

PHYSIOLOGICAL RESPONSES TO TEMPERATURE  
IN THE LIZARD, SCELOPORUS UNDULATUS

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## ABSTRACT

Temperature affects all organisms differently. Physiological processes, such as metabolism, interact with temperature to determine a minimal rate of energy loss. Physiological limits, such as heat and cold tolerances, likely constrain the activity and survival of organisms. Ultimately, these physiological processes and limits determine a species' geographical distribution. Through experiments, I sought to understand specifically how temperature affects the physiology of the lizard *Sceloporus undulatus*. This species is a model organism to answer such questions, as it is geographically widespread species and well-described phylogenetically. In the first experiment, I compared standard metabolic rates of lizards from three locales and interpreted these rates in the context of the metabolic theory of ecology—a set of models that describes the effects of body mass and body temperature on metabolic rate. My findings indicate that metabolic rate increases with body size, but that the exact nature of this relationship depends on temperature; this result contrasts a major assumption of the metabolic theory of ecology, which stresses the need to evaluate this theory through by examining intraspecific variation. In my second experiment, I measured the preferred body temperatures and critical thermal limits of *S. undulatus*. Existing theory indicates that physiological traits associated with temperature may be evolutionary static or labile. By measuring these traits from seven populations, which cover the majority of this species' range, I have provided one of the most comprehensive comparisons of thermal physiology in a single biological species. My results are consistent with the static

view of thermal physiology, suggesting that thermal physiology has not adapted to local conditions in this species.

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CHAPTER 1  
STANDARD METABOLIC RATES OF SCELOPORUS UNDULATUS FROM  
FLORIDA, INDIANA AND NEBRASKA

INTRODUCTION

Studies of metabolism are crucial in understanding the physiological ecology of organisms. Metabolic rate represents an animal's greatest component of its energy budget (Congdon et al. 1982). In ecology, general patterns of metabolism at the organismal level may indicate baseline measures from which to compare energy fluxes within cells, populations, communities or ecosystems (Marquet et al 2004). The recent development of a quantitative theory of metabolic patterns (West et al. 1997; Gillooly et al. 2001) has created some tension between physiologists and ecologists (see the forum on the metabolic theory of ecology in Ecology 2004). Proponents of the metabolic theory of ecology claim that body mass and body temperature explain the vast majority of variation in metabolic rate among organisms, and that these covariations can be described by very simple mathematical relationships. Many physiologists have argued that the metabolic theory is too simple to account for real mechanisms that underlie patterns of metabolic scaling among species and ignores many important sources of variation within species. However, the perspectives of physiologists and ecologists should be complementary. Physiologists strive to understand factors that influence metabolic rates of individuals, which should relate to ecological processes. In a very basic sense, one may describe an ecologist's approach to metabolism as a top-down move toward describing observations;

while physiologists could be similarly described to have a bottom-up approach where specific mechanisms help to describe general observations.

About a decade after the seminal model of West, Brown and Enquist (1997), the debate over their “metabolic theory of ecology” has inspired a closer look at what influences metabolic rate in organisms. Time and again, the original authors and their collaborators have argued the two fundamental patterns of metabolism their theory assumes (West et al. 1997; Brown et al. 2004; Savage 2004). First, the theory assumes that metabolic rate scales with body mass at a power of 0.75 in nearly all organisms (West et al. 1997). Second, the theory assumes a direct link between temperature and metabolism described by the Boltzmann factor (Gillooly et al. 2001). Both of these assumptions have undergone criticisms, due to their excessive conceptual simplicity and poor empirical support (Irlich et al. 2009; van der Meer 2006; Clarke 2006; Etienne et al. 2006; Clarke 2004; Clarke and Frazer 2004; Cyr and Walker 2004). Glazier (2005) and Clarke (2006) noted the plethora of factors that influence metabolism, and its scaling with mass and temperature. These authors repeatedly referred to studies that contrast the predictions of the metabolic theory about allometric scaling (Clarke 2006; Glazier 2005). Clarke and Frazer (2004) and Clarke (2004) argued that the “universal temperature dependence” described by the Boltzmann factor is simply a statistical fit to patterns at the organismal level and do not reflect mechanisms operating at the cellular level. Although the proponents of the metabolic theory argue for the elegance of simplicity, Clarke and Glazier emphasized the need to consider variation at the organismal and population levels for such a theory to have any real value (Clarke 2006; Glazier 2005). In reptiles, for example, factors such as time of day or year (e.g. Niewiarowski and Waldschmidt 1992; Angilletta 2001; Beaupre and Zaidan III 2001; Tsuji 1988a), age (e.g. Angilletta 2001; Beaupre and Zaidan III 2001; McCue and Lillywhite 2002),

and environmental history (e.g. Tsuji 1988b) can cause variation within species. Such studies representing an awareness of differences in physiological performance within and among species may reveal important patterns obscured by interspecific analyses.

This intraspecific study answers questions about temperature and size affecting metabolic rate in a species of lizards, *Sceloporus undulatus*. This species is geographically widespread throughout most of the United States and northern Mexico, and recently has been described through phylogenetic analyses (Leache and Reeder 2002; Leache and Cole 2007; Leache 2009). This species ranges over a substantial latitudinal gradient and exhibits latitudinal patterns of life-history strategies (Niewiarowski et al. 2004; Angilletta et al. 2004; 2006). I compared the thermal sensitivities of metabolic rate among three populations spanning the northern and southern limits of the geographic range. In addressing specific questions, such as effects of temperature and size on metabolism, standard metabolic rate is most appropriate to focus on any physiological effects within a controlled setting. Other methods (e.g. field metabolic rate, resting metabolic rate) may obscure findings due to additional variables that are important in different contexts (Niewiarowski and Waldschmidt 1992; Beyer and Spotila 1994). Pointing toward conservatism amongst populations, my results describe how each temperature separately influences metabolic rate with respect to lizard size. Finally, I consider these results in the context of the metabolic theory of ecology.

## METHODS

### ANIMAL COLLECTION AND CARE

In 2008, I collected lizards from populations of *Sceloporus undulatus* within Floyd County, Indiana (late April, n = 40), Escambia County, Florida (late April, n = 45), and Keith and Garden Counties in Nebraska (n = 45). Lizards from each site were collected by noosing and comprised of a mixture of ages and sexes, excluding neonates. Immediately upon capture, lizards were placed inside of a cloth bag (Hubco, Inc., Hutchinson, Kansas, USA). After I concluded with the collection (3-7 days), lizards were shipped overnight to Indiana State University's animal care facility. Each lizard was removed from the cloth bag and individually housed in a 30-L opaque, plastic terrarium on non-silica-based sand with 3-5 small rocks and one piece of wood for basking and hiding. Lighting was maintained on a 12-hour cycle by fluorescent (48" Reptisun 10.0 UVB™, ZooMed Laboratories, San Luis Obispo, California, USA), dual-bulb shop lights at approximately 30 cm distance. Heating was regulated by using heat tape (Flexwatt™, Calor/Que Ltd., West Wareham, Massachusetts, USA) and a rheostat device (Reptitemp Rheostat Deluxe 500™, ZooMed Laboratories, San Luis Obispo, California, USA). A thermal gradient of 22° – 39°C was maintained for the substrate of each enclosure. Lizards had daily access to water and were offered crickets, *Acheta domestica*, 3-4 days each week.

### MEASUREMENT OF STANDARD METABOLIC RATE

Standard metabolic rate measurements were made using a flow-through respirometry system (TR-3, Sable Systems International, Henderson, Nevada, USA). I measured standard

metabolic rate at two times of day at five temperatures (29°, 36°, 21°, 25°, and 33°C, respectively). Measurements at each temperature were separated by four days permitting lizards to feed. Each lizard was fasted for 48 hours before measure of metabolic rates to ensure lizards were in a post-absorptive state. Lizards were weighed and then placed into a 120-mL chamber at 1500-hours on each day. Chambers were placed into a programmable incubator set at the appropriate test temperature (Model KB115, Binder Inc., Great River, New York, USA) After six hours, to ensure that lizards were habituated to the chambers; the respirometry system began recording the concentrations of CO<sub>2</sub> in each chamber. The air entering the chambers emerged from a purge gas generator (Model 75-45; Parker Hannifin Corp., Haverhill, MA, USA), which removed CO<sub>2</sub> and water. A CO<sub>2</sub> analyzer (Model LI-6251, LI-COR Inc., Lincoln, Nebraska, USA) was used to measure the concentrations of CO<sub>2</sub> in the air leaving each chamber. Baseline recordings were made in an empty chamber before and after each measurement. During measurements, the flow rate of the air was determined by a mass-flow meter (v1.0, Sable Systems International). Prior to the study, the mass-flow meter was calibrated using a mass-flow controller valve (Sidetrak™, Sierra Instruments Inc., Monterey, California, USA). Each day, analyzers were calibrated using standard gases. After two measurements of standard metabolic rate for each lizard (roughly 16 hours each), lizards were removed from the chambers and returned to their terraria. Food and water was provided *ad libitum* prior to fasting for the next period of measurements.

## DATA ANALYSIS

Data were analyzed using the program ExpeData, ver.1.1.7 (Sable Systems International, 2002-5). Each recording of CO<sub>2</sub> production was accepted after subtracting the mean baseline

CO<sub>2</sub> value for each measurement period. Rates of CO<sub>2</sub> production (mL h<sup>-1</sup>) were averaged for the lowest 30 seconds from the final four minutes of each 10-minute recording to give a single estimate of each lizard's standard metabolic rate. (The initial six minutes of the recording was ignored to ensure the system was completely flushed between recordings). Since the air entering each chamber was purged of CO<sub>2</sub> and water, estimates of metabolic rate derived from CO<sub>2</sub> provided a very high signal to noise ratio (see Angilletta 2001).

I used Multivariate Analysis of Variance (MANOVA) for repeated measures to examine the effects of population, sex, body mass, body temperature, and time of day on metabolic rate. Descriptive statistics are reported as mean  $\pm$  95% confidence interval.

## RESULTS

In all three populations (Florida, Indiana and Nebraska), standard metabolic rate increases with body mass ( $MS = 58.12$ ,  $F_{1,111} = 28.86$ ,  $P < 0.000$ ; Table 1), however, the exact relationship depended on temperature ( $F_{4,108} = 6.108$ ,  $P < 0.000$ ; Table 1). Figure 2 demonstrates this effect and shows the scaling relationship for each population. At some temperatures, the allometric scaling exponent varies among populations. Yet, no clear and consistent thermal effect on the mass-scaling exponent was observed for any population (Table 2). As expected, standard metabolic rate increased as temperature increased; the thermal sensitivity of metabolic rate was similar among the three populations (Fig. 1). Additionally, the two recordings for each temperature showed females lizards increased their metabolic rate between the second and third measurement, relative to males (i.e., significant interaction between sex and time; see Table 1). Interestingly, no large and consistent differences in metabolic rate were observed among populations, when metabolic rates were averaged across temperatures and times (Table 1).

Table 1. MANOVA with repeated measures of the effects of size, population, sex, temperature and time on metabolic rate ( $\text{ml CO}_2 \text{ h}^{-1}$ ) of *Sceloporus undulatus*.

<i>Effects</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>Wilk's <math>\lambda</math></i>	<i>P</i>
Between-subjects:					
Log mass (size)	<b>1, 111</b>	<b>58.12</b>	<b>28.86</b>		<b>0.000</b>
Population (pop)	2, 111	1.01	0.50		0.606
Sex	1, 111	4.99	2.48		0.118
Pop x sex	2, 111	0.96	0.48		0.622
Within-subjects:					
Temperature (temp)	4, 108			0.402	0.807
Temp x size	<b>4, 108</b>			<b>6.108</b>	<b>0.000</b>
Temp x pop	8, 216			1.887	0.063
Temp x sex	4, 108			1.897	0.116
Temp x pop x sex	8, 216			0.614	0.766
Time period (time)	1, 111			0.327	0.569
Time x size	1, 111			1.823	0.180
Time x pop	2, 111			0.007	0.993
Time x sex	<b>1, 111</b>			<b>5.486</b>	<b>0.021</b>
Time x pop x sex	2, 111			0.999	0.372
Temp x time	4, 108			0.712	0.585
Temp x time x size	4, 108			0.935	0.447
Temp x time x pop	<b>8, 216</b>			<b>2.140</b>	<b>0.033</b>
Temp x time x sex	<b>4, 108</b>			<b>4.350</b>	<b>0.003</b>
Temp x time x pop x sex	8, 216			1.095	0.368

Note. Significant effects are designated by bold typeface.

## DISCUSSION

This study did not find any variation in standard metabolic rate across populations, but such variation has been noted in most major groups of vertebrate organisms (Garland and Adolph 1991; Table 1). Within one clade of *Sceloporus undulatus*, Angilletta (2001) noted variation of seasonal resting metabolic rate from New Jersey and South Carolina. Tsuji (1988a) also noted seasonal variation in metabolic rate of *Sceloporus occidentalis* from California and Washington. Beaupre and colleagues (1993) did not observe variation between two populations of *Sceloporus merriami* at two different elevations within Big Bend National Park, Texas. However, Beaupre (1995) sampled *Crotalus lepidus* from the same two locations in Big Bend National Park, TX as the study with *S. merriami*, and did detect differences in resting metabolic rate. One population was in an area lower in elevation and hotter than the other, limiting diurnal surface thermoregulation due to high temperatures. Beaupre concluded that the thermal environment affects growth rate and body size, and suspected energetics as key mediator of this effect (Beaupre 1995). These two studies illustrate the importance of intraspecific comparisons. Additionally, Beaupre and Zaidan (2001) detected differences in metabolism of *Crotalus horridus* between populations in Arkansas and Virginia, roughly 1500km apart. While a separation of only 30 km was enough to detect variation in resting metabolic rate for two species, *Crotalus lepidus* and *Crotalus mollosus* (Beaupre et al. 1993). McCue and Lillywhite (2001) did not find a difference between two Florida populations of *Agkistrodon piscivorous subsp.*, but noted variation exists among other populations in the species' range. In a relatively broad study spanning a 1500km gradient, Lindgren and Laurila (2009) found no variation in the metabolism of tadpoles (*Rana temporaria*).

By comparing my results to those of previous studies (Angilletta 2001; Hughes et al. 1982), I conclude that thermal effects on standard metabolic rate vary among populations of *S. undulatus*. Previous studies uncovered the existence of metabolic plateaus, in which metabolic rate remains relatively stable over some range of high temperatures. For populations from New Jersey and South Carolina, Angilletta (2001) detected a metabolic plateau between 30° and 36°C or between 33° and 36°C, depending on the season. Similarly, Hughes and colleagues (1982) detected a metabolic plateau between 30° and 36°C in *S. undulatus* from Kansas. In contrast to these studies, I did not detect a metabolic plateau within any range of temperatures (Fig. 1). Instead, I observed an accelerating (i.e., exponential) increase in metabolic rate with standard metabolic rate. Because I observed no metabolic plateau, thermal sensitivities of metabolic rate exceeded those recorded previously. The mean  $Q_{10}$  for the three populations was  $\approx 4.5$ . Angilletta (2001) obtained a  $Q_{10}$  value near 2.0, which is closer to average values ( $Q_{10} \approx 2.0-3.0$ ) of vertebrates (Bennett and Dawson 1976, K. Schmidt-Nielsen 1975, Andrews and Pough 1985).

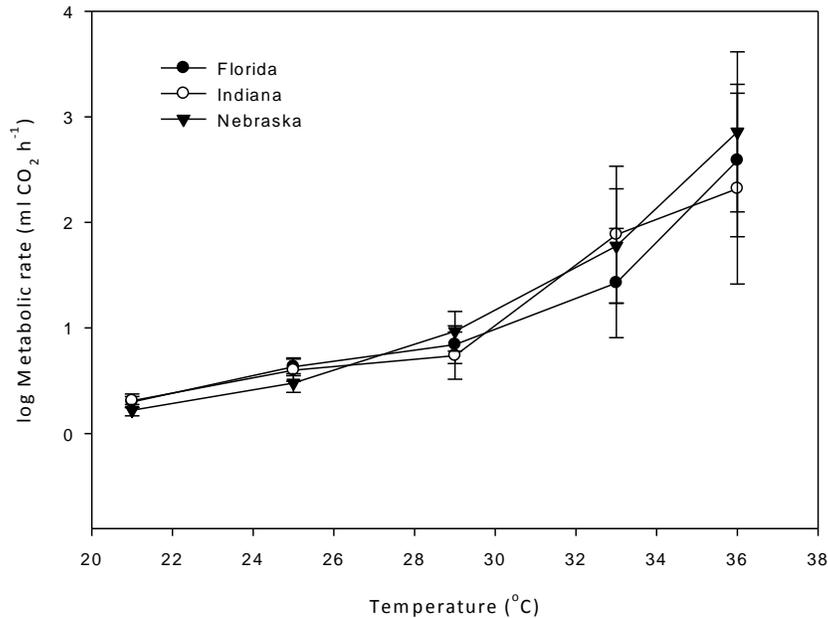


Figure 1. Thermal sensitivities of metabolic rate ( $\text{ml CO}_2 \text{ h}^{-1}$ ) in lizards from Florida (filled circles), Indiana (open circles), and Nebraska (inverted triangles) populations of *Sceloporus undulatus*. No significant variation was observed amongst populations ( $P > 0.05$ ).

Differences in thermal scaling among these studies might have resulted from errors in measurement or factors associated with populations. In the latter case, both genetic and environmental factors could have influenced the thermal scaling of metabolic rate. Beyer and Spotila (1994) showed that the metabolic rate of *S. undulatus* responds to seasonal acclimatization and to laboratory acclimation. For three seasons, lizards were collected from a New Jersey population and resting metabolic rate was measured within two days. Following these measurements, lizards were allowed to acclimate to laboratory conditions at roughly  $33^\circ\text{C}$  for two weeks before resting metabolic rate was measured again. Beyer and Spotila (1994) reported significant variation between acclimatized and acclimated resting metabolic rate of *S.*

*undulatus*, as well as detecting seasonal effects only from acclimatized individuals. Given the various sampling and maintenance protocols used in studies of metabolism, it is not unreasonable to assume that environmental effects can account for the discrepancies between my results and those of previous studies. However, a common garden experiment (e.g., Ferguson and Talent 1992) would be required to rule out genetic effects.

In the three populations studied here, temperature and size had an interactive effect on standard metabolic rate (Fig. 2). The interaction of temperature and size has been observed somewhat before in *S. undulatus*, from different populations. Angilletta (2001) evaluated seasonal effects on resting metabolic rate in New Jersey and South Carolina populations, and measured the temperature and age effects; where age corresponded to size. Interestingly, significant differences in resting metabolic rate due to temperature and age were only detected in the fall (Angilletta 2001). Differences between this study and Angilletta (2001) may be due to genetic and environmental differences (Leache and Reeder 2002); however, this does not seem most likely, because variation in metabolic rate between acclimatized and acclimated individuals has been detected previously in this species (Beyer and Spotila 1994).

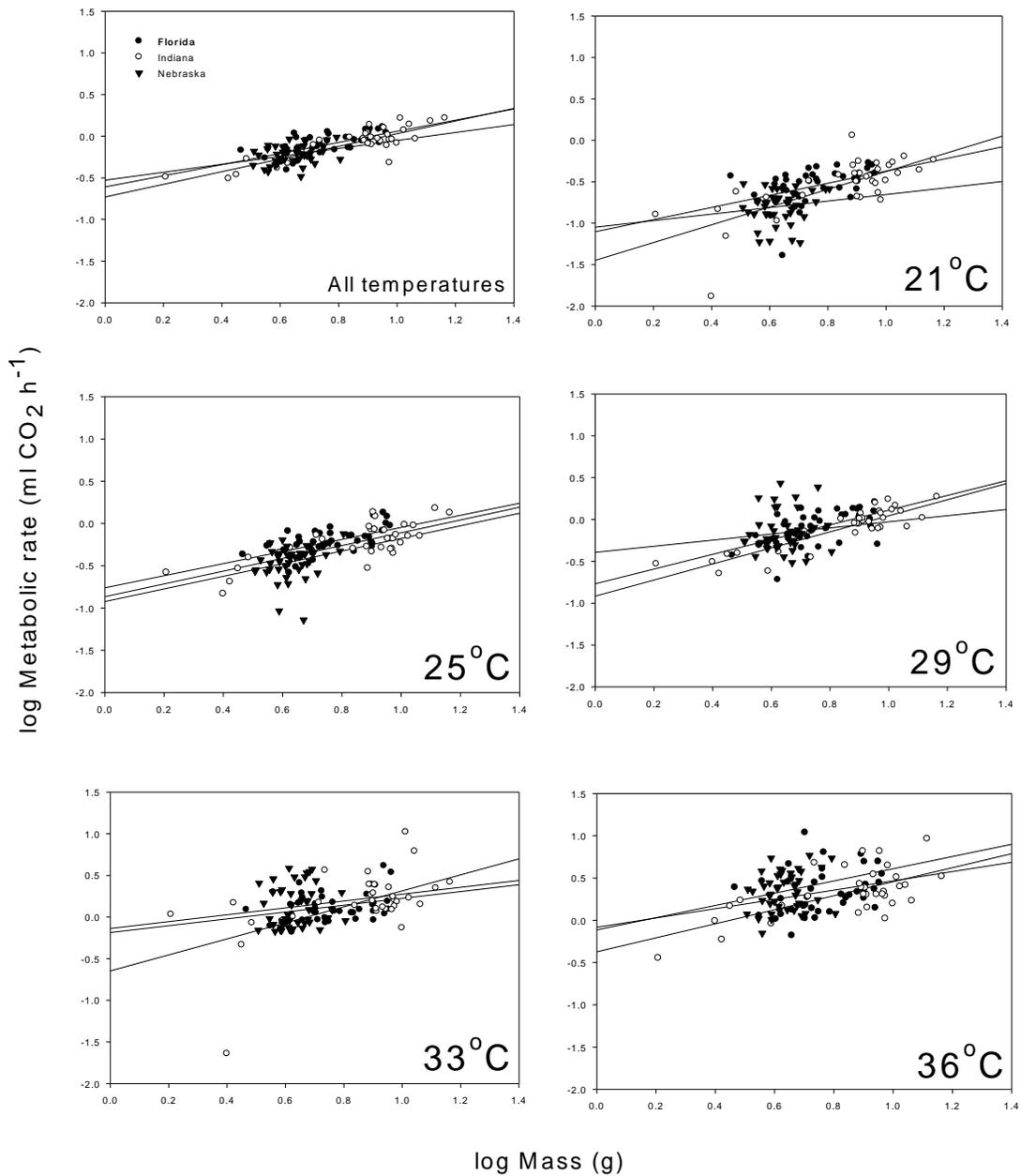


Figure 2. Relationship between body mass and metabolic rate (ml CO<sub>2</sub> h<sup>-1</sup>) of lizards in Florida, Indiana, and Nebraska populations of *Sceloporus undulatus*. Metabolic rates are the average rates from both measured time periods at each temperature. Slopes of the relationship between body mass and metabolic rate are shown for each population (Florida – filled circles, Indiana – open circles, Nebraska – inverted triangles).

Table 2. Mean allometric scaling exponents ( $b$ ) for SMR at five temperatures for three populations of *Sceloporus undulatus*.

Population	21 °C	25 °C	29 °C	33 °C	36 °C	All
Florida	0.730	0.715	0.879	0.410	0.549	0.667
Indiana	1.070	0.757	0.959	0.962	0.829	0.760
Nebraska	0.390	0.747	0.365	0.411	0.724	0.470

The relationship of temperature and size with metabolism in this study is not consistent with predictions of the metabolic theory of ecology. The metabolic theory of ecology simply states that metabolic rate scales with body temperature according to the Boltzmann factor. The scaling observed in this study followed an exponential form, which is similar to that assumed by the metabolic theory of ecology. However, the metabolic theory of ecology emphasizes that organisms follow a  $3/4$ -power allometric relationship between body size and metabolic rate. My data demonstrates variation in the scaling of metabolic rate with body size, which is not predicted by the metabolic theory. In particular, body temperature affected the scaling of metabolic rate with body mass (Fig. 2). While patterns adhering to metabolic theory of ecology predictions do exist in reptiles (e.g. Maxwell et al. 2003; Kratochvil and Frynta 2003; McCue and Lillywhite 2001), there are also considerable deviations from predicted patterns (e.g. Glazier 2009b; Zaidan 2003; Beaupre and Zaidan 2001, Thompson et al. 1995; Cruz-Neto and Abe 1994; Beaupre et al. 1993). For example, allometric scaling exponents varied across the range of temperatures in *Crotalus horridus* from 0.727 – 0.825, but had a mean = 0.78 (Beaupre and Zaidan 2001). When curves are generalized by a simple mean, one loses sight of the actual relationship between body size and metabolic rate within species. Generalizing this nonlinear pattern in an attempt to fit into an extremely broad model would likely accomplish very little by ignoring a host of factors, including population, diel, and seasonal effects on metabolism. In the case of this study, allometric exponents vary among populations, ranging from 0.36 – 1.07 across

the five temperatures (Table 2). The mean of these values obscures the significant variation in scaling within this species and should not be used to generalize patterns of metabolism among reptiles or ectotherms. Not only is intraspecific variation in allometric scaling ignored by authors of the metabolic theory of ecology, but so is interspecific variation within vertebrates and invertebrates (e.g. Glazier 2009a; Glazier 2009b; Sieg et al. 2009; Seibel 2007; Clarke and Johnston 1999; Berrigan and Partridge 1997; Lighton and Fielden 1995). Variation in metabolic rate can be associated with the life-history of the organism and can vary across taxonomic levels (Secor and Diamond 1998; Killen et al. 2010). As Bennett and Dawson (1976) noted, snakes may alter metabolic rate by several times due to feeding and ecology. Using a simple model, such as the one at the heart of the metabolic theory of ecology, to describe organismal tendencies could be helpful in limited forums, but creates a greater risk of obscuring meaningful patterns and misleading future research.

CHAPTER 2  
EVOLUTIONARY CHARACTERISTICS OF THERMAL PHYSIOLOGY IN  
SCELOPORUS UNDULATUS

INTRODUCTION

The survival and reproduction of every organism depends strongly on temperature (Huey & Stevenson 1979; Huey 1982; Angilletta et al. 2002a). Therefore, it should come as no surprise that most organisms regulate their body temperature through behavior, physiology and morphology (Angilletta 2009). A collection of theoretical models (e.g. Huey and Slatkin 1976; Hertz et al. 1983; Martin and Huey 2008) and conceptual reviews (e.g. Huey 1982; Huey and Bennett 1987; Angilletta et al. 2002b; 2006a; Seebacher 2005) have led to more refined views of the factors that determine strategies of thermoregulation. In particular, Huey and Slatkin (1976) developed a model of optimal thermoregulation from which a key prediction was made: individuals from cooler environments should have lower mean body temperatures than individuals from warmer environments. The prediction stems from the fact that both the cost and the constraint of thermoregulation should increase as the mean operative environmental temperature drops below the preferred body temperature. Therefore, individuals in cold (or hot) environments would have less access to preferred microclimates and would expend more energy locating these microclimates (Huey and Slatkin 1976; Huey 1982). The predicted body temperatures could result in two ways: (1) individuals in cooler environments can maintain lower body temperatures when they are active, or (2) individuals in cooler environments can maintain

similar body temperatures but do so for shorter durations (see Angilletta et al. 2006a). In the first case, one might expect that individuals from colder environments prefer lower body temperatures. In the second case, individuals would prefer the same body temperature but would differ in the duration of activity (e.g., individuals in cooler environments may be active for less time each day or each year).

Imperfect thermoregulation imposes selective pressures on the thermal sensitivity of physiological performance. This thermal sensitivity is generally quantified in terms of thermal optima and critical thermal limits. The current theory predicts that genotypes from cold environments are predicted to tolerate low body temperatures better and high body temperatures worse than genotypes from hot environments (reviewed by Angilletta et al. 2006a; Angilletta 2009). This prediction leads to the hypothesis that indices of thermal tolerance should vary predictably along latitudinal or altitudinal clines. Specifically, populations at high latitudes or altitudes should comprise individuals that tolerate cold better and heat worse than do populations at low latitudes or altitudes.

Evolutionary patterns of thermal physiology may be best understood by studying geographically widespread species. Such species encounter thermal clines that should impose the selective pressures described by current models. Indeed, partial support for the predicted patterns of thermal physiology comes from interspecific comparisons along latitudinal clines (e.g. Tsuji 1988b; Andrews 1998; Youssef et al. 2008; Rodrigues-Serrano et al. 2009). Surprisingly, relatively few studies of intraspecific variation have been conducted, and these few studies suggest that thermal physiology can sometimes be evolutionarily conservative (reviewed by Angilletta 2009). This perspective from intraspecific comparisons might be misleading when one considers that such studies often focus on few populations and typically lack a phylogenetic

perspective (e.g. Angilletta 2001; Angilletta et al. 2002a; Crowley 1985; Winne and Keck 2005). In such cases, broadening our comparisons to include additional populations, sampled in a phylogeographic context, might better resolve patterns of thermal physiology. For example, Crowley (1985) noted the conservatism of thermal physiology in a lizard, *Sceloporus undulatus*, from two western populations (Colorado and New Mexico). Almost 15 years later, Angilletta and colleagues (2002a) conducted similar measurements in a population of *S. undulatus* in South Carolina and observed differences in thermal physiology compared to the population studied by Crowley.

Recognizing a paucity of studies for widespread species, I conducted a large-scale study of intraspecific variation in the lizard, *Sceloporus undulatus*. *Sceloporus undulatus* constitutes a single biological species comprising four distinct clades with limited gene flow (Leache and Reeder 2002; Leache and Cole 2007; Leache 2009). Using phylogeographic information, I chose seven locales (and multiple populations) to include in my study of thermal physiology. I measured the preferred body temperature and critical thermal limits of lizard from each locality. This study represents the first comprehensive look at a species' thermal physiology throughout the majority of its geographic range. My findings support the traditional view that this species possess a relatively conservative thermal physiology, which fails to reflect the predicted pattern of geographic variation.

## MATERIALS AND METHODS

### ANIMAL COLLECTION AND CARE

In the springs (May) and summers (July/August) of 2007 and 2008, I collected *Sceloporus undulatus* from 26 populations (Table 1). These populations were sampled from the following areas: Tonto National Forest in Gila and Pinal Counties, Arizona); Canyon Rims Recreation Area in San Juan County, Utah; Wharton State Forest in Burlington and Camden Counties, New Jersey; Sumter National Forest in Edgefield County, South Carolina; three populations in Floyd County, Indiana; Blackwater State Forest in Escambia County, Florida; and three populations in Keith and Garden Counties in Nebraska. Approximately 40 lizards from each population were collected by noosing; these lizards comprised of a mixture of ages and sexes. Immediately upon capture, each lizard was placed inside of a cloth bag (Hubco, Inc., Hutchinson, Kansas, USA). After I concluded with collections from each population (3-7 days), lizards were transported to an animal care facility at Indiana State University. Here, lizards were housed individually in 6 L plastic terraria with a substrate of sand. A few small rocks and a piece of wood were provided to facilitate basking and hiding. Fluorescent lighting (40 W, 48" Reptisun 10.0 UVB™, ZooMed Laboratories, San Luis Obispo, California, USA) was provided for 12 hours each day. One side of the terrarium was heated by Flexwatt™ tape ( Calor/Que Ltd., West Wareham, Massachusetts, USA), which was controlled by a rheostat (Reptitemp Rheostat Deluxe 500™, ZooMed Laboratories, San Luis Obispo, California, USA). A surface temperature gradient of 22°–39°C existed within each enclosure. Lizards had access to water daily and were offered domestic crickets (*Acheta domestica*) 3-4 times per week. By adapting the methods of Angilletta (2001), lizards from AZ and UT were measured simultaneously in the laboratory

during June 2007, as were NJ and SC in August 2007, FL and IN in June 2008, and NE in August 2008. After a two week acclimation period, preferred body temperatures were measured. Critical thermal limits were measured approximately one month following.

Table 1. Demographics and collection information for seven populations of *Sceloporus undulatus*.

Locality	Collection date	Males	Females	Unsexed juveniles
Arizona	May, 2007	22	15	—
Florida	May, 2008	25	20	—
Indiana	April/May, 2008	22	14	—
Nebraska	July/August, 2008	23	21	—
New Jersey	July/August, 2007	12	17	12
South Carolina	August, 2007	14	11	14
Utah	May, 2007	20	15	7

## PREFERRED BODY TEMPERATURE

I measured the preferred body temperatures of lizards from each population. I constructed a thermal gradient in a lab at an ambient temperature of 19.0°-23.0°C using eight, 85 L (Model 1865, Sterilite Corp., Townsend, Massachusetts, USA) containers. Cardboard barriers were used between each container to eliminate territorial displays between lizards that could influence the area a lizard chooses to bask. Each container held approximately 3 cm of sand as a substrate. A 250 W ceramic infrared heat bulb (Pearlco™, RAM Network, Reseda, California, USA) was suspended above one end of the container. Operative temperatures within the gradients were adjusted to range from 24.0 to 41.0°C, using a rheostat (Reptitemp Rheostat Deluxe 500™, ZooMed Laboratories, San Luis Obispo, California, USA); operative temperatures were measured using a hollow copper model connected to an electronic thermometer (Model HHM31,

Omega Engineering Inc., Stamford, Connecticut, USA). Fluorescent lighting was provided for 12 hours each day, in addition to overhead room lighting for 8 hours daily. Lizards were placed inside the gradient between 1500 hours and 1600 hours, and then removed 24 hours later. Two measurements of body temperature were made daily (0800-0900 hours and 1500-1600 hours) using a quick-reading cloacal thermometer (Miller & Weber, Inc., Queens, New York, USA).

### CRITICAL THERMAL LIMITS

I determined the critical thermal minimum and the critical thermal maximum by following the methods commonly used for lizards (e.g., see Angilletta et al. 2002a). To determine critical thermal maximum of juveniles and adults, I placed each lizard in a 0.47 L container, (Ziploc® Twist ‘n Loc®, SC Johnson, Racine, WI, USA) and then floating in a water bath (Fisher-Scientific® IsoTemp® 228, Thermo Fisher Scientific Inc., Pittsburgh, PA, USA) at 50°C. This arrangement caused lizards to heat by approximately 1°C per minute. During warming, I assessed the lizard’s righting response several times per minute. If an individual could not right itself on two successive assessments, I considered its body temperature to equal the critical thermal maximum. I used a quick-reading cloacal thermometer (Miller & Weber, Inc., Queens, New York, USA) to measure this temperature at the end of each trial.

A similar protocol was used for determining the critical thermal minimum. Lizards were placed in a 0.47 L container, (Ziploc® Twist ‘n Loc®, SC Johnson, Racine, WI, USA) set into a container (63 L (Model 1758, Sterilite Corp., Townsend, Massachusetts, USA) of ice. This arrangement caused lizard to cool by approximately 1°C per minute. Righting response was assessed as described above. The body temperature at which righting response was lost was considered the critical thermal minimum.

## STATISTICAL MODELING

I used an information-theoretic approach to identify the most likely statistical models to describe the variations in preferred body temperature, critical thermal minimum, and critical thermal maximum. A set of candidate models were generated including combinations of the following independent variables and their interactions: snout-vent length, population, and sex. Each model was fit to the data using the General Linear Modeling module of Statistica 6.0 (Statsoft 2002). For each model, I calculated the second-order Akaike Information Criterion ( $AIC_c$ ) and the Akaike weight (Anderson and Burnham 2002). Models were ranked according to  $AIC_c$  to identify those models most worthy of consideration.

## RESULTS AND DISCUSSION

The most likely model of preferred body temperature included snout-vent length, sex, and time of day as independent variables but excluded an effect of population (Table 1). This model was only slightly more likely than a model that included only the effect of time of day. Thus, time of day clearly impacted preferred body temperature, with lizards preferring higher body temperatures in the afternoon than they did in the morning (Fig. 1). Interestingly this diel effect on preferred body temperature was not observed in a previous study of only two populations of *Sceloporus undulatus* (Angilletta 2001). Yet clear diel effects on preferred body temperature have been observed in other species of lizards (e.g., see Angilletta et al. 1999). Models that included population as an independent variable were extremely unlikely, implying that preferred body temperature has been conserved throughout the geographic range despite very different environmental conditions among populations (see Fig. 1). Diel pattern of this nature are less

extreme but reminiscent of those contemplated by Regal (1967), which he described as voluntary hypothermia. Regal noted that individuals of *Sceloporus magister* remained active for days, but at temperatures less than 20°C and clearly indicated no correlation between thermoregulation and photophase. Furthermore, Tracy and colleagues (2005) proposed that an herbivorous diet triggered voluntary hypothermia in the lizard, *Dipsosaurus dorsalis*. These patterns likely have a different cause than the pattern revealed by my study, because I found that preferred body temperature was associated with the photoperiod and I kept lizards on a diet of crickets throughout the study.

Previous studies also failed to uncover any significant variation in preferred body temperature between populations. For instance, Angilletta (2001) noted preferred body temperatures of *S. undulatus* from New Jersey and South Carolina to be 32.8°C and 32.9°C, respectively. The mean preferred body temperatures measured in this study (31-32°C; Table 8) were similar to, but slightly lower than, those reported previously. Nevertheless, variation in preferred body temperature among populations seems trivial compared to the variation in mean environmental temperature (ranging from 9.8°C to 19.2°C among the populations studied here; Angilletta et al. 2006b). This stability of thermal preference suggests that *S. undulatus* deals with geographic variation in its thermal environment by adjusting the timing of activity rather than its body temperature during activity (Sears and Angilletta 2004). Furthermore, one might expect that physiological processes proceed most rapidly at temperatures near the preferred body temperature (Huey and Bennett 1987). Indeed, the rates of energy assimilation by lizards from New Jersey and South Carolina were maximal at a body temperature very close to the preferred body temperatures recorded in this study (Angilletta 2001). A less likely alternative is that

thermal physiology varies among population but that no genetic variation in preferred body temperature exists.

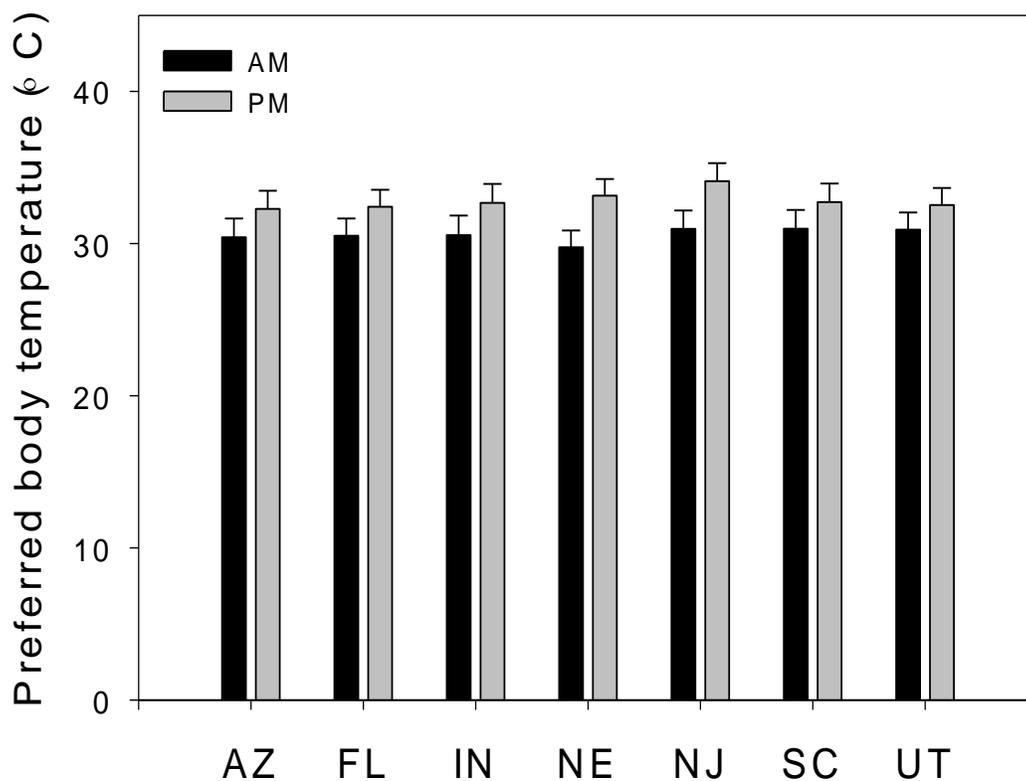


Figure 1. Preferred body temperatures of *Sceloporus undulatus* from seven populations.

Darker-colored bars represent morning measurements and lighter-colored bars represent afternoon measurements.

Table 2. Comparison of statistical models for preferred body temperature describing the relationships among population, time, sex and snout-vent length. Bold typeface denotes the most likely model in the set.

<i>Models</i>	<i>RSS</i>	<i>Variance</i>	<i>K</i>	<i>AIC<sub>c</sub></i>	<i>Δ<sub>i</sub></i>	<i>w<sub>i</sub></i>
L, P, S, T, L*P, L*S, P*S, L*P*S, P*T, S*T, P*S*T	5795.46	24.35	56	907.10	110.80	0.000
L, P, S, T, P*S, P*T, S*T, P*S*T	6156.50	25.87	31	845.84	49.54	0.000
P, S, T, P*S, P*T, S*T, P*S*T	6440.32	27.06	29	851.30	55.01	0.000
L, P, T, P*T, L*P	6129.87	25.76	29	839.55	43.25	0.000
L, P, T, P*T	6272.81	26.36	17	815.45	19.15	0.000
P, T, P*T	6540.54	27.48	15	820.78	24.48	0.000
L, S, T, L*S	6296.11	26.45	9	798.34	2.04	0.167
<b>L, S, T, S*T</b>	<b>6356.26</b>	<b>26.71</b>	<b>7</b>	<b>796.30</b>	<b>0.00</b>	<b>0.464</b>
S, T, S*T	6600.27	27.73	5	801.04	4.74	0.043
T	6603.79	27.75	3	797.01	0.71	0.326

L = snout-vent length; P= population; S = sex; T = time of day

Table 3. General linear model relating snout-vent length and sex to preferred body temperature. Bold typeface denotes  $P < 0.05$ .

<i>Effects</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P</i>
Between-subjects:				
<b>snout-vent length (SVL)</b>	<b>1</b>	<b>101.994</b>	<b>6.317</b>	<b>0.013</b>
sex	1	14.288	0.885	0.348
Within-subjects:				
time of day (time)	1	5.851	0.494	0.483
time x SVL	1	0.823	0.069	0.792
time x sex	1	1.520	0.128	0.721

Despite the stability of preferred body temperature throughout the range, lizards in northern and southern populations undoubtedly experience differences in extreme temperatures during inactivity. Extreme body temperatures might impose selection on cold and heat tolerances, which would be reflected as geographic variation in the critical thermal limits of performance. Therefore, I expected critical thermal limits to vary among populations, such that lizards from northern population had lower critical thermal minima and lower critical thermal maxima than lizards from southern populations. Both variations in the critical thermal maximum and the critical thermal minimum were best described by a model that included snout-vent length, population and the interaction of these variables (Tables 5 and 7). The interaction term indicated that snout-vent length affected the critical thermal limits in some populations but not in others. When snout-vent length did affect the critical thermal limits, larger lizards had higher critical thermal maxima and lower critical thermal minima (Fig. 2). Consequently, the variation in critical thermal limits among populations was greatest for small lizards (Fig. 2). Nevertheless, the pattern of variation bore no resemblance to the predicted pattern. For example, lizards from the relatively warm environment of South Carolina had the lowest critical thermal minimum. Overall, the mean critical thermal limits did not differ by more than 2°C between populations. Thus, the large variation in environmental temperatures was not reflected in the small variation in cold and heat tolerances. Possibly, critical thermal minima did not vary among populations, because I sampled population at times of the year when these populations do not experience extremely low temperatures. However, I expected some variation in the critical thermal maximum in summer based on the variation in maximal environmental temperatures during this season.

Table 4. Comparison of statistical models for critical thermal minimum describing the relationship among population, sex and snout-vent length. Bold typeface denotes the most likely model in the set

<i>Models</i>	<i>RSS</i>	<i>Variance</i>	<i>K</i>	<i>AIC<sub>c</sub></i>	<i>Δ<sub>i</sub></i>	<i>w<sub>i</sub></i>
L, P, S, L*P, L*S, P*S, L*P*S	395.2	1.89	29	219.72	68.9	0.000
L, P, S, P*S	469.52	1.94	16	194.81	44	0.000
P, S, P*S	547.58	2.26	15	229.74	78.92	0.000
<b>L, P, L*P</b>	<b>456.36</b>	<b>1.63</b>	<b>15</b>	<b>150.81</b>	<b>0</b>	<b>1.000</b>
L, P	493.5	2.04	9	191.22	40.41	0.000
P	568.4	2.35	8	223.26	72.44	0.000
L, S, L*S	527.51	2.18	5	198.83	48.01	0.000
L, S	540.98	2.24	4	202.84	52.03	0.000
S	618.65	2.56	3	233.24	82.43	0.000

L = snout-vent length; P= population; S = sex

Table 5. General linear model relating population and snout-vent length to the critical thermal minimum. Bold typeface denotes  $P < 0.05$ .

<i>Effects</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P</i>
<b>population</b>	<b>6</b>	<b>6.0686</b>	<b>2.9256</b>	<b>0.009136</b>
<b>snout-vent length (SVL)</b>	<b>1</b>	<b>20.261</b>	<b>9.7675</b>	<b>0.002016</b>
<b>population x SVL</b>	<b>6</b>	<b>6.1907</b>	<b>2.9844</b>	<b>0.008016</b>

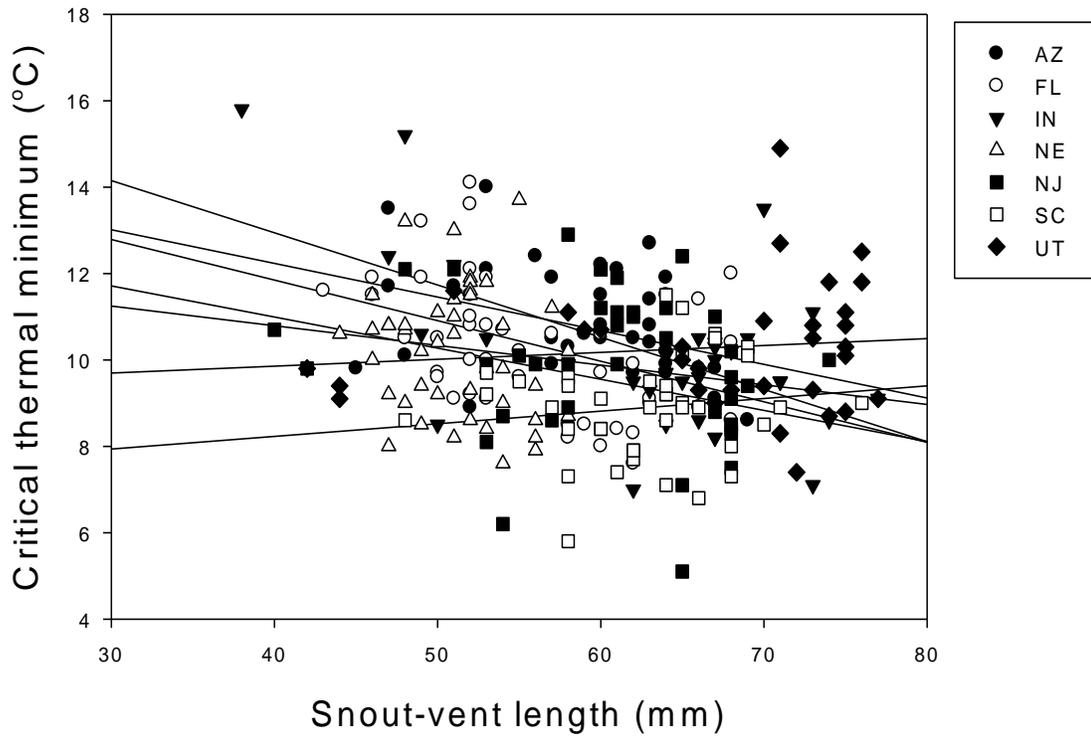


Figure 2. The interaction between snout-vent length and population on critical thermal minimum in seven populations of *Sceloporus undulatus*. Filled circles represent individuals from Arizona, open circles represent Florida, inverted-filled triangles represent Indiana, open triangles represent Nebraska, filled squares represent New Jersey, open squares represent South Carolina, and filled diamonds represent Utah.

Table 6. Comparison of statistical models for critical thermal maximum describing the relationship among population, sex and snout-vent length. Bold typeface denotes the most likely model in the set.

<i>Models</i>	<i>RSS</i>	<i>Variance</i>	<i>K</i>	<i>AIC<sub>c</sub></i>	<i>Δ<sub>i</sub></i>	<i>w<sub>i</sub></i>
L, P, S, L*P, L*S, P*S, L*P*S	256.99	1.07	29	82.70	26.27	0.000
L, P, S, P*S	280.23	1.17	16	71.64	15.20	0.000
P, S, P*S	294.51	1.23	15	81.26	24.83	0.000
<b>L, P, L*P</b>	<b>265.56</b>	<b>1.11</b>	<b>15</b>	<b>56.43</b>	<b>0.00</b>	<b>0.942</b>
L, P	287.45	1.20	9	62.08	5.65	0.056
P	299.03	1.25	8	69.40	12.97	0.001
L, S, L*S	419.38	1.75	5	144.21	87.78	0.000
L, S	420.07	1.75	4	142.52	86.09	0.000
S	430.54	1.79	3	146.36	89.93	0.000

L = snout-vent length; P= population; S = sex

Table 7. General linear model relating population and snout-vent length to the critical thermal maximum. Bold typeface denotes  $P < 0.05$ .

<i>Effects</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P</i>
<b>population</b>	<b>6</b>	<b>3.824</b>	<b>3.139</b>	<b>0.005686</b>
snout-vent length (SVL)	1	0.762	0.625	0.429937
<b>population x SVL</b>	<b>6</b>	<b>3.648</b>	<b>2.995</b>	<b>0.007844</b>

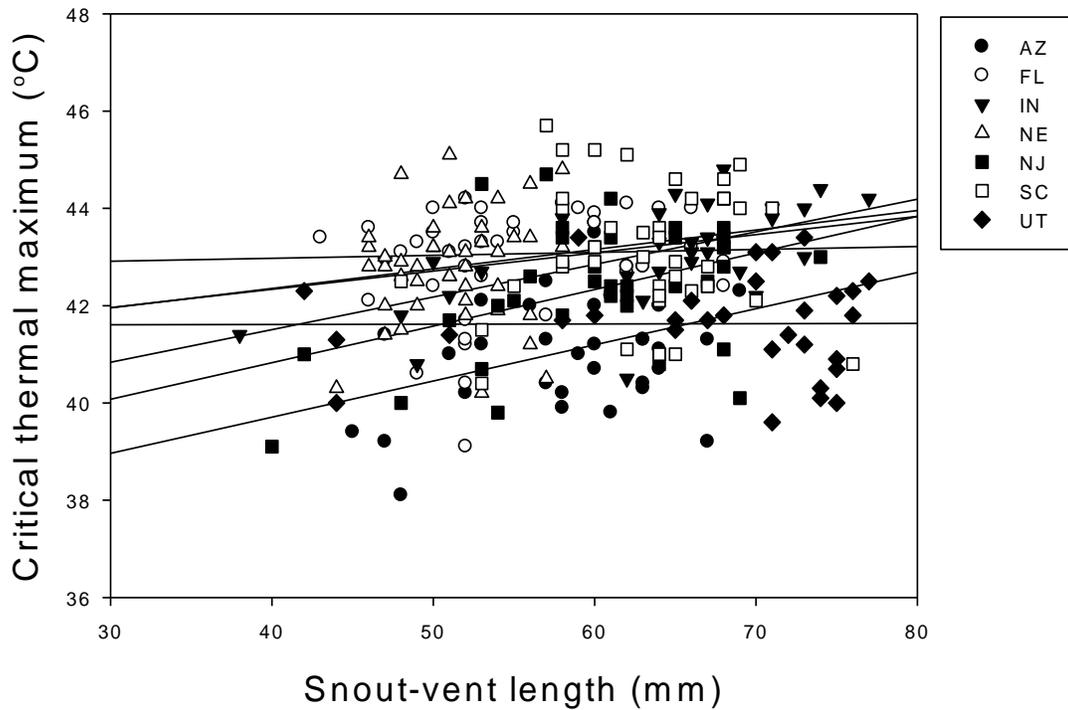


Figure 3. The interaction between snout-vent length and population on critical thermal maximum in seven populations of *Sceloporus undulatus*. Filled circles represent individuals from Arizona, open circles represent Florida, inverted-filled triangles represent Indiana, open triangles represent Nebraska, filled squares represent New Jersey, open squares represent South Carolina, and filled diamonds represent Utah.

By sampling many populations of a geographically widespread species, I sought to determine whether thermal physiology was static or labile (Hertz et al. 1983). Coming from a widely-distributed genus, *S. undulatus* is itself a geographically widespread species, and thus makes an excellent model organism for addressing such a theoretical question. Overall, the preferred body temperatures and critical thermal limits of this species appear to be evolutionarily conserved, such that little variation in these traits exists throughout the species' range. This finding shows that individuals from cooler environments do not necessarily have to have lower preferred body temperatures. Access to preferred temperatures, though limited annually, may be sufficient for an organism with a conservative thermal physiology to persist. Likewise, the evolution of cold and heat tolerances does not seem necessary for populations to persist at the northern and southern edges of the range, respectively. Thus, my study confirms the conclusions drawn from interspecific comparisons and less extensive intraspecific comparisons of sceloporine lizard: thermal preferences and thermal physiology are evolutionarily conserved among these lizards. Future work should focus on the ecological consequences of this conservatism, including the potential impacts of anthropogenic climate change.

Table 8. Mean preferred body temperature (PBT) with standard deviation, mean critical thermal minimum ( $CT_{min}$ ) and maximum ( $CT_{max}$ ) with standard deviation, and set-point ranges for seven populations of *Sceloporus undulatus*. Numbers in parentheses represent the number of individuals used for each experiment.

<i>Population</i>	<i>PBT</i>	<i>Set-point range</i>	<i>CT<sub>min</sub></i>	<i>CT<sub>max</sub></i>
Arizona	31.3°C ± 2.0°C (36)	28.5°C – 34.0°C	10.8°C ± 1.3°C (37)	42.8°C ± 1.2°C (36)
Florida	31.5°C ± 3.6°C (42)	26.2°C – 35.9°C	10.4°C ± 1.5°C (42)	42.9°C ± 1.1°C (42)
Indiana	31.6°C ± 3.3°C (33)	27.3°C – 34.8°C	10.1°C ± 2.0°C (32)	43.1°C ± 1.0°C (32)
Nebraska	31.5°C ± 3.2°C (44)	27.2°C – 35.3°C	10.2°C ± 1.5°C (44)	42.8°C ± 1.1°C (44)
New Jersey	32.5°C ± 3.2°C (41)	28.5°C – 36.6°C	9.9°C ± 1.8°C (40)	42.4°C ± 1.3°C (40)
South Carolina	31.6°C ± 2.3°C (39)	29.0°C – 35.2°C	8.9°C ± 1.2°C (39)	43.1°C ± 1.3°C (39)
Utah	31.7°C ± 2.2°C (42)	28.6°C – 34.4°C	10.2°C ± 1.6°C (38)	41.7°C ± 1.0°C (36)

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