; N35.7420, W82.2716, elevation
114 1850m, mean temperature 7.5 °C) and the low elevation site (LOW; N35.7500, W82.2198,
115 elevation 900m, mean temperature 10.8 °C) was greater than 900m.

116 We collected 11 *P. albagula* from the SOUTH site and 9 *P. albagula* from the NORTH
117 site. We collected 9 *P. montanus* from the HIGH site and 7 *P. montanus* from the LOW site.
118 Only adult individuals were used in these experiments. At all stages of this study we followed
119 taxon-specific guidelines approved by the American Society for Ichthyologists and
120 Herpetologists for the care and maintenance of wild animals (Beaupre et al. 2004). We housed
121 salamanders individually in 20x13x5cm plastic enclosures lined with moist paper towels; each
122 also contained a crumpled paper towel providing a moist refuge. Prior to testing, salamanders
123 were maintained at 15 °C, a common maintenance temperature for *Plethodon* studies, for 30 days
124 to minimize stress from capture and allow acclimation to the laboratory (Anthony 1993; Gabor
125 and Jaeger 1995; Mathis et al 2000).

We defined assimilated energy as the difference between energy acquired and energy lost:

$$\text{Assimilated Energy} = E_a - E_f$$

where E_a is energy acquired through foraging and E_f is energy lost through feces (Fitzpatrick 1972; Angilletta 2001). We quantified energy assimilation using feeding trials (Merchant 1970). We fed salamanders a known quantity of fruit flies (*D. hydei*), a common food source for lab-based physiological experiments with *Plethodon* (Crump 1979; Bobka et al. 1981; Feder and Arnold 1982). Each day the number of flies eaten were counted and replaced with fresh flies to the original number. All flies were removed after five days of feeding. At the conclusion of feeding, salamanders were fasted and all feces collected until the digestive tract was clear (3-5 days of no feces production). The duration of feces collection was inversely related to temperature, with low temperature requiring up to two weeks from the start of the feeding trial to obtain all feces collected. By subsampling *D. hydei* and burning them in a bomb calorimeter, we determined the average energy content per fly to be 0.0125kJ. We multiplied this value by the number of flies consumed over the five days to represent E_a . We determined that the energy content of feces (via bomb calorimetry) did not differ among temperatures and *Plethodon* species (species $P = 0.1292$, $\beta = 0.61$; temperature $P = 0.4364$, $\beta = 0.99$; TA Clay unpublished data) and resulted in a conversion factor of 36.95kJ/gram of feces. We multiplied the mass of feces produced by this conversion factor to determine E_f for each trial. For each individual, we determined the average daily E_a and E_f at 5 ecologically relevant temperatures: 5, 10, 15, 20, and 25 °C. Prior to thermal performance curve production, energy assimilation was divided by the number of days (5) and the mass of the individual to determine daily assimilation rates per gram of body mass.

We assumed a critical thermal minimum of 0 °C for each population and a critical thermal maximum of 33 °C based on averages from other *Plethodon* (T.A. Clay unpublished data; Spotila 1972; Layne and Claussen 1982a, b). Statistical procedures and GIS were completed using the statistical platform R version 3.1.2 (R Development Core Team 2014) and packages *lme4*, *lsmeans*, *raster*, and *rgdal* (Hijmans and van Etten 2012; Bates et al. 2015; Bivand et al. 2015; Lenth 2016). For each individual, thermal performance measures were combined with the critical thermal measures and fitted to a range of potential curves using non-linear curve-fitting (Table 1; Angilletta 2006). A best-fit curve was chosen using Akaike Information Criterion corrected (AICc) and from it individual thermal optimum, maximal performance, and performance breadth were estimated. We used ANOVA to test for differences in thermal metrics between populations within species. We used a regression analysis to determine the relationship between thermal optimum and performance maximum. We extracted 50-year averages of annual mean temperature (Bio1), and temperature seasonality (Bio4) from the Worldclim dataset for each of our collection sites (Hijmans et al. 2005; Table 2).

Results

A beta-curve provided the best fit for our data (Table 1). Thermal metrics extracted from performance curves differed between northern and southern sites in *P. albagula*, but did not differ between high and low elevation sites in *P. montanus* (Table 2, Figs. 1, 2). In *P. albagula*, the thermal optimum for energy assimilation was higher in the southern population compared to the northern population ($P = 0.04$; $F_{1,18} = 4.84$; Table 2; Fig. 1). Similarly, maximum performance tended to be greater in the southern population compared to the northern population ($P = 0.05$; $F_{1,18} = 4.25$; Table 2; Fig. 1). Thermal performance breadth was greater in the northern

population compared to the southern population ($P = 0.04$; $F_{1,18} = 5.02$; Table 2; Fig. 1). Thermal optimum ($P = 0.67$; $F_{1,14} = 0.19$), maximal performance ($P = 0.08$; $F_{1,14} = 3.62$), and performance breadth ($P = 0.62$; $F_{1,14} = 0.25$) did not differ between populations of *P. montanus* (Table 2; Fig. 2).

To test the hypothesis that “hotter is better”, performance maximum was log transformed and plotted against the inverse of the product of thermal optimum and the Boltzmann Constant. The resulting relationship was negative and significant ($P < 0.01$; $R^2 = 0.55$; $F_{1,34} = 44.52$; Fig. 3). A single regression, as opposed to species or population specific regressions, was performed on the relationship because an ANCOVA showed that species and populations did not differ in how maximal performance responded to thermal optimum ($P = 0.15$).

Average annual temperature (Bio1) was lower for our low latitude site and low elevation site relative to our high latitude site and high elevation site (Table 2). Temperature seasonality (Bio4) was greater at the high latitude site compared to the low latitude site (Table 2). The magnitude of difference in temperature seasonality between the high and low elevation sites was less and opposite of our expectations (Table 2). Due to the nature of the Worldclim dataset, temperature data from collection sites lack measures of variation, however, these data points represent 50 year averages.

Discussion

Plethodon montanus from the high and low elevation sites showed no differences in thermal sensitivities. Previous studies on ectotherms have found that elevation can produce different thermal sensitivities (van Berkum 1986) even across a narrower elevation range (600m; Navas 1996). However, these studies compared variation among species and the species were

194 confined to different elevational ranges, thereby increasing the chances for genetic adaptation to
195 the differing thermal environments. Similar to our study, Crowley (1985) found that thermal
196 physiology (thermal optimum) within a lizard species did not differ between elevational sites.
197 Site-specific genetic adaptation to local environmental conditions could be prevented by gene
198 flow between our populations. However, gene flow tends to be limited even across small spatial
199 scales in *Plethodon* (Cabe et al. 2007) due in part to their relatively small home ranges
200 (Nishikawa 1990; Marvin 1998) and very short dispersal distances (30-40m; Marsh et al. 2004;
201 Liebgold et al. 2011).

202 The ability to effectively thermoregulate has been shown to influence the thermal optima
203 between species or populations. More effective thermoregulators show fewer differences in
204 thermal optima than those that are less effective (Crowley 1985; van Berkum 1986). The family
205 Plethodontidae are generally thought to be thermoconformers and limited in their opportunities
206 for thermoregulation, suggesting that average environmental temperatures should be highly
207 correlated with their thermal optima. From climatic data, we have shown that average
208 temperatures at both the high and low elevation sites differ in a similar fashion as the two
209 latitudinal sites. Despite the sites having different average annual temperatures, the field body
210 temperatures of salamanders at the elevationally separated sites examined may not be different.
211 If this were the case, it could produce limited selection pressure driving local adaptation. Similar
212 field body temperatures, despite differing annual average temperatures, could arise in
213 salamanders at the elevationally separated populations if activity occurs during different times of
214 day or during different seasons. This would allow the populations to exploit similar temperatures
215 during slightly different parts of the year. Shifted phenologies due to altitudinal gradients are not
216 uncommon and occur in many taxa including *Plethodon cinereus* (Howard and Wallace 1985;

Petranka 1998; Fielding et al. 1999). Furthermore, the thermal sensitivity of locomotor performance is similar between northern and southern populations of the tree frog *Pseudeacris crucifer*, and this similarity has been proposed as a reason behind their shifted phenologies (John-Alder 1989). It is unknown if there are different phenologies between the high and low elevation populations of *P. montanus*, but among and within species of *Plethodon*, activity and abundance appear to be highly dependent on temperature (Petranka 1998; Peterman and Semlitsch. 2013).

Optimal temperature for energy assimilation was lower in the higher latitude population, and higher in the lower latitude population of *P. albagula*. This correlates with the cooler temperatures experienced by salamanders at higher latitudes, and warmer temperatures experienced by salamanders at lower latitudes (Table 2). The environmental temperatures and thermal optima, although exhibiting a pattern consistent with thermal adaptation, were dissimilar. This is likely an artifact of our environmental data being produced from 50 year averages of weekly average temperatures and not from nightly temperatures during parts of the year when individual salamanders would likely be active. Similar studies comparing latitudinal responses have produced conflicting results, suggesting that some species are inflexible with regard to their thermal sensitivities of physiological traits (John-Adler 1989). However, geographic variation in thermal performance of the marsh frog, *Lymnodynastes peronii*, suggests that the thermal optimum is correlated with latitude (Wilson 2001). Furthermore, using a common garden experiment, Wilson (2001) suggested that these differences in thermal sensitivities may have a genetic component.

Thermal performance breadth was also greater in the high latitude population compared to the low latitude population. The greater performance breadth in the high latitude population of

P. albagula potentially a response to the increase in temperature seasonality at this site (Stevens 1989). A larger performance breadth would be advantageous for an organism that thermoconforms and experiences a greater range of environmental temperatures (Janzen 1967; Frazier et al. 2006). A narrower performance breadth, as observed in the lower latitude population of *P. albagula*, could also arise as a side-effect of selection for increased performance (Gilchrist 1995). Generalist-specialist trade-offs predict that an increase in maximal performance can only be possible through the narrowing of performance breadth (Huey and Slatkin 1976; Gilchrist 1995). Alternatively, the narrow performance breadth may be an artifact of assuming the same critical thermal maximum for both populations. We adjusted the critical thermal maximum for each population, increasing it in the southern population and decreasing it in the northern population. Amelioration of differences in thermal performance breadths occurred when the difference between populations in critical thermal maximum differed by three degrees. The likelihood of our populations of *P. albagula* having critical thermal maxima that differ to this extent is unlikely for two reasons. First, within *Plethodon*, critical thermal maxima differ by less than one degree when acclimated to similar temperatures (Spotila 1972). Second, a sampling of plethodontid salamanders from across the families range exhibit critical thermal maxima that differ by less than three degrees (Spotila 1972).

Many aspects of both the organism and the environment can influence the optimal temperature of a physiological trait. If thermal sensitivity is strongly influenced by thermodynamic constraints on underlying biochemical processes, then an increase in optimal temperature should result in an increase in maximal performance; in other words “hotter is better”. When plotted on an Arrhenius scale the presence of a thermodynamic constraint should be detectable as a negative relationship between the thermal optimum and maximal performance

with a slope of approximately -0.6, the activation energy, E , of enzymes (Savage et al. 2004). We found strong support for this hypothesis with respect to energy assimilation at the individual level, suggesting that “hotter is better” for energy assimilation. When utilizing this approach with our data, we arrived at a slope of -1.11, suggesting a high degree of thermodynamic constraint on energy assimilation. This value exceeds the predicted value, and steeper slopes have been observed (-0.97, Frazier et al. 2006; -0.85, Knies et al. 2009) suggesting that hotter is even better. Furthermore, when standardized, our correlation coefficient of -0.97 is similar to values found in a broad meta-analysis of thermodynamic constraints on physiological and performance traits (correlation coefficients ranged from -0.96 to 0.76, Angilletta et al. 2010).

In summary, we tested whether the thermal sensitivity of energy assimilation differed among geographically separated populations of lungless salamanders. We demonstrated intraspecific differences in thermal sensitivity of energy assimilation among populations of *P. albagula* across latitude but not among populations of *P. montanus* across elevation, potentially suggesting local adaptation to prevailing environmental conditions in the former case. We cannot definitively rule out the role of developmental plasticity in generating the observed results; however, all salamanders were acclimated to laboratory conditions for 30 days prior to experimentation. These data suggest that geographic proximity between populations may limit the amount of local environmental adaptation, although shifts in behavior or differences in trait evolvability could produce similar effects and warrant further investigation. Furthermore, we found evidence for potential thermodynamic constraints on thermal performance in lungless salamanders.

Acknowledgements

286 We thank J. Chamberlain, W. Peterman, and A. Winters for their help in obtaining individuals
287 from the field and their help with laboratory experiments. We also thank two anonymous
288 reviewers for critical comments that greatly improved this manuscript. This work was carried out
289 under research permit number 12-SC00548 from the North Carolina Wildlife Resources
290 Commission, special use permit number FS-2700-4 from the U.S. Department of Agriculture,
291 and Missouri Wildlife Collector's Permit #15203. Financial support was provided by a grant
292 from the U.S. National Science Foundation (DEB-0949038, to M.E.G.) and by the University of
293 Arkansas at Little Rock. The University of Arkansas at Little Rock Institutional Animal Care and
294 Use Committee (IACUC) approved all experimental procedures (protocol: R-09-06)
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438 frog *Limnodynastes peronii*. *J. Exp. Biol.* 204, 4227–4236.

439

440 Table 1. Models and AICc scores used for the thermal performance curve selection for each
441 species.

Model	Formula	<i>P. albagula</i>	<i>P. montanus</i>
Beta	$Y = a * (((b - X) / (b - c)) * (X / c) ^ {c / (b - c)})$	-16.2	-15.6
Polynomial	$Y = a + b * X + c * X ^ 5$	-14.5	-14.0
Gaussian	$Y = a * \exp (-0.5 * ((\text{abs}(X - b)) / c) ^ 2$	-12.4	-15.1
Quadratic	$Y = a * X ^ 2 + b * X + c$	-10.8	-9.8

Table 2. Average and standard deviation for thermal optimum (T_{opt}), maximal performance (P_{max}), and performance breadth (B_{80}) for two populations of each species. 50-year average of annual mean temperature (mean of all weekly mean temperatures; Bio1), and temperature seasonality (standard deviation of the weekly mean temperatures multiplied by 100; Bio4). Temperature data was obtained from the WorldClim data set (Hijmans et al. 2005).

Species	Population	T_{opt}	P_{max}	B_{80}	Bio1	Bio4
<i>P. albagula</i>	NORTH	21.7 ± 2.2	0.09 ± 0.03	11.5 ± 1.6	12.1	935.0
	SOUTH	24.8 ± 1.9	0.13 ± 0.05	9.0 ± 1.6	15.0	797.7
<i>P. montanus</i>	HIGH	22.8 ± 1.0	0.10 ± 0.02	10.7 ± 0.7	7.5	689.8
	LOW	22.6 ± 1.9	0.13 ± 0.04	10.9 ± 1.3	10.8	695.8

482 Figure 1. Estimated population level thermal performance curves of energy assimilation for
483 southern (solid line, solid circles) and northern (dashed line, open circles) latitude populations of
484 *P. albagula*. Light shaded region indicates performance breadth range and dark shaded region
485 indicates overlap in performance breadth ranges between populations. Vertical lines represent
486 thermal optimum for each population.

496 Figure 2. Estimated population level thermal performance curves of energy assimilation for low
497 (solid line, solid circles) and high (dashed line, open circles) elevation populations of *P.*
498 *montanus*. Light shaded region indicates performance breadth range and dark shaded region
499 indicates overlap in performance breadth ranges between populations. Vertical lines represent
500 thermal optimum for each population.

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509 Figure 3. Arrhenius plot of maximal performance (natural log) against the thermal
510 optimum(expressed on an Arrhenius scale, $1/K \times \text{Thermal optimum}$) for *P. albagula* (solid circles)
511 and *P. montanus* (open circles). Regression line (solid line) represents a best-fit line for all
512 individuals. Dashed lines represent 95% confidence intervals.

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514

515 **Vitae**

516

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522

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