

**Ecology and Evolution of Geographic Range Size Variation
in North American Plethodontid Salamanders:
Perspectives from Thermal Physiology**

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DEDICATION

*For my mother, Judy Ann Markle. Thank you for your love and support,
and for instilling in me your strength, wisdom, and tenacity.*

ABSTRACT

Species exhibit remarkable variation in geographic range size. Understanding the causes of this variation is fundamental to the fields of ecology and evolution, and is central to understanding how species will respond to rapid climate change. Using eastern North America's species-rich salamander fauna, I explore whether seasonal variation in temperature and climatic tolerance evolution underlie geographic range size variation (as per the climate variability hypothesis). First, I determined critical thermal maximum (CTMax) and critical thermal minimum (CTMin) temperatures of 18 salamander species. I then tested for relationships between thermal tolerances, seasonality, and geographic range size. Localities with greater annual temperature ranges (seasonality) were found to have individuals with broader thermal tolerances, and correspondingly species with larger latitudinal extents. Intraspecific tests, however, found only one of six wide-ranging species to relate thermal tolerances to environmental temperature changes across the range. Next, I estimated acclimation ability of salamanders to see if species with larger distributions have greater physiological plasticity. Salamanders were acclimated to 14 and 22°C and results of a phylogenetically controlled MCMCglmm model indicated that there are significant differences in temperature adjusted standard metabolic rates (SMRs) of species with wide versus narrow latitudinal extents. Wide-ranging species showed a slight increase in SMR after acclimation, whereas narrow-ranging species showed a statistically significant drop in SMR. These results indicate that wide-ranging species have a greater thermal acclimation capacity than narrow-ranging species. Finally, using GIS-based climate data I included all available locality points to estimate species-level

thermal niche breadths. I found that CTMax and CTMin of species are strongly correlated with the maximum and minimum temperatures that occur within their geographic ranges. I also found that species' thermal tolerance breadths (CTMax – CTMin) are highly correlated with estimates of their thermal niche breadths. My general finding that wide-ranging species have broader physiological tolerances than narrow-ranging species supports key predictions of the climate variability hypothesis and the role of seasonality in the evolution of physiological traits. It also highlights the potential vulnerability of narrow-ranging montane salamanders.

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INTRODUCTION

Understanding the factors that limit species' geographic distributions is a fundamental objective of the fields of ecology and evolution. It is also central to predicting whether species' ranges will shift, contract, or remain stable in response to rapid climate change. Yet, despite far-reaching implications, it remains poorly understood why some species have restricted distributions while others are more widespread.

Temperature can have a profound influence on the geographic distributions of species (Merriam 1984; Gaston 2003; Calosi et al. 2010; Kaspari et al. 2014) and physiological specialization to subtle differences in climate may promote variation in range size. The climate variability hypothesis suggests that seasonal variation serves as an evolutionary driver of broader thermal tolerances, as survival at higher latitudes often requires individuals to endure harsh thermal extremes (Janzen 1967; Stevens 1989; Gaston et al. 1998; Cadena et al. 2012). Differences in thermal tolerances could help to explain the disparity in range size between species with similar ecologies, yet, with a few notable exceptions (see Snyder & Weathers 1975; Calosi et al. 2010; Sunday et al. 2011; Sheth & Angert 2014) there are a very limited number of studies that use physiological data to investigate large-scale biogeographic patterns among closely related species.

Understanding the factors that shape geographic distributions is even more pressing in light of global warming. For amphibians, climate change is likely to exacerbate problems in a group already facing numerous threats such as pollution, habitat loss, and disease (Whitton et al. 2012). As ectotherms are highly dependent on the temperature of their environment, they are likely to be greatly influenced by changes in

climate (Bozinovic et al., 2011; Whitton et al., 2012). For narrow-ranging montane species, sensitivities to warming temperatures are likely to be detrimental (Bernardo & Spotila 2006). With dry, hot valleys leaving few options for dispersal, and strong competition at lower elevations, their persistence will rely heavily on the ability to withstand or adapt to future climatic conditions. Given that many of these species are restricted to areas associated with cooler conditions, they may lack the physiological capacity to withstand higher temperatures (Bernardo & Spotila 2006; Gifford & Kozak 2012).

Using eastern North America's species-rich salamander fauna as a model system, the goals of my dissertation research are to disentangle the role that physiological constraints and climate play in shaping the geographic distributions of plethodontid salamanders. Chapter one explores whether seasonal variation in temperature and thermal tolerance evolution underlies variation in geographic range size, as predicted by the climate variability hypothesis (Brown 1984; Stevens 1989). First, critical thermal maximum (CTMax) and critical thermal minimum (CTMin) temperatures for 18 species of plethodontid salamanders were determined to approximate the thermal conditions that each species can withstand. After testing for differences in thermal tolerances among species, I tested the assumption that my higher latitude localities have a greater temperature range than southern localities. I then set out to explicitly test predictions of the climate variability hypothesis by looking for relationships between two measures of seasonality (temperature seasonality (standard deviation *100) and temperature annual range) versus thermal tolerance breadth. Further, I tested for an association between thermal tolerance breadth and latitudinal extent. Finally, I investigated whether any wide-ranging species had

intraspecific variation that would indicate a cline in thermal tolerance with temperature/latitude.

In chapter two, I explore the relationship between thermal acclimation and geographic range size. Because thermal acclimation enables a species to occupy a broader range of thermal conditions, it is thought to be an important factor influencing species' distributions (Fry 1958; Brattstrom 1968; Feder 1984; Christian et al. 1988; Spicer & Gaston 1999). Following predictions of the climate variability hypothesis, I anticipate that species experiencing greater seasonality should have greater physiological plasticity in their response to changes in temperatures (Fernandez & Vrba 2005; Navas 2006; Calosi et al. 2010). I tested this prediction by comparing standard metabolic rates (SMRs) of 17 salamander species acclimated to two different temperatures (14 and 22°C) to see if there is any adjustment in SMR after acclimation at a higher temperature. An increase in SMR (or even a lack of change) would be an indication of acclimation ability. Although negative compensation of SMR in response to high temperature can be viewed as an adaptation to survive short-term suboptimal conditions, recent work suggests that metabolic depression following exposure to high temperatures is likely a sign of physiological stress (Bernardo & Spotila 2006). In addition, I explored whether there are any apparent trade-offs between short-term acclimation ability and thermal tolerance.

Finally, in chapter three, I explore the relationship between thermal tolerances (min, max, and breadth) and patterns of temperature variation across localities in which species naturally occur. The spatial extent of a species' climatic niche is thought to play a fundamental role in limiting its geographic range (Soberón 2007; Fisher-Reid et al. 2012; Quintero & Wiens 2013a,b), and the availability of GIS-based climate data provides an

expedient way to quantify dimensions of species' climatic niches. However, a key assumption underlying this approach—that geographic distribution-based models of species' climatic-niches mirror their physiological-tolerances—is rarely addressed. Here, I used GIS-based climatic variables to calculate thermal-niche breadths for 18 species of plethodontid salamander and assess whether thermal tolerances measured in the laboratory relate to temperature variation where species occur in the wild.

Without a good understanding of what drives species distributions our understanding of which species will be hardest hit by climate change and how those species will respond remains very limited. This study is one of a small number that investigates physiological traits and biogeographic patterns in a group of ecologically similar species within a phylogenetic context (see also Quintero & Wiens 2013b; Sheldon & Tewksbury 2014; Sheth & Angert 2014). As such, our findings offer valuable insight into the role that thermal tolerance evolution plays in shaping species' geographic distributions.

The chapters of this thesis have been written as separate manuscripts, and either have been, or will be, submitted to peer-reviewed journals. The first chapter has been submitted to the *Journal of Biogeography*, the second will be submitted to the *Journal of Thermal Biology*, and the third chapter to *Ecography*. As such, the formatting of each chapter may reflect the requirements of the target journal. Plural pronouns (e.g. “we”) are used throughout, as the intended publications have multiple co-authors. However, as senior author on all manuscripts I am responsible for the content.

Chapter 1

Ecophysiological Analysis of Variation in Geographic Range Size

INTRODUCTION

Species exhibit remarkable variation in geographic range size. Even among closely related species range size can vary by many orders of magnitude, with most species occupying relatively small areas and comparatively few being widespread (MacArthur, 1972; Brown, 1995; Gaston, 2003). Understanding the factors that underlie this pattern is central to ecology and evolution, and is critical for establishing how species will respond to rapid climate change. However, despite its importance, the disparity in species' geographic range sizes remains poorly understood.

Given that the range limits of many species are associated with temperature isotherms (Salisbury, 1926; Andrewartha & Birch, 1954; Gaston, 2003), specialization to subtle differences in temperature is likely to play a key role in shaping species' geographic distributions (Hutchison, 1961; Janzen, 1967; Merriam, 1984; Gaston, 2003; Calosi et al., 2010; Kaspari et al., 2014). The climate variability hypothesis (CVH) postulates that greater seasonal variation towards the poles drives the evolution of broader thermal tolerances (Stevens, 1989; Gaston et al., 1998; Cadena et al., 2012). Species distributed at lower latitudes would not have such selective pressures (species would not adapt to conditions they do not experience) (Janzen, 1967; Gaston, 2003). Having a broad fundamental tolerance breadth would enable a species to survive in more places and expand their geographic range (Brown, 1984; Stevens, 1989; Gaston & Spicer,

2001), thus promoting latitudinal variation in range size among species. Understanding the relationship between temperature, thermal tolerance, and geographic range size is important for many topics in ecology, evolution, and conservation, and thus tests of the climatic variability hypothesis are a valuable contribution to many fields.

Further, wide-ranging species may be comprised of genetically differentiated populations that are locally adapted to climatic variation across their range (Avice, 2000; Davis et al., 2005; Angert et al., 2011). If populations are adapting to local thermal regimes as per the CVH, we should observe a cline in physiological tolerances associated with the temperature gradient of species' ranges (Davis et al., 2005; Gaston et al., 2009; Lee & Boulding, 2010; Angert et al., 2011). As population-level variation has the potential to affect profoundly the evolutionary and ecological dynamics of a species, investigating population-level genetic variation in traits is important when investigating a species as a whole, yet in many biogeographic studies it is often ignored (see Bolnick et al., 2003; Angert et al., 2011; Buckley et al., 2013).

To test predictions of the climate variability hypothesis we use salamanders of the family Plethodontidae, which provides an excellent study system for our tests. This group exhibits striking variation in range size, with species whose entire geographic distributions are confined to a single mountaintop, to species with massive ranges that encompass areas that were uninhabitable at the last glacial maximum (Highton, 1995). Thermal physiology of plethodontid salamanders is readily measured in the laboratory, moreover, the evolutionary relationships of North American species are well resolved (Kozak et al., 2009). Thus, all comparative analyses can be evaluated in a phylogenetic

framework and trait independence across the phylogeny is considered prior to all analyses.

Here, we focus on 18 species, representing the most speciose and abundant genera in eastern North America (7 species of *Desmognathus* and 11 species of *Plethodon*). *Desmognathus* species have an aquatic larval stage and are semi-terrestrial as adults, often remaining in the vicinity of streams and seeps. Species in the genus *Plethodon* have direct development and inhabit terrestrial forested areas at all life stages. Apart from these differences, salamanders in this study share similar ecologies, being nocturnal generalist predators with low dispersal rates (Petranka, 1998).

We test predictions of the climatic variability hypothesis by examining the relationships between thermal tolerances, seasonality, and range size. First, we determine critical thermal maximum (CTMax) and critical thermal minimum (CTMin) temperatures of individuals and test for differences among species. Next we test the assumption of broader seasonal temperatures at higher latitudes for our collection sites. We then explore whether greater seasonality (temperature seasonality (standard deviation *100) and temperature annual range) is associated with broader thermal tolerances and whether species with the broadest thermal tolerances have the largest latitudinal distributions. Finally, we quantify population-level variation in thermal tolerances across the ranges of wide-ranging species to determine whether there are clines in thermal tolerances associated with environmental temperature extremes. Other studies have investigated the relationship between thermal tolerances and geographic range size, including in amphibians (e.g. see Snyder & Weathers, 1975; Sunday et al., 2011; Whitton et al., 2012). However, this study is one of a small number that investigates physiological traits

and biogeographic patterns within and among ecologically similar and closely related species while considering phylogenetic relationships (see also Quintero & Weins, 2013b; Sheth & Angert, 2014; Sheldon & Tewksbury, 2014). As such, our findings offer valuable insight into the relationship between physiological tolerances and species' geographic distributions.

MATERIALS AND METHODS

Collections and phylogeny

We collected salamanders from 2009 to 2012 throughout the Appalachian Mountains of eastern North America (Fig. 1). States included New York, North Carolina, Pennsylvania, Virginia, and West Virginia. This region is a center of plethodontid salamander diversity and salamanders were readily found under logs, rocks, and leaf litter. Season of collection was standardized as much as possible and only mature salamanders were collected to avoid confounding effects due to age variation. A total of 53 localities were sampled, and from each locality, three to eleven individuals (target of 10) per species were collected. Wherever possible, multiple sites were sampled for each species to represent populations across the latitudinal extent of each range and approximate a “whole species” account of thermal tolerance. As our goal was to provide some geographic and species perspective, in a few instances we included sample sizes that were smaller than desired for species/populations where collections were challenging (e.g. $n < 10/\text{species}$).

The 18 species studied belong to four major clades that are strongly supported

based on phylogenetic analysis of mitochondrial- and nuclear-DNA sequences (Kozak et al., 2009): the genus *Desmognathus*, the *Plethodon cinereus* group, the *Plethodon glutinosus* group, and the *Plethodon wehrlei* group (Table 1, Fig. 2). For *P. sherando*, allozyme data were used to determine placement within the *cinereus* group, but sequence data was not currently available (Highton, 2004). Each of these clades contains species with very restricted southern ranges and species with extensive northern ranges (Fig. 3 a-d). Our sampling encompassed this variation in range size among species (Table 1).

Once collected, salamanders were transported to the laboratory and maintained in an environmental chamber where they were housed individually in plastic containers lined with moist paper towels and fed crickets weekly. Prior to physiology trials, salamanders were kept in a temperature-controlled room with a 12L:12D light regime and acclimated at 14°C for a minimum of four weeks.

Climatic data

To approximate seasonality and temperature annual range of each sampled locality, thermal data (averages 1950-2000) were obtained from the WorldClim online database (Hijmans et al., 2005) at *c.* 1 km resolution. Using the program DIVA-GIS (Hijmans et al., 2002), georeferenced salamander localities were mapped and data was extracted from four bioclimatic variables of interest. These four variables include: temperature seasonality (standard deviation *100) (Bio4), maximum temperature of the warmest month (Bio5), minimum temperature of the coldest month (Bio6), and temperature annual range (Bio5-Bio6). Bio 5 and Bio 6 will hereafter be referred to as Tmax and Tmin, respectively.

Thermal tolerances

We obtained CTMax and CTMin temperatures for individual salamanders through nonlethal laboratory tests similar to those used by Layne & Claussen (1982), where loss of righting response (LRR) is considered the end point. This point is achieved when the salamander turns over on its back (either independently, or by the experimenter) and is unable to right itself within 30 seconds (Hutchison, 1961). This closely follows the original definition of Cowles & Bogert (1944) where CTMax and CTMin are each defined as “the thermal point at which locomotory activity becomes disorganized and the animal loses its ability to escape from conditions that will promptly lead to its death.” Although LRR is a common and widely accepted measure of CTMax (Brattstrom, 1968; Layne & Claussen, 1982; Gonzalez, et al. 2010), Lutterschmidt & Hutchison (1997) have demonstrated that onset of spasms is a more accurate endpoint. Here, however, we present LRR results as it provides a standard measure for both CTMax and CTMin (salamanders are unlikely to go into spasms at low temperatures), to then calculate thermal tolerance breadth. Further, it has been found that some salamanders do not go into spasms at all (Brattstrom, 1968) and onset of spasms causes greater physiological stress and higher incidence of death, which is not ideal for individuals that are needed for additional thermal trials.

Deep body temperatures of salamanders (including several larger sized species used in this study) have been found to closely follow water temperature with no measurable lag at a heating rate of 1.0°C per minute (Hutchison, 1961; Feder & Lynch, 1982). As our rate of temperature increase/decrease is half of this (0.5°C / minute), all

salamanders (regardless of mass) should have a deep body mass approximating water temperature at any given time, and any actual differences should be negligible. As such, we follow many other studies and define CTMax and CTMin as the water temperature at the endpoint of the trial (as measured by digital thermometer, Fluke 51 II, Everett, USA) (see Brattstrom, 1968; Lutterschmidt & Hutchison, 1997). The majority of salamanders in this study were tested for both CTMax and CTMin, with the treatment order assigned randomly to each individual. Salamanders were given a minimum of four weeks between tests to recover, based on Hutchison's (1961) study showing that a minimum of two weeks is required for repeated values to approximate the originals. In addition, repeated trials on a small number of individuals ($n = 8$) had CTMax and CTMin results within 0.2°C of the original test value. As diet and mass can affect thermal physiology, salamanders were not fed for six days prior to measurements of CTMax and CTMin (Hutchison 1961), and were weighed before the trial to within 0.001g.

To obtain CTMax, individuals were placed into a small plastic container with 2 cm of water and an open top. Salamanders were fully immersed in the water to prevent desiccation, although they were permitted to hold their heads above the water. Starting water temperature for all CTMax trials was 21°C and salamanders were permitted to adjust to room temperature (range $20\text{-}22^{\circ}\text{C}$) for approximately 20 minutes before the start of each trial. A 150-watt infrared-heat lamp placed 27 cm from the surface of the water was used to increase water temperature by $0.5^{\circ}\text{C}/\text{minute}$ until the end point was reached. An air bubbler circulated the water and ensured consistent water temperature, as well as additional oxygen.

For CTMin, trials were conducted in a temperature-controlled room at 14 °C to achieve low water temperature at a steady rate. Salamanders were placed into an insulated plastic chamber with 1.5 cm of water on top of a cold plate (Thermoelectric TCP-2, USA), which cooled the water in the chamber at 0.5 °C/minute until the end point was reached. Starting water temperature for CTMin trials was 13 °C. An air pump was also used to circulate the water, maintain an even temperature distribution, and help to prevent the water from freezing. Once LRR was achieved, salamanders were transferred to room temperature water where they quickly recovered. Mean thermal tolerance breadth for each species was calculated by subtracting CTMin from CTMax for each individual and then taking the mean.

Phylogenetic consideration

In consideration of the potential affect of phylogenetic non-independence in comparative analyses of species, we tested the influence of phylogeny for all measured traits used in the analysis (CTMax, CTMin, thermal tolerance breadth, latitude, range size, and mass), by comparing AIC scores of Brownian motion versus lambda models using the “geiger” package in R v.3.0.2 (R Development Core Team, 2013). In all cases lambda models were chosen as the best model (with most traits having lambda scores of < 0.0001), indicating that these traits have little to no phylogenetic signal. Thus, in all of the analyses below, we present and focus on the results of non-phylogenetic GLMs.

Statistical analyses

Critical thermal maximum temperatures were determined for 525 salamanders, and critical thermal minima for 493. The number of individuals per species ranged from 8 in *P. punctatus* and *P. virginia* to 75 in *P. cinereus* and *D. ochrophaeus* (Table 1). Data are from both male and female salamanders, and as such, sex was included as a covariate in all models. However, sex was not found to be significant factor on CTMax or CTMin within any given species ($p > 0.10$ all tests). Mean body mass ranged from 0.89 g in *P. cinereus* to 6.11 g in *P. teyahalee* (Table 1). As body mass has the potential to significantly influence thermal tolerances, and was found to vary among (and in some cases within) species, it was also included as a covariate in each analysis. All statistical analyses in our study were conducted in R v.3.0.2 (R Development Core Team, 2013).

To first test for differences among species for response variables CTMax and CTMin, post hoc Tukey HSD tests were performed on each ANCOVA, including mass and sex as covariates. Sex was not found to be a significant explanatory variable ($p > 0.15$) and was removed from the models.

Our first test of the climate variability hypothesis was to confirm whether greater seasonality is found at higher latitudes for the localities in our study. Tmax and Tmin were used in separate linear regressions to specifically relate temperature extremes of each locality with its corresponding latitude. Next, we used linear regression to test for a relationship between temperature annual range (i.e. seasonality) and latitude.

Our second test of the climate variability hypothesis was to determine whether broader thermal tolerances are associated with greater seasonality. Here, we used multiple regressions for the response variable thermal tolerance breadth (CTMax-CTMin)

versus two measures of “seasonality”. The first being temperature seasonality (standard deviation *100) and the second being temperature annual range (Tmax-Tmin).

Further, to explore whether salamanders show physiological adaptation to the thermal conditions of their specific locality, regressions were generated using CTMax versus Tmax and CTMin versus Tmin. Species, mass, and sex were included in all models as additional explanatory variables.

Next, to test whether thermal tolerance breadth is related to range size (as predicted by the CVH), we ran a multiple regression with response variable thermal tolerance breadth versus explanatory variables latitudinal extent, mass, and sex.

Latitudinal extent for each species was obtained by subtracting the southernmost known latitudinal point from the most northern, based on distributional maps and occurrence data including the Global Amphibian Assessment database (IUCN *et al.*, 2004).

Latitudinal extent (decimal degrees: dd) ranges from 0.07 dd in *P. hubrichti* to 16.44 dd in *P. glutinosus*, (Table 1). Further, the same analysis was performed independently for *Plethodon* and *Desmognathus* to test whether patterns and statistical significance remain within each genus.

Finally, intraspecific regressions were used to determine whether differences in CTMax or CTMin exist among populations across the temperature range of the environment. Of the 18 species in this study, we had six “wider-ranging” species with four or more sampled populations that could be used for analysis (Table 2). Mass and sex were included as additional explanatory variables in each analysis.

RESULTS

Thermal tolerances

Mean CTMax ranged from 31.6°C in *P. sherando* to 33.2°C in *D. ochrophaeus*. Mean CTMin ranged from -1.5°C in *P. cinereus* to -0.3°C in *Desmognathus santeetlah* (Table 1). Maximum and minimum critical thermal limits were found to differ significantly between species of plethodontid salamanders (CTMax: p-value < 2.2e-16, $F_{17, 507} = 27.58$, $R^2 = 0.46$, $n = 525$ and CTMin: p-value < 2.2e-16, $F_{18, 474} = 21.32$, $R^2 = 0.426$, $n = 493$) and specific differences between species are indicated by post hoc Tukey HSD tests (Fig. 4 a,b). Body mass was found to influence CTMin ($p = 0.031$), but not CTMax ($p = 0.934$), therefore it only remained as a covariate in the CTMin model. Sex was not found to be a significant factor in either model ($p > 0.25$) and was removed.

Temperature range and latitude

For our sampled localities, the relationship between the maximum temperature of the warmest month (Tmax) and latitude falls just outside significance limits (p-value = 0.063, $R^2 = 0.0479$) (Fig. 5a), yet there is a strong association between the minimum temperature of the coldest month (Tmin) and latitude (p-value = 4.45e-13, $R^2 = 0.639$) (Fig. 5b). Overall, higher latitudes are found to be strongly associated with greater temperature annual range (i.e. seasonality) (p-value < 2.e-16, $F_{1, 51} = 159.6$, $R^2 = 0.753$, $n = 53$) (Fig. 5c).

Seasonality and thermal tolerances

Mean species thermal tolerance breadths range from 32.1°C in *D. santeetlah* to 34.3°C in *D. fuscus* (Table 1). For individuals, those with greater thermal tolerance breadths are found to be strongly associated with localities that have greater seasonality: both temperature seasonality (standard deviation *100) (p-value = 0.000685, $R^2 = 0.637$, n=366) and temperature annual range (p-value = 0.000920, $R^2 = 0.624$, n= 366) (Figs 6 a,b). Covariates species, mass, and sex were also included in each model. In both models, mass was not a significant variable ($p > 0.67$) and was removed.

Further, regressions of CTMax versus the maximum temperature of the warmest month (Tmax) and CTMin versus the minimum temperature of the coldest month (Tmin) find some relationship between physiological tolerances and environmental temperature extremes. CTMax and Tmax had no correlation ($p = 0.8303$, $n = 468$, with species as an additional explanatory variable) (Fig. 7a), however, Tmin was found to be a significant explanatory variable of CTMin ($p = 0.0123$) in a model along with other significant explanatory variables mass and species ($R^2 = 0.46$, $n = 439$) (Fig. 7b).

Thermal tolerance breadth and latitudinal extent

Thermal tolerance breadth is positively correlated with latitudinal extent (p-value $< 2e-16$, $R^2 = 0.28$, $F_{1, 410} = 157.2$, $n = 412$) (Fig. 8). Mass and sex were not statistically significant covariates and were removed from the model. In addition, when the same model was performed separately for *Plethodon* and *Desmognathus* salamanders, the results remained significant and the trend the same (for *Plethodon*, $p < 2e-16$, $R^2 = 0.33$ and for *Desmognathus*, $p < 2e-16$, $R^2 = 0.36$).

Intraspecific thermal tolerances

Intraspecific population-level tests found only one out of six of the species tested, *D. ochrophaeus*, to demonstrate a significant cline in thermal tolerance with temperature ($p = 0.005439$ and $R^2 = 0.21$ for CTMin versus Tmin). Mass was also found to be a significant covariate in the model ($p < 0.05$). For the remaining five species, no within species relationships were found between CTMax versus Tmax and CTMin versus Tmin (see Table 2 and Figs 9 a,b). Although low statistical power may explain a lack of relationship for some species with lower sample numbers, we still fail to see a clear pattern for the majority of wide-ranging species where many individuals and populations were sampled (e.g. *P. cinereus* with 11 populations and 75 individuals).

DISCUSSION

Temperature is believed to be a major factor limiting species' geographic distributions, and differences in physiological tolerances may explain some of the great disparity in range size found among species. Here, we find environmental temperature extremes and latitudinal extent to be strongly associated with thermal tolerances of North American Plethodontid salamanders, a finding that supports key predictions of the climate variability hypothesis (Stevens, 1989; Gaston et al., 1998; Parmesan, 2005). If thermal tolerances are selected for by seasonality of the environment, species in more variable environments should have broader thermal tolerance breadths than those in more stable locales (Janzen, 1967; Stevens, 1989; Ghalambor et al., 2006). Broader thermal tolerances would subsequently enable species to occupy wider geographic distributions.

Our first series of tests confirmed that significant differences in CTMax and CTMin exist among Plethodontid salamanders. We then confirmed that our higher latitude localities have a greater range of temperature (i.e. greater seasonality) than more southern localities. Although thermal tolerance differences between species are relatively small, we find strong and consistent relationships between thermal tolerance, seasonality, and range size. As such, there is an indication that even slight differences in thermal physiology may equate to large ecological and biogeographic effects. Temperature can affect virtually all amphibian physiological systems, including metabolism, muscle contraction, enzymatic digestion, solute transport, growth, and reproduction. (Angilletta, 2009; Hillman et al., 2009). As physiological functions are temperature dependent, small differences in thermal tolerance could have profound effects on the survival and fitness of individuals. For instance, loss of locomotor or muscle response can result in feeding reduction and increased predator vulnerability, and reduction in digestion efficiency can impact rates of energy assimilation (Hillman et al., 2009). Ultimately, temperature effects on physiology may determine where a species is able to occur.

Our first major test of the CVH was to determine whether greater seasonal temperatures are associated with broader thermal tolerances. Here we find a strong relationship with thermal tolerance breadth for two measures of seasonality: temperature seasonality (standard deviation *100) and temperature annual range. When pieced apart further, we find a strong association between CTMin and the coldest environmental temperatures, but little association between CTMax and the warmest temperatures. A number of other studies on terrestrial ectotherms have also found CTMin to show a stronger association with latitude and environmental temperature than CTMax. These

include investigations on amphibians (Brattstrom, 1968; Snyder & Weathers, 1975; Sunday et al., 2011), lizards (Van Berkum, 1988; Hoffmann et al., 2013), and insects (Addo-Bediako et al., 2000; Calosi et al., 2010). This is likely due in part to cold winter temperatures being a stronger driver of seasonality (i.e. a steeper slope with latitude) at higher latitudes than warm temperatures. Further, our study finds that high temperatures have much more scatter (and consequently a very low R-squared) in their relationship with latitude than cold temperature extremes. A better fit of the line would promote stronger directional selection for thermal tolerances to fit environmental temperatures. The greater tolerance of wide-ranging species to cold temperatures suggests that cold tolerance an important physiological trait for northern range expansion and survival (Brattstrom, 1968; Ghalambor et al., 2006). Finally, it remains possible that current CTMax tolerances may reflect selection during warmer climates or while in southern glacial refugia, and exceed what is necessary at higher latitudes. If there are no energetic costs associated with retaining higher CTMax, then a lack of correlation with recent annual maximum temperatures could simply represent retention of thermal tolerances associated with ancestral thermal regimes (Sunday et al., 2011).

In some taxa, physiological mechanisms that regulate upper and lower thermal tolerances appear to be decoupled (e.g. Hoffmann et al., 2002; Terblanche et al., 2005). Thus, selection pressures can drive tolerance to hot or cold separately, and differences between species (and even within species) may not be uncommon (Calosi et al., 2010). For many groups, the mechanisms underlying thermal tolerances and the heritability of these traits are largely unknown. Cellular functions such as the production of heat shock proteins, rates of enzymatic reactions, physiology of heat and cold tolerance, and

influences on the cellular membrane require further study (see Spicer & Gaston, 1999; Angilletta, 2009; Huey et al., 2009).

Our next test of the climate variability hypothesis finds that broader thermal tolerances are strongly associated with larger latitudinal extents. If seasonality drives the evolution of broader thermal tolerances then the greater tolerance capacity of more northern species would enable individuals to survive in more places and further promote range expansion (Brown, 1984; Stevens, 1989; Gaston & Spicer, 2001). This method could help to explain why there is such great disparity in geographic range size among closely related species in this group.

Further, species' ranges often consist of phenotypically and genetically distinct populations that have traits adapted to their local environments (Avice, 2000; Hereford, 2009). Although population-level variance in thermal tolerance is rarely investigated, adaptation to local climatic conditions across a species' range may have significant ecological effects, including the ability of a species to adapt to a changing climate (Bolnick et al., 2003; Quintero & Weins, 2013b). Our results, however, show little support for this aspect of the CVH, as only one out of six wide-ranging species in this study show any population-level variation in thermal tolerance in relation to environmental temperature. This result is unexpected, as salamanders have low dispersal rates and exceedingly small home ranges (Petranka, 1998; Wells, 2007), a combination that typically results in low levels of gene flow and potential for rapid adaptation to local conditions. The one notable exception is *D. ochrophaeus*, where for CTMin this species retains a strong relationship with latitude. Why this species is somewhat of the exception remains unknown, but warrants further study. Although few relationships between

latitude and thermal tolerance were found within species in our study, results showing intraspecific variation in physiological tolerances have been observed for several other species of plants and animals (e.g. Brattstrom, 1968; Lacey, 1988; Klok & Chown, 2003; Etterson, 2004; Angert et al., 2011; Koehler et al., 2012). Finally, we found a fair amount of within-population variation in thermal tolerance, suggesting that local selection on thermal tolerances is not particularly strong.

When investigating thermal tolerances in North American species, some consideration must be given to the dynamic climatic history of the continent. Climatic shifts and glacial cycles have offered multiple opportunities for range expansions and contractions that have shaped the extant ranges of North American species (Pielou, 1991; Parmesan et al., 2005). Selection for broad tolerances in leading-edge populations could explain the lack of variation in thermal traits among populations of wide-ranging salamander species. However, numerous other studies have demonstrated that adaptive differentiation to local conditions since the last glacial maxima is possible (see Lacey, 1988; Rehfeldt et al., 2002; Davis et al., 2005). For example, Scots pine (*Pinus sylvestris*) in Finland is found to be locally adapted to diverse elevations and latitudes in its current range (Hurme et al., 1997). Another possibility is that genetic variation was lost during population bottlenecks and rapid post-glacial expansion from Pleistocene refugia (see Sage & Wolff, 1986; Zink & Dittmann, 1993). Thus, current thermal tolerances could reflect what was preserved in refugial populations and the system may not yet be in equilibrium (Parmesan et al., 2005; Sunday et al., 2011). Thermal homogeneity within species could also be the result of strong gene flow between populations causing maladaptation to local conditions (Kirkpatrick & Barton, 1997; Paul et al., 2011).

However, for low dispersing salamanders across vast geographic areas, this seems unlikely.

Further, microclimates available to salamanders might allow populations to experience similar temperatures across the range, thus reducing local adaptation of thermal tolerance. For instance, Quintero and Weins (2013b) found plethodontid salamanders, hylid frogs and phrynosomatid lizards to be surprisingly homogeneous in their climatic niche breadths across their geographic ranges. Therefore, the lack of a thermal tolerance cline in our study could result from similar climatic conditions among localities (see also Woods et al., 2015). Although this needs to be explored further, our climatic locality data show that the minimum temperatures of localities sampled decrease with latitude, and therefore are not uniform. In addition, it is possible that the populations sampled simply do not encompass all of the variation within each species. However, as data were acquired for populations from across much of the latitudinal extent of each species' range, it should encompass at least some population-level variation in thermal tolerance if it exists.

Another major limitation is that this experiment does not control for the environment of the salamanders at the development stage, which can potentially influence thermal tolerances at maturity (Angilletta, 2009). Therefore, any differences along gradients could just reflect environmental effects unrelated to adaptation. Although a cross-generation breeding design is of importance, this would be a very difficult undertaking in salamanders. Eggs can be a challenge to rear and encouraging females to reproduce in the laboratory is a major obstacle (Bernardo & Arnold, 1998).

A final consideration is that these salamanders move underground when thermal conditions become too hot or cold (Wells, 2007). As such, they are buffered from the full extent of seasonal temperature extremes, and body temperatures are likely to decouple from environmental temperatures making them more constant across latitude (Angilletta, 2009; Huey et al., 2009; Kearney et al., 2009). Such thermoregulatory behaviour is likely to influence the degree of selection for thermal tolerances, yet there is little way to quantify such behavioural adaptations in this study. However, CTMin tolerances are highly correlated with minimum environmental temperatures, indicating that salamanders are still responding to thermal conditions and are not fully buffered.

As ectotherms, salamanders are highly dependent on the temperature of their environment and are likely to be greatly influenced by changes in climate (Bozinovic et al., 2011; Whitton et al., 2012). Range contractions and extinctions in amphibians have already occurred (Parmesan, 2006), and climate change is likely to exacerbate problems in a group already facing numerous threats (e.g. pollution, habitat loss, disease) (Whitton et al., 2012). The magnitude of the impact will depend on many factors, including the speed and degree of warming, the availability of alternative suitable habitat, physiological sensitivity to changes in temperature, and the potential for behavioural or physiological evolution and plasticity (Davis et al., 2005; Bernardo & Spotila, 2006). As thermal tolerances (and likely other physiological properties) are found to vary significantly even among closely related species, the results of this study highlight the need for species-specific physiological studies and the inappropriateness of developing conservation strategies solely on data from close relatives. Further, given that many of these species are restricted to montane regions, they may be specialized to cool

microclimates and lack the physiological capacity to withstand high temperatures. There is already evidence that some narrow-ranging salamanders may be experiencing climatic conditions near their physiological limits (Bernardo & Spotila, 2006; Gifford & Kozak, 2012).

In summary, we find some support for the role of seasonal variation in temperature driving broader thermal tolerances in North American salamanders and consequently variation in geographic range size. Individuals with broader thermal tolerances are found from localities with greater seasonal variation and such species are associated with greater latitudinal extents. The greater cold tolerance of higher latitude individuals is likely an important factor in northern range expansions, and would be a main target of selection in more seasonal northern climates.

Table 1. Salamander groups and species, detailing mean body mass (g), mean CTMax, number of individuals used to determine CTMax (with number of populations in brackets), mean CTMin, number of individuals used to determine CTMin (with number of populations in brackets), mean thermal tolerance breadth (CTMax – CTMin of individuals), and latitudinal extent. Note that thermal tolerance breadth is only taken from individuals where results were available for both CTMax and CTMin.

Species	Mean body mass (g)	Mean CTMax (°C)	n CTMax (# popns)	Mean CTMin (°C)	n CTMin (# popns)	Mean thermal tolerance breadth (°C)	Latitudinal extent (degrees)
Desmognathus group (7 species)							
<i>D. carolinensis</i>	1.10	32.25	11 (2)	-0.87	10 (2)	33.3	1.26
<i>D. fuscus</i>	2.07	33.12	26 (4)	-1.19	24 (4)	34.3	13.65
<i>D. monticola</i>	3.66	33.06	54 (6)	-0.86	45 (6)	33.9	9.12
<i>D. ochrophaeus</i>	1.01	33.15	75 (8)	-1.03	72 (9)	34.2	8.94
<i>D. ocoee</i>	1.33	32.68	24 (3)	-0.64	23 (3)	33.2	3.05
<i>D. orestes</i>	1.13	32.81	36 (4)	-0.72	35 (5)	33.5	1.75
<i>D. santeetlah</i>	1.35	31.81	10 (1)	-0.28	11 (1)	32.1	1.18
Plethodon cinereus group (5 species)							
<i>P. cinereus</i>	0.89	32.47	72 (11)	-1.47	75 (11)	33.9	14.28
<i>P. hubrichti</i>	1.18	32.12	12 (1)	-1.06	10 (1)	33.1	0.07
<i>P. richmondi</i>	1.07	32.66	20 (4)	-1.16	18 (3)	33.8	3.59
<i>P. sherando</i>	1.05	31.6	11 (1)	-1.29	11 (1)	32.9	0.15
<i>P. virginia</i>	1.28	31.97	9 (1)	-0.99	8 (1)	32.9	0.93
Plethodon glutinosus group (4 species)							
<i>P. cylindraceus</i>	4.90	32.76	27 (5)	-0.94	24 (5)	33.7	5.77

<i>P. glutinosus</i>	4.85	33.01	63 (9)	-1.0	60 (8)	34.0	16.44
<i>P. montanus</i>	1.95	32.51	37 (4)	-0.73	30 (4)	33.3	1.84
<i>P. teyahalee</i>	6.11	32.56	18 (2)	-0.74	20 (2)	33.3	1.44
Plethodon wehrlei							
group (2 species)							
<i>P. punctatus</i>	3.81	31.86	11 (2)	-1.1	8(2)	32.8	1.66
<i>P. wehrlei</i>	1.75	32.34	9 (1)	-1.32	9 (1)	33.7	6.27

Table 2. Regression results for population-level intraspecific tests of wide-ranging species. Thermal tolerances of individuals (critical thermal minima and maxima) versus average annual temperature range of population localities. ** indicates highly significant relationship.

Species	n populations	CTMax vs. Tmax p-value	CTMin vs. Tmin p-value
<i>D. fuscus</i>	4	0.679	0.330
<i>D. monticola</i>	6	0.131	0.585
<i>D. ochrophaeus</i>	8	0.160	0.005439 **
<i>P. cinereus</i>	10	0.930	0.914
<i>P. cylindraceus</i>	4	0.292	0.291
<i>P. glutinosus</i>	9	0.307	0.430

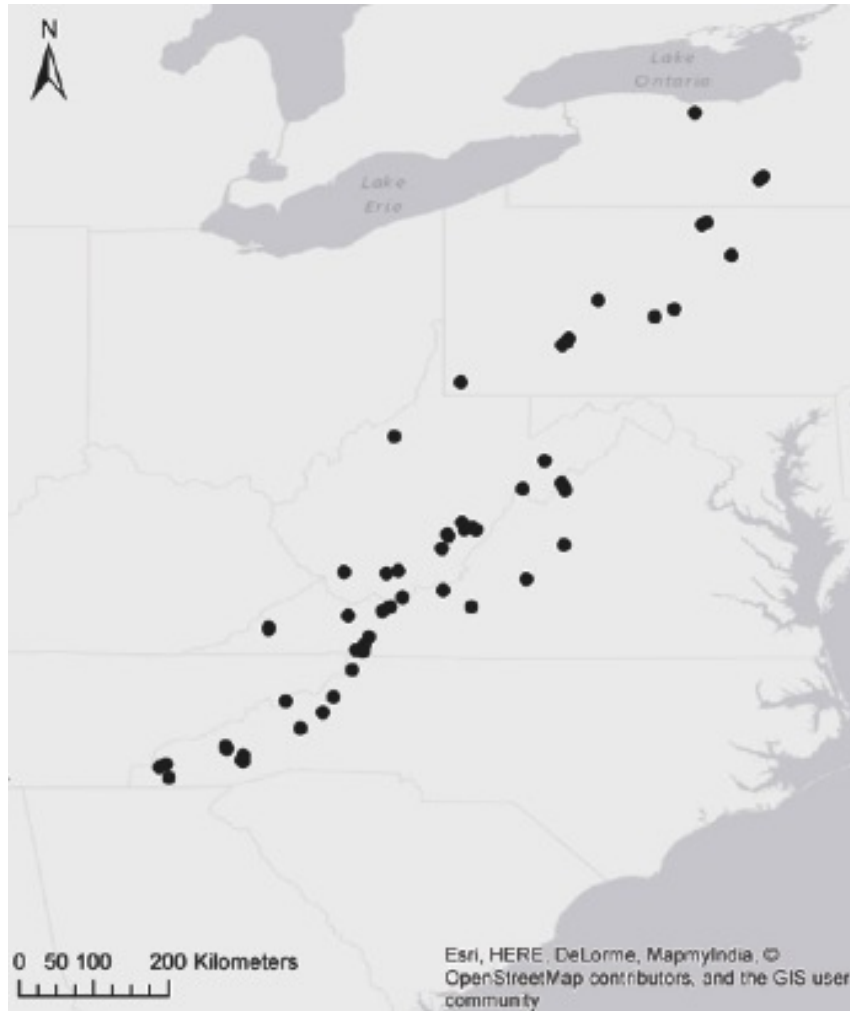


Figure 1. Site localities of salamanders collections in eastern North America (states sampled = North Carolina, Virginia, West Virginia, Pennsylvania, and New York) (n=53).

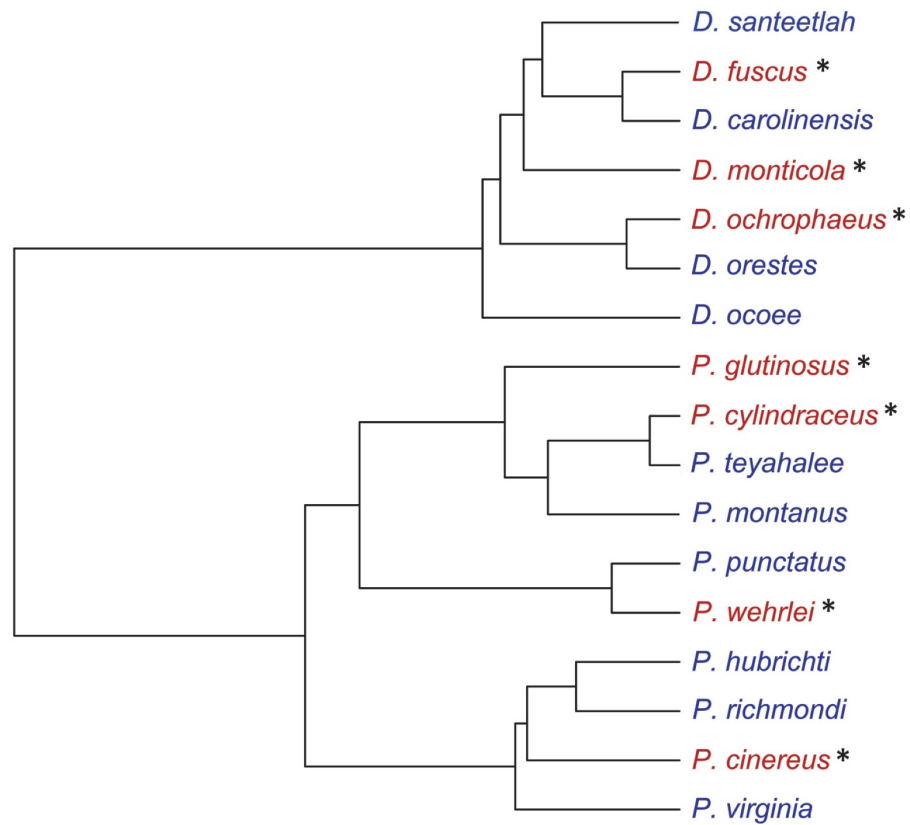


Figure 2. Estimated phylogeny for 17 of the 18 Plethodontid salamanders in this study based on mtDNA and nuclear DNA from Kozak et al. (2009). Sequence data not currently available for *P. sherando*. Wide-ranging species indicated by * (latitudinal extent > 5 degrees), whereas, remainder are narrow-ranging (latitudinal extent < 5 degrees).

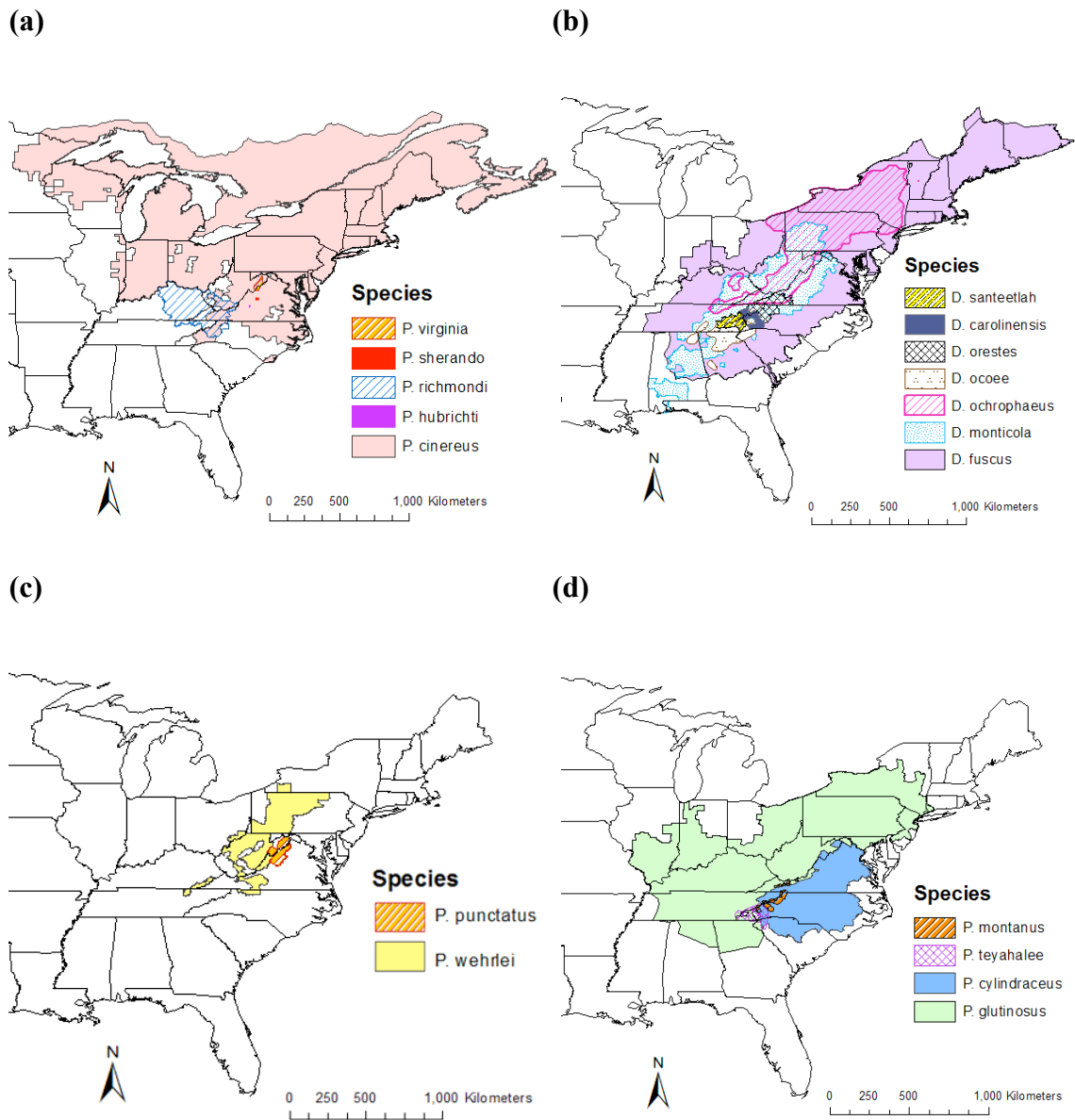


Figure 3. Distributions of salamander species in the four groups: (a) *P. cinereus*, (b) *Desmognathus*, (c) *P. wehrlei*, and (d) *P. glutinosus*. Maps illustrate the large variation in geographic range size among closely related species.

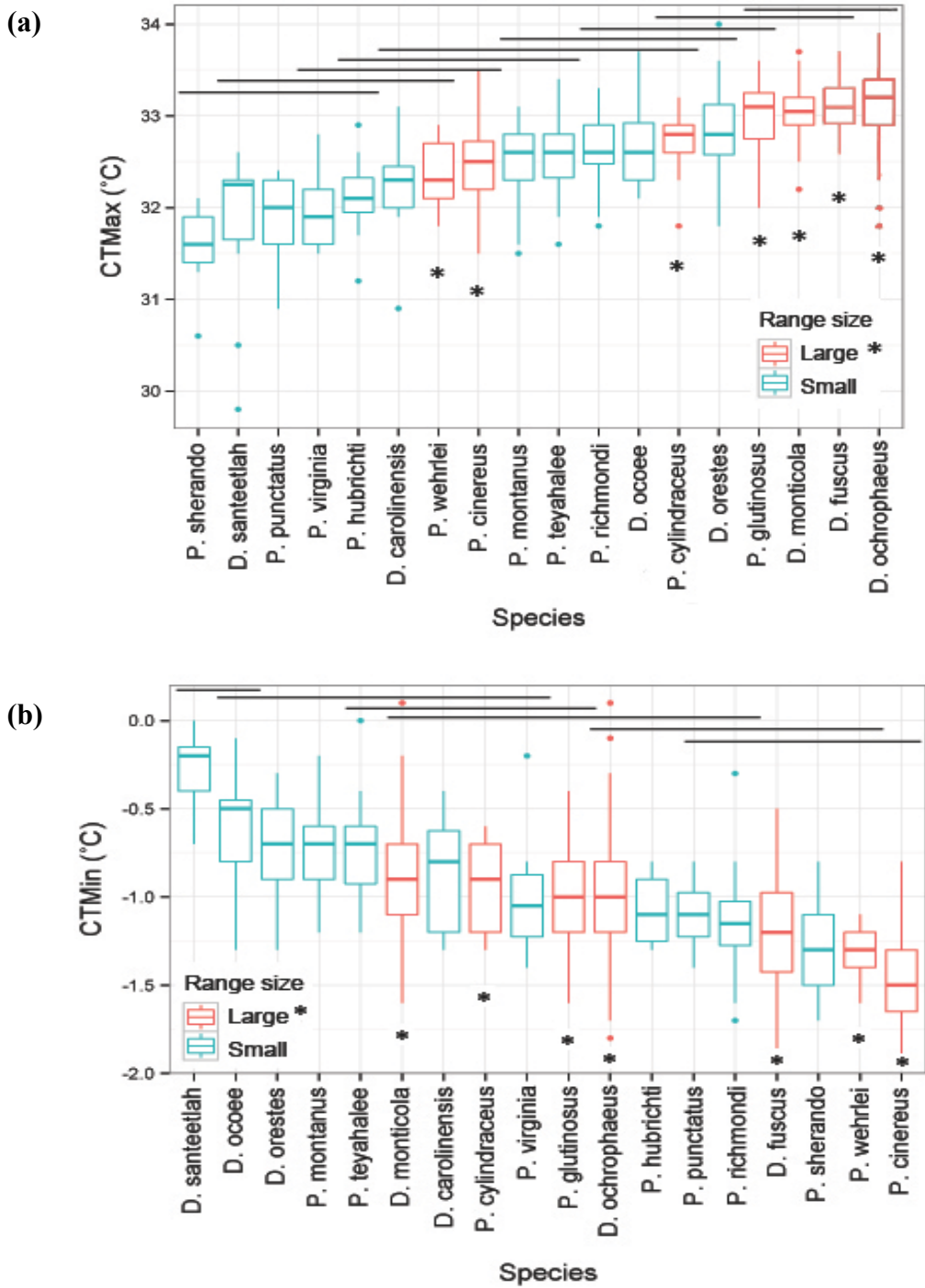


Figure 4. Boxplots of (a) CTMax (n = 525) and (b) CTMin (n = 493) by salamander species. Wide-ranging species indicated by *. ANOVAs indicate significant differences between species (p -value $< 2.2e-16$) and horizontal lines near top of each figure indicate Tukey HSD test results. Species sharing the same line are not significantly different from one another ($p > 0.05$).

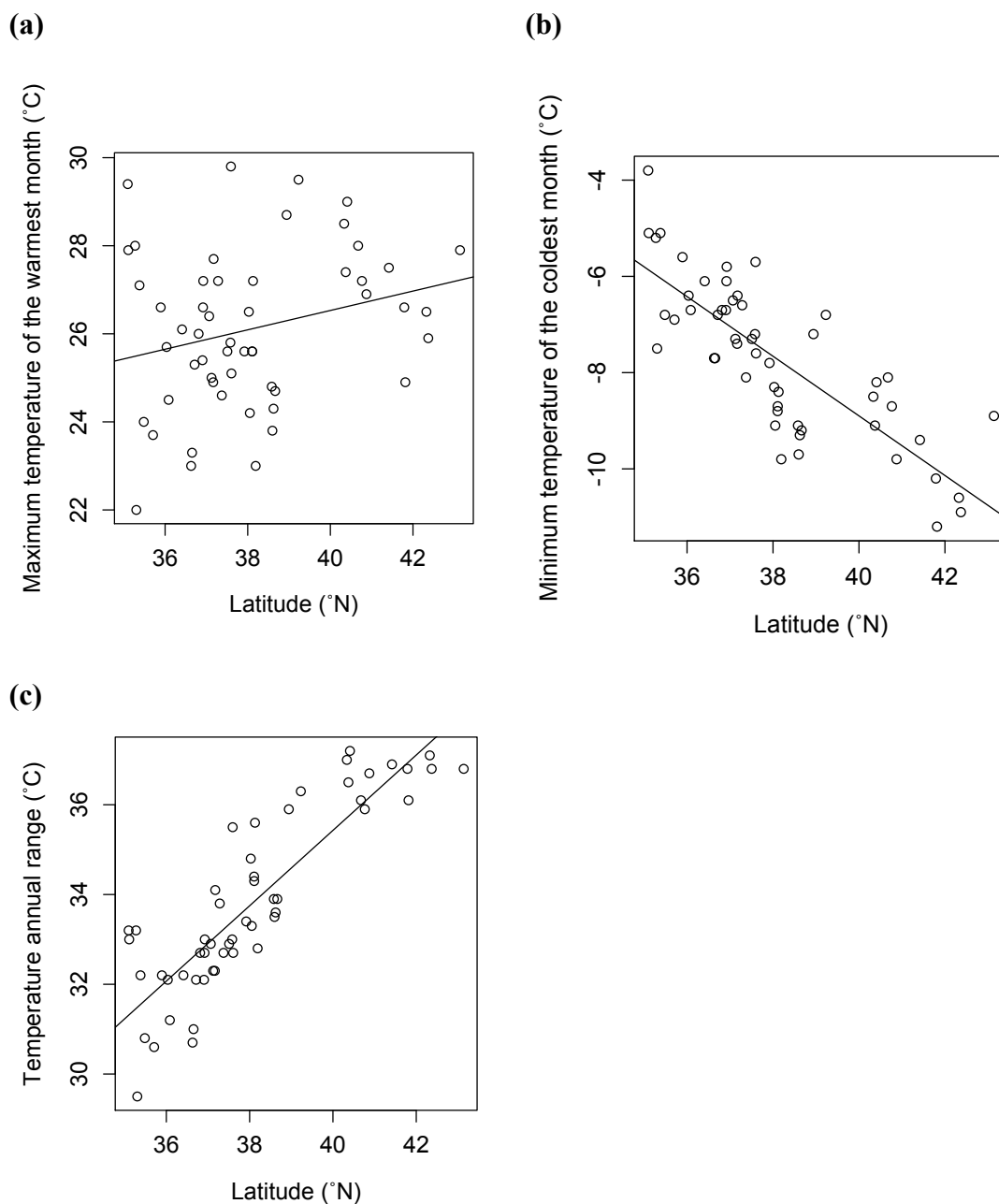


Figure 5. Linear regressions of site locality latitude versus temperature (n=53). (a) Maximum temperature of the warmest month versus latitude (p-value = 0.062972, $R^2 = 0.0476$), (b) minimum temperature of the coldest month versus latitude (p-value = 4.45×10^{-13} , $R^2 = 0.639$), and (c) temperature annual range versus latitude (p-value $< 2 \times 10^{-16}$, $R^2 = 0.753$).

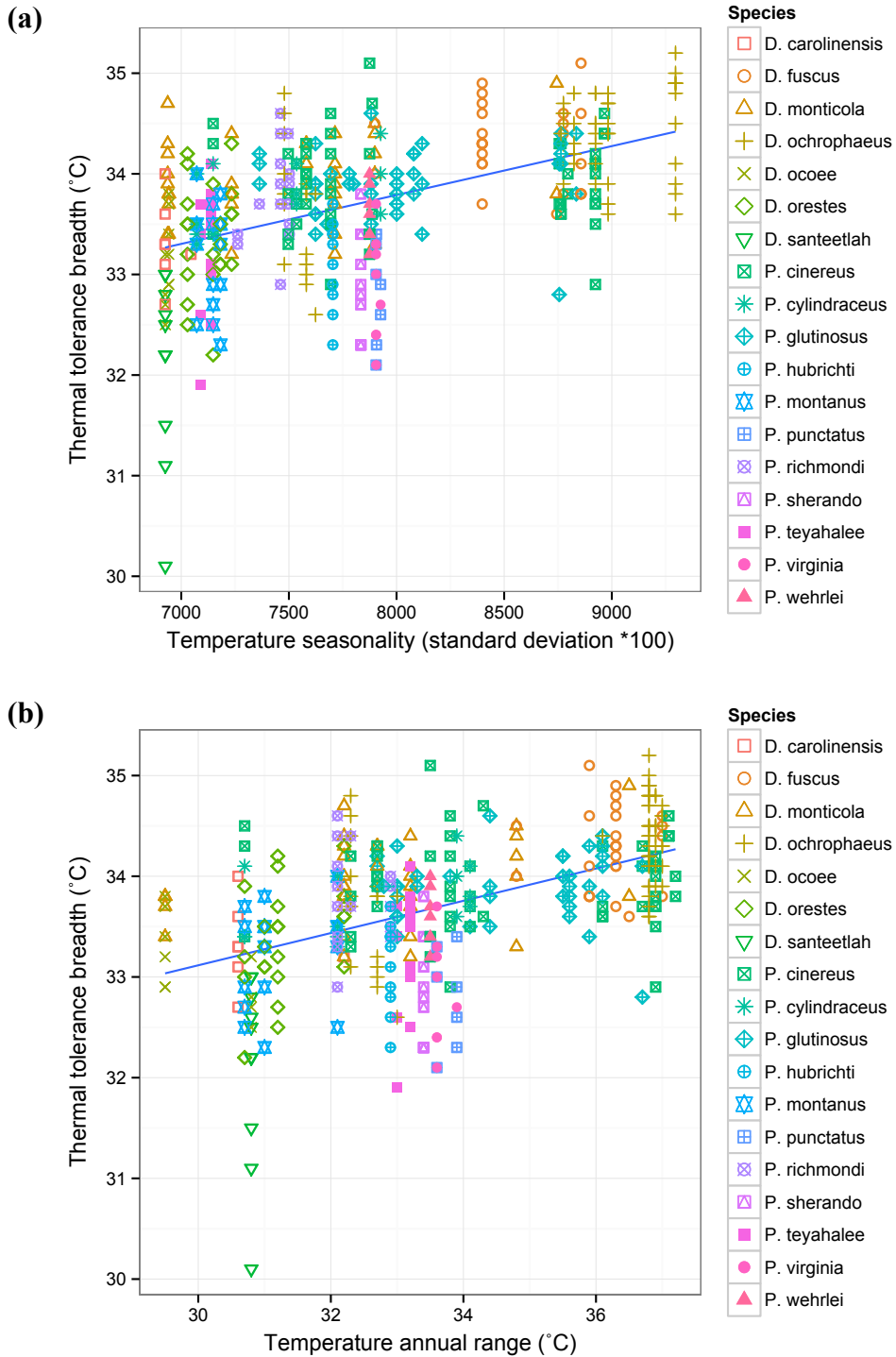


Figure 6. Thermal tolerance breadth versus measures of seasonality using individual-level results of salamanders: (a) thermal tolerance breadth versus temperature seasonality (standard deviation * 100) ($p = 0.000685$, $R^2 = 0.637$, $n=366$) and (b) thermal tolerance breadth versus temperature annual range (p -value = 0.000920 , $R^2 = 0.624$, $n= 366$).

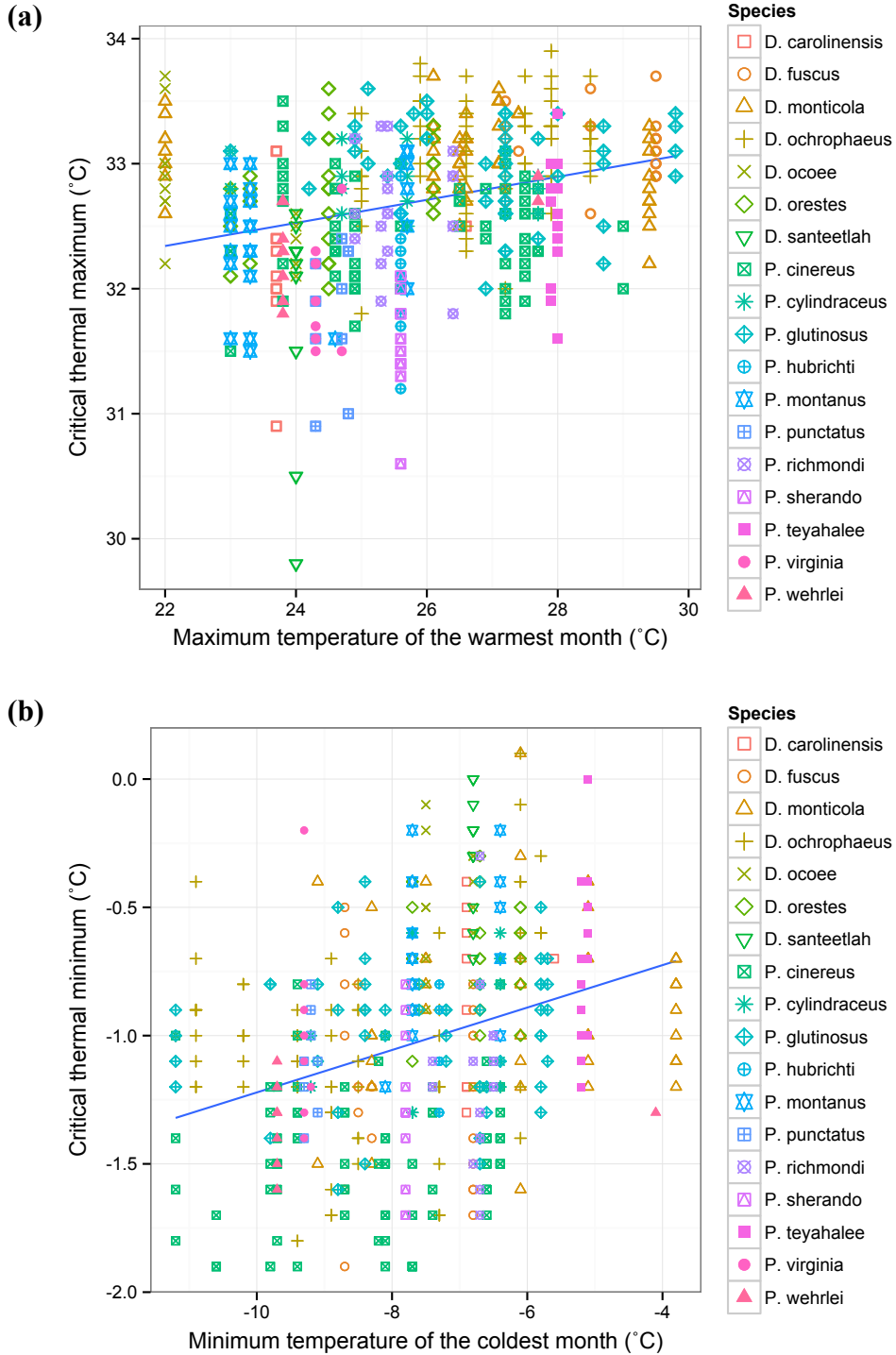


Figure 7. Thermal tolerances versus temperature max and min for individual salamanders: (a) CTMax versus Tmax (maximum temperature of the warmest month) ($p = 0.8303$, $R^2 = 0.47$, $n = 468$) and (b) CTMin versus Tmin (minimum temperature of the coldest month) ($p = 0.0123$, $R^2 = 0.46$, $n = 439$).

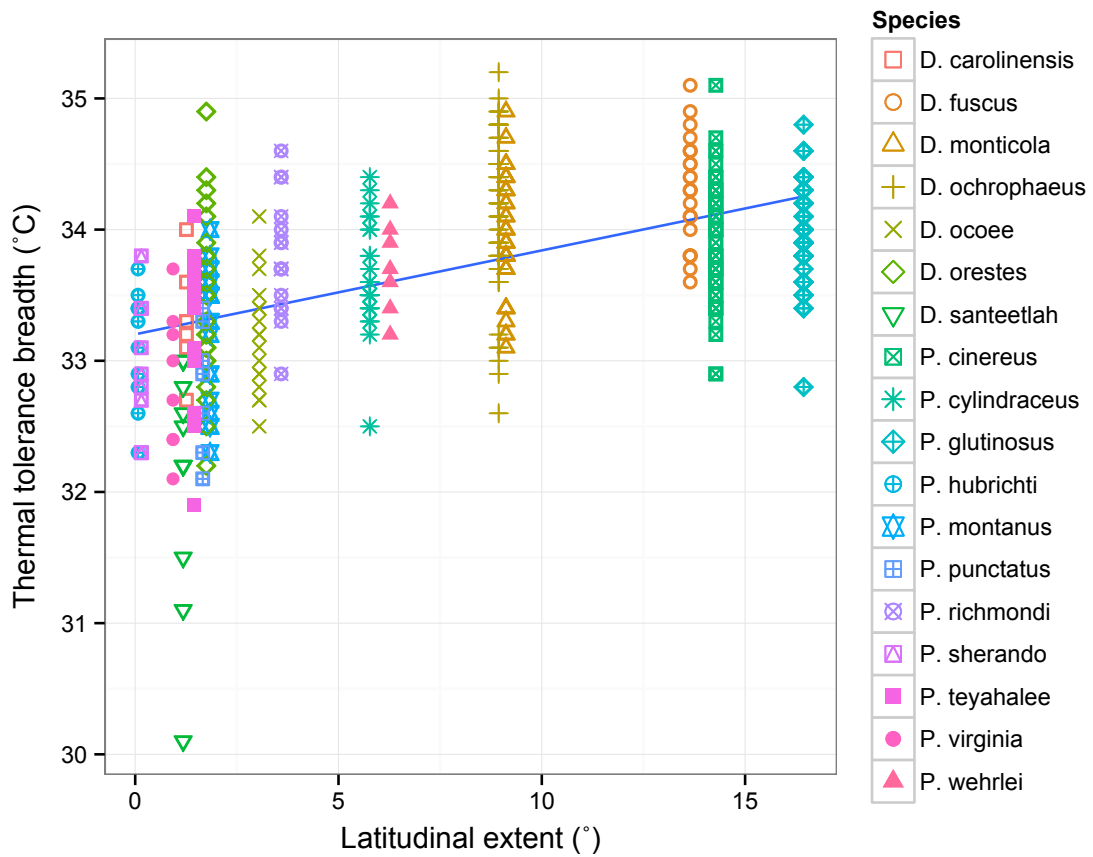


Figure 8. Individual-level thermal tolerance breadth versus latitudinal extent by salamander species ($p < 2e-16$, $R^2 = 0.28$, $n = 412$).

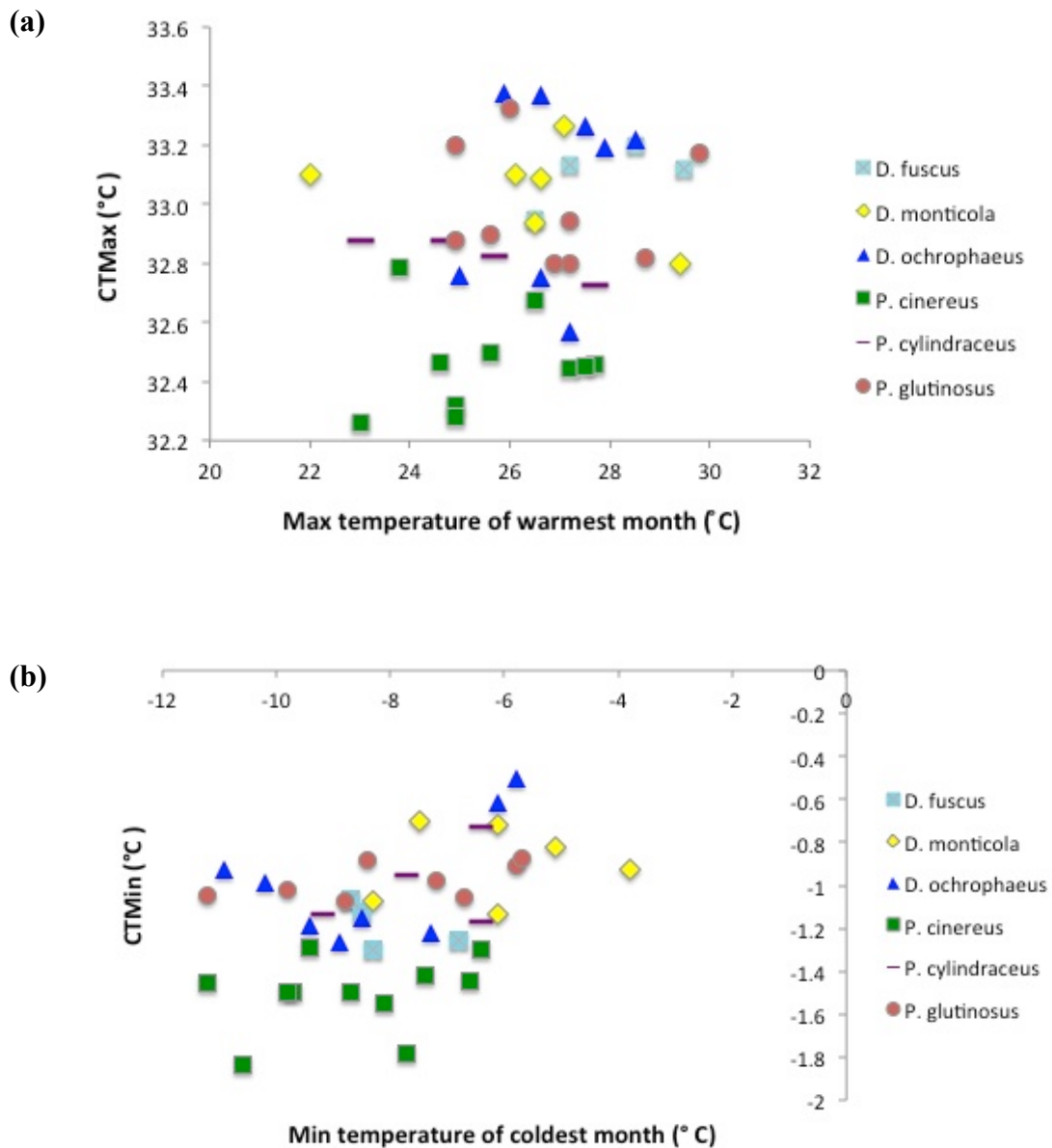


Figure 9. Intraspecific regression results for 6 species of wide-ranging North American plethodontid salamanders (figure indicates population means). (a) CTMax (critical thermal maximum) versus Tmax (maximum temperature of the warmest month) and (b) CTMin (critical thermal minimum) versus Tmin (minimum temperature of the coldest month). The only species with a statistically significant relationship is *D. ochrophaeus* for CTMin versus Tmin ($p = 0.005439$, $R^2 = 0.209$).

Chapter 2

Thermal Acclimation, Range Size Variation, and the Fate of Thermal Specialists Under Climate Change

INTRODUCTION

Animals adapt to thermally heterogeneous environments in numerous ways. Behavioural adjustments (such as moving to a warmer or cooler location) help to mitigate temperature impacts, whereas over the long-term, natural selection provides physiological adaptation to specific conditions (Hertz 1981; Christian et al. 1988). Another key short-term response of many species is thermal acclimation (physiological adjustment in response to temperature change), which enables an animal to express a wider range of physiological tolerances (Fry 1958; Brattstrom 1968; Christian et al. 1988; Spicer & Gaston 1999; Calosi et al. 2008). Such physiological plasticity may be critical for ectotherms, which rapidly take on the temperature of their environment (Fitzpatrick 1973a; Feder 1976). As temperature influences physiology and metabolic rate (Hochachka & Somero 1973; Bennett & Dawson 1976), it can affect numerous life-history traits including maintenance, growth rate, digestion, reproduction, and development (Berven 1982; Feder 1985; Clarke 1993; Ashby 1997; Dunham & Beaupre 1998). Thermal acclimation can therefore favorably influence fitness by helping an individual to maintain a positive energy balance in all seasons (Feder 1984; Feder 1978).

Species living in temperate habitats often face large seasonal fluctuations in temperature, requiring adaptive physiological strategies to survive. As an extension of the

climate variability hypothesis, a correlation should exist between the capacity for thermal acclimation and habitat seasonality (Scholander et al. 1950; Brattstrom 1968; Prosser 1975; Stevens 1989; Cadena et al. 2012). Greater physiological plasticity could enable species to occupy wider geographic ranges and may be critical for the invasion of higher latitudes (Navas 2006; Fernandez & Vrba 2005). Empirical studies have shown that tropical amphibians and reptiles that remain in relatively stable or narrow habitats show poor acclimation ability, whereas temperate species are more commonly found to adjust their physiologies in response to temperature change (Brattstrom 1968; Feder 1978; Huey et al. 2009; Young & Gifford 2013). Physiological adjustment to temperature would have little value in more constant climates, and narrow-ranging species would therefore fail to evolve such traits (Janzen 1967; Feder 1978; Stevens 1989). If acclimation ability is more prominent in wide-ranging species, it could help to explain the great disparity in geographic range size found among many closely related, ecologically similar species (Gaston 2003).

One common way of measuring thermal acclimation in ectotherms is to test for differences in standard metabolic rate (SMR) for individuals acclimated at higher and lower temperatures (Feder 1978; McKechnie 2008; Barcelo et al. 2009). When SMR is then measured at the same test temperature, species with acclimation ability should have higher oxygen consumption at the higher acclimation temperature, indicating a positive response shift in physiology (Feder 1985; Angilletta 2009; Hillman et al. 2009). A drop in metabolic rate (metabolic depression) after acclimation at a higher temperature, however, could be an indication of physiological stress (Bernardo & Spotila, 2006). Here,

we ask whether wide-ranging species of North American salamanders have a greater propensity for thermal acclimation than narrow-ranging species.

Further, evolutionary trade-offs in physiological tolerances are rarely investigated and are likely to have important implications for species' geographic range limits and vulnerability to climate change (Pörtner et al. 2006; Calosi et al. 2008). Stillman (2003) proposes that tradeoffs should exist between acclimatory capacity and thermal tolerance. This follows results that porcellanid crabs with the greatest thermal limits had the lowest acclimatory ability for those traits. Calosi et al. (2008), however, find that in European diving beetles, those species with the lowest acclimatory ability also have the lowest tolerance to warm temperatures. Using thermal tolerance data from Chapter one, we will test whether species with broad thermal tolerances also have broad acclimation ability, or whether as Stillman (2003) proposes, there are tradeoffs between these traits.

We focus on 17 ecologically similar salamander species that vary in geographic range size. All species are from the family Plethodontidae, with known evolutionary relationships (Kozak et al. 2009), enabling results to be analyzed in a phylogenetic context. A relationship between thermal acclimation ability and latitudinal extent would provide support for the climate variability hypothesis (i.e. wide-ranging species should exhibit greater thermal acclimation of SMR). We also investigate whether there is a trade-off between thermal tolerance and acclimation ability. Although acclimation ability and thermal tolerance are key traits in physiological ecology, few other studies have examined their interrelationships within a group of closely related species. By quantitatively testing the broader patterns of physiological traits and possible trade-offs

between traits, we will gain a better understanding of the ability of species to respond to environmental change (Bozinovic et al. 2011).

METHODS

Collection and maintenance of salamanders

Live salamanders were collected throughout the Appalachian Mountains of eastern North America from 2009 to 2012. Seventeen species of plethodontid salamanders representing four clades were examined: the genus *Desmognathus*, the *Plethodon cinereus* group, the *Plethodon glutinosus* group, and the *Plethodon wehrlei* group (Table 1). The family Plethodontidae represents a diverse group of lungless salamanders and the phylogenetic relationships among species in this study are strongly supported based on phylogenetic analysis of mitochondrial- and nuclear-DNA sequences (Kozak et al., 2009). Species were chosen to represent a wide variety of range sizes, and mature salamanders were collected from throughout the latitudinal extent of each species' range to provide a "whole species" estimate of metabolic acclimation.

Once collected, salamanders were transported back to the laboratory and maintained at 14°C in an environmental chamber until ready for testing. A light:dark photoperiod of 12L:12D was implemented. Salamanders were kept in individual plastic containers lined with moist paper towels and fed crickets on a weekly basis.

Experimental design (SMR measurements)

Conspecific salamanders were sorted by weight and then randomly split into two equivalent groups to be acclimated for a minimum of 2 weeks at 14°C or 22°C (see Hutchison 1961; Feder et al. 1984). Environmental chambers maintained air temperature within 1°C of the desired acclimation temperature. Acclimation temperatures are representative of fairly typical late spring through early fall evening temperatures that surface-active salamanders within each range would experience (Brattstrom 1963), although 22°C would be at the higher end for some montane endemics. Both mature male and female salamanders were included in the analyses, however, gravid salamanders were not used. Sample sizes per species ranged from 6 to 44 with a summed total of 305 salamanders (see Table 1). Before each trial, salamanders were measured to the nearest 0.001g.

To approximate standard metabolic rate (SMR), we recorded oxygen consumption (VO_2) at rest (see Fitzpatrick et al. 1972; Homyack et al. 2010). Automated closed-system respirometry (Sable Systems International, Hendersonville, NV) was used to measure oxygen consumption at three test temperatures of 5, 15, and 25°C. As diet influences metabolic rate, salamanders were not fed for 7 days prior to the start of the first respirometry trial (Lagerspetz 1977; Feder 1982). Oxygen consumption measurements were made at one temperature per day, with the order of test temperature assigned randomly to individuals and alternating every week. Salamanders were placed inside individual tubes (with two-way stopcocks) within a digitally controlled incubator. Up to seven animals could be measured during the same trial, with each chamber

recording independently. An empty chamber identical to the others was used as the baseline and control.

For each trial run, oxygen consumption was recorded for each chamber for 75 seconds at 10-minute intervals and then repeated for two to three hours. Air entering the salamander chambers was scrubbed of CO₂ and entered at a known flow rate of 250 ml/min. Before entering the respirometry tubes, air passed through a water air bubbler to control humidity and prevent desiccation of the salamanders. Air leaving the chambers passed through dryrite and ascarite to remove both water vapor and CO₂ before entering the oxygen analyzer where concentrations were recorded each second by data acquisition software (FC-10a, Sable Systems International, Las Vegas, NV). Rates of oxygen consumption ($\mu\text{l VO}_2/\text{hr}$) were calculated based on equations from Withers (1977).

Measurements were taken between 9am and 4pm, during the time when nocturnal salamanders would naturally be inactive. As salamanders inhabit underground retreats or spaces beneath rocks and logs during the day, they are well suited to moist respirometer vessels and are assumed to remain inactive for the most part (Feder et al. 1984).

Salamanders were allowed to habituate inside the test chambers for the first hour, and as such, data from this period were not included in the analysis. From the remaining runs, the mean of the values from the lowest two runs per test temperature was taken as the approximated SMR for each individual.

Once all trials were complete for individuals at a given acclimation temperature, salamanders were given a multi-week rest period at 14°C before being acclimated at the remaining temperature. Each individual therefore had a total of 6 trials (5, 15, and 25°C for each acclimation treatment at 14 and 22°C). Our experimental design attempted to

control for many of the extrinsic factors that can affect metabolic rate, e.g. feeding, photoperiod, acclimation length, activity, season, and reproductive state (see Lagerspetz 1977; Feder 1982). Using individual-level data, average SMRs were then calculated for each species.

Statistical analyses

To determine whether there are differences in SMR between wide and narrow-ranging species when acclimated at different temperatures, we ran a phylogenetically controlled Markov chain Monte Carlo generalized linear mixed model (MCMCglmm) with repeat measures. All modeling was conducted in R ver. 3.1.2 using packages APE and MCMCglmm (See Appendix 1 for code). Metabolic rates were \log_{10} transformed prior to analysis for data to meet the assumption of normality. Fixed factors to examine the influence on \log_{10} VO_2 included: test temperature (5, 15, 25°C), acclimation temperature (14 and 22°C), and range size (latitudinal extent, as well as species grouped as wide or narrow-ranging). To assign species as wide or narrow-ranging, a natural break was found between species with latitudinal extents greater than or less than 5 degrees of latitude. Sex, and mass were also included as covariates in the model. The initial model also included several interactions: test temperature*acclimation temperature, test temperature*range size, acclimation temperature*range size, and test temperature*acclimation temperature*range size. Individual salamander was used as a random factor and as a repeated measure. The three-way interaction, as well as the two-way interaction for test temperature*range had p-values > 0.30 and were removed.

To examine where differences in SMR occur within narrow and wide-ranging

species, additional MCMCglmm analyses were performed for each test and acclimation temperature within each group. Additionally, species-level tests including sex, species, and mass as covariates, were performed to observe species-level trends in thermal acclimation at each test temperature. Although mass is included as a covariate in all models, for display purposes many of our figures present weight-corrected metabolic rates. Regardless of method used, p-values remain essentially unchanged. To correct SMR for the influence of body mass, we used the formula $M=A.W^b$, where M =metabolic rate in $\mu\text{l O}_2$ consumed per hour; W =body weight in g; and A and b are constants (Hart 1971; Feder 1976). The constant b reflects the degree to which metabolic rate is affected by body mass, and is the slope of the line for the \log_{10} - \log_{10} plot of SMR versus mass. Slopes for this relationship depend on test temperature. Calculated average slopes used in the equation are $b=0.59$ for 5°C and $b= 0.71$ for measurements at 15 and 25°C .

Finally, to investigate if there are trade-offs between physiological abilities, a linear regression was performed between thermal acclimation ability (based on individual-level differences in SMR between acclimation temperatures) and thermal tolerance breadth of species, including covariates mass and sex. Thermal tolerance data come from earlier physiological tests of these species. For discussion of these methods see Chapter one of this thesis. Further, phylogenetic relatedness was considered for SMR and thermal tolerance breadth in this analysis. AIC scores of Brownian motion versus lambda models were compared and lambda models were chosen in all cases. This indicates that these traits have no phylogenetic signal and do not need to be corrected for phylogenetic non-independence in this test.

RESULTS

MCMCglmm analyses of the full data set revealed significant 2-way interactions between acclimation temperature and geographic range size: for latitudinal extent ($p = 0.0436$) and for wide- versus narrow-ranging species ($p < 0.004$) (Fig. 1). In addition, a significant two-way interaction was found for test temperature by acclimation temperature ($p < 0.05$ for both variations of range size) (Fig. 2). Sex and mass remained as significant covariates in all models.

For data grouped by range size, MCMCglmm analyses revealed that narrow-ranging species have a significant drop in LVO_2 at the higher acclimation temperature for test temperatures 5°C ($p < 0.004$) and 25°C ($p < 0.004$) (Fig. 3a). For wide-ranging species, there is a significant increase in LVO_2 at the higher acclimation temperature for test temperature 15°C ($p = 0.0073$) (Fig. 3b).

At the species-level, SMR results (Table 2) and MCMCglmm tests revealed some trends of interest (Figs. 4 a-j & 5 a-g). For the ten narrow-ranging species, the majority (7 out of 10) showed metabolic depression at the higher acclimation temperature, especially for the highest test temperature. Four of the ten species (*D. orestes*, *P. montanus*, *P. punctatus*, and *P. richmondi*) show a significant drop in SMR with increased acclimation temperature at the 25°C test temperature (p -values all < 0.05). For wide-ranging species there is a mixed response, with some species increasing SMR with increased acclimation temperature, others decreasing SMR, and some remaining essentially unchanged. For instance, *D. monticola*, is found to have a significant increase in VO_2 at the 25°C test temperature with increasing acclimation temperature ($p = 0.00039$), whereas,

P. glutinosus is found to have a significant decrease ($p = 0.026$). Taken together, these results indicate that many narrow-ranging species exhibit metabolic depression at lower temperatures than other salamanders. Further, although wide-ranging species have large variation in their SMR response, the overall trend is for the SMRs of wide-ranging species to increase slightly with acclimation temperature.

Finally, we find no relationship/trade-off between acclimation ability and thermal-tolerance breadth ($p = 0.273$, $R^2 = 0.017$) (Fig. 6). Mass was found to be a significant covariate (p -value = 0.017) but sex was not (p -value = 0.292).

DISCUSSION

Thermal acclimation provides a beneficial physiological adjustment in response to temperature. As acclimation offers a selective advantage in places with greater temperature variation, species with wider latitudinal extents are expected to have a greater capacity for thermal acclimation than narrow-ranging species. Results of our MCMCglmm analyses indicate that wide and narrow-ranging species are significantly different in how their standard metabolic rates respond to temperature. On average, wide-ranging species acclimated at the warmer temperature have an increase in mean VO_2 at higher test temperatures, whereas narrow-ranging species held at 22°C have a sharp drop in VO_2 compared to those at 14°C.

When narrow and wide-ranging species are investigated at the species-level, we find the majority of wide-ranging species (five out of seven) have a trend of increasing VO_2 consumption at the higher acclimation temperature, although only one

(*D. monticola*) has a statistically significant increase at the highest test temperature. These results indicate that these species are potentially capitalizing on energy afforded by warmer temperatures and increased metabolic rates (Angilletta 2009; Hillman et al. 2009). For narrow-ranging species, seven out of ten have trend of decreasing SMR at higher acclimation and test temperatures, while four have a statistically significant drop. These results support predictions of the climate variability hypothesis, as wide-ranging species are able to physiologically adjust and maintain regular functions with increasing temperature, whereas the majority of narrow-ranging species are not (Brattstrom 1968; Feder 1978; Navas 2006; Calosi et al. 2008). Although a reduction in SMR is an evolutionary response to conserve energy during short-term bouts of suboptimal conditions, substantial metabolic depression can be a sign of physiological stress (Bernardo & Spotila 2006). It should also be noted that a small number of otherwise healthy montane individuals died over the course of acclimation at 22°C, further suggesting metabolic distress at this temperature. Thus, narrow-ranging montane species appear to be more sensitive to warm temperatures than other species of salamanders that we examined in this study.

Physiological specialization to cooler habitats has been hypothesized to be a trade-off at the expense of larger distributions and lowland dispersal (Huey & Kingsolver 1993; Gilchrist 1995; Bernardo & Spotila 2006). Many temperate amphibians (including salamanders) are adapted for activity at mild/cooler temperatures, typically at field body temperatures below 20°C (Brattstrom 1963; Navas 2006). Although behavioral thermoregulation can offer some reprieve from temperature extremes, salamanders may be especially sensitive to rapid or prolonged changes in their environment (Huey &

Stevenson 1979). For species not well adapted to warm temperatures, even moderate increases in temperature can have large effects on physiological systems. High temperatures can impair enzymatic function and disrupt membrane structure, causing reduced locomotion ability, digestive inefficiencies, and reduced growth (Angilletta 2009; Hillman et al., 2009). For lungless salamanders in particular, cutaneous respiration on its own may not provide sufficient oxygen at warmer temperatures (Whitford & Hutchison 1965, 1967). Ultimately, these effects will lower the fitness and survival of individuals. Although some species of *Plethodon* inhabit a wide range of elevations, recent work suggests that the ancestor of this group was restricted to a montane climate (Kozak & Wiens 2010). As such, adaptation to cooler, higher elevation climates may be constraining low-elevation dispersal and geographic ranges of many salamander species (Gifford & Kozak 2012).

One question that remains is whether there are any evident trade-offs in adaptive thermal physiology for these species. For instance, higher thermal tolerances could come at the cost of reduced acclimation or growth. Other studies have shown that trade-offs do exist (see Stillman 2003; Calosi et al. 2008) and some have indicated that acclimatory capacity may be more important than thermal tolerance per se in determining vulnerability to climate change (Stillman 2003). Our results, however, find no relationship between acclimation ability and thermal-tolerances, and therefore no evidence of trade-offs for these traits.

Conclusion

Thermal acclimation may enable a species to occupy more seasonal habitats, and is thought to be an important factor determining life histories and distributions of species (Angilletta 2009; Angert et al. 2011). Here, we find a significant difference in acclimation ability between wide and narrow-ranging temperate salamanders. Wide-ranging species have a trend of increasing SMR for those acclimated at a higher temperature, whereas many narrow-ranging species show significant metabolic depression at higher test and acclimation temperatures. This could be an indication that narrow-ranging montane species are more likely to suffer metabolic distress under a warming climate than low- elevation/broad-ranging salamander species.

A better understanding of species' physiological tolerances is important in the face of rapid climatic change. How a species will fare will depend on the plasticity and strength of their thermal tolerances, their ability to adapt, and their accessibility to alternative suitable habitat (Kozak & Wiens 2010; Davis & Shaw 2001). Plasticity may be important, as species that are unable to acclimate thermally in response to changes in temperature may be at a decided disadvantage. There are already indications that some species are near their thermal limits (Bernardo & Spotila 2006; Gifford & Kozak, 2012) and to predict which species are likely to be most vulnerable we need quantitative physiological data for multiple species. Unfortunately, few studies to date have provided the necessary data linking species' physiology and vulnerability to climate change (cf. Calosi et al. 2008). This research helps to highlight the genetic components of physiological tolerances and such data is likely to be critical in making predictions of

how future climates will influence geographic ranges (see Chown et al. 2004; Bernardo et al. 2007).

Table 1. Salamander groups and species, detailing number of individuals (n), mean body mass (g), CTMax (Critical thermal maximum), CTMin (Critical thermal minimum), thermal-tolerance breadth (CTMax – CTMin), and latitudinal extent (degrees).

Species	n	Mean body mass (g)	Mean CTMax (°C)	Mean CTMin (°C)	Thermal tolerance breadth (°C)	Latitudinal extent (degrees)
Desmognathus group (7 species)						
<i>D. carolinensis</i>	8	1.10	32.2	-0.87	33.1	1.26
<i>D. fuscus</i>	20	2.07	33.1	-1.2	34.3	13.65
<i>D. monticola</i>	24	3.66	33.1	-0.86	34.0	9.12
<i>D. ochrophaeus</i>	36	1.01	33.1	-1.0	34.1	8.94
<i>D. ocoee</i>	12	1.33	32.7	-0.64	33.3	3.05
<i>D. orestes</i>	18	1.13	32.8	-0.72	33.5	1.75
<i>D. santeetlah</i>	8	1.35	31.8	-0.28	32.1	1.18
Plethodon cinereus group (4 species)						
<i>P. cinereus</i>	44	0.89	32.5	-1.5	34.0	14.28
<i>P. hubrichti</i>	11	1.18	32.1	-1.1	33.2	0.07
<i>P. richmondi</i>	14	1.07	32.7	-1.2	33.9	3.59
<i>P. virginia</i>	8	1.28	32.0	-0.99	33.0	0.93

Plethodon						
glutinosus group						
(4 species)						
<i>P. cylindraceus</i>	18	4.90	32.8	-0.94	33.7	5.77
<i>P. glutinosus</i>	33	4.85	33.0	-1.0	34.0	16.44
<i>P. montanus</i>	22	1.95	31.7	-0.73	32.4	1.84
<i>P. teyahalee</i>	16	6.11	32.6	-0.74	33.3	1.44
Plethodon						
wehrlei group						
(2 species)						
<i>P. punctatus</i>	6	3.81	31.9	-1.1	33.0	1.66
<i>P. wehrlei</i>	7	1.75	32.4	-1.3	33.7	6.27

Table 2. Effect of acclimation temperature (AT) on weight-corrected oxygen consumption ($\mu\text{l VO}_2/\text{hr}$) \pm SE for each test temperature (TT). Weight correction follows formula $M=A.W^b$.

Species	TT = 5C		TT = 15C		TT = 25C	
	14C AT	22C AT	14C AT	22C AT	14C AT	22C AT
<i>D. carolinensis</i>	28.27 \pm 11.29	19.17 \pm 7.36	60.27 \pm 19.91	85.43 \pm 26.53	129.4 \pm 28.26	117.53 \pm 24.71
<i>D. fuscus</i>	35.02 \pm 6.89	34.7 \pm 6.92	77.71 \pm 16.11	90.33 \pm 14.78	128.1 \pm 18.28	145.0 \pm 26.69
<i>D. monticola</i>	30.37 \pm 5.63	18.14 \pm 4.26	65.09 \pm 6.31	73.91 \pm 7.7	111.0 \pm 11.09	140.8 \pm 13.03
<i>D. ochrophaeus</i>	26.02 \pm 3.61	23.99 \pm 3.49	64.94 \pm 7.65	77.41 \pm 9.17	134.4 \pm 7.91	140.7 \pm 14.63
<i>D. ocoee</i>	29.17 \pm 7.65	32.30 \pm 4.88	59.81 \pm 13.85	71.82 \pm 18.02	144.8 \pm 30.52	154.3 \pm 30.59
<i>D. orestes</i>	27.16 \pm 5.88	29.46 \pm 3.31	73.05 \pm 11.84	60.29 \pm 11.39	129.6 \pm 19.02	98.96 \pm 17.13
<i>D. santeetlah</i>	33.72 \pm 13.24	21.4 \pm 6.04	83.12 \pm 18.54	74.29 \pm 25.43	126.9 \pm 25.58	147.6 \pm 28.98
<i>P. cinereus</i>	43.13 \pm 15.9	24.41 \pm 2.91	53.47 \pm 7.98	60.1 \pm 8.59	137.1 \pm 13.9	152.9 \pm 16.98
<i>P. cylindraceus</i>	22.15 \pm 4.24	29.8 \pm 6.45	44.25 \pm 6.72	50.30 \pm 7.54	110.5 \pm 8.75	114.9 \pm 12.26
<i>P. glutinosus</i>	22.73 \pm 3.55	21.73 \pm 3.98	47.19 \pm 4.86	49.09 \pm 6.2	123.0 \pm 15.32	105.1 \pm 10.04
<i>P. hubrichti</i>	34.39 \pm 9.87	26.88 \pm 5.58	38.83 \pm 6.19	52.07 \pm 20.88	148.9 \pm 32.2	133.2 \pm 26.37
<i>P. montanus</i>	22.43 \pm 4.04	21.14 \pm 3.22	53.14 \pm 8.35	45.17 \pm 7.04	125.8 \pm 20.84	93.88 \pm 18.12
<i>P. punctatus</i>	19.46 \pm 3.31	26.13 \pm 6.41	37.33 \pm 14.53	43.69 \pm 10.89	149.9 \pm 30.28	111.4 \pm 16.21
<i>P. teyahalee</i>	24.92 \pm 6.24	25.29 \pm 5.04	48.1 \pm 10.19	48.68 \pm 10.6	118.2 \pm 18.63	119.9 \pm 11.8
<i>P. richmondi</i>	21.48 \pm 3.43	18.46 \pm 7.45	39.71 \pm 5.06	24.57 \pm 4.55	110.6 \pm 12.83	89.19 \pm 13.91
<i>P. virginia</i>	31.2 \pm 10.07	20.8 \pm 6.35	52.24 \pm 21.65	46.69 \pm 15.51	160.6 \pm 43.05	136.7 \pm 43.37
<i>P. wehrlei</i>	13.8 \pm 4.99	27.58 \pm 2.58	30.39 \pm 9.39	38.24 \pm 6.88	132.7 \pm 31.07	123.5 \pm 48.23

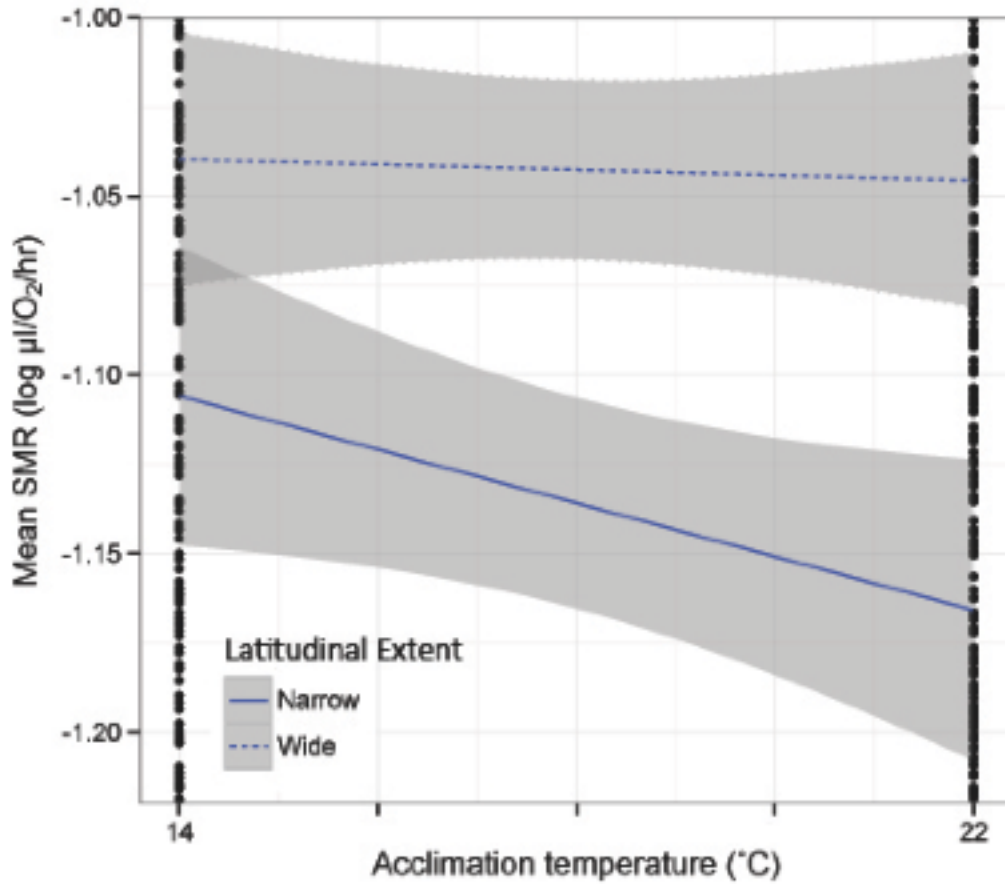


Figure 1. Acclimation temperature * Latitudinal extent two-way interaction from MCMCglmm model (p-value = 0.004, n=17 species). Wide-ranging species have little overall change in SMR between acclimation temperatures, whereas narrow-ranging species have a sharp drop in SMR at the higher acclimation temperature. Shaded areas indicate 95% confidence intervals.

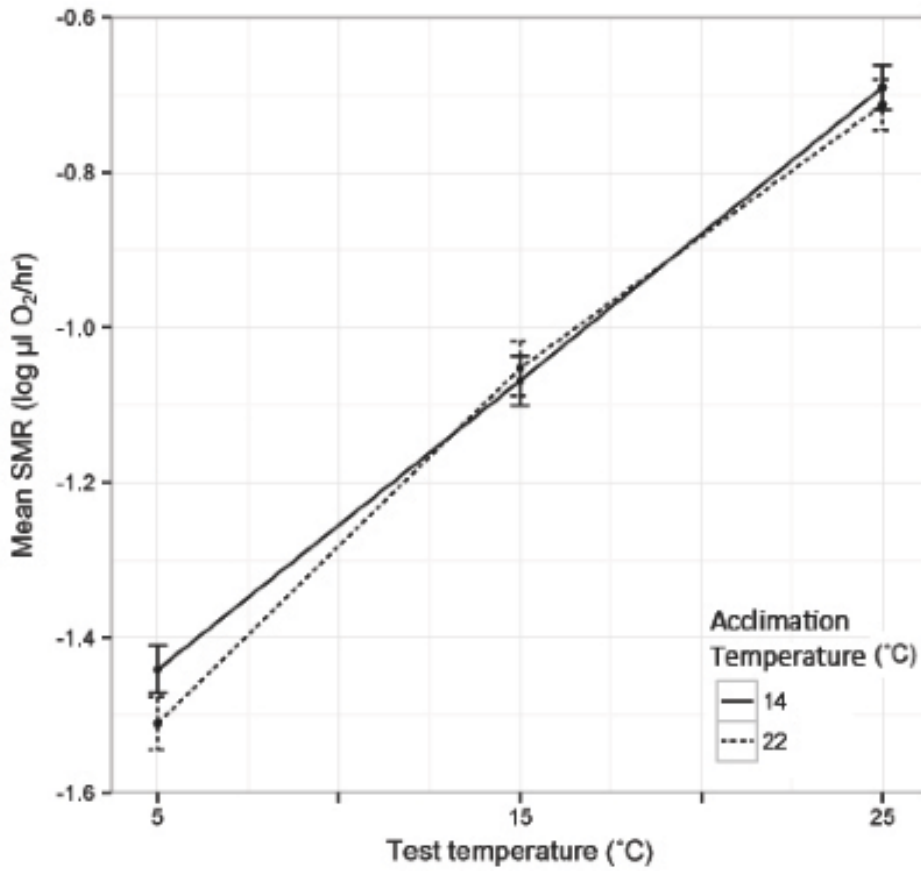


Figure 2. Test temperature*Acclimation, two-way interaction from MCMCglmm model (n = 17 salamander species). Interaction is significant ($p < 0.05$) for all model variations using latitudinal extent. Error bars indicate 95% confidence intervals.

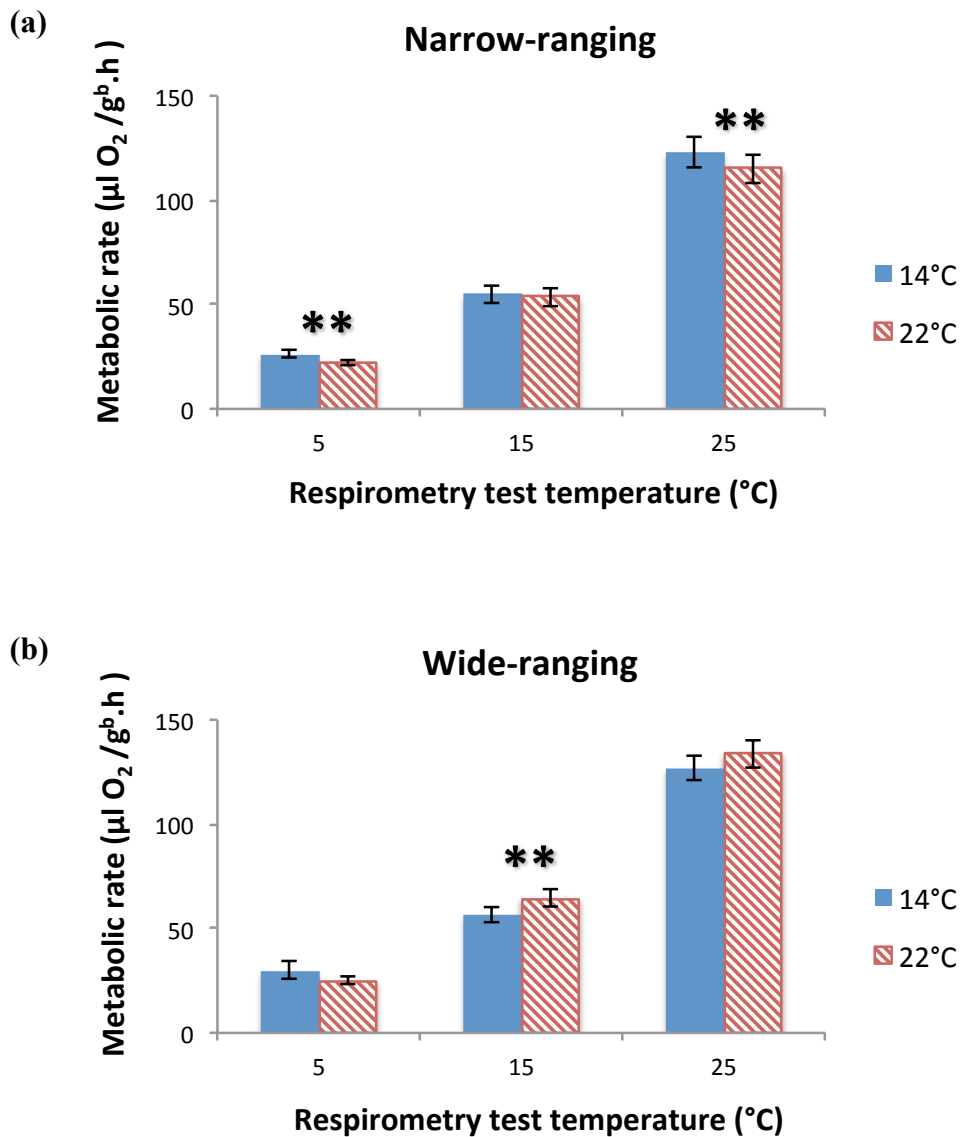
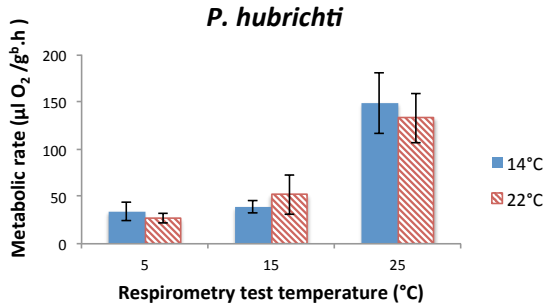
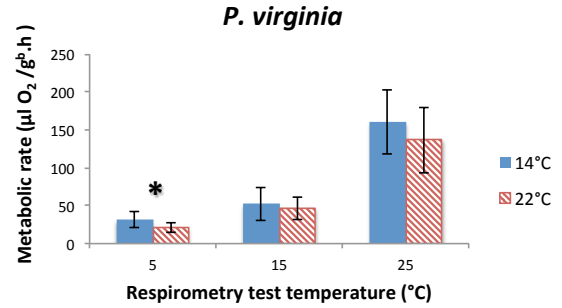


Figure 3. Effect of acclimation temperature on weight-corrected standard metabolic rates of salamanders. Species grouped into (a) narrow and (b) wide-ranging based on latitudinal extent $>$ or $<$ 5 degrees (17 species: 10 narrow and 7 wide-ranging). MCMCglmm to test for differences between acclimation temperatures for each test temperature. Means are plotted \pm 95% confidence limits. Significance of difference between 14°C and 22°C is given above each test temperature: * = $p < 0.05$, ** = $p < 0.01$; no symbol = $p > 0.05$.

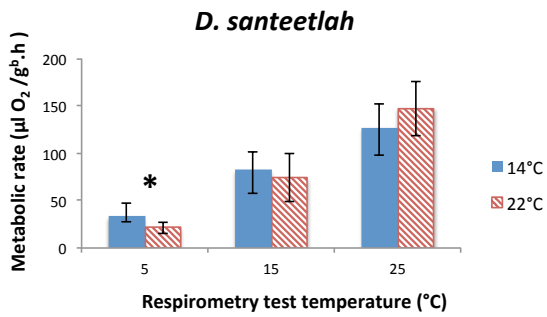
(a)



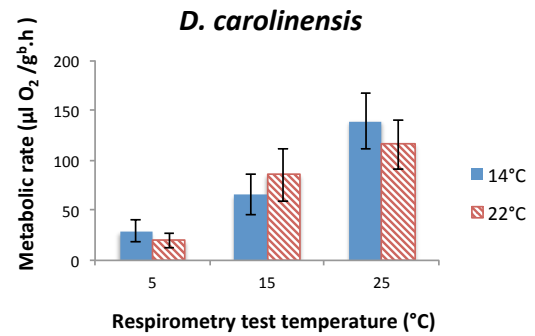
(b)



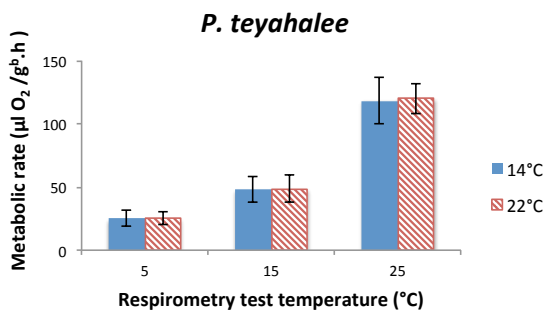
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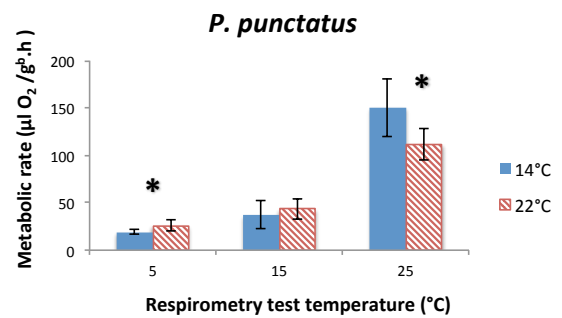
(d)



(e)



(f)



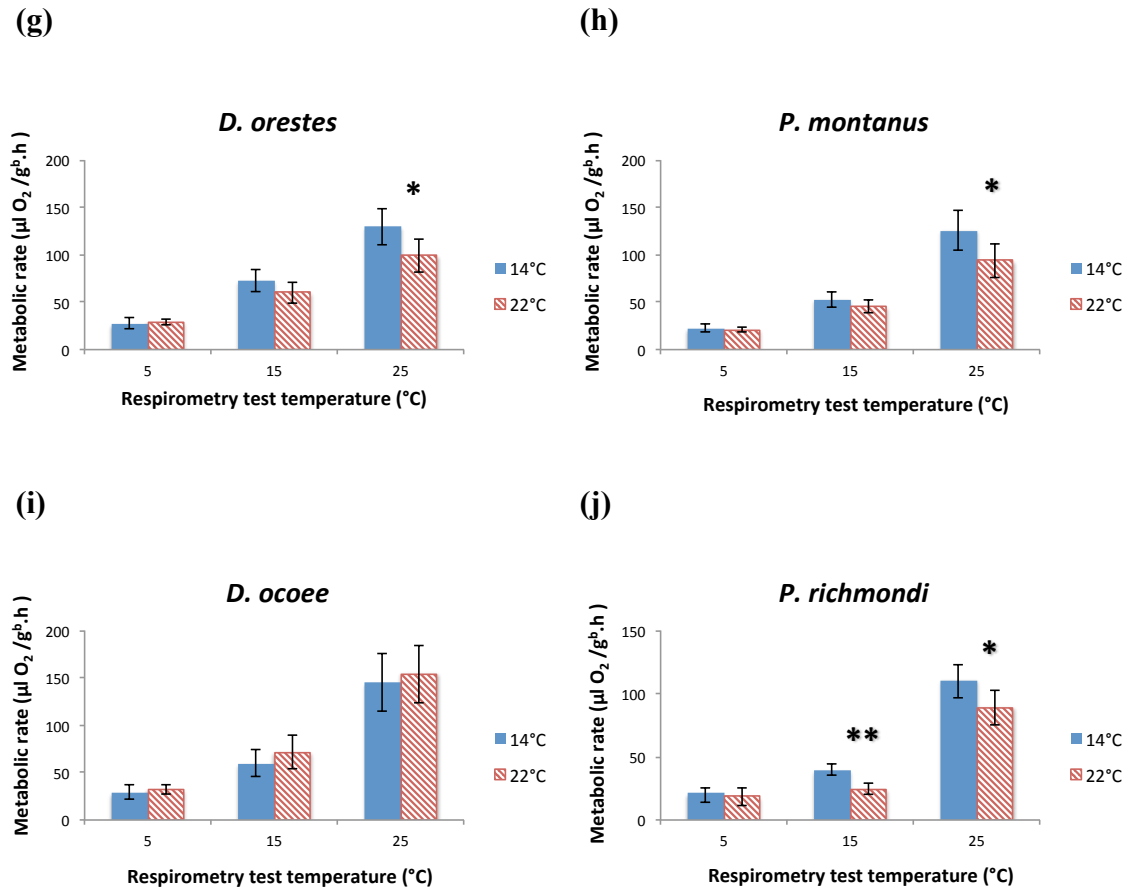
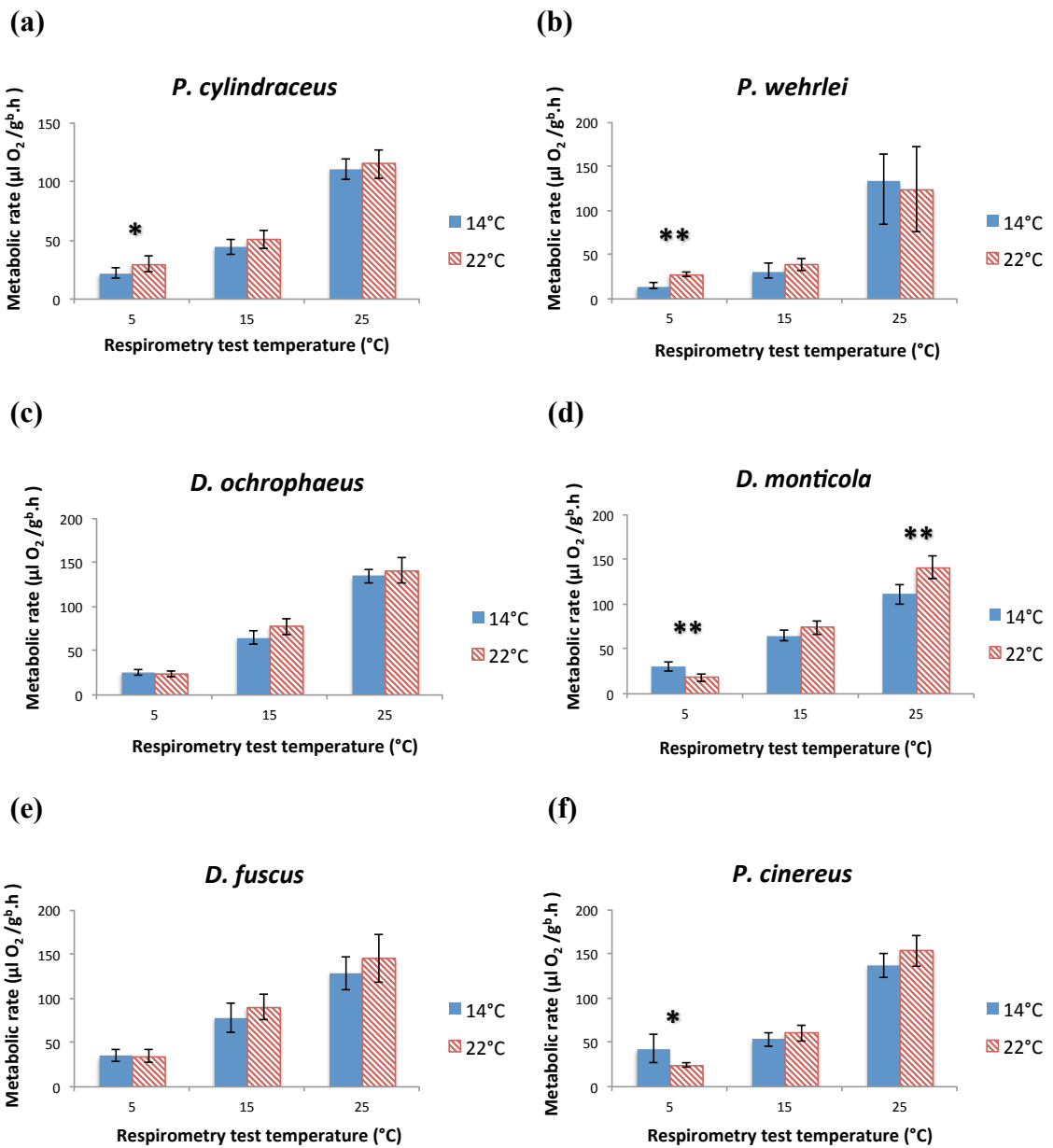


Figure 4. Narrow-rangin. Effect of acclimation temperature (14 vs. 22°C) on weight-corrected standard metabolic rates for 10 narrow-rangin salamander species. Species ordered from smallest to largest based on latitudinal extent (a)-(j). MCMCglmm tests for difference in acclimation at each test temperature (5, 15, and 25°C). Means are plotted +/- 95% confidence limits. Significance of difference between 14°C and 22°C is given above each test temperature: * = $p < 0.05$, ** = $p < 0.01$, no symbol = $p > 0.05$.



(g)

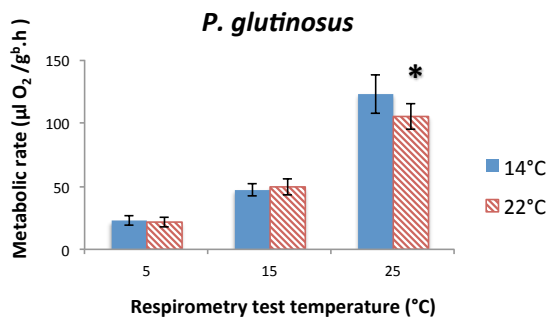


Figure 5. Wide-ranging. Effect of acclimation temperature (14 vs. 22°C) on weight-corrected standard metabolic rates for seven wide-ranging salamander species. Species ordered from smallest to largest based on latitudinal extent (a)-(g). MCMCglmm tests performed for acclimation at each test temperature (5, 15, and 25°C). Means are plotted +/- 95% confidence limits. Significance of difference between 14°C and 22°C is given above each test temperature: * = $p < 0.05$, ** = $p < 0.01$, no symbol = $p > 0.05$.

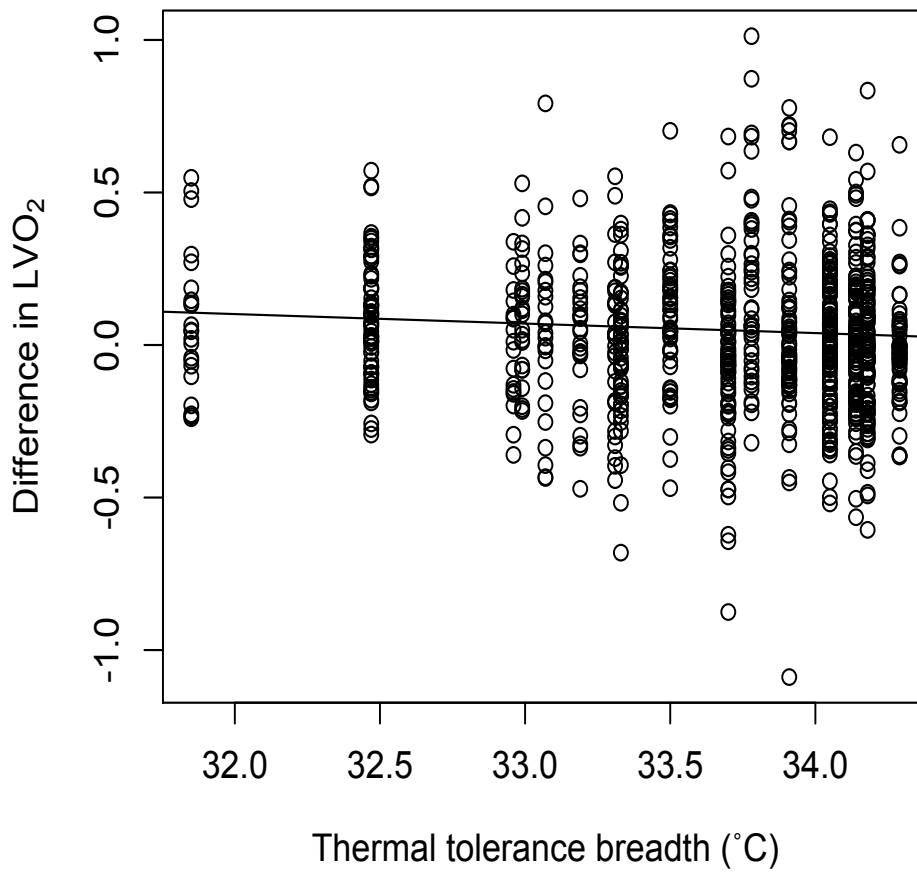


Figure 6. Acclimation ability (difference in LVO₂ between acclimation temperatures) versus thermal-tolerance breadth for 17 species of Plethodontid salamander. No apparent relationship/trade-off between acclimation ability and thermal tolerance breadth ($p = 0.273$, $R^2 = 0.017$).

Chapter 3

Relationship between Thermal Tolerance and Climatic Niche in North American Salamanders

INTRODUCTION

The climatic niche – the set of climatic conditions in which an individual/species can persist (Hutchinson, 1957; Soberón, 2007; Quintero & Wiens, 2013a), is thought to play an important role in limiting species' ranges. Quantifying dimensions of species' climatic niches across space and time is critical for understanding biotic responses rapid environmental change. Yet, the factors that regulate species' distributions remain poorly understood (Gaston, 2003; Thuiller et al., 2004). Moreover, the relationship between the climatic niche and range-limiting physiological traits has rarely been investigated (see Soberón, 2007; Fisher-Reid et al., 2012; Quintero & Wiens, 2013b).

Geographic variation in temperature is thought to play a fundamental role in limiting species' ranges (Andrewartha & Birch, 1954; Hutchison, 1961; Janzen, 1967; Merriam, 1984; Gaston, 2003), as many species have geographic ranges that correspond with thermal isotherms (Salisbury, 1926; Caughley et al., 1987; Root, 1988). Species from more temperate climates (that experience greater seasonality) are expected to have broader thermal tolerances than those from areas with more limited seasonality (i.e. those in the tropics) (Janzen, 1967; Stevens, 1989), leading to broader climatic niches and larger geographic ranges (Brown, 1984; Quintero & Wiens, 2013a; Slatyer, 2013). Such

variation in geographic range size is thought to be at least partly attributed to the evolution of physiological traits in response to climatic variability (Bozinovic et al., 2011; Quintero & Wiens, 2013a). However, despite a growing literature on using species' physiological traits to map species' climatic niches (Kearney & Porter, 2004; 2009; Monahan, 2009; Gifford & Kozak, 2012), few studies have examined the relationship between species' thermal tolerances and the climatic niche (Thuiller et al., 2004; Bernardo & Spotila, 2006; Bozinovic et al., 2011; Sunday et al., 2011). Further, the geographic ranges of species do not always correspond with the spatial extent of the climatic niche, suggesting that factors other than climate and physiology may underlie range limits (see Monahan, 2009; Barve et al., 2011; Peterson et al., 2011).

Here, we use thermal tolerance data from lab-based trials of North American salamanders (family Plethodontidae), along with GIS-based climate data from points where individuals are found, to examine the relationship between species' thermal tolerances and their estimated climatic (thermal) niches. Thermal tolerances are readily estimated for salamanders in the laboratory, and the availability of many closely related species with varying range sizes makes for an ideal study system. We collected critical thermal maximum (CTMax) and minimum (CTMin) data for 18 species of salamanders from laboratory trials and used these to estimate the thermal tolerance breadth of each species (defined as CTMax minus CTMin). To approximate the climatic (thermal) niche of each species' natural environment, climate data were extracted from known species localities. We calculated the thermal niche for each species by subtracting the coldest temperature of the coldest month from the maximum temperature of the hottest month across all localities based on averages from 1950-2000.

Using these data, we address the following questions:

1. Do mean thermal tolerances (CTMin and CTMax) of species correspond with maximum and minimum temperatures of species' ranges?
2. Do thermal tolerances of species relate to minimum and maximum latitudes of species' ranges or elevational positions?
3. Do species' thermal tolerances determine whether they will have a wide or narrow climatic niche? (i.e. is there a correlation between thermal tolerance breadth and thermal niche breadth at the species level?)
4. Is the climatic (thermal) niche breadth of a species related to its latitudinal extent?
5. Can laboratory thermal tolerances be used to predict the impacts of a rapidly warming climate on salamander distributions? (e.g. can they tell us which species are more likely to be imperiled?)

MATERIALS AND METHODS

Study System and Collections

The Appalachian Mountains are a well-known hotspot of salamander diversity, with the family Plethodontidae (lungless salamanders) being the most speciose family (60+ species) (Petranka, 1998; Wells, 2007). Here, we focused on 18 species of Plethodontid salamander for which thermal tolerance, climatic, and phylogenetic data were available. Species were chosen to provide representation across the family and to offer a variety of geographic range sizes within closely related groups (Table 1).

For thermal tolerance data, salamanders were collected from five states across eastern North America (North Carolina, Virginia, West Virginia, Pennsylvania, and New York) from 2009 to 2012. From each locality, three to ten individuals of a given species were collected by hand. Only mature salamanders were used for physiological trials, and wherever possible collections were made from multiple populations throughout the latitudinal extent of each species' geographic range. To control for possible variation in thermal physiology related to environment/seasonality, salamanders were acclimated in a temperature-controlled room with a 12L:12D light regime at 14.5°C for a minimum of four weeks prior to physiology trials. Collection and laboratory care of the salamanders followed all field permit and university protocols.

Thermal Tolerances

Critical thermal maximum temperatures were determined for 525 salamanders and critical thermal minima for 493 using standard nonlethal methods and loss of righting response (LRR) as the end point (Hutchison, 1961; Brattstrom, 1968; Layne & Claussen, 1987; Lutterschmidt & Hutchison, 1997) (Table 1). This measurement closely follows the original definition of Cowles & Bogert (1944) where CTMax and CTMin are defined as “the thermal point at which locomotory activity becomes disorganized and the animal loses its ability to escape from conditions that will promptly lead to its death.” As deep body temperature of salamanders closely follows water temperatures at heating rates of 1°C per minute (Hutchison, 1961), and our rate is 0.5°C per minute, CTMax and CTMin temperatures are taken as the water temperature when LRR is reached. Thermal tolerance breadth was calculated by subtracting CTMin from CTMax of individuals and then taking

the mean for each species. For specific details of the methods used to determine CTMax and CTMin see Chapter one of this thesis and Layne & Claussen (1987).

Locality and Climatic Data

All available georeferenced occurrence data was compiled for each salamander species in North America (records were obtained from HerpNet, the United States Museum of Natural History, the Bell Museum of Natural History, and personal records). Distributions are fairly well known for the salamanders in this study, and georeferenced samples span the known ranges of each species, ensuring that climatic variation is adequately represented. Further, points were plotted in ArcGIS v.9.3.1 (ESRI, 2009) to ensure that georeferenced localities fit within the known geographic distribution of each species. Finally, it was ensured that points were reasonably spread across the extent of the known range and not clustered within particular areas.

In total, 10,405 localities were identified for the 18 species. Climatic data, based on averages from 1950-2000, were obtained from the WorldClim online database (Hijmans et al., 2005) at *c.* 1 km resolution. Using DIVA-GIS (Hijmans et al., 2002), georeferenced salamander localities were mapped and data were extracted from bioclimatic variables related to temperature, as well as elevation and latitude. As salamanders have low dispersal abilities, and retain small home ranges, climatic niches should be fairly accurately identified (Petranka, 1998; Vitt & Caldwell, 2013). The best available data to estimate a thermal niche come from the maximum temperature of the warmest month (Bio 5) and the minimum temperature of the coldest month (Bio 6).

Climatic (Thermal) Niche Breadth

The thermal niche breadth for each species was estimated by subtracting the minimum temperature of the coldest month (Bio 6) from the maximum temperature of the warmest month (Bio 5) (i.e. max Bio 5 – min Bio 6) from across all localities. We first investigated results using the maximum value of Bio 5 and minimum value of Bio 6 for each species. We then further estimated the thermal niche breadth by using the mean values for Bio 5 and Bio 6 calculated across the sampled localities. The latter may help to remove influence from potential outliers, as well as climatic influences from localities at the edges of the range, which may be less representative of the range as a whole.

Statistical Tests and Phylogenetic Consideration

In comparative analyses among species, phylogenetic relationships need to be considered to ensure that data points remain independent despite structured phylogenies (Bolnick et al., 2003; Gaston et al., 2009). For all measured traits in our analysis (Bioclimatic variables 5 & 6, thermal niche breadth, CTMax, CTMin, thermal tolerance breadth, latitude, and range size) we tested for the influence of phylogeny by comparing AIC scores of Brownian motion versus lambda models using the “geiger” package in R v.3.0.2 (R Development Core Team, 2013). Lambda models were chosen in all cases (with most traits having lambda scores < 0.0000), indicating that these traits have little to no phylogenetic signal. Therefore there is no need to correct for phylogenetic non-independence and it is most appropriate to use the original data in all analyses (see Garland et al., 2005).

We used linear regression to examine the relationship between salamander thermal tolerances (i.e. CTMax, CTMin, and thermal tolerance breadth) and environmental temperatures (i.e. temperature min, max, and range). As body mass was found to vary significantly between species (ANOVA $p < 2.2e-16$, $R^2 = 0.706$), average species' body mass from the physiological trials was included as a covariate in each analysis. However, mass was not found to be significant in any of the regressions and was removed from all models.

For CTMax, linear regressions were performed versus the maximum and mean of the warmest temperature (Bio 5), minimum latitude, and minimum elevation. For CTMin, regressions were performed for the minimum and mean of the coldest temperatures (Bio 6), maximum latitude, and maximum elevation. As both latitude and elevation have an influence on temperature, we performed additional regressions using adjusted values of latitude and elevation to provide more accurate comparisons of the distributional data (Miller & Packard, 1977; Cruz et al., 2005). For localities where elevation was greater than 600m, a constant correction factor of 1.75 degrees was added for every 200m of elevation (Cruz et al., 2005). In regressions testing against elevation, elevational data were corrected to account for differences in latitude between points using the inverse of the relationship above. Finally, we examined the relationship between thermal tolerance breadth (CTMax-CTMin) versus thermal niche breadth (Bio5-Bio6) across species, as well as the relationship between thermal niche breadth and latitudinal extent. All statistical analyses were conducted in R v.3.1.2 (R Development Core Team, 2014).

RESULTS

Critical thermal maxima of species were found to have a positive relationship with the maximum temperature of the warmest month (Bio5) (Fig. 1 a,b). This includes both the highest temperature at a single locality per species ($R^2 = 0.337$, p-value = 0.00683), along with the mean of highest temperatures across the sampled localities for each species ($R^2 = 0.192$, p-value = 0.0391). A relationship was also found between CTMin and minimum environmental temperature, regardless of whether the coldest sampled locality ($R^2 = 0.293$, p-value = 0.012) or the mean values for species were used ($R^2 = 0.231$, p-value = 0.0252) (Fig. 1 c,d).

Minimum and maximum latitudinal points were also found to correspond with species' thermal tolerances. Species with localities at the lower latitudes were found to have higher estimates for CTMax ($R^2 = 0.476$, p-value = 0.000916) and this relationship remained after latitudinal points were adjusted for the influence of elevation ($R^2 = 0.381$, p-value=0.00379) (Fig. 2 a,b). Species at higher latitudes tend to have lower estimates of CTMin ($R^2 = 0.443$, p-value = 0.00154), which also remained significant when adjusted for latitude ($R^2 = 0.328$, p-value = 0.00769) (Fig. 2 c,d).

We further found a relationship between CTMax versus minimum elevation ($R^2 = 0.228$, p-value = 0.0259), which remained after adjusting for influence of elevation ($R^2 = 0.185$, p-value = 0.0426) (Fig. 3 a,b). For CTMin versus maximum elevation, we also found a significant relationship ($R^2 = 0.172$, p-value = 0.0494), however, when elevation was adjusted for differences in latitude between sites the relationship disappears ($R^2 = 0.09$, p-value = 0.121) (Fig. 3 c,d).

In addition, salamanders with the largest thermal tolerance breadths (CTMax-CTMin) exhibited the largest thermal niche breadths ($R^2 = 0.32$, p -value = 0.00852) (Fig. 4). Finally, a regression between thermal niche breadth and latitudinal extent (p -value = 0.0011, $R^2=0.466$) (Fig. 5) indicates that species with greater latitudinal extents encompass greater thermal niche breadths than species with more restricted distributions.

DISCUSSION

The degree to which environmental temperature influences geographic distributions remains a key question in biogeography (Osmond et al., 1987; Root, 1988; Hawkins & Felizola Diniz-Filho, 2006; Bozinovic et al., 2011; Quintero & Weins 2013b). Here, we find that thermal tolerances of 18 salamander species are strongly correlated with the temperature range across environments where these salamanders occur. Such links provide evidence that species' thermal tolerances mirror the thermal dimensions of their climatic niches (Monahan, 2009; Sunday et al., 2011). In addition, we find very strong associations between CTMin and CTMax of species and the latitudinal extremes across which they are found. This pattern remains unchanged after latitudinal values are adjusted for the influence of elevation. These results further suggest that environmental temperature plays a strong role in shaping species' geographic distributions and that thermal tolerance evolution is an important factor for surviving in more seasonal habitats.

For elevation, the picture is less clear. Although species that occur at lower elevations have greater tolerance to warm temperatures, no relationship between CTMin

and maximum elevation was found after correcting for the influence of latitude. This result is surprising, as latitude and CTMin are strongly related and it is expected that species found at higher elevations should be better adapted to cold conditions (Gaston, 2003). One potential reason that we failed to find a relationship is that we use mean CTMax and CTMin data per species and not data specific to each locality. Although there is little indication of intraspecific variation in thermal tolerances across the latitudinal extent of these species (see Chapter one), it remains possible that the available data are not sufficient to address this relationship. Further, the Appalachian Mountains are taller at more southern latitudes, and despite the high elevations, cold extremes may be somewhat mitigated in the south compared to more northerly latitudes.

The strong links between CTMax/CTMin and environmental temperature range in these species helps to validate the use of GIS-based climate data to model species' distributions and in comparative-phylogenetic studies on the evolution of the niche (see Kearney & Porter, 2004; Mustin et al., 2007; Sheth & Angert, 2014). Study of thermal limits alone does not provide information on many other important aspects of a species' niche (e.g. other physiological traits/environmental variables, biotic interactions, habitat, diet, etc.), but it does provide insight into what is likely a major factor regulating salamander distributions (Salisbury, 1926; Caughley et al., 1987; Root, 1988). Used in combination with correlative models, thermal tolerance data are likely to provide better predictions of species' current and future distributions by defining more accurately the geography of physiological limits (Kearney & Porter, 2004). Integrating physiological traits is essential for developing the best models of species' geographic range limits, yet how to best do this remains unclear (see Hijmans & Graham, 2006; Bozinovic et al.,

2011). Difficulties stem from the need for a basic understanding of how each organism functions and which factors are most critical to be included in the model (Kearney & Porter, 2004; Buckley et al., 2013). Further, as even closely related species can have vastly different distributions due to differences in life history, physiology, and other attributes, even if general patterns are found among certain groups, species-specific tests will still be needed.

Our findings that thermal niche breadth (Bio5-Bio6) has a strong positive relationship with thermal tolerance breadth (CTMax-CTMin), as well as with latitudinal extent, fits well with predictions of earlier hypotheses pertaining to patterns in geographic range size (Brown, 1984; Stevens, 1989; Gaston & Spicer, 2001). As thermal niche breadth increases with latitude, we expect thermal tolerances to follow (Quintero & Wiens, 2013b). Brown (1984) argues that inhabiting a wider variety of conditions could enable some species to adapt to environmental extremes and become more widespread. The result would be a positive relationship between niche breadth and geographical range size (Brown, 1984; Gaston & Spicer, 2001; Slatyer et al., 2013). It is believed that these relationships are driven by seasonality and in smaller part by differences in climatic conditions among localities (Janzen, 1967; Stevens, 1989; Gaston et al., 1998; Spicer & Gaston, 1999). The greater the degree of seasonality, the greater the breadth of thermal tolerance needed for individuals to survive in such conditions. Such adaptation to local environments can help to explain the large disparity in geographic range size between closely related species with similar ecologies. Further, factors regulating climatic niche breadth remain poorly understood. The strong relationship between climatic niche breadth and thermal tolerance breadth indicates that physiological tolerances can be used

to help determine whether a species will have a wide or narrow climatic niche (Brown, 1984; Quintero & Wiens, 2013b).

This data can further be useful in highlighting which species are more likely to be negatively impacted by future climate change. Species with narrow thermal niche breadths are likely to be most vulnerable to extinction, as they are shown to have narrower thermal tolerances. Those species unable to adapt or exhibit phenotypic plasticity may have to track preferred climates to survive (Huey et al., 2009; Kearney et al., 2009). This is particularly concerning for many mountaintop endemic salamanders, which have very limited options for tracking suitable habitat in a warming climate (Kozak & Wiens, 2006; Gifford & Kozak, 2012;).

When estimating climatic niches from GIS-based climate data a number of caveats need to be considered (Kearney & Porter, 2009). For instance, difficulties in estimating thermal niches can arise due to a species' ability to behaviorally thermoregulate. As salamanders move underground when climatic conditions become unfavorable, such responses can weaken the relationship between thermal tolerances and climatic data (see Kearney & Porter, 2009; Fisher-Reid et al., 2012). This factor is difficult to control for, yet, despite the potential ability to behaviorally avoid extreme environmental temperatures, species thermal tolerances still mirror variation in macroclimate. As such, it seems unlikely that behavior is masking much of the influence of temperature.

Another caveat is that known localities are not likely to include the entire realized niche of a species and therefore the estimated thermal niche is only as good as the data available. Additionally, some species may be able to tolerate climatic conditions outside

of the area where they naturally occur (Monahan, 2009; Peterson et al., 2011), especially if a part of the range is restricted by non-climatic factors such as competition (see Barve et al., 2011). Monahan (2009), for instance observed that realized niches in focal bird species are considerably smaller than potential or fundamental niches. As a result, the thermal niche breadth calculated in this study may be an underestimate of the true breadth. Fortunately, the low vagility and small home range of salamanders in general enables more accurate estimates of a species' climatic niche than may be afforded for other taxa (Petranka, 1998; Wells, 2007).

Finally, although temperature is believed to be a critical factor in delimiting species' distributions (Merriam, 1984; Caughley et al., 1987; Root, 1988; Whitton et al., 2012), our analysis did not consider other variables known to be important for this group. It remains possible that a factor not examined here may better describe the distributions of these species. For example, moisture and precipitation are important to amphibian survival (Wells, 2007) and an obvious choice for investigating the climatic niche of a species. However, precipitation alone was not found to be a significant predictor of distribution in other studies looking at salamanders (Quintero & Wiens, 2013b). This association can be complicated as terrestrial salamanders are not likely to suffer from too much precipitation, and lack of rain during periods when salamanders are underground and inactive may be less impactful (Quintero & Wiens, 2013b). In addition, we did not run physiological tests on different development stages of salamanders and juvenile or larval phases may be more or less vulnerable to temperature extremes (Angilletta, 2009). Taxa with different physiologies (e.g. aquatic species, endotherms, plants) may also have different patterns, so our results need to be compared with other groups to see if a broad

pattern emerges. Yet, even within a single species, different climatic variables may limit different parts of the range (Fisher-Reid et al., 2012; MacArthur, 1972). This can make it especially challenging to decipher patterns and determine which factors are most critical in shaping species' geographic ranges.

Conclusion

There is an implicit assumption that species' climatic niches reflect their physiological tolerances, and this is one of the first studies to show that they do (see also Sunday et al., 2011). The strong relationship found between thermal tolerances and environmental temperature extremes gives us confidence in the validity of geographic distributions approximated through bioclimatic data. Thermal tolerances alone are not able to account for all the factors defining a species' niche, but may provide insight where correlative models are otherwise lacking.

Table 1. Salamander groups and species detailing number of individuals n (with number of populations in brackets), mean body mass (g), CTMax (Critical thermal maximum), CTMin (Critical thermal minimum), thermal tolerance breadth (CTMax – CTMin), Tmax = mean maximum temperature of the warmest month (Bio5), Tmin = mean minimum temperature of the coldest month (Bio6), thermal niche breadth (max Bio 5 – min Bio 6), and latitudinal extent for each species.

Species	n (popns)	Mean body mass (g)	Mean CTMax (°C)	Mean CTMin (°C)	Thermal tolerance breadth (°C)	Tmax (°C)	Tmin (°C)	Thermal Niche Breadth (°C)	Latitudinal extent (degrees)
Desmognathus group (7 species)									
<i>D. carolinensis</i>	11 (1)	1.10	32.2	-0.87	33.1	26	-6	32	1.26
<i>D. fuscus</i>	26 (4)	2.07	33.1	-1.2	34.3	28	-8.02	36.1	13.65
<i>D. monticola</i>	61 (7)	3.66	33.1	-0.86	34.0	27.8	-5.84	33.6	9.12
<i>D. ochrophaeus</i>	81 (8)	1.01	33.1	-1.0	34.1	26.8	-9.2	36	8.94
<i>D. ocoee</i>	32 (3)	1.33	32.7	-0.64	33.3	26.3	-5.29	31.6	3.05
<i>D. orestes</i>	42 (5)	1.13	32.8	-0.72	33.5	24.9	-6.73	31.6	1.75
<i>D. santeetlah</i>	11 (1)	1.35	31.8	-0.28	32.1	24.8	-6.56	31.4	1.18
Plethodon cinereus group (5 species)									
<i>P. cinereus</i>	81 (12)	0.89	32.5	-1.5	34.0	27.8	-7.67	35.5	14.28
<i>P. hubrichti</i>	11 (1)	1.18	32.1	-1.1	33.2	27.1	-6.28	33.3	0.07
<i>P. richmondi</i>	22 (5)	1.07	32.7	-1.2	33.9	26.9	-6.38	33.3	3.59
<i>P. sherando</i>	12 (1)	1.05	31.6	-1.3	32.9	26.8	-7.11	33.9	0.15
<i>P. virginia</i>	9 (1)	1.28	32.0	-0.99	33.0	26.2	-8.47	34.6	0.93

Plethodon									
glutinosus group									
(4 species)									
<i>P. cylindraceus</i>	27 (4)	4.90	32.8	-0.94	33.7	27.7	-6.24	33.9	5.77
<i>P. glutinosus</i>	69 (13)	4.85	33.0	-1.0	34.0	27.8	-7.63	35.4	16.44
<i>P. montanus</i>	39 (4)	1.95	31.7	-0.73	32.4	24.9	-6.71	31.6	1.84
<i>P. teyahalee</i>	21 (2)	6.11	32.6	-0.74	33.3	26.3	-5.8	32.1	1.44
Plethodon									
wehrlei group									
(2 species)									
<i>P. punctatus</i>	12 (2)	3.81	31.9	-1.1	33.0	24.8	-9.12	33.9	1.66
<i>P. wehrlei</i>	10 (1)	1.75	32.4	-1.3	33.7	26.4	-8.78	35.2	6.27

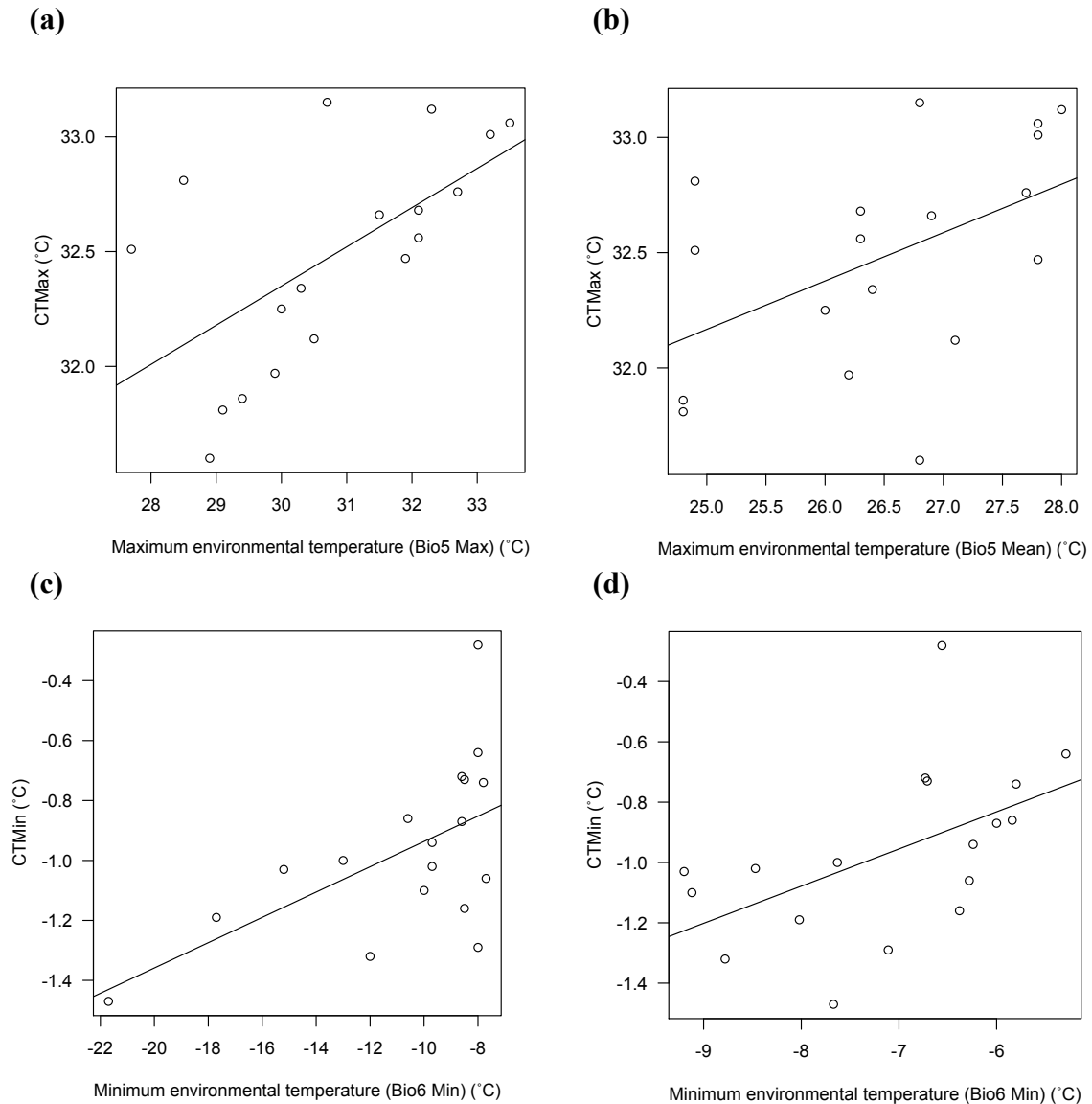


Figure 1. Mean thermal tolerances versus maximum and minimum range of temperatures (Bio 5 and Bio 6) for 18 salamander species. (a) CTMax vs. locality with hottest temperature ($R^2 = 0.34$, p-value = 0.0068), (b) CTMax vs. mean of maximum temperatures across all localities per species ($R^2 = 0.19$, p-value = 0.039), (c) CTMin vs. locality with coldest temperature ($R^2 = 0.29$, p-value = 0.012), and (d) CTMin vs. mean of minimum temperatures from across all localities per species ($R^2 = 0.23$, p-value = 0.025).

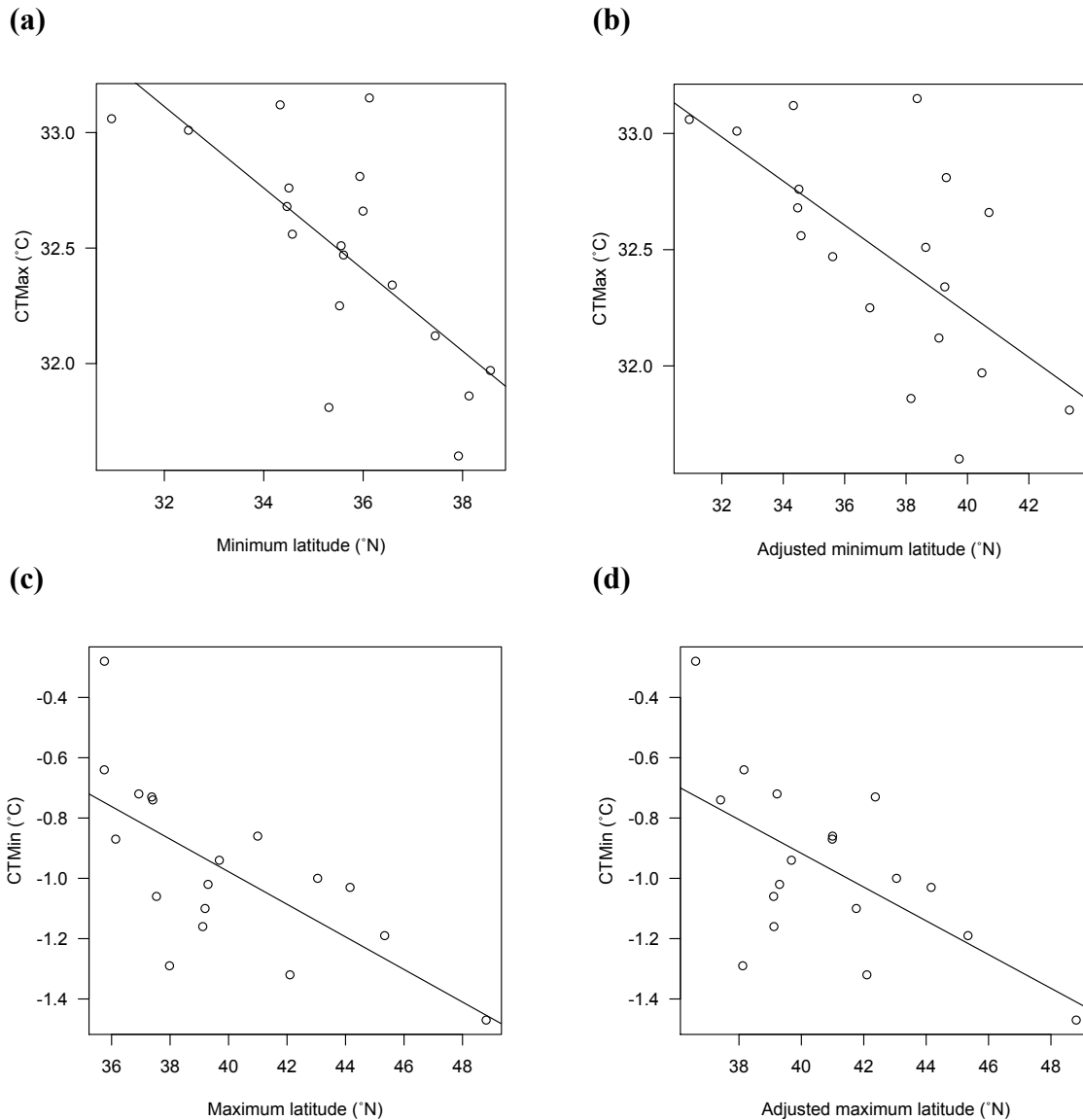


Figure 2. Mean thermal tolerances as a function of latitudinal extremes for 18 salamander species. (a) CTMax versus lowest latitude locality per species ($R^2 = 0.48$, p-value = 0.00092), (b) CTMax versus minimum latitude adjusted for elevation ($R^2 = 0.38$, p-value=0.0038), (c) CTMin versus maximum latitude locality for each species ($R^2 = 0.44$, p-value = 0.0015), (d) CTMin versus maximum latitude adjusted for elevation ($R^2 = 0.33$, p-value = 0.0077).

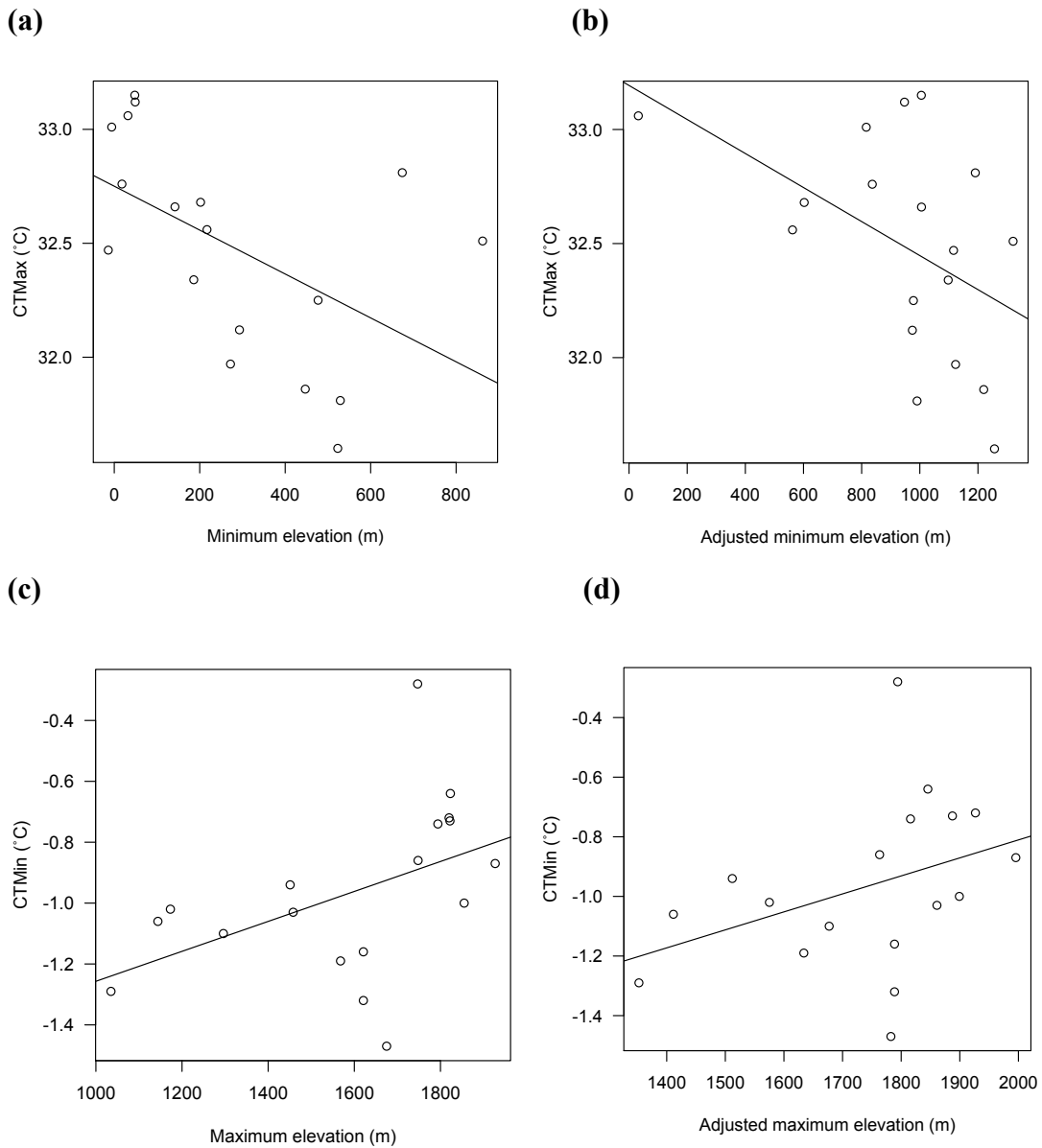


Figure 3. Thermal tolerances as a function of elevation extremes for 18 species of salamanders. (a) CTMax versus lowest elevation locality ($R^2 = 0.23$, p -value = 0.026), (b) CTMax versus minimum elevation adjusted for latitudinal ($R^2 = 0.19$, p -value = 0.043), (c) CTMin in relation to maximum elevation locality ($R^2 = 0.17$, p -value = 0.049), and (d) CTMin versus maximum elevation adjusted for latitude ($R^2 = 0.09$, p -value = 0.12).

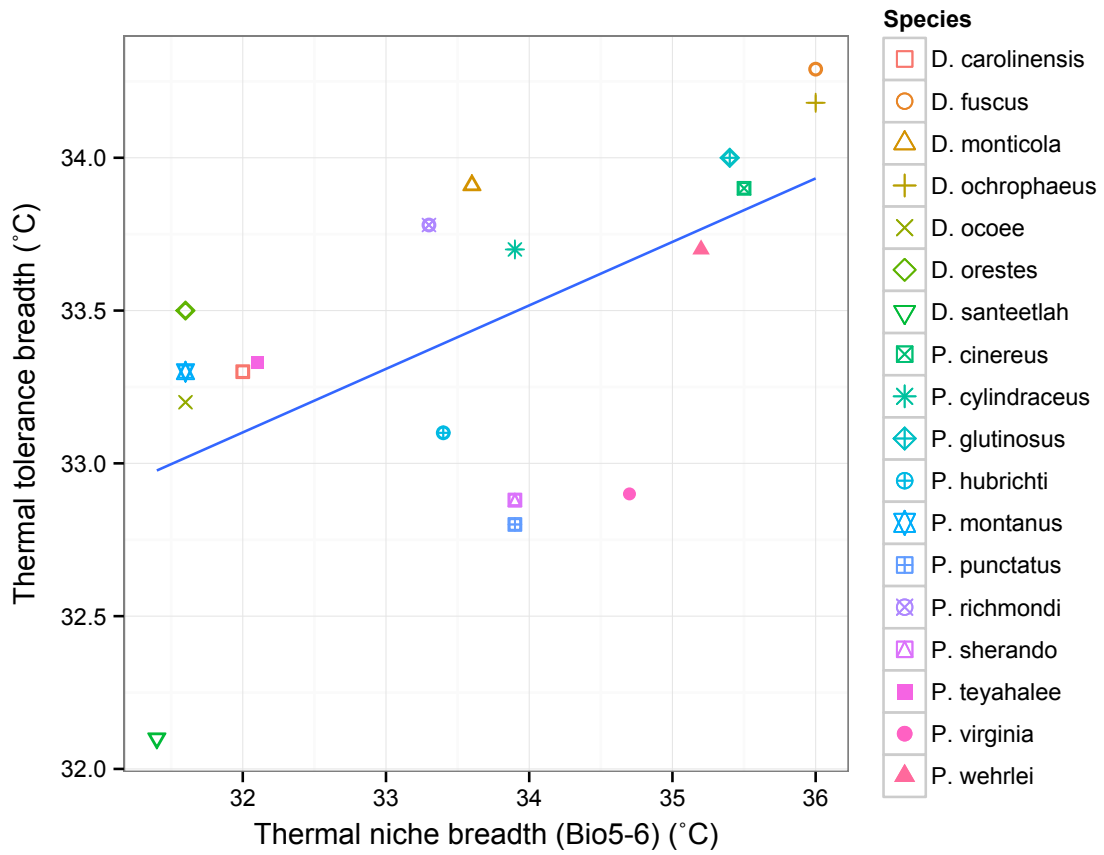


Figure 4. Mean thermal tolerance breadth (CTMax – CTMin) versus thermal niche breadth (Bio5 – Bio6) for 18 species of salamanders ($R^2 = 0.32$, $p\text{-value} = 0.0085$).

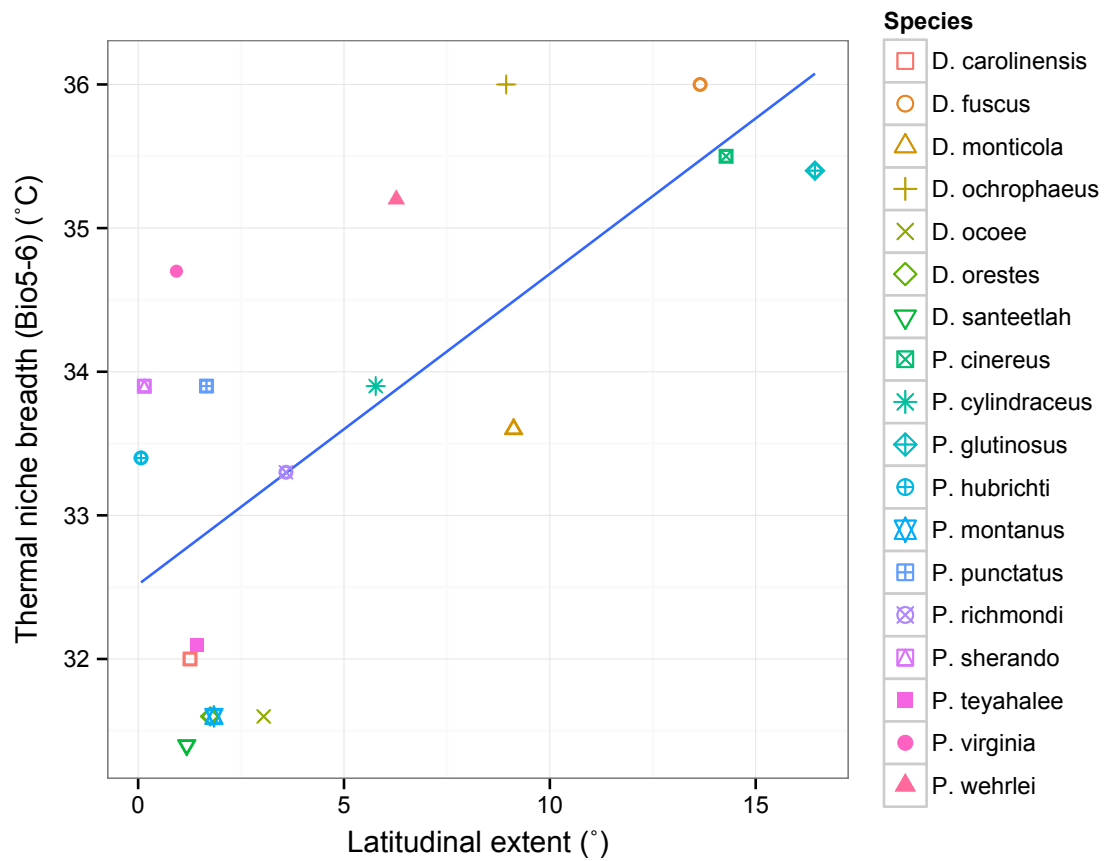


Figure 5. Relationship between thermal niche breadth (Bio5 - Bio6) and latitudinal extent for 18 salamander species (p-value = 0.0011, $R^2=0.47$).

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APPENDICES

Appendix 1: Chapter 2 MCMCglmm R Code

```
install.packages("ape")
install.packages("MCMCglmm")
library(ape)
library(MCMCglmm)
dataset<-read.csv(file="RespData.csv", head=TRUE)
attach(dataset)
dataset$Range<-as.factor(dataset$Range)
str(dataset)

#Phylogeny Component
tree<-read.tree("Plethodontidae_comb61_PL.phy")
species<-c("D._carolinensis_KHK103", "D._fuscus_KHK142",
"D._ochrophaeus_WKS05", "D._ocoee_B_KHK62", "D._orestes_KHK129",
"D._monticola_A", "D._santeetlah_11775", "P._cinereus", "P._cylindraceus",
"P._glutinosus", "P._hubrichti", "P._montanus", "P._punctatus", "P._richmondi",
"P._teyahalee", "P._virginia", "P._wehrlei")
pruned.tree<-drop.tip(tree,tree$tip.label[-match(species, tree$tip.label)])# Prune tree to
include only species of interest
sptree<-makeNodeLabel(pruned.tree, method="number", prefix="node") #rename nodes
to be unique
treeAinv<-inverseA(sptree, nodes="TIPS")$Ainv
prior<-list(G=list(G1=list(V=diag(2), nu=2, alpha.mu=c(0,0), alpha.V=diag(2)*1000)),
R=list(V=diag(1), nu=0.002))
random=~us(1+Temp):ID

#Final Model: Range size (Range)
model1<MCMCglmm(LVO2~1+Range+Acclm+Temp+Mass+Sex+Temp*Acclm+Accl
m*Range, random=random, data=dataset, family="gaussian",
ginverse=list(species=treeAinv), prior=prior, nitt=300000, burnin=25000, thin = 1000,
verbose=FALSE)
```