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Comparative Energetics of Mammalian Thermoregulatory Physiology

Ana M. Breit
University of Maine, ana.breit@maine.edu

COMPARATIVE ENERGETICS OF MAMMALIAN THERMOREGULATORY PHYSIOLOGY

By

Ana M. Breit

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Advisory Committee:

Danielle L. Levesque, Associate Professor of Mammalogy and Mammalian Health, Advisor

Kristina Cammen, Assistant Professor of Marine Mammal Science

José Meireles, Assistant Professor of Plant Evolution and Systematics

Andy Rominger, Assistant Professor of Ecological Bioinformatics

Nishad Jayasundara, Assistant Professor of Environmental Toxicology and Health

COMPARATIVE ENERGETICS OF MAMMALIAN

THERMOREGULATORY PHYSIOLOGY

By Ana M. Breit

Dissertation Advisor: Prof. Danielle L. Levesque

Endothermy allows species to decouple body temperature from environmental temperatures but does not equate to endothermic species maintaining those constant temperatures. Instead, heterothermy fluctuating body temperatures, both in and outside of torpor is common and allows endotherms to expand the limits of thermoneutrality. Thermolability is likely to be more common in the tropics and subtropics, where species live within or above their thermoneutral zone. My dissertation research focused on the heterothermic-homeothermic continuum, specifically quantifying where on the continuum different species fall at certain times and why those species have evolved to be at those points. I quantified the thermal profile of *Sundamys muelleri*, a tropical, nocturnal rodent. *S. muelleri* increased evaporative water loss and subcutaneous body temperature at ambient temperatures of 33°C, indicating an upper thermal limit, although metabolic rate showed no increase up to the highest ambient temperature (38.2°C), suggesting this species will tolerate future climatic changes. I then studied the extremely thermally-labile *Tenrec ecaudatus.* Although there was a wide range of intraindividual variability in metabolic rate and body temperature at both the higher and lower ambient temperatures, I identified a lower thermal limit thermoneutrality of 19.1°C. I then investigated how detailed climate variables may affect thermolability in Chiropteran species across biogeographical zones. I quantified how thermolability phenotypes in bats are globally distributed but found no relationship between thermoregulatory variables of body temperature and lower thermal limits and

climatic variables. The unique thermoregulatory adaptations in Chiroptera gives us insights into the physiology and evolution of thermolability in endotherms. Finally, I focused on thermolability at the limits of thermoneutrality in Rodentia, the most diverse and specious clade of Mammalia. Body temperatures at the upper limits of thermoneutrality and the differential between the upper limit and the body temperature at that limit increase with latitude. However, the thermoneutral zone was wider for species with ranges at latitudes closer to the equator than the poles. Lower latitude species have lower temperatures at upper thermal limits and maintain their body temperatures closer to those limits. Through my research we can see how thermolability is shaped by evolutionary history, phylogeny, biogeographical patterns, and climate.

DEDICATION

To Ben and Dorothy Maleski, and Ray and Cina Breit, who taught me how to work hard

and be patient, but also how to be bullheaded, headstrong, anxious, and stubborn.

Finishing this without you sucked.

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CHAPTER 1

INTRODUCTION

1.1   Overview

As climate change progresses and global temperatures continue to rise, there is a greater push to understand how organisms interact with tenvironmental temperatures. Mechanistic models require an accurate understanding of the thermoregulatory physiology of a species (Buckley and Kingsolver, 2012; Seebacher and Franklin, 2012). Most models currently focus on ectothermic species (Angilletta et al., 2010), which have a more direct relationship with environmental temperatures than endothermic species. However, contrary to endothermic species that generate heat metabolically, ectotherms rely predominantly on external sources of heat, therefore their relationship between body temperature and ambient temperatures is generally more direct and predictable (Angilletta and Angilletta, 2009; Buckley and Kingsolver, 2012; Tattersall et al., 2012). Endothermic species have more complex interactions with ambient temperature, as they can produce internal heat, uncoupling body temperature from ambient temperature more readily (Bartholomew, 1982), and thus have a wide range of thermoregulatory capabilities. By manipulating their metabolic heat production, fluctuating insulation, and varying rates of evaporative water loss, endotherms can balance heat production with heat loss to maintain constant body temperature (Scholander et al., 1950a; Mitchell et al., 2018). Endotherms range from extreme homeotherms (maintaining constant body temperatures) to obligate hibernators at the extreme heterothermic end of the continuum (Boyles et al., 2013; McKechnie and Wolf, 2019).

Homeothermic endotherms maintain relatively constant body temperatures either year-round (obligatory homeothermy) or only during their reproductive season (facultative homeothermy; Lovegrove, 2012). By employing homeothermy during reproduction, facultative homeotherms are better able to support certain physiological functions, such as

faster fetal growth rates (Farmer, 2000), and thus potentially greater reproductive fitness (Farmer, 2003). It has been hypothesized that homeothermy evolved from heterothermy because maintaining a constant body temperature above ambient temperature would have allowed early mammals to forage more widely in the nocturnal niche (Crompton et al., 1978). High metabolic rates were not necessary to body temperatures in early mammals, as they were relatively low (28-30°C; Crompton et al., 1978; Lovegrove, 2012) and close to environmental temperatures during the daytime restphase. As mammals evolved heightened senses to cope with the nocturnal niche, brain size also increased, and with it, higher metabolic rates became necessary (Armstrong, 1983; Genoud et al., 2017; Isler and Schaik, 2006). Some nocturnal mammals became able to sustain higher body temperatures, increasing the temperature differential between body and ambient temperatures. This adjustment allowed for passive heat dissipation, reducing the need for heat dumping mechanisms such as panting or sweating, enabling them to invade the diurnal niche in multiple different events (Crompton et al., 1978). Many extant nocturnal insectivorous species, however, are descendants of clades that never became diurnal, and thus some may retain those ancestral characteristics of low body temperature associated with low metabolic rate (Crompton et al., 1978; Lovegrove, 2012).

Heterothermic animals, at the other end of the thermoregulatory continuum from homeotherms, escape the energetic demands of homeothermy and instead fluctuate body temperatures with ambient temperatures. Periodically, some heterotherms lower their internal body temperature to near-ambient temperatures and employ facultative hypothermia, or torpor, a state of reduced body temperature and metabolic rate (Geiser and Ruf, 1995). Torpor is considered the most efficient energy-saving mechanism for birds and mammals, especially when used during low food or water availability or inclement weather (Geiser and Ruf, 1995; Ruf and Geiser, 2015). Alternatively, some heterotherms also exhibit non-torpid heterothermy, allowing their body temperature to track ambient temperature, but maintaining normal activity, essentially altering their normothermic body temperature (Levesque et al., 2014).

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Studies focusing on mammalian thermoregulation and energetics often center on characterizing the thermal profile by quantifying the upper and lower thermal limits (T_{uc} and T_{lc}), which delineate the thermoneutral zone (TNZ). These thermal profiles, also called Scholander-Irving models, require measuring resting metabolic rates (using oxygen consumption as a proxy) in fasted, non-reproductive adult individuals across environmental temperatures to determine the range of ambient temperatures over which metabolic rate remains minimal, referred to as the thermoneutral zone (TNZ; Scholander et al., 1950b). The thermoneutral zone is the area where the minimal amount of energy is required for thermoregulation. At ambient temperatures below the thermoneutral zone, thermal conductance (the rate of heat lost to the environment, the inverse of insulation) is at a minimum, and resting metabolic rate increases as different mechanisms, like shivering and, in some mammals, nonshivering thermogenesis, is used to increase heat production and maintain stable body temperature. At warmer temperatures above the thermoneutral zone, thermal conductance is maximized, and evaporative cooling (panting and sweating) is used to lower body temperature (Tattersall et al., 2012). By identifying the upper and lower bounds of the thermal neutral zone, we can identify the temperatures beyond which energy and water costly physiological means are implemented to maintain stable body temperature. The shape of the profile can give an indication of the physiological adaptations and energy use of a species in relation to climate (Scholander et al., 1950b). Many studies have attempted to use thermal profiles to make predictions of endotherms' physiological responses to climate change (Buckley et al., 2018; Fristoe et al., 2015; Khaliq et al., 2017). However, many variables can confound the bounds of the thermoneutral zone or make it obsolete when transferring the assumptions onto free-ranging animals in a dynamic environment, making the findings of some of these predictive models inaccurate or incorrect (Levesque et al., 2016). Misconceptions and biases of thermoregulatory physiology undermine attempts to form predictive mechanistic models of endothermic thermoregulation (Boyles et al., 2019; Mitchell et al., 2018).

THERMAL PROFILE

Thermoneutral Zone

Body Temperature

Evaporative Water Loss

Thermal Conductance
(C_{wet})
(C_{dry})

Metabolic Rate

Ambient temperature
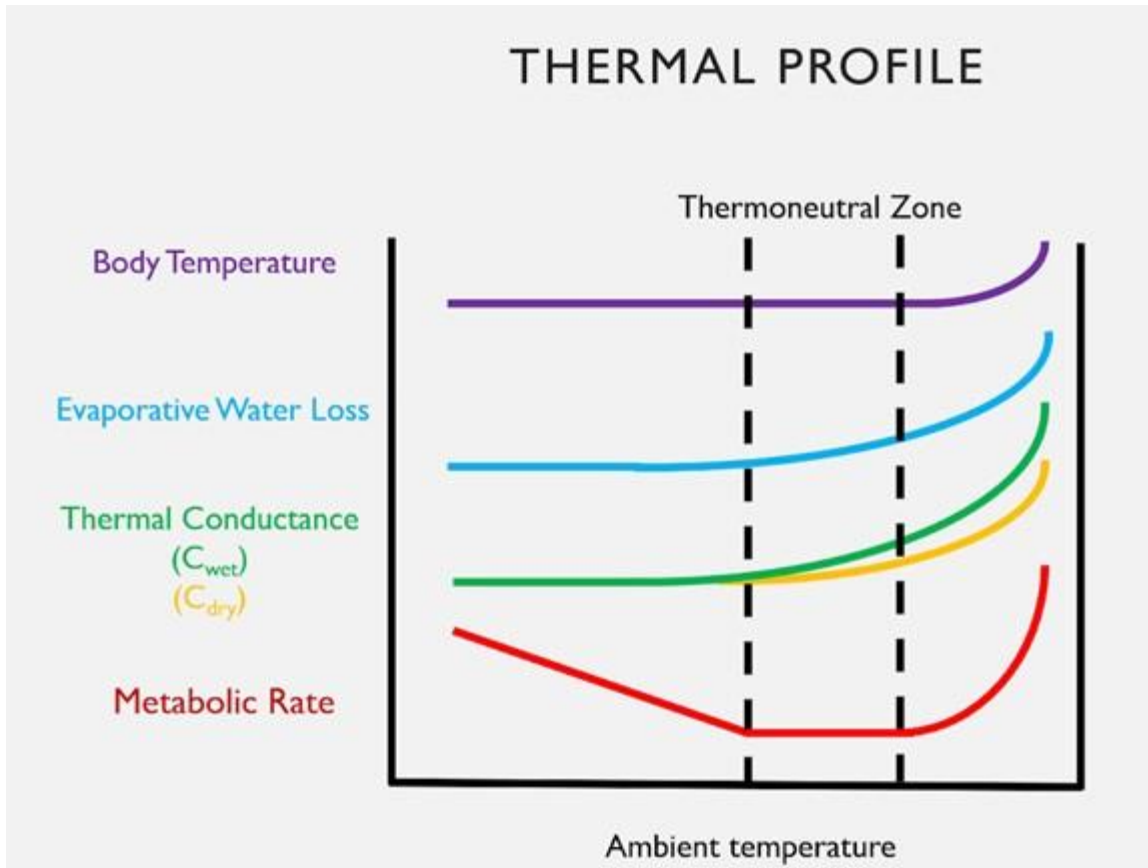
Figure 1.1: Traditional thermal profile, or Scholander-Irving curve, depicting the relationships between metabolic rate and body temperature as functions of ambient temperature. Adapted from Scholander et al., 1950, Tattersall et al., 2012, and Tomlinson, 2016.

The limits of the thermoneutral zone are plastic and can vary seasonally (Lovegrove, 2005). By altering their degree of insulation (fat or fur), endotherms manipulate their thermal conductance, the rate of heat transfer with the external environment (Chappell, 1980; Scholander et al., 1950; Webb and McClure, 1988). Above and below the thermoneutral zone, endotherms adjust the rates of energy required by altering their thermal conductance or insulation (Scholander et al., 1950). At temperatures below the thermoneutral zone, more insulation (less thermal conductance) equates to less energy being used on shivering and nonshivering thermogenesis, allowing metabolism to remain low and shifting the lower limit of the thermoneutral zone lower (Lovegrove, 2005; Scholander et al., 1950; Webb and McClure, 1988). At temperatures above the thermoneutral zone, animals can maximize their thermal conductance, enabling animals to

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dissipate heat to the environment, effectively expanding the upper limit of the thermoneutral zone. The limits of the thermoneutral zone are essentially phenotypically plastic, and the range of flexibility may be a function of latitude and body size (Lovegrove, 2005).

Early studies of mammalian thermoregulation focused predominantly on species found in the northern hemisphere and relatively few physiological studies have been conducted in the tropics, leading to broad generalizations that fail when applied to non-Holarctic mammals (Levesque et al., 2016; Lovegrove, 2000; Nowack et al., 2020). Because of an early bias of physiological research being done in the northern hemisphere, strict homeothermy was thought to be the norm in mammals, and heterothermy was seen as the exception, but as more studies are conducted, it is becoming clear that homeothermy may actually be the more derived state (Canale et al., 2012; Lovegrove, 2012b; Nowack et al., 2020). As the ancestral mammal evolved in tropical climates and likely exhibited torpor, studying thermolability in tropical mammals may provide insights into the evolution of endothermy and more specifically how homeothermy evolved from a heterothermic ancestor (Crompton et al., 1978; Grigg et al., 2004; Lovegrove, 2012a).

Thermal profiles provide a means of measuring the relationship between ambient temperature and a species' physiological responses, resting on the assumption that the animal maintains constant body temperature (Scholander et al., 1950). However, many species fluctuate their body temperatures, both over daily and seasonal time scales (Boyles et al., 2013). By allowing their body temperature to be labile, the limits of the thermoneutral zone shift, requiring less energy to maintain body temperatures at temperatures beyond the traditional thermoneutral zone (Boyles et al., 2013; Levesque et al., 2016). Some endotherms exhibit daily fluctuations, either altering body temperature with activity levels, with higher body temperatures during activity, and lower body temperatures during rest (Chappell and Bartholomew, 1981; Levesque et al., 2018; Thonis

et al., 2020; Wells, 1978), or with circadian rhythms independent of activity (Aschoff, 1983). Additionally, some species thermoconform, increasing or decreasing in normothermic body temperature tracking ambient temperature (Gerson et al., 2019; Ruf and Geiser, 2015; Staples, 2016; Turner, 2020). At ambient temperatures above body temperatures, evaporative cooling can be used to lower internal body temperatures, resulting in water loss (Gerson et al., 2019). To reduce water loss and avoid dehydration, some species instead actively raise their body temperature setpoint to above ambient temperature and employ facultative hyperthermia (Gerson et al., 2019; Tieleman and Williams, 1999). By increasing their internal temperatures to warmer than ambient temperatures, animals maintain a positive thermal gradient with ambient temperature, passively dissipating heat to the environment via convection (Gerson et al., 2019; Turner, 2020). This passive increase in body temperature effectively expands the upper bound of the thermoneutral zone and raises the threshold at which an increase in metabolic rate and evaporation water loss is needed to lower the body temperature, reducing both energy and water demands of the animal (Gerson et al., 2019; Tieleman and Williams, 1999). Most studies focusing on the upper bounds of the thermoneutral zone have been conducted in birds or desert mammals, and still relatively little is known about water loss when body temperature is greater than ambient temperature (Levesque et al., 2016). Additionally, studies extrapolating lab findings to the environmental temperatures species are experiencing have been lacking (Levesque et al., 2016).

By fluctuating their normothermic body temperature with ambient temperature, thermally-labile heterotherms can minimize the gradient between body temperature and ambient temperature, minimizing their rates of heat loss to the environment and therefore maximizing their energy and water use Angilletta et al. (2010). By increasing the flexibility of body temperature regulation, or thermolability, some species may effectively expand their thermoneutral zone (Brice et al., 2002; Levesque et al., 2014). Currently, thermolability has been documented in many mammalian species (*Echinops telfairi*,

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(Dausmann et al., 2020); *Setifer setosus* (Levesque et al., 2014); *Tadarida aegyptiaca*, (Toussaint et al., 2009); *Microcebus griseorufus* (Kobbe et al., 2011) spanning several orders, which may indicate that thermolability is more widespread than current literature indicates, and more thermally labile species may be identified as the tropical thermoregulatory gap is filled (Nowack et al., 2020). Additionally, many of the studies identifying plastic changes to the limits of the thermoneutral zone emphasize fluctuations in insulation, and relatively little focus has been put on the actual changing of body temperature setpoints as the main driver of the expansion of the thermoneutral zone (Boyles et al., 2012; Holloway and Geiser, 2001; Porter and Kearney, 2009). Additionally, analyses of the thermal profile can only provide limited insight into energy and water budgets of species within their natural environment (Porter and Kearney, 2009) and more focus needs to be put on understanding how mammals thermoregulate in the environment using thermoregulatory data from free-ranging animals.

1.2 Thesis summary

My dissertation research focused on the heterothermic-homeothermic continuum, specifically quantifying where on the continuum different species fall at certain times and why those species have evolved to be at those points. I started my research program by quantifying the thermal profile of a common yet understudied Sundaland murid rodent and then comparing the metabolic rates, body temperature, and thermolability to other tropical and nocturnal species. I then studied a thermally-labile basoendotherm, the tailless tenrec (*Tenrec ecaudatus*), and quantified their metabolic rate and limits of their thermoneutral zone. For my third data chapter, I investigated the effects of microclimate variables on thermolability characteristics and basal metabolic rate Chiropteran species.
For my fourth and final data chapter, I assembled a comprehensive thermoregulatory database for rodent species to identify biogeographical patterns of thermolability. I first quantified the thermolability of all rodent species for which we had measured thermal

profiles and identified phylogenetic and biogeographical patterns of the distribution of thermoregulatory traits. I also identified critical areas needing more research, such as more studies quantifying upper critical limits and thermoregulatory studies conducted on tropical and non-holarctic species. Through my research I sought to understand the fundamental principles of mammalian thermoregulation, specifically how thermolability is shaped by evolutionary history, phylogeny, biogeographical patterns and climate, and how it in turn affects species' complex interactions with their environment. My findings will serve as a foundation for more accurate mechanistic models incorporating stronger data with appropriate assumptions of the biophysics of thermoregulatory physiology.

1.3   Contribution of co-authors and remarks on style

This dissertation is formatted as a series of manuscripts, of which I am the primary author, following the styles of the journals of intended submission when not conflicting with the University's formatting requirements. The first data chapter will be submitted to Biotropica, with Andrew Alek Anak Tuen, Awang Khairul Ikhwan, and Marcellinus Isaac from Universiti Malaysia- Sarawak, and Tal Kleinhaus-Goldman Gedalyahou and Danielle L. Levesque from the University of Maine as co-authors. The second data chapter will be submitted to the Journal of Experimental Biology, with Gilbecca Smith and Michael Treat, and Frank van Breukelen from the University of Nevada Las Vegas, Marshall D. McCue and John Lighton from Sable Systems International, Las Vegas, Nevada, and Danielle Levesque as co-authors. My third data chapter will submitted to Functional Ecology, with Dr. Ofir Levy from Tel Aviv University and Danielle L. Levesque as co-authors. My fourth and final data chapter will be consolidated with data from my third chapter, as well as future work on other mammalian thermoregulatory data and submitted as one manuscript.

The study was conceived and designed with input at all stages from D.L. Levesque. The data for the first data chapter were collected with the help of my field assistants, T. Kleinhaus-Goldman Gedalyahou, A.K. Ikhwan, and M. Isaac, and with logistical support from A.A.A. Tuen, all who provided editorial comments on the manuscript for which they are co-authors. The data for the second data chapter was collected with the help of M. Treat, G. Smith, and M. McCue, with logistical support and mentoring provided by F. van Breukelen, M.D. McCue, and J.R.B. Lighton, all of whom provided editorial comments on the manuscript for which they are co-authors. O. Levy provided modeling and conceptual support for the third data chapter. All data analyses and the initial drafting of the manuscripts were performed by myself with guidance from D. L. Levesque.

CHAPTER 2

HIGH TEMPERATURE TOLERANCE IN A NOCTURNAL TROPICAL MAMMAL

2.1   Chapter Abstract

With rising average global temperatures, understanding how animals will cope with their changing environment is an increasingly pressing issue. Despite tropical biomes harboring the highest level of mammalian biodiversity, relatively little is known about their thermoregulatory physiology and even less so for those from the Indomalayan region. As climate change progresses, it is crucial we understand how species in equatorial regions thermoregulate under stressful thermal conditions. Using flow-through respirometry we measured the thermoregulatory characteristics, (subcutaneous body temperatures, metabolic rate, and evaporative water loss, in relation to ambient temperatures up to temperatures exceeding current maximas) of a common, murid rodent, Mueller's giant Sunda rat (*Sundamys muelleri*, Jentink, 1879). Contrary to our predictions, that tropical rodents would have a comparatively low basal metabolic rate, the values for *S. muelleri* fell within the average for similarly sized mammals. We observed that subcutaneous body temperatures and evaporative water loss increased at ambient temperatures above 33°C, but no increases in metabolic rate were observed up to ambient temperatures as high as 38.2°C. Our data show *S. muelleri* are able to budget water and energy to maintain homeostasis at environmental temperatures exceeding their current habitat temperatures, and therefore should be able to tolerate climatic changes in the future. Unlike tropical ectotherms, which have been predicted to experience declines in thermal performance and fitness in the face of climate change, we anticipate that nocturnal tropical rodents such as *S. muelleri* will be more resilient.

2.2 Introduction

As global temperatures continue to rise and deforestation advances at an unprecedented pace, the need to understand how these changes pose risks to the survival of species is greater than ever (Tuff, Tuff, & Davies, 2016; Urban et al., 2016). Large-scale physiological estimates of how resilient species may be to climate change are largely based on patterns first demonstrated in ectotherms, species that rely predominantly on behavioral rather than physiological means of thermoregulation. These estimates predict that tropical species will face dire consequences in the face of climate change (Deutsch et al., 2008; Dillon, Wang, & Huey, 2010; Huey et al., 2012; Sunday et al., 2014; Tewksbury, Huey, & Deutsch, 2008). However, patterns derived from ectotherms are unlikely to hold for endothermic species which instead rely primarily on physiological means of thermoregulation (Pollock, Brawn, & Cheviron, 2021; Thonis, Ceballos, Tuen, Lovegrove, & Levesque, 2020). Due to their ability to regulate body temperature through internal heat production, endotherms are able to decouple their body temperature from environmental temperature. Thus, endotherms' interactions with environmental temperatures are complex, making it difficult to predict the range of environmental temperatures that species are able to tolerate from physical and physiological principles alone (Levesque & Marshall, 2021; Mitchell et al., 2018). Understanding why thermoregulatory patterns exist can inform climate change predictions (Huey et al., 2012), however most macrophysiological datasets are biased towards temperate species (Fristoe et al., 2015; Khaliq, Hof, Prinzinger, Böhning-Gaese, & Pfenninger, 2014). Relative to the immense biodiversity of the species from equatorial regions (Burgin, Colella, Kahn, & Upham, 2018), the number of thermoregulatory studies remain sparse (Lovegrove, 2000, 2003; Mitchell et al., 2018; Welman, Tuen, & Lovegrove, 2017b). For example, the Indomalayan zoogeographic zone has the highest density of mammals among the six major zoogeographical zones, 127.2 species/km² (Burgin et al., 2018), yet only 18 of the 487 species from an extensive database of mammalian basal metabolic rates (BMR, Lovegrove, 2000) were from the Indomalayan region. Since 2000, more thermoregulatory studies have been conducted in the region

(Levesque, Tuen, & Lovegrove, 2018; Thonis et al., 2020; Welman, Tuen, & Lovegrove, 2017a; Welman et al., 2017b) but our knowledge still lags significantly behind what is known for higher latitude species. Species-specific patterns have not yet been widely observed and as global temperatures continue to rise, it is crucial we further understand adaptations to extreme temperatures. Latitude is likely a better predictor of thermoregulatory traits than proximal climatic measurements, as it both incorporates other, unassessed values (wind speed, day length, etc), and is a better predictor of the long-term climatic pattern in which the species likely evolved (Naya, Spangenberg, Naya, & Bozinovic, 2012). Extrapolating predictions based primarily on data from temperate species or from ectotherms to equatorial tropical endotherms leads to drastic misrepresentations of species' responses to climate change (Pollock et al., 2021; White et al., 2021).

Species that evolved in the tropics have their own unique evolutionary history, evolving in the absence of large, seasonal temperature fluctuations and the cold winters that dominate the evolution of thermoregulatory phenotypes in the holarctic (Janzen, 1967; Lovegrove, 2000, 2003, 2012; Naya et al., 2012). However, their current rate of niche evolution is predicted to be vastly outpaced by the rapid pace of climate change (Quintero & Wiens, 2013). Unlike higher-latitude species (Humphries & Careau, 2011), tropical mammals may live most of their lives within, or even above, their thermoneutral zone, encountering different thermoregulatory selective pressures than temperate species (Levesque, Lobban, & Lovegrove, 2014; Levesque et al., 2018; Reher & Dausmann, 2021; Welman et al., 2017b). Continuous living in warm environmental temperatures allows species to forgo the need for high thermogenic capacity, allowing for the existence of species with lower and more variable body temperatures, particularly nocturnal species (Boyles et al., 2013; Grigg, Beard, & Augee, 2004; Levesque et al., 2014; Lovegrove, Lobban, & Levesque, 2014; Welman et al., 2017b). When environmental temperatures are greater than body temperature and ambient-body temperature differential approaches zero, the capacity

to cool via conduction and radiation diminishes (McKechnie & Wolf, 2019) and therefore endothermic species must resort to using evaporative cooling. Some warm climate, desert-adapted species of birds and mammals have been shown to raise their body temperature above environmental temperature to use passive cooling to dissipate heat (Degen, 2013; Gerson et al., 2019), saving both energy and water (McKechnie & Wolf, 2004; Tattersall et al., 2012). We currently lack information on whether tropical species rely on increased body temperature to facilitate passive cooling or if they have other means of coping with heat.

One of the most common ways to quantify the relationship between ambient temperature and body temperature in endotherms has been by using Scholander- Irving curves (also known as thermal profiles or SI curves; Scholander, Hock, Walters, Johnson, & Irving, 1950). Changes in either heat production at colder experimental temperatures or heat loss at higher temperatures often result in either a hockey-stick or U-shaped pattern in metabolic rate depending on the species (Withers, 1992; Withers, Cooper, Maloney, Bozinovic, & Cruz-Neto, 2016). BMR is the metabolic rate within the thermoneutral zone, the range of ambient temperatures over which metabolic rate remains minimal and constant when measured in resting, non-reproductive individuals, during their rest-phase. The upper thermal limit has several contending definitions: the more common approach defines the upper thermal limit as the ambient temperature at which metabolic rate increases (Khaliq et al., 2014; Wolf, Coe, Gerson, & McKechnie, 2017), while others define it as the ambient temperature at which rates of evaporative water loss (EWL) increase (IUPS Thermal Commission, 2003; Mitchell et al., 2018; Withers et al., 2016). Regardless of the definition used, the upper limit has been measured in far fewer species than lower limits, (93 vs. 204 species, Riek & Geiser, 2013; 101 vs. 210, (Thonis et al., 2020) and of those measurements, most are from high-latitude and nocturnal species (65/93 Bennie et al. 2014). Furthermore the majority of these studies lack information evaporative water loss (24/101, (Thonis et al., 2020) which is vital in understanding endotherm responses to

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heat (McKechnie & Wolf, 2019). Although caution should be taken in using the parameters of the TNZ to infer responses to climate change, it does provide a useful indication of the energy and water costs during the rest phase (reviewed in Levesque & Marshall, 2021).

Diurnal mammals are predicted to experience shrinking habitable space because of increasing global temperatures butting against their physiological limits at high environmental temperatures (Bonebrake, Rezende, & Bozinovic, 2020; Levesque, Nowack, & Stawski, 2016; McCain & King, 2014). A majority of mammals (70%) maintain the ancestral characteristic of nocturnality (Bennie, Duffy, Inger, & Gaston, 2014; Crompton, Taylor, & Jagger, 1978; Gerkema, Davies, Foster, Menaker, & Hut, 2013), especially in warm environments where nocturnality can drastically reduce the energetic and water costs of thermoregulation (Crompton et al., 1978; Levesque et al., 2014; Lovegrove, 2017). Warm daytime temperatures during the rest-phase allow species to maintain normothermic body temperatures with low energetic costs, and activity during the cooler night phase offsets thermoregulatory costs (Crompton et al., 1978; Humphries & Careau, 2011; Levesque et al., 2014). The energetic benefits of nocturnality become more dramatic the lower the normothermic body temperature of that species is maintained. For example, Welman et al. (2017b) found frugivorous bats *Cynopterus brachyotis* in Malaysian Borneo had low normothermic body temperatures compared with other bat species (32.5°C, Welman et al., 2017b). Additionally, the nocturnal primate, western tarsiers (*Cephalopachus bancanus*), also studied in Malaysian Borneo, have low body temperatures of 31°C and have difficulty maintaining body temperatures at environmental temperatures below 30°C despite increases in metabolism (Welman et al., 2017a). However, during their rest phase, arboreal nocturnal species in particular may be challenged by warming daytime temperatures, the degree to which will depend on microclimate and how well their nests are buffered from environmental temperatures (Lovegrove, Lobban, et al., 2014; Reher, Rabarison, Nowack, & Dausmann, 2022; Welman et al., 2017a, 2017b).

Thus far, in the Indomalayan tropics robust thermoregulatory studies have only been conducted on endemics clades, such as western Tarsiers (*Cephalopachus bancanus*; Welman et al., 2017a), and treeshrews (*Tupaia* spp. Levesque et al., 2018; Thonis et al., 2020). To broaden our understanding of more common and cosmopolitan species we chose to study the abundant murid rodent *Sundamys muelleri* (Jetnik, 1879) on the island of Borneo. *S. muelleri* is one of four members of the *Sundamys* genus, all of which are endemic to Sundaland, and for which no previous metabolic studies exist. Due to their global distribution, importance to food webs, major role in crop destruction (John, 2014; Singleton, Belmain, Brown, Aplin, & Htwe, 2010) and as reservoirs for zoonotic diseases (Bordes et al., 2013; Meerburg, Singleton, & Kijlstra, 2009; Morand, Jittapalapong, & Kosoy, 2015) it is critical we understand how murid rodents will be impacted by climate change. Our objective was therefore to measure the potential impacts of warming temperatures on the energetics of a nocturnal rodent by measuring metabolic rate and water loss during their rest phase. We hypothesized that the small, tropical, nocturnal *S. muelleri* should have a low BMR for its body mass, similar to what has been observed in lower-latitude birds (Londoño, Chappell, Castañeda, Jankowski, & Robinson, 2015) and mammals (Lovegrove, 2003). Additionally, we hypothesized *S. muelleri* should maintain lower and more flexible body temperatures compared to both temperate species and diurnal tropical species (Lovegrove, 2003). By comparing the relationship between physiological characteristics and the environmental temperatures they experience during their daytime rest phase we can make predictions on how nocturnal murid rodents as well as other small tropical mammals will respond to climate change.

2.3 Methods

Our study took place between June and August 2019 in the Bidayuh community forests of Kampung Tanjong Bowang (1°30'16" N, 110°10'40" E) in the district of Bau, Sarawak, Malaysia. The forest at the base of Mt. Singai is a secondary agroforest used for

recreation, food gathering, and hunting (Tuen et al., 2014). Our research was conducted following our approved University of Maine IACUC protocol: (A2019-05-03) and Sarawak Forestry Department and the Malaysian Ministry of Natural Resources permit No. (289)JHS/NCCD/600-7/2/107). We used locally-made cage traps baited with banana and checked the traps at 0600 h, 1200 h, and 1800 h. Upon capture, we took biometric measurements and examined the pelage to identify the animals to species using the Phillips and Phillips Field Guide (2016). We weighed each individual to the nearest 0.1 g using a digital scale, and then identified the sex and age (adult or juvenile). Only nonreproductive adults were used in the study; all other individuals were released at their capture site. To continuously measure the subcutaneous temperature (T_{sub}) and identify recaptured individuals, we inserted a small, temperature-sensitive transponder (BioThermo13, Biomark, Boise, ID) subcutaneously into the loose skin in the interscapular region of the animal. We compared temperature readings of a handful of the temperature-sensitive transponders and temperature data loggers (described below) to a mercury thermometer traceable to a national standard in a water bath between 20°C and 40°C following Toussaint & McKechnie (2012) and found no significant differences. We used T_{sub} as an estimate of core body temperature (T_{core}), which, while potentially underestimating T_{core} at lower temperatures and overestimating it at high temperatures, generally provides a good proxy (Oswald, Evlambiou, Ribeiro, & Smit, 2017).

Respirometry measurements were conducted under conditions similar to those described in Thonis et al. (2020), but are briefly summarized here. We ran tests during the animal's rest phase from 0700 h to 2000 h. During each test, we measured responses to two different ambient temperatures, selected at random, between 20° and 37°C. Exposure to each test temperature was between 4 to 6 hours, and, due to constraints of the test temperature control system, was always ramped from cool to warm. After initial processing at the capture site, we placed the individual in a 4.45-L sealed container (Lock and Lock, Seoul, South Korea) modified with two air-ports (John Guest Male Connector NPTF, Greenville,

SC, USA), one for each incurrent and excurrent air flow. A raised metal grate rested on the bottom of the container, through which urine and feces could collect below the animal and be absorbed by mineral oil, preventing spikes in water vapor measurements. The respirometry chamber was then placed into a large modified cooler box in which we manipulated ambient temperature using a Pelt5 Temperature Controller (Sable Systems, Las Vegas, NV). Ambient air was pulled through a column of silica gel and Drierite, removing incurrent water vapor, and was then continuously pumped through the respirometry chamber using a factory-calibrated mass flow meter (MC-10SLPM-D, Alicat Scientific, Tucson, AZ) and pump (KNF Micro DIAPHRAGM GAS Pump UNMP830, KNF Neuberger, Inc., Trenton, NJ, USA). We used high flow rates of 1400 mL/min to keep chamber humidity lower than outside jungle conditions. Air from the respirometry chamber and from a reference airstream flowed into a BL-2 baseline unit (Sable Systems), which was set to sample the reference airstream for five minutes both before and after the 40 minutes of the chamber airstream collection.

Using a field gas analysis system (FoxBox, Sables Systems, Las Vegas, NV), a subsample of air (at 150 mL/min) was pulled through an H_2O and CO_2 analyser (LI-840A CO_2/H_2O gas analyzer, LI-COR Biosciences, Lincoln, NE), then dried using Drierite (W.A. Hammond Drierite Co LTD, Xenia, OH, USA) before entering the O_2 analyzer on the FoxBox. Fractional concentrations of H_2O, CO_2, and O_2, respirometer flow rate, and barometric pressure were automatically digitally recorded every second by the LI-COR program and ExpeData (Sable System's data acquisition software,Ver 1.9.7 Sable Systems). T_{sub} was recorded every second using a Biomark HPR tag reader (Biomark, Boise, ID). To monitor the ambient temperature in the respirometer, we used a Bluetooth Tempo Disc (Blue Maestro, London, UK) placed on the underside of the lid of the chamber and four temperature data loggers (DS1922L Thermochron iButtons, Dallas Semiconductor, Dallas, TX) placed in opposite corners of the respirometry chamber and set to record the temperature at 10-second intervals. To monitor the activity and position of the animal, we

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used an infrared USB security camera (ELP, Guangdong, China). After completing the experiment, all animals were released back at their capture site.

At the start of each trial, the O_2 span on the Foxbox was set to 20.95 percent and O_2 measurements were further drift-corrected using the drift correction function in the Expedata program. The CO_2 and H_2O analyser was factory-calibrated one month before field experiments began and the O_2 analyzer was zeroed using dry CO_2-free air. As the O_2 analyzer malfunctioned during four of the 20 experimental days, we selected segments for analysis based on CO_2 values instead of O_2 values. Within each 40-minute experiment, we selected the 10-minute section with the lowest CO_2 measurement within the 20-minute segment with the most stable CO_2 measurements to use for calculating VO_2. To test for lag between the two analyzers, we compared the start of each baseline and found the FoxBox lagged behind the LI-COR by 10 seconds. We then aligned the 1-second intervals obtained from the LI-COR CO_2 and H_2O analyser with Foxbox readings that were 10 seconds later. In this way we were able to use the same exact 10-minute interval for water vapor density (WVD), and the excurrent flow of CO_2 and O_2, measurements. Data from within the first two hours of each test were not used in final calculations to ensure the animal was fully adjusted to the new conditions. As the animals were not given food prior to starting the experiment, we can reasonably assume that they were close to a post-absorptive state within the first two hours of the experiment (Speakman, McDevitt, & Cole, 1993).

Using equations from Lighton (2019), we calculated VO_2, VCO_2, and RQ values. We calculated evaporative water loss (EWL) by multiplying the water vapor density by the flow rate in mL/min. To convert EWL to evaporative heat loss (EHL), we first converted EWL to mg/sec and then multiplied by the latent heat of evaporation (expressed in Joules/mg of H_2O; Withers, 1992). We calculated metabolic heat production (MHP) by multiplying VO_2 (mLO_2/s) by the oxyjoule equivalence (expressed in Joules/mg of O_2). Using MHP, EHL, ambient temperature (T_a), and T_{sub}, we calculated the wet thermal conductance (C_{wet}; and the dry heat transfer coefficient (C_{dry}; McNab, 1980). Video

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footage and T_{sub} readings were also used to validate that the animal was resting during the selected intervals. Respiratory quotient (RQ) values were calculated from tests where both the O_2 and CO_2 measurements were deemed accurate. On July 6 and July 16, the FoxBox O_2 sensor showed inaccurate fluctuations in O_2, therefore VO_2 was calculated from VCO_2 using an RQ of 0.7. All statistics were performed using R version 4.0.1 (R Development Core Team 2011) and linear mixed modeling was implemented using the R package *nlme* (Pinheiro et al., 2013). We ran preliminary analyses comparing a generalized linear model with T_a and mass to a second model which additionally included animal identity, and a third model additionally including time of measurement. As the model including only mass and T_a was not significantly different from models including animal identity or timing, we only included mass in the final analyses. To determine the limits of the thermoneutral zone (inflection points in VO_2 as a function of T_a) we used piecewise linear regressions (Crawley, 2007) with mass as a covariate using the segmented package in R (Muggeo, 2017). We repeated this analysis for T_{sub}, EWL, the ratio of EWL to MHP, C_{wet}, and C_{dry}. To determine BMR for *S. muelleri*, we took the average metabolic rate per individual above the estimated T_{lc}. We then compared the BMR to other rodents using phylogenetic generalized least squares (PGLS). Using the "pgls" function in the R package *caper* (Orme et al., 2013), which uses maximum likelihood to calculate the branch-length transformation, and a recent comprehensive mammalian phylogeny trimmed to just rodent species (Upham, Esselstyn, & Jetz, 2019), we tested the correlation between BMR, and body mass using data from 213 rodent species (Genoud, Isler, & Martin, 2017; Lobban, Lovegrove, & Rakotondravony, 2014) and activity period (nocturnal, diurnal, crepuscular, and cathemeral) using data from Bennie et al. (180 rodent species; (Bennie et al., 2014). To calculate confidence and prediction intervals for the OLS model we used "gls.ci" and "gls.pi" functions in the R package *evomap* (Smaers & Mongle, 2014; Smaers & Rohlf, 2016).

2.4 Results

Over the two-month field study, we caught a total of eight individuals: seven males, and one female, and conducted experiments on six of the adult males over 10 days of respirometry trials for a total of 20 RMR values. Mean body mass was 188.09 ± 29.28 g (range $154.15 - 234.47$ g). The T_as tested ranged from $22.1°C$ to $38.2°C$. All animals, except two (see below), rested for the duration of the respirometry measurements as confirmed via a sustained drop in T_{sub} and the video. During two of the tests at high T_a ($T_a = 35°C$ and $37°C$), the animals showed increases in T_{sub} either greater than $41°C$, or that had increased T_{sub} by $2°C$ in less than 10 minutes. We therefore ended the metabolic measurement and released the animal back at the capture site, as per our animal safety protocol. Due to a low number of samples (N= 6 individuals, n=20 measurements) the T_a at which metabolic rate increased (VO_2 inflection point/ T_{lc}) could not be accurately estimated by the segmented package ($24.9°C$ with a 95% confidence interval of 19.2-30.5°C; Figure 2.1). We therefore opted to use $31.0°C$, the T_a below which MR first started to increase, as a loose estimate for the lower limit of the TNZ. No inflection points in VO_2 above the TNZ were identified up to our highest measured test temperature of $38.2°C$. Conversely, there were measured increases in T_{sub}($33.0°C$; Figure 2.1), EWL ($34.7°C$; Figure 2.2), C_{wet}($35.2°C$; Figure 2.2), C_{dry}($35.3°C$; Figure 2.2), and the ratio of evaporative heat loss/metabolic heat production (EHL:MHP in W; $33.4°C$).

T_{sub} was elevated during handling but dropped after being in the respirometry chamber for 30 minutes and at steady-state ranged from $33.7°C$ to $40.8°C$, (mean $T_{sub}= 36.9°C \pm 1.1$). Minimum EWL, calculated as the average EWL recorded for each individual below the EWL inflection point, $33.7°C$, was 1.55 ± 0.68 mg/h/g. Minimum C_{wet} was 0.19 ± 0.06 W/°C, and minimum C_{dry} was 0.31 ± 0.57 W/°C. BMR, calculated as the mean of the mean RMR value for each individual (n=6) for all T_as greater than $31.0°C$, was 181.783 ± 23.878 mlO$_2$/h, or 0.986 ± 0.167 mlO$_2$/h/g. *S. muelleri* had a BMR close to that predicted by its body mass (avg mass = 188.09 g) and phylogeny, as calculated by the best-fitting

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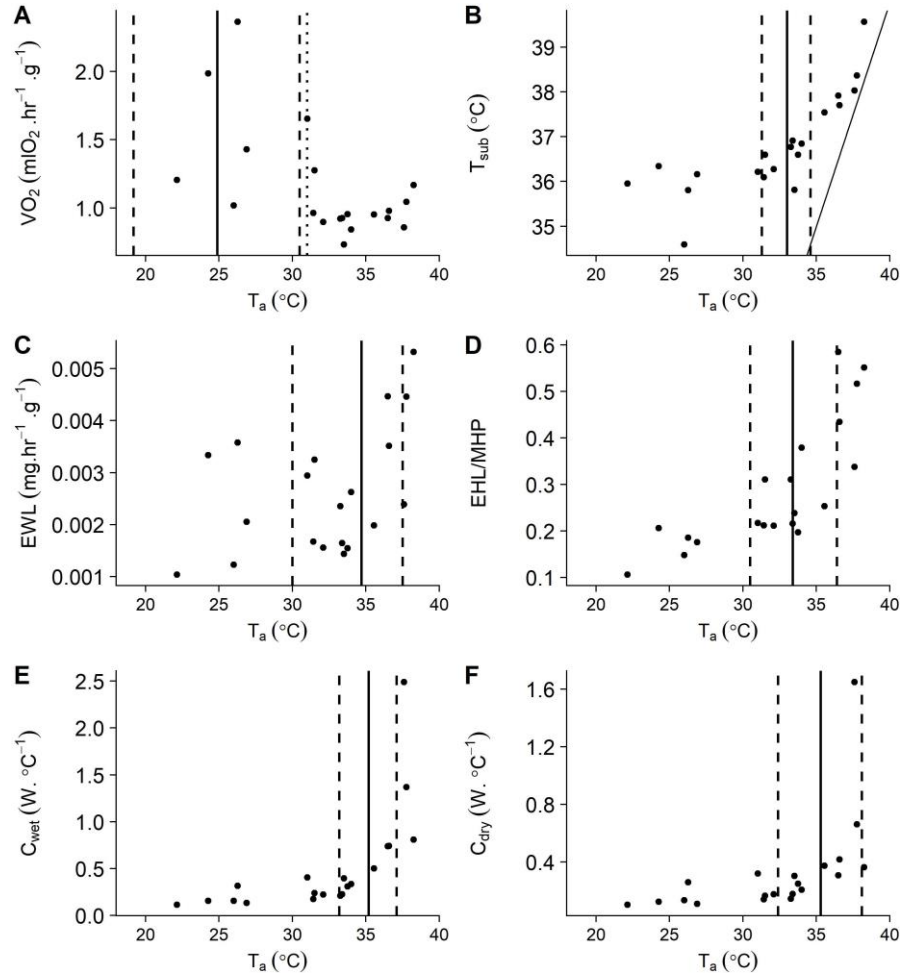
Figure 2.1: VO₂ (A), Subcutaneous body temperatures (B), evaporative water loss (C), the ratio of evaporative heat loss to metabolic heat production (D), wet thermal conductance (E), and dry thermal conductance (F) of *S. muelleri* across a range of ambient temperatures. Solid lines delineate the mean inflection point and dashed lines delineate the 95% confidence intervals of predicted inflection points. In (A) the dotted line represents the selected T_{lc}. In (B) the solid diagonal line represents the 1:1 relationship between T_a and T_{sub}.

PGLS model, and was within the prediction intervals calculated from the OLS model (Figure 2.2). According to the best-fit PGLS model, there was a significant relationship between log-transformed body mass and log-transformed basal metabolic rate (p<0.0001, $R_2 = 0.8898$). Activity phase was not found to be significant (p>0.05, $R_2 = 0.8862$).
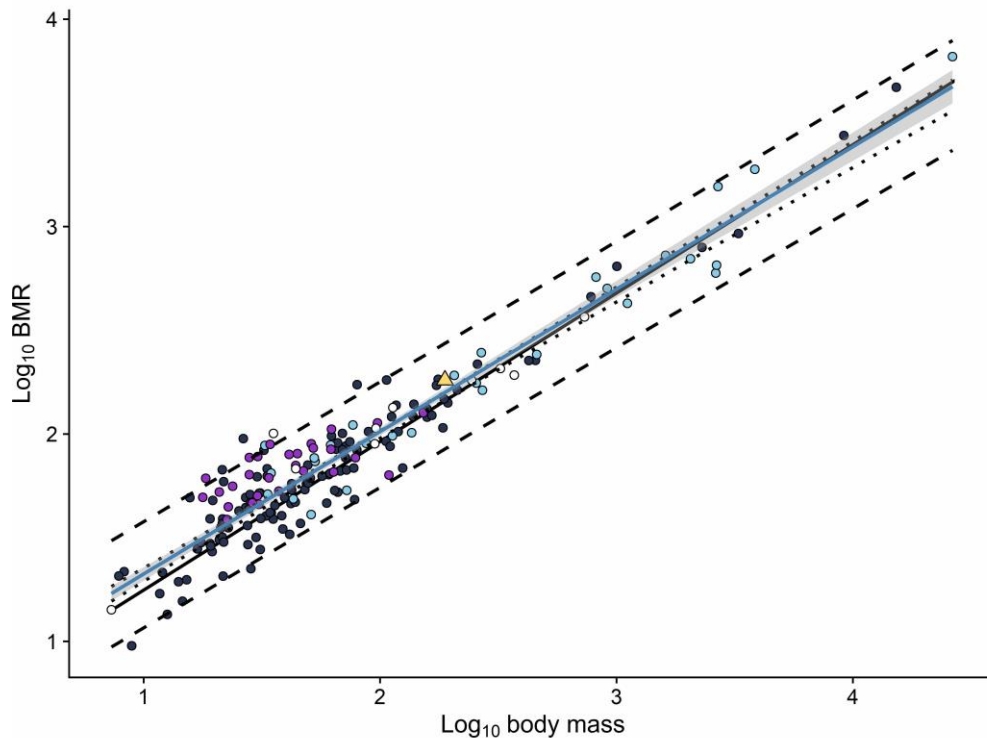
Figure 2.2: Log-transformed basal metabolic rate as a function of log-transformed body mass for 180 rodent species (Genoud et al., 2017). The blue line represents the OLS model trendline, with upper and lower 95% confidence intervals delineated with dotted lines, and upper and lower prediction intervals delineated with black dashed lines. The solid black line represents the results of the phylogenetic generalized least squares analysis of rodent species. *S. muelleri* is represented as the yellow triangle, nocturnal species as dark blue points, diurnal as light blue points, crepuscular as purple points, and cathemeral as white circles (Bennie et al. 2014). Differences between nocturnal and diurnal species were not significant (p>0.05).

2.5   Discussion

Our findings suggest that *S. muelleri*, as a nocturnal murid rodent, may be well-adapted for an incoming warmer climate, contrasting findings from tropical ectotherms as well as nocturnal arboreal tropical mammals (Deutsch et al., 2008; Welman et al., 2017a, 2017b). We could not, however, accurately determine the limits of the thermoneutral zone at either the lower or upper end due to a small sample size. The lowest test temperature at which metabolic rate appeared to increase (31°C, Figure 2.1), falls within average daily maximum environmental temperatures at the study site (27.9°C, 24.1°C - 33.9°C; Levesque et al., 2018) indicating that *S. muelleri* currently spends the majority of its time

below the thermoneutral zone. We could not determine a clear upper critical limit of the thermoneutral zone for *S. muelleri*, but did find changes in subcutaneous body temperature, evaporative water loss, and both wet and dry conductance between 33.0°C - 35.3°C. Subcutaneous body temperatures increased starting at ambient temperatures of 33°C. Evaporative cooling also increased above 33.7°C, indicating a switch in thermoregulatory state (Figure 2.1) Additionally, both wet and dry thermal conductance increased around 35°C, well above the 33°C point where subcutaneous temperature began to increase (Figure 2.1). These findings mirror the increases in evaporative cooling above 32°C and metabolic heat production below 31°C found for the Bornean endemic, *Tupaia minor* (Thonis et al., 2020). *S. muelleri* was also found to have the ability to tolerate environmental temperatures above body temperature. No previous metabolic studies had been conducted on any of the members of the *Sundamys* genus, which consists of four species, all of which are endemic to Sundaland (Camacho Sánchez, 2017; Musser & Newcomb, 1983). The only study describing thermoregulatory parameters reported body temperatures ranging from 34.5°C-39.2°C for *S. muelleri*, which match the subcutaneous temperatures observed in our study, and 36.1°C -38.2°C for the closely related *Rattus annandalei* (Rudd, 1966). Although life history and pathological studies have been conducted, relatively little is known about the thermoregulatory physiology of any murid rodents in the Indo-Malayan region (Lobban et al., 2014; Lovegrove, 2000).

When comparing the metabolic rate of *S. muelleri* to other rodents we found that they fall within the normal range for their body mass (Figure 2). Nocturnality, an ancestral mammalian trait, is still retained by a majority of rodent species (Bennie et al., 2014). During the day when nocturnal mammals are at rest, their body temperature is maintained at an elevated temperature, using convective heat from the warmer environment. During the cooler nighttime environmental temperatures, nocturnal animals' body temperature is raised above environmental temperature by heat produced during activity. Some tropical

arboreal species have been found to exhibit hyperthermia during daytime rest phases (Lovegrove, Canale, et al., 2014; Reher & Dausmann, 2021) and it has been further hypothesized that tropical, nocturnal species may experience increases in overall energy expenditure as climate change progresses, as the Arrhenius effect will directly cause increased metabolic rates with increased temperatures (Lovegrove, Canale, et al., 2014). However, the average maximum daily temperature at the site was usually below 31°C (Levesque et al., 2018), below the point in which the metabolic rate of *S. muelleri* appeared to increase to produce metabolic heat (31°C, Figure 2.1), indicating *S. muelleri* can likely tolerate higher temperatures than what they currently experience during their rest phase. Additionally, as a non-arboreal species, the temperatures Bornean rodents experience within below-ground burrows during the daytime rest-phase (Wells, Lakim, & Pfeiffer, 2006) is presumably much lower than the daily above-ground temperatures (Pike & Mitchell, 2013).

We found that *S. muelleri* delayed changes in thermoregulatory state until relatively warm environmental temperatures (33-35.3°C) and did not significantly increase their metabolism even up to the hottest temperature measured: 38.2°C (Figure 2.1). Similarly the diurnal lesser tree shrew (*Tupaia minor*) had a relatively high lower thermal limit of thermoneutrality of 31°C compared to other mammal species (Thonis et al., 2020), which is still above the current daily maximum environmental temperature tree shrews experience in their habitat, suggesting some tropical mammals may be better at coping with rising environmental temperatures than previously thought (Thonis et al., 2020). We currently lack representative data from tropical species, and despite the significant biodiversity of the tropics, most global climate change predictions rely primarily of temperate species (White et al., 2021). We did measure an increase in T_{sub} at experimental temperatures above 33.0°C, above which *S. muelleri* maintained elevated subcutaneous temperatures through to the warmest test temperature measured (38.2°C, Figure 2.1). By allowing T_{sub} to increase with T_a instead of maintaining strict homeothermy, animals can increase the

ambient-body temperature differential, increasing their ability to dump heat to the environment and thereby reducing energy and water costs. Together, these delays of using evaporative cooling or adjusting body temperature setpoints until a relatively high experimental temperature indicates an adaptability to warming environmental temperatures, well above current environmental temperatures measured in their current habitat (daily average environmental temperature of 24.9°C, range: 23.3° - 26.4°; (Levesque et al., 2018).

More pressing than climate change, the greatest threat to tropical mammals is likely habitat loss. Over the last few decades, Southeast Asia has experienced high rates of deforestation (Achard et al., 2002; Sodhi, Koh, Brook, & Ng, 2004; Vijay, Pimm, Jenkins, & Smith, 2016; Wells, Lakim, & O'Hara, 2014). As of 2004, deforestation rates were higher in Borneo than any other rainforest biome (Curran et al., 2004; Sodhi et al., 2004). To clear large areas of jungle in a short amount of time, fire is often employed, destroying the habitat for hundreds of native species, and resulting in severely reduced biodiversity (Dennis et al., 2005). As species are exposed to more variable and fragmented habitats, only some native species will be able to adapt to the degraded environment (Wells, Kalko, Lakim, & Pfeiffer, 2007). Specifically, in Sarawak, on the island of Borneo, *S. muelleri* has been found to inhabit heavily disturbed areas throughout rural, developing, and urban settings (Blasdell, Morand, Perera, & Firth, 2019; Blasdell, Perera, & Firth, 2019; Charles & Ang, 2010; Hamdan et al., 2017), including fragmented old growth forest, fallow, city parks, and rubber plantations (Madinah, Mariana, Fatimah, & Abdullah, 2013; Nakagawa, Miguchi, & Nakashizuka, 2006). A recent study conducted in northeast Borneo found native murid species to be associated with less disturbed patches of rainforest, whereas the invasive *Rattus rattus* was more likely to be found in degraded forests (Cusack, Wearn, Bernard, & Ewers, 2015). *R. rattus* poses a threat to native rodent species as both a competitor for resources and direct interference (Cusack et al., 2015). Additionally, a study conducted in Kota Kinabalu, Sabah, Malaysia, found that in urban settings, most native

small mammal species had been replaced by invasive and commensal species, with *S. muelleri* as the only native species with occurrences closer to humans, but still with a decreasing abundance from forest to urban settings (Wells et al., 2014). In addition to creating a degraded habitat and increasing the risk of invasive species, deforestation also drastically affects the microclimate species experience (Cusack et al., 2015). As the rainforest is thinned, solar radiation and air flow permeate the understory, reducing evapotranspiration rates resulting in warmer and dryer microclimates (Hardwick et al., 2015; Senior, Hill, González del Pliego, Goode, & Edwards, 2017). As *S. muelleri* likely nest in belowground burrows buffered from high daily temperatures, they will likely be less affected by hot daytime temperatures than their arboreal counterparts as both climate change and habitat destruction continue. As deforestation drastically affects the microclimate species experience, increases exposure to invasive species (Cusack et al., 2015), and inevitably reduces habitat, coupled with the fact that Borneo has some of the highest rates of deforestation in the world (Achard et al., 2002; Sodhi et al., 2010), we anticipate habitat disturbances to be a greater threat to *S. muelleri* than climate change.

The lack of a clear increase in metabolic rate at even the highest measured temperatures (38.2°C), as well as increases in evaporative cooling and T_{sub} at relatively high experimental temperatures (>33.0°C), indicate that *S. muelleri* are likely well equipped for the incoming increase in environmental temperatures predicted for the tropics. Our study indicates that tropical endotherms may not follow the predictions set forth by global predictions derived from datasets lacking in good representation from tropical environments. More species, habitat, and latitudinal specific studies are therefore needed if we which to have a full understanding of mammalian vulnerability to climate change.

2.6 Acknowledgments

CHAPTER 3

THERMOLABILITY OBSCURES BOUNDS OF THE THERMONEUTRAL ZONE IN A BASOENDOTHERM

3.1   Chapter Abstract

The evolution of endothermy allowed the global geographic expansion of birds and mammals by enabling the decoupling of body temperature from environmental temperatures. Extant mammals demonstrate a continuum of thermoregulatory patterns ranging from hibernators, to daily heterotherms, to rigid homeotherms. The common tenrec (*Tenrec ecaudatus*) are extremely thermally labile allowing us to quantify how heterothermy affects the shape of the relationship between metabolic rate and ambient temperature in non-torpid animals. Using flow-through respirometry over a range of ambient temperatures, we identified an inflection point in metabolic rate at 19.1°C, indicative of upper and lower limits of thermoneutrality. However, we found a high degree of inter-individual variability in normothermic body temperatures and metabolic rates, indicating a range of thermolability which may be advantageous in the hot environments to which tenrecs are endemic and where endothermy in mammals is hypothesized to have evolved. Tenrecs retain many of the ancestral mammal characteristics such as insectivory, nocturnality, and relatively unspecialised limbs. A further understanding of thermoregulation in this unique mammal may therefore give us insight into the evolution of endothermy.

3.2   Introduction

As climate change progresses and global temperatures continue to rise, there is a greater push to understand how organisms interact with environmental temperatures (Tuff, Tuff, & Davies, 2016; Urban et al., 2016). Endothermic species effectively balance heat production with heat loss to maintain constant body temperatures by manipulating their

metabolic heat production and varying insulation and evaporative water loss (Gerson et al., 2019; Tattersall et al., 2012). Whole-body endotherms (birds and mammals) have complex interactions with environmental temperatures, as they are capable of metabolic heat production, allowing them to uncouple body temperature from ambient temperature (Clarke, Rothery, & Isaac, 2010; Grigg, Beard, & Augee, 2004). Endothermy exists as a continuum, ranging from strict homeothermy (maintaining constant body temperatures) to extreme heterothermy (varying body temperature to save energy; (Angilletta, Cooper, Schuler, & Boyles, 2010; Boyles et al., 2013; Clarke & Pörtner, 2010; Lovegrove, 2012a). Heterothermic endotherms are able to enter periods of torpor, a controlled decrease in body temperature, resulting in relatively low metabolic rates saving vast amounts of energy (Bartholomew & Cade, 1957; Heldmaier & Ruf, 1992; Ortmann & Heldmaier, 1997). In addition to torpor, heterotherms may also allow their body temperature to fluctuate with ambient temperature and still maintain normal activities such as foraging and grooming, a notable adaptation for life in environments where strict homeothermy would be energetically limiting (Boyles et al., 2013; Brice, Grigg, Beard, & Donovan, 2002; Clarke et al., 2010; Levesque, Lobban, & Lovegrove, 2014). In cool conditions, allowing body temperature to track ambient temperature saves energy that would otherwise be required to maintain a body temperature elevated above cold ambient temperatures, the cost of which increases as ambient temperature decreases and the defended body temperature remains high. In hotter conditions, maintaining a body temperature cooler than high ambient temperatures requires high evaporative water loss. Instead some species in hot, dry environments may use facultative heterothermy, and allow body temperature to climb above ambient temperature and instead use the temperature differential between ambient and water to dissipate excess body heat to the environment (Gerson et al., 2019; Reher & Dausmann, 2021).

Endothermy evolved in warm, humid conditions similar to today's tropics (Crompton, Taylor, & Jagger, 1978; Grigg et al., 2004; Lovegrove, 2012a, 2012b). Rather than

maintaining a constant elevated temperature, early endotherms instead fluctuated their body temperature, resulting in large variations between active and resting body temperatures (Lovegrove, 2017). Thus, the large amount of homeothermy observed in today's mammal species likely evolved from a more heterothermic ancestral state (Grigg et al., 2004; Lovegrove, 2012a). Heterothermy, both as torpor use and thermolability, may provide an evolutionary advantage in the tropics. Tropical and subtropical species are adapted to live in a range of ambient temperatures within, rather than below, their thermoneutral zones, the range of ambient temperatures where a minimal metabolic rate is required to maintain a constant body temperature (Levesque, Tuen, & Lovegrove, 2018; Thonis, Ceballos, Tuen, Lovegrove, & Levesque, 2020; Welman, Tuen, & Lovegrove, 2017). In such environments, being thermally-labile and fluctuating body temperatures with

ambient temperatures (i.e. maintaining a low T_b - T_a differential) during the resting phase can reduce the costs of thermoregulation, saving vast amounts of energy, allowing tropical species to invest more energy in activities such as reproduction (Boyles, Smit, & McKechnie, 2011; Gerson et al., 2019; Lovegrove et al., 2014; Turner, 2020). This low-energy lifestyle facilitated by warm ambient temperatures might have been a common strategy for early mammals (Crompton et al., 1978).

The interaction of body temperature, ambient temperature, and metabolic rate in endotherms often results in a distinct pattern called the thermal profile (Scholander, Hock, Walters, Johnson, & Irving, 1950). At low ambient temperatures, metabolic rate must be elevated to defend a body temperature warmer than ambient temperature. The thermoneutral zone is a range of ambient temperatures between a lower and upper critical temperature within which metabolic rate is constant and minimal to defend a constant body temperature. At the upper limit of thermoneutrality, metabolic rate increases again (Khaliq, Hof, Prinzinger, Böhning-Gaese, & Pfenninger, 2014; Wolf, Coe, Gerson, & McKechnie, 2017) along with evaporative water loss (IUPS Thermal Commission, 2003; Mitchell et al., 2018; Withers, Cooper, Maloney, Bozinovic, & Cruz-Neto, 2016) lowering

body temperature to its normothermic level (Withers, 1992; Withers et al., 2016). Thermal profiles, specifically the limits of the thermoneutral zone, have been used to compare species physiological responses to changes in ambient temperature. This pattern of the thermal profile, specifically the limits of the thermoneutral zone, can be used to compare species' physiological responses to changes in ambient temperature (Riek & Geiser, 2013; Thonis et al., 2020; Wolf et al., 2017) but see caveats in (Boyles et al., 2019).

In some species, termed basoendotherms (Lovegrove, 2012a), there is a high level of overlap between active and torpid body temperatures. Basoendotherms possess endothermic characteristics from Cretaceous and Early Cenozoic mammals such as low body temperature and corresponding low metabolic rates. Some basoendotherms, such as the greater hedgehog tenrec, *Setifer setosus*, have been shown to defend a normothermic body temperature below the lower limits of thermoneutrality only when pregnant and lactating (Levesque & Lovegrove, 2014), presumably to increase milk production and development of their young, leading to an increase in overall fitness (Farmer, 2000). Free-ranging individuals of the heterothermic short-beaked echidna, *Tachyglossus aculeatus*, have been shown to regularly reduce body temperature during periods of inactivity (Augee, Ealey, & Spencer, 1970; Brice et al., 2002; Nicol & Andersen, 2006). However, reproductive females were found to defend a constant body temperature across fluctuating daily ambient temperatures, allowing for the quantification of a thermoneutral zone (Grigg & Beard, 2000). Additionally, under laboratory conditions, T. *aculeatus* maintained homeothermy, albeit at low normothermic body temperatures (T_b = 30°C; Schmidt-Nielsen, Dawson, & Crawford, 1966). Basoendotherms maintain less rigid body temperatures, so their thermal profiles may not have as clear of inflection points in metabolic heat production and evaporative water loss that would be required for more strict and less-flexible maintenance of constant body temperature.

The Afrotherian subfamily of Tenrecinae (Everson, Soarimalala, Goodman, & Olson, 2016) have some of the lowest metabolic rates and the lowest body temperatures while also

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having some of the largest variability of active body temperatures of any eutherian mammal (Lovegrove, 2003; Treat et al., 2018). Currently, we know that lesser hedgehog tenrec (*Echinops telfairi*) has the capacity to maintain an elevated body temperature above ambient temperatures (Oelkrug et al., 2013) and can hibernate up to several months in both fluctuating and constant T_a in lab settings (Dausmann, Levesque, Wein, & Nowack, 2020). Additionally, the greater hedgehog tenrec, *Setifer setosus*, while also highly heterothermic, delays increases in wet thermal conductance, a mechanism of heat dissipation, until ambient temperature is around 32.5 - 32.8°C (Levesque & Lovegrove, 2014). The wide range in ambient temperatures between the lower limit of thermoneutrality and wet thermal conductance, along with the associated increase in body temperature with warmer ambient temperatures indicates extreme thermolability, in that heat dissipation mechanisms are only employed beyond certain body temperatures to avoid hyperthermia. However, we do not yet know the heat tolerance of tenrec species, nor their capacity for evaporative cooling and their reliance on it as a heat dissipation mechanism (Levesque & Lovegrove, 2014). The uniqueness of tenrec thermoregulatory patterns and their reliance on thermolability at both high and low ambient temperatures make them a perfect candidate on which to quantify how thermolability may affect the confines of the thermoneutral zone. The tailless tenrecs have one of the lowest eutherian mammalian body temperatures (Nicoll, 1985, 1986) and can hibernate for months without interbout arousals (Lovegrove & Génin, 2008). Additionally, *Tenrec ecaudatus* at 1003.1 g are significantly larger than 147.1-272.3g *Setifer* (Levesque & Lovegrove, 2014) and 135g, *Echinops* (Eisenberg & Gould, 1969) and should thus both gain heat faster and lose heat slower than the smaller *Setifer* and *Echinops* species. The ratio of peripheral tissues where heat exchange takes place to an animal's overall volume is greater in larger species, causing them to retain heat and potentially enable them to maintain a higher body temperature (Geiser & Baudinette, 1990). Alternatively, thermal inertia also makes arousals from cool body temperatures more energetically expensive for larger-bodied species (Geiser & Baudinette, 1990). Thermal inertia has thus been used to predict a more constant body temperature for larger species (McNab, 1970).

We sought to fully characterize thermoregulation in *Tenrec ecaudatus* by measuring metabolism and evaporative water loss (EWL) to demonstrate the flexibility with which

tenrecs regulate body temperature across a range of ambient temperatures. We hypothesized that tenrecs would be thermally-labile and minimally defend body temperatures above cooler ambient temperatures and below hot ambient temperatures. We hypothesized that this thermal flexibility would potentially obscure any clear inflection points of VO_2, indicative of a lower limit of thermoneutrality or EWL, indicative of an upper limit of thermoneutrality. At high ambient temperatures we expected tenrecs to exhibit facultative hyperthermia and elevate their body temperature above ambient temperature in order to mitigate EWL. For our thermography experiment, we hypothesized areas of minimal insulation and high vascularization would be exposed at high temperatures to facilitate heat dumping to the environment, and obscured at cold temperatures to minimize heat loss.

3.3 Methods

The tenrecs (*Tenrec ecaudatus*) were part of a captive colony housed at the University of Nevada-Las Vegas (Treat et al., 2018). For this experiment, we selected 16 adult tenrecs from the captive colony (13 male, 3 female). All animals had already been implanted with temperature data loggers as part of a previous study (Tempo Disc Bluetooth Temperature Sensor Beacon and Data Logger DSCTEMP001, Blue Maestro, London, UK, Treat et al., 2018) from which data could be downloaded via Bluetooth. Individuals were housed at 28°C, subject to 16:8 light:dark photoperiod, and fasted 12 hours before the start of each experiment. The experiments took place at the Sable Systems Headquarters in North Las Vegas, where we transported the 16 individual tenrecs each morning (25 minute drive in light traffic) before bringing them back to the UNLV animal facility at the end of the daily session. We used a Sable Systems Promethion (Las Vegas, USA) flow-through respirometry system with a temperature-control cabinet to measure O_2 consumption (VO_2)as well as H_2O production (VH_2O,or "EWL"), and CO_2 (VCO_2)production of the 16 tenrecs in each trial. The Promethion system was calibrated the morning of each experiment using H_2O span gas of known concentration and zeroed using 100% N_2. The temperature-controlled

cabinets had the capacity to test 16 animals at a time with two multiplexed banks each with 8 cages and one baseline. Each bank sampled either one of the cages or the baseline at a time, with sampling at one-second intervals. We ran experiments during the daytime, the rest phase of *Tenrec ecaudatus* (Nicoll, 1985) and kept the lights on inside the environmental cabinet during tests to mimic the normal housing unit at UNLV. Each trial lasted 8 hours and consisted of one tested ambient temperature. We conducted trials at seven different temperatures: 12°C, 14°C, 17°C, 22°C, 27°C, 32°C, and 34°C, plus an additional day of acute heat exposure (see below). Corn cob bedding about 2 cm thick covered the bottom of each of the 16 respirometry chambers within the temperature-controlled cabinet. Raw data were collected using Expedata (Sable Systems International, Las Vegas) and later analyzed using custom macros in Expedata. We monitored body temperature using the surgically implanted temperature data-loggers for all experiments and opened the door to the temperature chamber at regular one-hour intervals during the test at 34°C to monitor the animals. If the animal showed signs of distress (attempting to escape, lying on their back, or with body temperatures over 36°C) the individual was removed from the experiment for that day. After each 8-hour experiment, individuals were removed from the respirometry system, given access to water, and transported back to UNLV animal housing where they were weighed, returned to their housing boxes, and fed their normal daily amount. To further investigate thermoregulation of *T. ecaudatus* in response to heat, we quantified evaporative water loss and VO_2 during acute heat exposure and compared to the internal body temperature at those temperatures. After all of the 8-hour respirometry tests were complete, we used the same respirometry setup to measure the tenrecs' acute responses to heat by exposing them to increasing temperatures in short intervals. Starting at 30°C, we measured tenrecs in the Promethion setup for one hour, then increased the temperature by 1°C/hour until we saw a significant increase in their VO_2 and/or a decrease in VCO_2. At the end of each test, all individuals were given food and water and placed back in their housing area.

3.3.1 Thermal Windows

To quantify potential thermal windows as well as whole-body heat loss, we conducted thermography experiments in a temperature-controlled environmental chamber, increasing or decreasing the ambient temperatures by 2°C at 1.5-hour intervals. We began experiments in the morning at 0700 or in the afternoon at 1300 and each trial lasted about 6 hours. Animals to be tested were fasted 12 hours before the experiments began (overnight). During each trial, each individual was placed in its own tall plastic container (an empty, black kitchen garbage bin; 37cm x 28cm x 46cm) and the four containers were taped together at their open tops to keep them close together. The factory calibrated FLIR p640 thermal camera (Teledyne Oregon, USA), was mounted directly above the 4 bins on a tripod, facing downwards and focused to see all bins. Two temperature data loggers were placed in a temperature-controlled room; one was placed on 3cm-thick the styrofoam flooring and one temperature and humidity sensor was hung from the ceiling. At the beginning of each trial, the tenrecs were left for one hour at 20°C in the testing bins to get acclimated to the new environment. Both warm and cool trials started at 20°C, then either decreased to 18°C, then to 16°C (cold trials), or increased to 24°C, 28°C, and finally 32°C (warm trials). Animals were tested at either the warming or cooling temperatures on any given day. Thermal images were taken after animals had been exposed to a steady temperature for 30 minutes. No animals showed signs of distress at any point in the thermography experiments. After all temperatures had been tested for the four individuals, they were given access to water and food and returned to their housing area. The above procedure was conducted on 12 of the same individuals that were used for metabolic measurements.

3.3.2 Statistical analysis

VO_2, VCO_2, and EWL were calculated using equations from Lighton (2019) within Expedata. All statistical analyses were conducted using R version 4.2.0. To determine the limits of the TNZ, we averaged the measurements of the lowest 20-minutes of the flattest hour of each ambient temperature test for each individual and constructed a thermal profile

for *Tenrec ecaudatus*, identifying inflection points for both VO_2, EWL, wet and dry thermal conductance (C_{wet} and C_{dry}), and T_b. However, later tests showed that the bedding used to keep the tenrecs calm during experiments interfered with water measurements, so EWL, EHL:MHP, and C_{dry} were not usable. Inflection points were calculated with piecewise linear regressions (Crawley, 2007) using the *Segmented* R package (Muggeo, 2017), and linear mixed modeling was implemented using the R package *nlme* (Pinheiro et al., 2013). To quantify the metabolic rate at high ambient temperatures during the ramping experiments, we averaged the values of the last 10 minutes of each one-hour exposure to the ramped temperature.

3.3.3   Thermal Images

Exposed surface area and average temperature for different body segments were analyzed using ResearchIR software (Version 4.40.11.35, FLIR Systems, Portland, Oregon). We calibrated the images by measuring the known distance of two visible points within the experimental containers. Overall visible surface area was calculated for each body segment using the outline tool, which then calculated both area and average temperature of the segment. We ran linear mixed models on the data using the *lm* function in the *nlme* R package with body mass as a covariate (Pinheiro et al., 2013).

3.4   Results

Respirometry experiments were conducted on 15 individuals (13 male and 2 female) at seven different test temperatures ranging from 11.1°C to 34.9°C. Mean body mass ranged from 1008 ± 111 g (range 778 - 1252 g, N = 13) for males and 958 ± 201 g (range 688 - 1116 g, N = 2) for females. One of the original 16 individuals gave birth on the day of the ramping experiment therefore the data pertaining to that individual were not included in this study. Additionally, the female with the lowest body mass entered hibernation after the fourth experiment and was therefore not measured for the final three test temperatures at 10, 20, and 25°C, the ramping experiment, or the thermography experiment. Two individuals were removed from the study at 32°C and nine at 34°C, because their body temperature increased beyond 36°C, or increased by more than 2°C in less than 10 minutes, indicating potential heat stress. The water measurements and subsequent calculated values of EWL, EHL:MHP, and C_{dry} were not usable because of interference from the corncob bedding.

We found the lower inflection point of VO_2, indicative of the lower limit of thermoneutrality, at 19.1°C (95% confidence interval: 16.9 - 21.3°C, Figure 3.1A). Inflection points in T_b were found at 19.4°C (95% confidence interval: 18.1 - 20.8°C, Figure 3.1B), and wet thermal conductance at 29.4 °C (95% confidence interval: 20.5 - 38.4°C, Figure 3.1C). Minimum C_{wet}, calculated as the minimum of the average C_{wet} recorded for each individual, was -5.54 ± 2.0 W·°C^{-1}, and the overall minimum C_{wet} was -43.6 W·°C^{-1}. BMR, calculated as the mean of the mean RMR value for each individual (N=15) for all T_as greater than 19.1°C (VO_2 inflection point), was 195.7 ± 60.1 mlO$_2$,·h^{-1} or 0.20 ± 0.07 mlO$_2$h^{-1}·g^{-1}. During the ramping experiments, we found no inflection points in either VO_2, or T_b at any of the ambient temperatures (p > 0.05, Supplementary Materials).

Twelve individual tenrecs were used in thermography experiments at seven experimental temperatures, for a total of 84 thermal images. Each group was exposed to three experimental temperatures during cool experiments (16.0°C - 20.9°C), and four
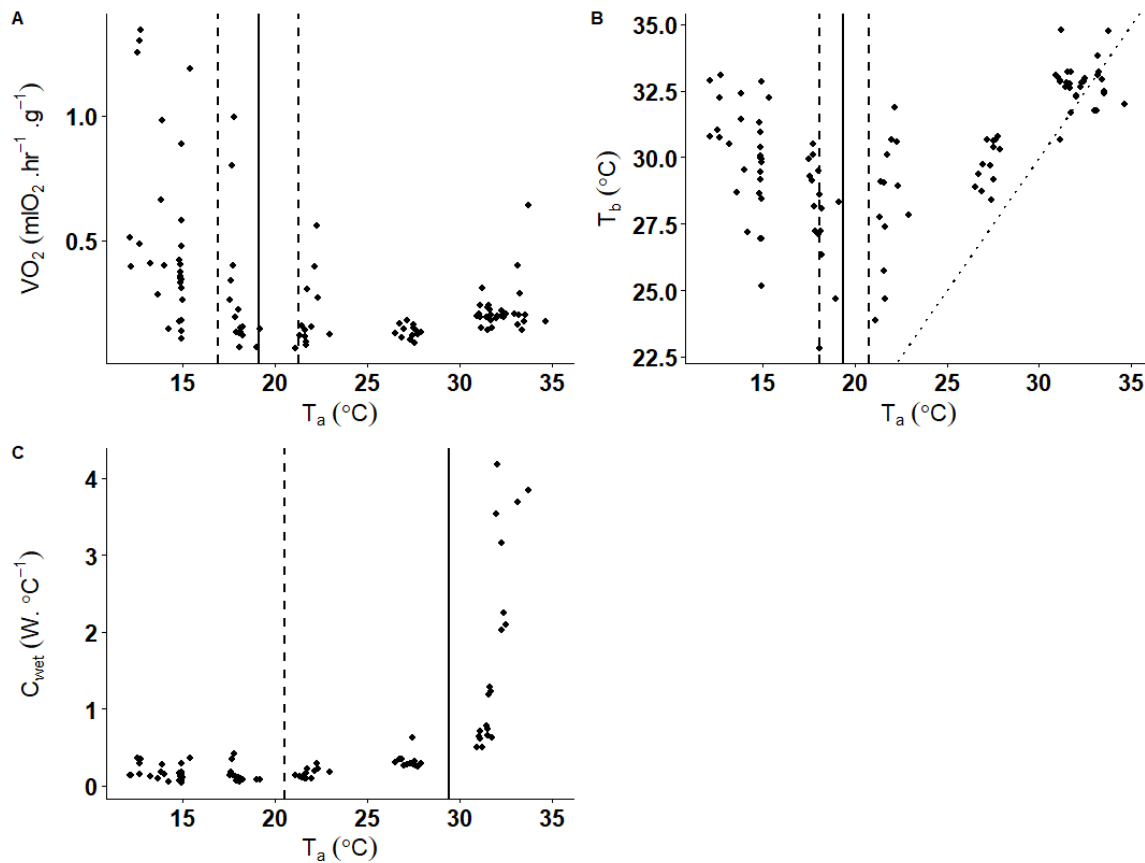
Figure 3.1: Metabolic rate (A, VO_2 in $mlO_2\ h^{-1}\ g^{-1}$;), body temperature (B, T_b), and wet thermal conductance (C, C_{wet} in $W\ °C^{-1}$) in *Tenrec ecaudatus* all show inflection points when measured across a range of ambient test temperatures, indicative of limits of thermoneutrality. For visual clarity only positive values for wet conductance were included. Solid vertical lines represent calculated breakpoint, dashed vertical lines represent the calculated 95% confidence intervals, and the diagonal dotted line shows where $T_a = T_b$.

experimental temperatures during warm experiments (19.8°C - 31.6°C). The total surface body temperature and the total surface area of the exposed body parts of the tenrecs increased as ambient temperatures increased ($p<0.05$, $R^2 = 0.90$, and $p<0.05$, $R^2 = 0.57$, Figure 3.3a). When the snout, paws, and ears were exposed and measurable, they all had significant, positive relationships with T_a. Positive relationships were found between T_a and T_{snout} ($p<0.05$, $R^2 = 0.81$), T_{paws} ($p<0.05$, $R^2 = 0.83$), and T_{ears} ($p<0.05$, $R^2 = 0.93$, Figure 3.3b). Specifically, these thermal windows were cooler than T_b at cool temperatures and warmer than body temperature at warm temperatures (Figure 3.3b).
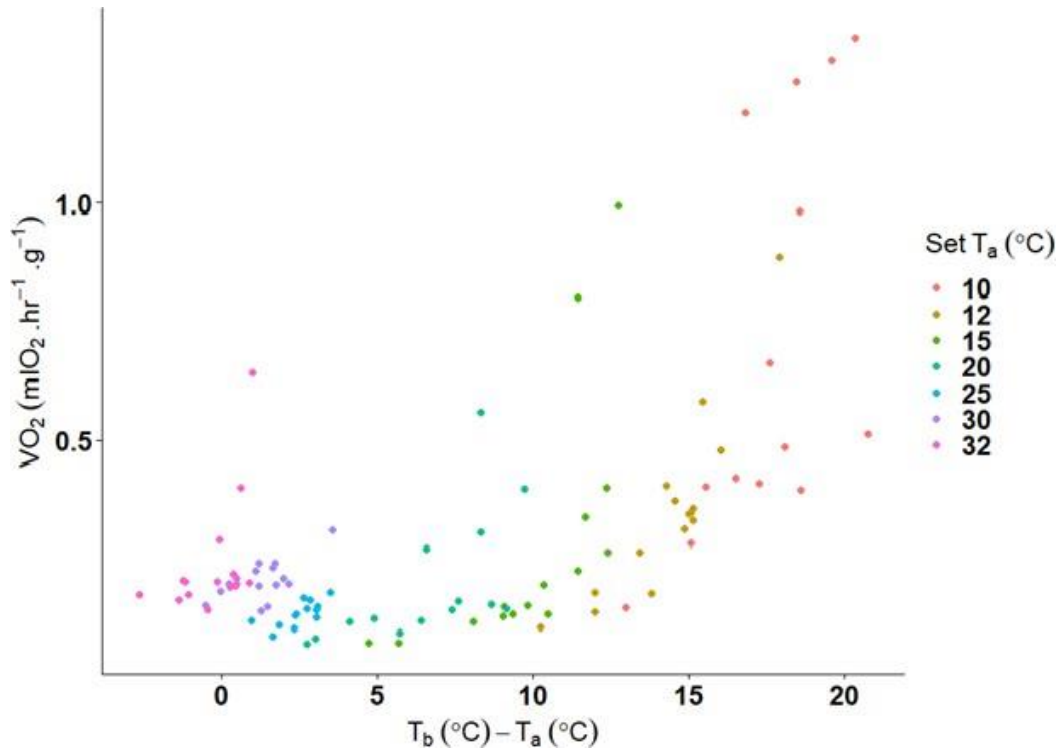
Figure 3.2: Metabolic rate (VO_2 in $mlO_2 \cdot hr^{-1} \cdot g^{-1}$) and the variability of VO_2 in *Tenrec ecaudatus* increased as the differential between body and ambient temperatures increased. Colors indicate the set T_a of the specific tests in which the values were measured. Individuals maintaining similar temperature differentials still varied in their VO_2, primarily at the higher temperature differentials.

3.5   Discussion

Throughout all experiments, tenrecs showed a high degree of thermolability, demonstrating extreme flexibility in T_b regulation. Although current definitions of the TNZ specify the limits as increases in metabolic rates and EWL to maintain a constant, normothermic body temperature (Withers 1992, Withers et al. 2016), tenrecs instead demonstrated extreme variation outside of the standard TNZ; at low temperatures, there was large variation between individual tenrecs ranging from relatively high to relatively low metabolic rates (Figure 3.1), but these differences in metabolic rates were not correlated to changes in body temperatures (Figure 3.2). We found the TNZ to be more clearly defined as the zone where the variation in both metabolic rate and T_b are extremely reduced
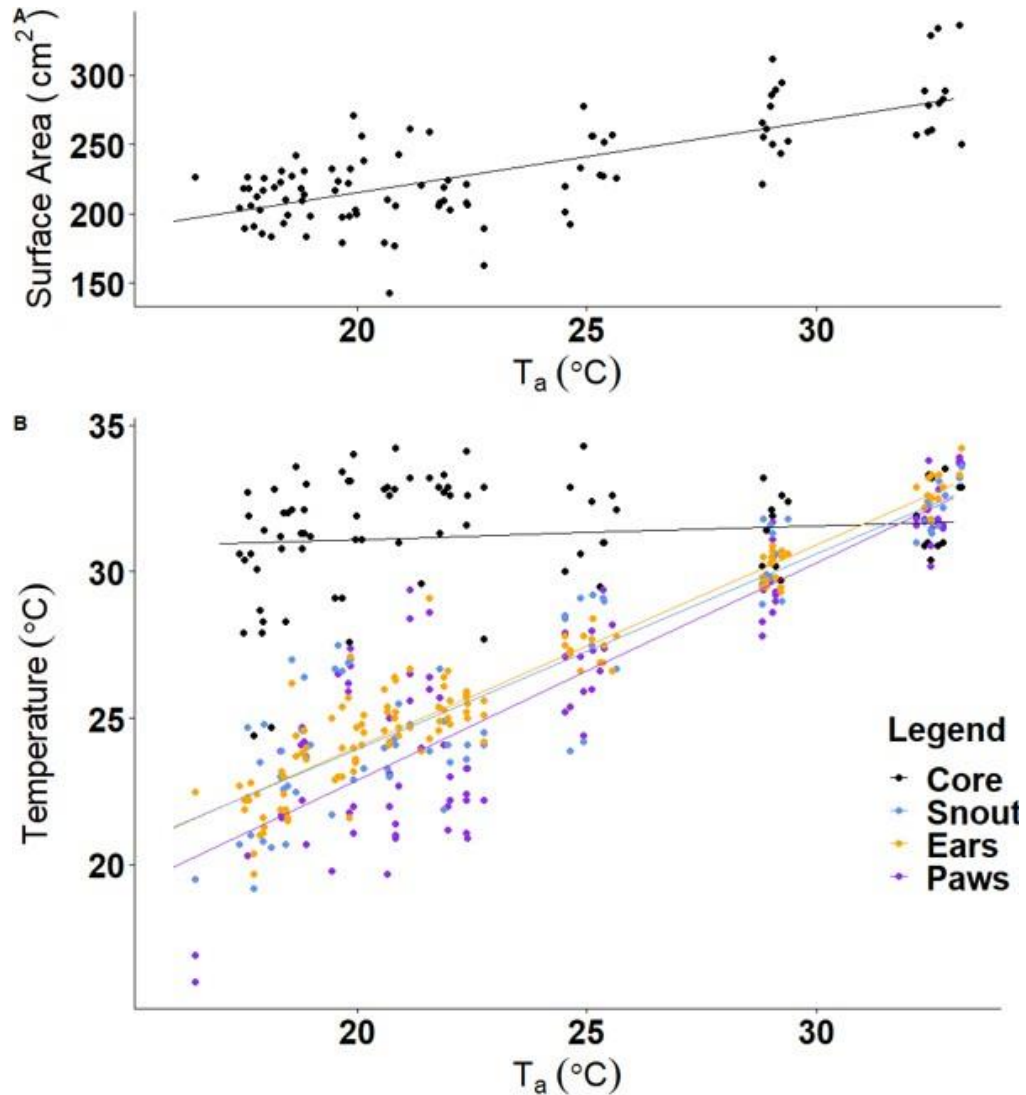
Figure 3.3: (A) Total visible body surface area and (B) Temperature of core and body surfaces increase with ambient temperatures during thermography experiments.

compared to areas beyond the newly-defined thermoneutral zone (Figure 3.1). With T_bs either at normothermic levels or tracking ambient temperature outside of the TNZ, tenrecs exemplify the extreme end of thermolability (Figure 3.1). Additionally, individuals maintaining T_bs significantly above ambient temperature did not always have an associated high metabolic rate (Figure 3.2). For instance, at the 10°C measurements, individuals maintaining almost the same T_b - T_a differential between 17.5°C and 18.5°C had corresponding metabolic rates varying almost three-fold from 0.40 $mlO_2\ h^{-1}{\cdot}g^{-1}$ to 1.25

mlO$_2$ h^{-1}·g^{-1}. At cool ambient temperatures, animals with higher metabolic rates did not necessarily have high body temperatures and animals with lower metabolic rates did not necessarily have corresponding low T$_b$s (Figure 3.2). This pattern holds when controlling for set T$_a$ and the differential between T$_a$ and T$_b$ (Figure 3.2). It seems as though different individuals may have different normothermic set points at which they begin actively employing metabolic heating or cooling to maintain that specific T$_b$, while some individuals tolerate a greater range of ambient temperatures before thermoregulating.

Mechanistic models require an accurate understanding of the thermoregulatory physiology of a species. The current definition of the TNZ, which assumes animals are defending a constant euthermic temperature, does not account for non-torpid heterothermy in thermally-labile species such as the tailless tenrec (Withers, 1992; Withers et al., 2016). Our findings confirm that beyond the traditional TNZ, sub-tropical mammals do not defend normothermic T$_b$s. Instead, what we found in *Tenrec* is increased variation in VO$_2$, values at the extremes of ambient temperatures (Figure 3.1). Thermolability is advantageous at cool ambient temperatures where more metabolic heat would need to be produced in an individual maintaining T$_b$ elevated above T$_a$, or at ambient temperatures above T$_b$ where water loss would need to be elevated to keep T$_b$ below ambient temperature. Instead, thermally-labile species could allow T$_b$ to track T$_a$ and save energy and water outside of the TNZ. In light of these findings, we strongly advocate for an effort to increase the representativity of mammalian thermoregulatory phenotypes by collecting data from underrepresented taxa and geographical regions, such as the tropics and subtropics. Such an effort would not only contribute towards a more solid understanding of the underlying biophysical assumptions of thermoregulatory physiology, but also provide data for more accurate mechanistic models.

We found extreme variation among individual tenrecs in metabolic rate and T$_b$. Due to differences in surface-area to body size ratios, larger individuals may lose heat at slower rates in cooler ambient temperatures than smaller conspecifics (Geiser & Baudinette, 1990; McNab, 1970). Although mass was found to be non-significant as a covariate in linear models of VO$_2$ and T$_b$ ($p > 0.05$), thermal inertia may still play a role in the

thermoregulatory strategies between different species of tenrec that vary in size. We found BMR in *Tenrec ecaudatus* (1003g) to be 0.20 ± 0.07 mlO$_2 \cdot$h$^{-1} \cdot$g^{-1}, lower than that of male greater hedgehog tenrecs (*Setifer setosus*, 172g), with resting metabolic rates within the TNZ of 0.36 ± 0.11 mlO$_2$h$^{-1} \cdot$g^{-1} (Levesque & Lovegrove, 2014) and that of the highland streaked tenrecs (*Hemicentetes nigriceps*, mass = 114.7g) and lowland streaked tenrec (*Hemicentetes semispinosus*, mass = 100.2g) with summer resting metabolic rates in males of 0.28 mlO$_2$ h$^{-1} \cdot$g^{-1} and 0.42 mlO$_2$h$^{-1} \cdot$g^{-1} respectively (Stephenson & Racey, 1994). The C$_{wet}$ of *Tenrec ecaudatus* was -0.02 W$\cdot$°C$^{-1} \cdot$g^{-1}, lower than the $9.2 * 10\text{-}6$ W$\cdot$°C$^{-1} \cdot$g^{-1} reported for male *Setifer setosus* (Levesque & Lovegrove, 2014) and supporting our hypothesis that the larger *Tenrec ecaudatus* loses heat at lower rates than other smaller tenrec species.

Using thermography across a range of ambient temperatures, we found tenrecs to vary their position to either expose or restrict the amount of exposed surface area. At cooler temperatures, tenrecs were curled into tighter balls with much lower measurable surface area, and their paws and snout were less likely to be exposed and visible (Figure 3.3). By decreasing the exposure of more vascularized appendages, tenrecs retain more heat. Alternatively, at warmer test temperatures, more body parts were not only more likely to be visible, but also in a position that increased surface area. These exposed surfaces (snout, paws, and ears) were significantly warmer than both core body temperature and the temperature of the surface of the body as measured within the thermal image (Figure 3.3). These exposed vascularized areas allow tenrecs to dump heat to the environment and aid in thermoregulation. Although heat loss is not the primary function of these body parts, their use as thermal windows is nonetheless advantageous, as tenrecs both manipulate the amount of exposed surface area and these thermal windows are significantly different temperatures from the body core.

We found tenrecs to fluctuate both heat production and heat loss through means of metabolic heat production, and thermal windows in an attempt to maintain somewhat constant body temperatures. However, T_b still appeared flexible outside of the TNZ, where body temperature of some individuals fluctuated with ambient temperature, resulting in a form of thermolability that reduces energy use. Thermolability is an advantageous adaptation to harsh environments, similar to those where endothermy is hypothesized to have evolved (Crompton et al., 1978; Grigg et al., 2004; Lovegrove, 2012a, 2012b). Tenrecs retain many of the ancestral mammal characteristics (nocturnal, insectivores, and relatively unspecialised limbs) and a better understanding of the physiology of thermolability may give us insight into how endothermy evolved and the role of metabolism in thermoregulation (Treat et al., 2018). By identifying the exceptions to the traditional rules of thermoregulation, like thermally-labile species maintaining plesiomorphic thermoregulatory phenotypes, we can obtain insights as to how mammals made the transition from ectothermic-like heterothermy to homeothermy.

3.6 Acknowledgements

3.7 Supplementary Materials

Figure 3.4: Body temperature (T_b; A), and oxygen consumption (B), in *Tenrec .ecaudatus* all have no significant relationship to T_a, but do have inflection points when measured across a range of ambient test temperatures. The diagonal dotted line in panel A indicates the isotherm of $T_b = T_a$.
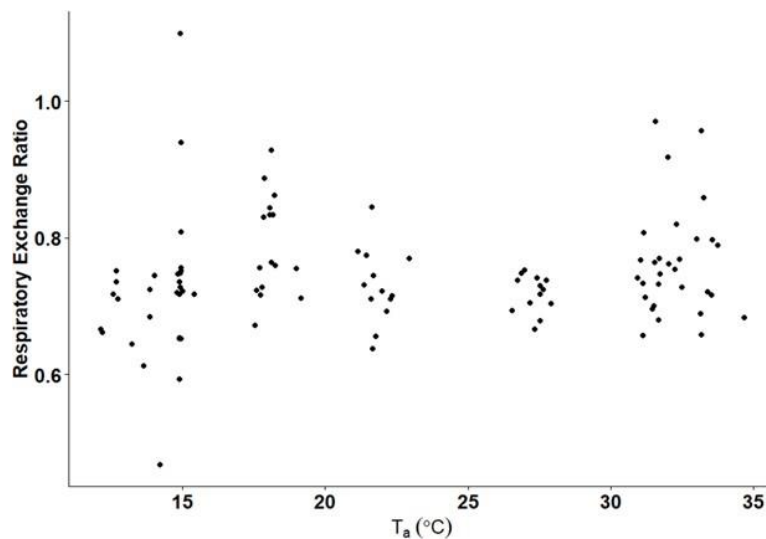
Figure 3.5: The Respiratory Exchange Ratio (RER; the ratio of VCO_2 to VO_2) of *Tenrec ecaudatus* across a range of T_a. An RER of 0.7 indicates fats are primarily being burned, while an RER of 1.0 indicates that carbohydrates are primarily being burned.

CHAPTER 4

MACRO AND MICROCLIMATE AFFECTS GLOBAL DISTRIBUTION OF THERMOREGULATORY PHENOTYPES IN CHIROPTERA

4.1 Chapter Abstract

Analyses of the distribution of thermoregulatory phenotypes have focused on coarse, global patterns or local, species-specific microsite variables. In Chiroptera, fine-scale climate variability in ambient temperatures and precipitation has been shown to affect body temperature regulation patterns and the limits of the thermoneutral zone in a few species, yet to our knowledge, no large-scale analyses have been conducted. We found several detailed climatic variables predictive of residual basal metabolic rate when controlling for phylogeny: annual mean temperature, minimum temperature of coldest month, temperature annual range, temperature monthly range, and temperature seasonality. However, no significant correlations were found between any specific climatic variables and any of the parameters of the thermoneutral zone or body temperature even after controlling for phylogeny. Chiroptera are often excluded from macro studies of BMR and thermoregulation in mammals due to their highly variable energetic patterns. However, these unique thermoregulatory adaptations can give us invaluable insights into the physiology and evolution of thermolability when bats are considered as more than just problematic outliers.

4.2 Introduction

Understanding the relationship between a species' physiology and their environment is one of the fundamental lines of inquiry of biology. An important aspect of this endeavor with repercussions for conservation, biogeography and evolutionary sciences is the understanding of how animals' thermoregulatory characteristics, and by extension their energetics, vary according to environmental factors. Understanding the linkage between

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precipitation, humidity, and temperature and their effects on thermoregulatory patterns and energy and water balance is fundamental for models predicting species-range shifts with climate-change (Duffy et al., 2015) and to understand animals' capacity to cope with anthropogenic climate change (Czenze et al., 2022). Mass die-offs of species as a direct result of increased intensity and frequency of heat waves makes it urgent for us to further understand heat-related mortality events in small endotherms such as birds (McKechnie et al., 2021) and bats (Welbergen et al., 2008; Ratnayake et al., 2019; McKechnie et al., 2021). Furthermore, the global distribution of phenotypes and thermoregulatory strategies can be used to disentangle the role of physiological constraints and climate in evolution.

   With an increase of absolute latitude there is a decrease in the variability of both temperature and precipitation, a decrease in average yearly temperature, and an increase in yearly temperature extremes (Scholander et al., 1950; Lovegrove, 2000). Lovegrove (2000) conducted a meta-analysis of 487 mammal species and found that in more variable environments (i.e. with high interannual variability of ambient temperatures and rainfall), small mammal species were more likely to have lower metabolic rates than species in more stable environments. He attributed this pattern to resource limitations, as lower metabolic rates and body temperatures have lower maintenance costs and are thus likely adaptations to a less predictable resource availability. Additionally, a follow-up study of 267 small mammals (< 1Kg) found that both basal metabolic rate (BMR) and body temperature increased with latitude; high BMR was found to be associated with higher latitudes, where BMR was affected by ambient temperature and mean annual rainfall, and species in zoogeographical regions with high variability of rainfall, like the Indomalayan and Afrotropical zones, tended to have lower BMR (Lovegrove, 2003). These low BMRs represent the slow end of the slow-fast metabolic continuum; species with less energetically expensive maintenance physiology should be better able to cope with the extreme conditions such as variable water availability or extreme temperature fluctuations (Lovegrove, 2000, 2003). Thermoregulatory scope (mean active body temperature - lowest ever body temperature

for the species) was also shown to be affected by absolute latitude, with small, high latitude species demonstrating greater range of thermoregulatory patterns than low latitude species (Boyles et al., 2013). However, animals are unable to lower body temperature below ambient temperatures when using heterothermy, therefore, at higher absolute latitudes, the minimum body temperatures species can reach is lower than that of their tropical counterparts. It is therefore difficult to separate the absolute ability of a species to decrease their body temperature (their fundamental physiological niche *sensu* Landry-Cuerrier et al., 2008) from their realized body temperature patterns. During summer months, however, tropical species were more heterothermic than higher latitude species, exhibiting a combination of deeper and longer torpor bouts than species at higher latitudes (Boyles et al., 2013). This pattern of increased heterothermy in the tropics and subtropics and decreased use in higher latitude species during the summer months could be a result of the more variable availability of resources during the summer at lower latitudes or the importance of breeding seasons and reproductive urgency at higher latitudes during the short summers (Boyles et al., 2013; Levesque, Nowack and Stawski, 2016). Specifically, the subtropical northern long-eared bat, *Nyctophilus bifax*, was found to enter nightly torpor bouts during the summer, even those individual bats that were in good body condition (Stawski and Geiser, 2010a). Conversely during the summer, species at higher latitudes are often in the midst of their short breeding season. The little brown bat (*Myotis lucifugus*) does still employ torpor during the reproductive season, but those torpor bouts are significantly shorter in pregnant females, likely because the delayed parturition due to torpor use during pregnancy diminishes an individual's reproductive success (Dzal and Brigham, 2013).

The aforementioned emerging patterns, however, might be biased by the low resolution of available data, which forced researchers to use broad variables like latitude and zoogeographic regions to represent predictability of rainfall and temperature regimes (Lovegrove, 2000, 2003). The relationship between thermoregulatory phenotypes and environmental variables are often analyzed using coarse data, such as latitude and

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precipitation patterns, across large areas or entire bioregions for whole families or phyla (Lovegrove, 2003). On the other side of the spectrum many studies detail the location-level relationship between individual species and microsites (Baudinette et al., 2000; Lausen and Barclay, 2006; Langwig et al., 2012; Rajasegaran, Shazali and Khan, 2018; Czenze et al., 2022), yet only a handful of studies explicitly study the relationships between environmental variables at the local level, like roost sites, in specific locations and corresponding physiological characteristics (e.g. Czenze et al., 2022). The dichotomy between broad-scale generalizations and extremely local, species-specific correlations can lead to two extremes of interpretations, each with its own benefits and shortcomings. Although computing technology has greatly advanced in recent years, the corresponding fine-scale, high quality ecological data has lagged behind (Levy et al., 2014). A meta-analysis of earlier models of species distribution relied on microclimate grids, on average, 10,000 times larger than the animals they were studying (Potter, Woods and Pincebourde, 2013). These issues can be overcome by using global patterns of micro-climate data, combining the two extremes to provide a fine-scale, yet globally accurate description of the relationship between climatic variables and thermoregulatory patterns (Levy et al., 2014). Furthermore, the use of standardized data collection techniques with comparable variables for many species will allow us to evaluate how thermoregulatory physiology has evolved with microclimate preferences.

Bats are an excellent model to study the relationship between microclimate variability and thermoregulatory phenotypes due to their cosmopolitan distribution and wide range of active and resting body temperatures (Arends, Bonaccorso and Genoud, 1995; Reichard et al., 2010; Doty, 2012). The order Chiroptera counts over 1386 species divided across 227 genera, and its members are found on all continents except Antarctica (Teeling et al., 2005; Burgin et al., 2018). As such, bats showcase large variations in size, habitat selection, and ultimately thermoregulatory phenotypes (e.g. strict homeotherms, daily torpidators, and seasonal hibernators), which appear to be a product of climate and roost selection (Stawski, Willis and Geiser, 2014; Nowack et al., 2020). In the temperate

and polar regions, bats must contend with the cold winter temperatures using one of two strategies (or a hybrid of the two); migration or hibernation for months at a time in roosts buffered from outside temperatures (such as caves; Weller et al., 2016; Klüg-Baerwald et al., 2017). Bats at higher latitudes with seasonal winters tend to be smaller and have thermoregulatory strategies ranging from short shallow bouts of daily torpor to hibernation throughout the winter with weeks of deep torpor broken up by interbout arousals (Geiser & Stawski 2012, Ruf & Geiser, 2015). Most bat species, however, inhabit warm climates where they use daily or multi-day torpor, with only a few tropical species hibernating during periods of inclement weather or low food availability (Stawski, Turbill and Geiser, 2009; Stawski and Geiser, 2010b). Furthermore, some larger tropical species appear to be homeothermic, as they have not been shown to use torpor in laboratory settings (Ransome 1990, McNab, 1989, Bonaccorso 1992, Riek Kortner Geiser 2010). Another potential advantage of torpor is predator avoidance, which would suggest a contribution of biotic factors affecting the thermoregulatory phenotype of a species in addition to known abiotic factors like environmental temperatures (Stawski and Geiser, 2010b; Nowack, Stawski and Geiser, 2017). Bats are also more exposed than other mammals to the environment as they possess a large surface area to volume ratio because of their highly vascularized wing membranes (Lyman, 1970) and are simultaneously more active at night (when ambient temperature is at its lowest). The large wing surface areas exposed to the environment provide an interface for gas exchange to take place (Makanya and Mortola, 2007), losing body heat rapidly to the atmosphere (Reichard et al., 2010).

Although these broad patterns of thermoregulation across latitudes and zoogeographic zones hold for a majority of species, variations exist across sympatric species. For example, species roosting in warmer roosts have been shown to have higher heat tolerance limits and evaporative cooling capacity (Cory Toussaint and McKechnie, 2012; Czenze et al., 2022). A study quantifying the upper critical limits in six small southern African bat species found

that the species occupying roosts less buffered against outside temperatures (showing greater temperature fluctuations) had greater evaporative cooling capacity and lower metabolic rates (Czenze et al., 2022). Lower metabolic rates coincide with lower metabolic heat production, an adaptation of small endotherms living in hot environments (van Jaarsveld et al., 2021; Wojciechowski et al., 2021), and may be the greatest limitation of bats' ability to cool. Additionally, bat species roosting in caves more sheltered from the heat, had lower evaporative cooling capacity than species roosting in hotter, more exposed microsites like building roofs and tree cavities (Czenze et al., 2022). Together, these findings support the hypothesis that thermal physiology co-evolved with roost microclimates, highlighting the importance of accounting for microclimate data when modeling thermoregulatory responses under climate change scenarios.

To test if using fine-scaled climatic data on a global scale would provide a better explanatory power in understanding bat thermoregulatory phenotypes, we conducted phylogenetic and biogeographical analysis of basal metabolic rate and body temperature measurements from chiropteran species. Chiroptera, the second-most specious order of mammals (Burgin et al., 2018), is often considered the outlier group of most physiological data, as patterns explaining mammalian thermoregulation become clearer when this speciose clade is removed from analyses, likely because of their flexibility in body temperature regulation and blurred division between torpor bouts and periods of low but active body temperature. We specifically investigated how the variability of environment (temperature and precipitation) affected body temperature and metabolic rate at the lower critical limits of Chiropteran species.

4.3   Methods

Physiological data were retrieved from previously assembled and published datasets on body temperature and metabolic rate. We first used the database from Clarke and O'Connor, (2014) subsetted for Chiropteran species. We then subset that dataset for

species that had also been part of the meta-data study from Genoud et al. (2017) to obtain quality-controlled BMR values. To find additional thermoregulatory variables, we first focused on those publications cited in the Genoud et al (2017) database to see if they had measured body temperature simultaneous to metabolic measurements, as those values should be the closest variables measured within the same setting as the metabolic measurements. After documenting any of the thermoregulatory variables and variables pertaining to the rigor with which the measurements were made, we ran an additional literature search using Google Scholar search terms "body temperature" + "species name" to find any other existing studies. We then documented each of the other studies that matched the criteria and assigned a score (good, ok, or poor) on the quality of the thermoregulatory data provided.

The specific thermoregulatory variables we documented for each study were the upper or lower critical limits (T_{uc} and T_{lc}) of the thermoneutral zone (TNZ), and body temperatures at those limits. When body temperature was not measured at those temperatures, we documented the body temperature and specified which ambient temperature at which it was measured, and whether or not it was within the species' thermal neutral zone, where basal metabolic rate is also measured. We also noted the maximum and minimum ambient and body temperatures measured in the study. The range of their TNZ was taken by subtracting the T_{lc} from the T_{uc}. We then also noted which conditions of body temperature and basal metabolic rate measurements were met, i.e. if measurements were taken on fasted, adult, non-reproductive individuals during their rest phase in the summer, whether the animals were free-ranging, captive, domestic, or wild caught, the method of body temperature measurement (rectal, implanted data logger, etc.), the average mass of the individuals, and the number of individuals measured in the study. The quality of the data was determined by how many of the previously mentioned criteria were met. For instance, a study was considered "good" if the body temperature was measured using an implanted data logger on a large number of individuals at their T_{lc}, only "ok" if measured using only rectal T_b or a lower sample size, and "poor" if the T_b

measurement was not taken at the T_{lc}. For our study, we used only data categorized as "good" or "ok."

Using the final physiological database, we identified as close as possible the GPS coordinates of the location where the thermoregulatory and metabolic data had been collected. In a majority of studies, either latitude and longitudinal values were provided, or specific landmarks were mentioned to identify the location within a reasonable distance. We then noted the latitude and longitude for each study and each year of fieldwork for the study. Some studies had multiple locations within each year, so we also kept those data. We downloaded ECMWF Reanalysis v5 (ERA5) microclimate data using the *Mcera*5 package in R (Klinges et al., 2022) in the form of .nc files. ERA5, through Copernicus Climate Change Service, provides hourly data covering the globe in a 30 x 30 km grid. When given the specific coordinates for our study, it automatically calculates distance matching. We downloaded microclimate data for the full year during which fieldwork had been conducted, from January 1 to December 31. For studies that did not specify the year, we chose one year before publication. Because *mcera*5 does not have data pre-1969, the one study with data from that time was removed. Once the files from each of the years and locations were downloaded, we extracted both the climate data and precipitation data using "extract_clim" and "extract_precip" functions in the *mcera*5 R package (Klinges et al., 2022). For each location we calculated the annual mean temperature (average of all hourly temperatures for the year), the maximum temperature of the warmest month, the minimum temperature of the coldest month, temperature annual range (TAR; maximum temperature of the warmest month - minimum temperature of the coldest month), temperature monthly range (TMR; the average of the differences between the maximum and minimum monthly temperatures), temperature seasonality (TS; the standard deviation of the average monthly temperatures), and precipitation seasonality (PS; the standard deviation of the average monthly precipitation) (Naya et al., 2012; Luna, Naya and Naya, 2017).

4.3.1 Phylogenetic Analyses

To quantify the effects of climatic and precipitation variables on thermoregulatory phenotypes, we used Phylogenetic Generalized Least Squares tests (PGLS) with the "pgls" function in the R package *caper*, which uses maximum likelihood to estimate the value of phylogenetic signal (λ) to optimally adjust the degree of phylogenetic correlation in the data (Orme et al., 2013). λ ranges from 0, indicating no correlation, and 1, indicating very strong correlation. We used the most up-to-date mammalian phylogeny trimmed to Chiropteran species downloaded from treeoflife.org (Upham, Esselstyn and Jetz, 2019). Our dependent variables (run as separate models) were BMR and log-transformed BMR while controlling for body mass, the body temperature (T_b) at T_{lc}, T_{lc}, the differential between T_{lc} and what T_b the species maintained (T_b at T_{lc} - T_{lc}). For each PGLS, we calculated the confidence and prediction intervals for the corresponding ordinary least squares (OLS) models using "gls.ci" and "gls.pi" functions in the *evomap* R package (Smaers and Mongle, 2014; Smaers and Rohlf, 2016).

4.4 Results

We found thermoregulatory data relating to 64 species of bats across 110 studies. 78 studies contained robust thermoregulatory data for 58 species of bats, resulting in 105 location files for studies containing multiple years and multiple locations. Of the 58 species investigated, 24 met the experimental requirements to be categorized as either good or passable T_b measurements at T_{lc}, and were thus able to be used in subsequent analyses (Figure 4.1 - 4.2). Mass of the 24 species followed a negative binomial distribution, with most species having a small mass (22 species < 100 g, min= 4.8g) and only two species with body mass greater than 300g (max = 598g).

As expected, logBMR was positively correlated with logmass and logmass explained 90% of the variance in BMR ($P < 0.001$, $R^2 = 0.90$). Residuals of BMR from this model were not affected by the annual mean temperature ($p > 0.05$), the minimum temperature of the
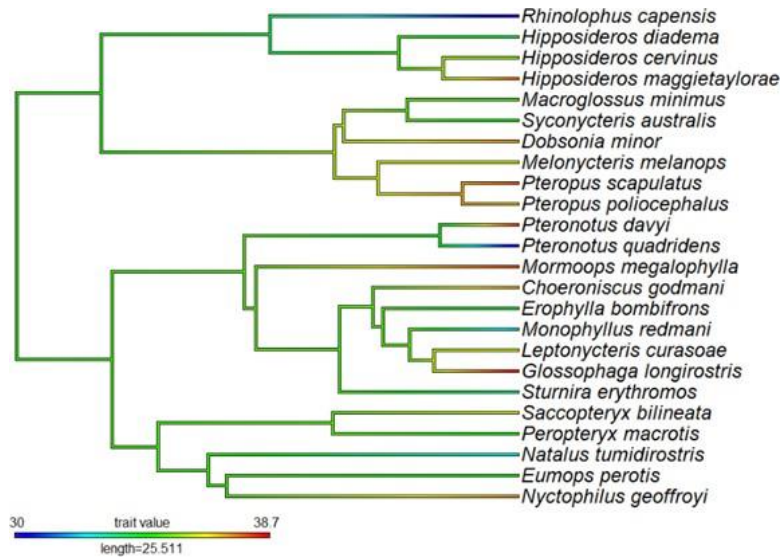
Figure 4.1: Phylogenetic tree showing the relationship between 24 bat species as a continuous trait map with body temperatures (T_b, °C) at their lower limit of thermoneutrality (T_{lc}).
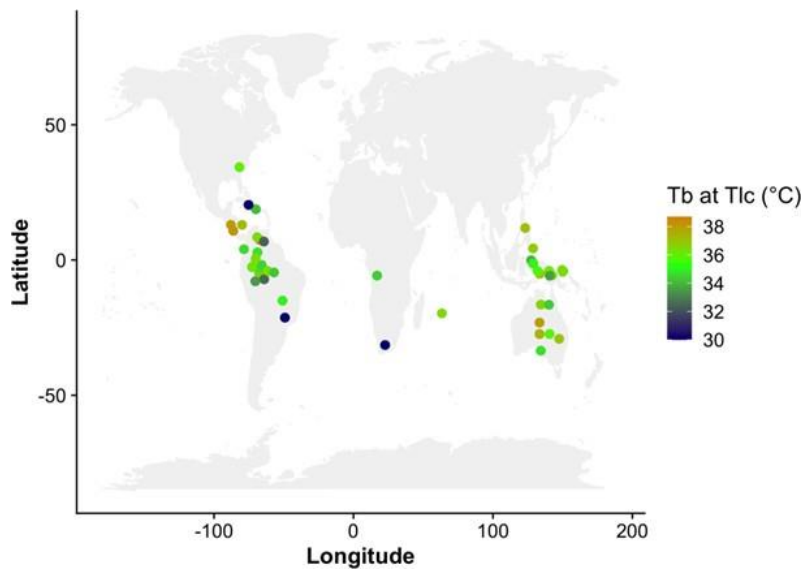
Figure 4.2: Midpoints of the ranges of 24 species of bat, with colors depicting body temperatures (T_b, °C) at their upper limit of thermoneutrality (T_{uc}).

coldest month ($p > 0.05$), the temperature annual range ($p > 0.05$), temperature monthly range ($p > 0.05$; Figure 4.3). T_b at T_{lc}, T_{lc}, and the difference between T_b at T_{lc} and the T_{lc} of each bat species all had no relationship with either climatic or precipitation microclimate variables when controlling for the effect of Brownian-motion phylogeny ($p > 0.05$; Figures 4.4 to 4.6). Additionally, both BMR and

Figure 4.3: The minimum temperature of the coldest month of the year (A), the temperature annual range (B), the temperature monthly range (C), and the temperature seasonality (the standard deviation of the average monthly temperatures; (D) have no effect on the residuals of logBMR as a function of log mass for 24 Chiropteran species.

Figure 4.4: The minimum temperature of the coldest month of the year (A), the temperature annual range (B), the temperature monthly range (C), and the temperature seasonality (the standard deviation of the average monthly temperatures; D) have no effect on the body temperature at the lower limit of thermoneutrality (T_b at T_{lc}, °C) for 24 Chiropteran species.

logBMR, when controlling for the effects of body mass and

Brownian-motion phylogeny, showed no relationship to either the climatic or precipitation

microclimate variables ($p > 0.05$; Figures 4.7 - 4.8).

4.5 Discussion

Endothermy allowed the dominance of Mammalia, and its importance is undoubted. Yet, attempts to describe the potential physiological drivers and constraints of the global distribution of endotherms are still struggling with many unanswered questions. We attempted to broaden our understanding of the relationship between detailed climatic variables and thermoregulatory phenotypes in Chiropteran species. As a clade, bats are often excluded from similar studies on BMR and thermoregulation in mammals due to

Figure 4.5: The minimum temperature of the coldest month of the year (A), the temperature annual range (B), the temperature monthly range (C), and the temperature seasonality (the standard deviation of the average monthly temperatures); D) have no effect on the difference between body temperature at the lower limit of thermoneutrality and the lower limit of thermoneutrality (T_b at T_{lc} - T_{lc}, °C) for 24 Chiropteran species.

their unusual and highly variable energetic patterns (Lovegrove, 2003), but see (Clarke, Rothery and Isaac, 2010; Lovegrove, 2012; Boyles et al., 2013). We found several climatic variables predictive of residual BMR when controlling for phylogeny: annual mean temperature, minimum temperature of coldest month, temperature annual range, temperature monthly range, and temperature seasonality. However, no relationships were found between any specific climatic variables and any of the purely thermoregulatory variables (T_b at T_{lc}, T_{lc}, the differential between T_b at T_{lc}, and T_{lc}) even after controlling for phylogeny. These findings were unexpected, as the relationship between latitude and other thermoregulatory phenotypes have been documented on a broad scale, and we anticipated microclimatic variables to have a direct descriptive power. Looking simply at T_b and T_{lc} (as has been done previously) instead of range of thermoregulatory scope or use of heterothermy may obscure
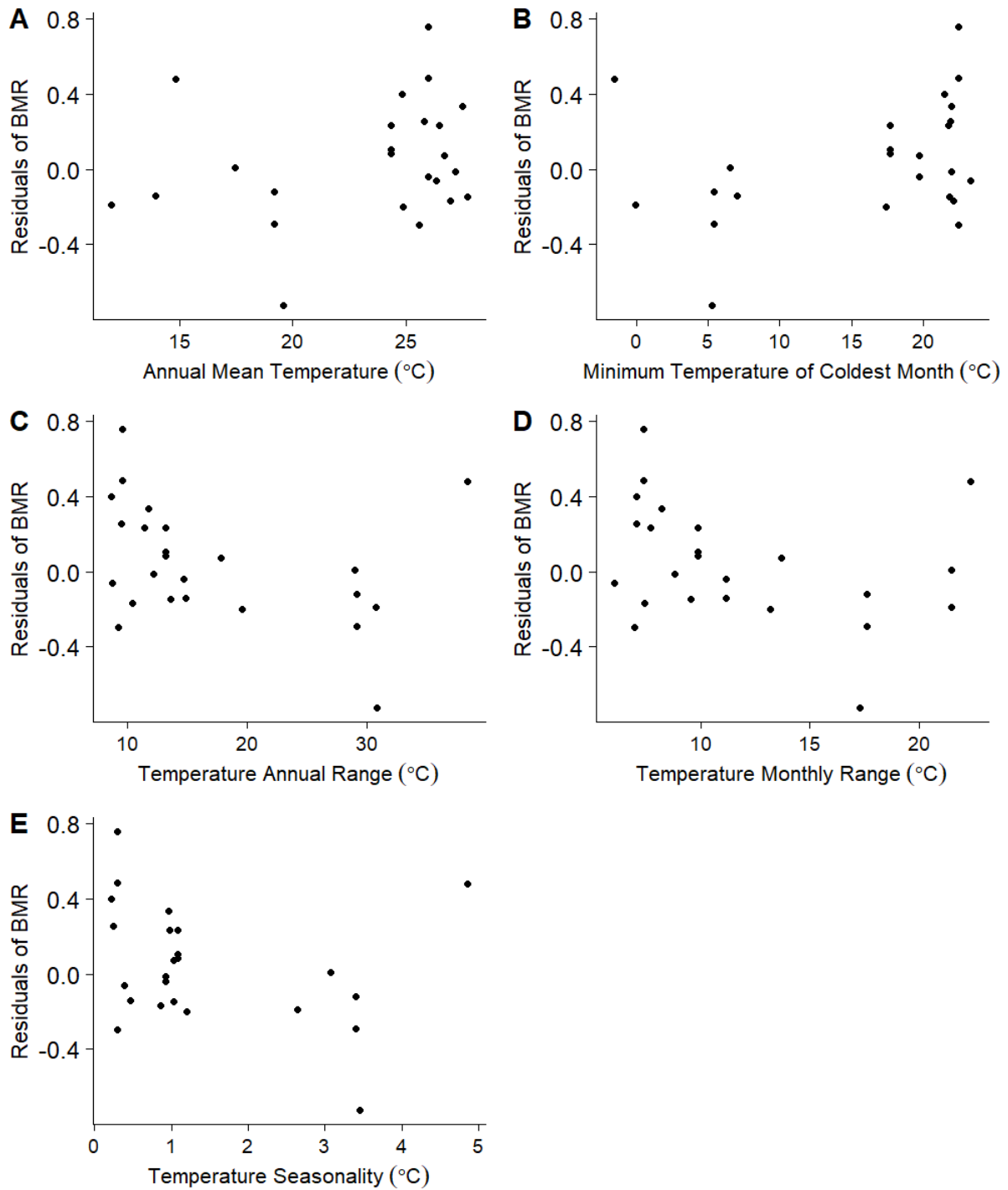
Figure 4.6: The minimum temperature of the coldest month of the year (A), the temperature annual range (B), the temperature monthly range (C), and the temperature seasonality (the standard deviation of the average monthly temperatures; D) have no effect on the lower limit of neutrality (T_{lc}, °C) for 24 Chiropteran species.

more complex thermoregulatory relationships species have with detailed climate variables (Welman, 2018). In particular, species' thermoregulatory scope, (average T_b - minimum T_b), as well as heterothermy index (the magnitude of a heterothermic response compared to an optimal T_b) have both been shown to increase with latitude (Boyles, Smit and McKechnie, 2011; Boyles et al., 2013), as higher latitudes tend to have more predictable temperature and precipitation regimes, lower average annual temperatures, and greater annual extremes (Scholander et al., 1950; Lovegrove, 2003). During heterothermy, a species' T_b cannot be below T_a, so higher latitudes have a greater range in which those species can thermoregulate, resulting in a greater thermoregulatory scope for those species (Boyles et al., 2013). During the summer, species closer to the equator have a greater variation in T_b, which may be due to fluctuations in resource availability, and/or the
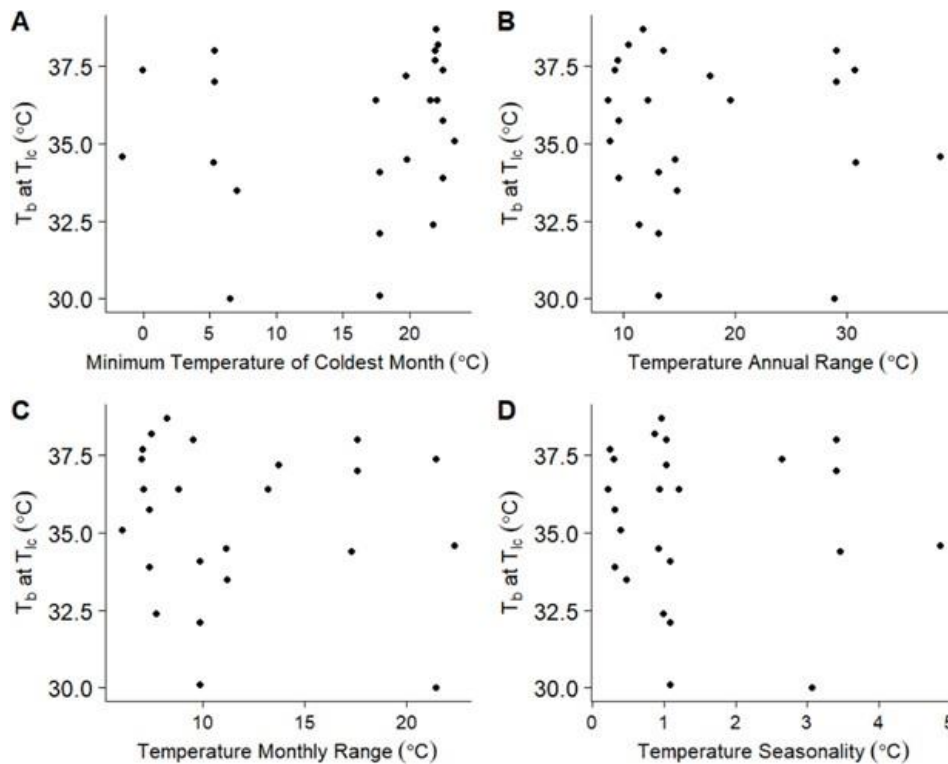
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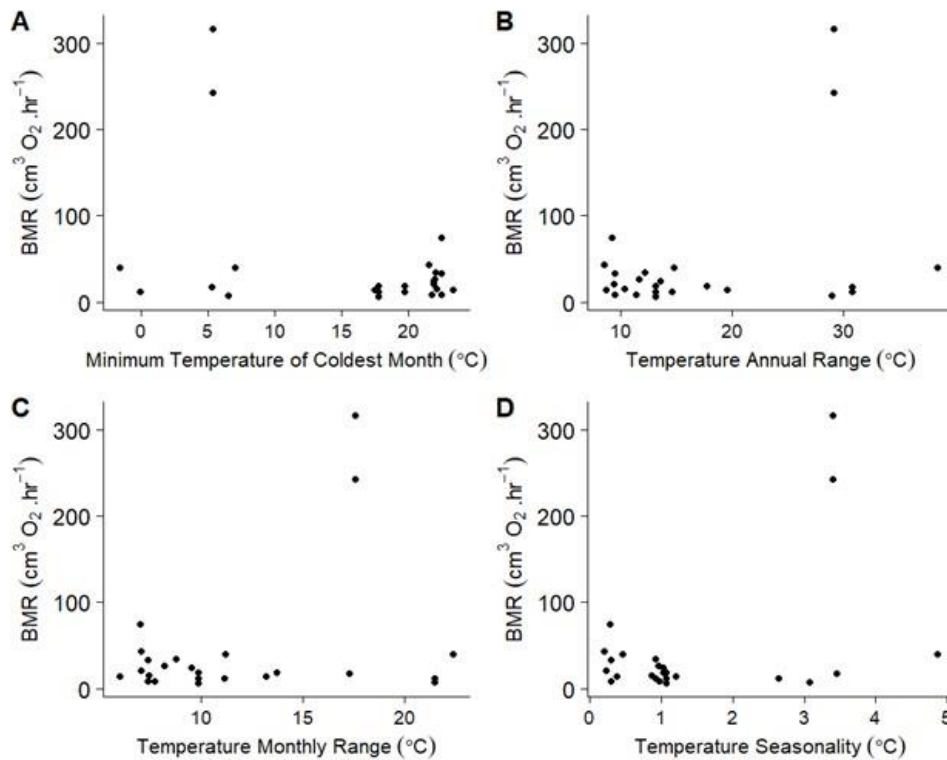
Figure 4.7: The minimum temperature of the coldest month of the year (A), the temperature annual range (B), the temperature monthly range (C), and the temperature seasonality (the standard deviation of the average monthly temperatures; D) have no effect on BMR ($cm^3 O_2 \cdot h^{-1}$) for 24 Chiropteran species.

necessity to maintain less variable T_b in species in higher latitudes due to their short reproductive window during the summer months (Boyles et al., 2013).

A body mass of around 100g in endotherms has been hypothesized to be a "transition zone" below which the effects of ambient temperature have an increasing effect on residual BMR (Lovegrove, 2003; Naya, Naya and White, 2018). As ambient temperature decreases, species can decrease their surface area to volume ratios (become larger), take measures to avoid the decreasing ambient temperature (behavioral: burrowing, migrating, changing activity pattern; physiological: using torpor or hibernation), decrease their heat loss to the environment (changing body coloration, increasing insulation through subcutaneous fat or fur thickness), or increase their metabolic heat production (via shivering or changes in the amount of metabolically active organs) (Naya, Naya and Lessa, 2016; Naya, Naya and
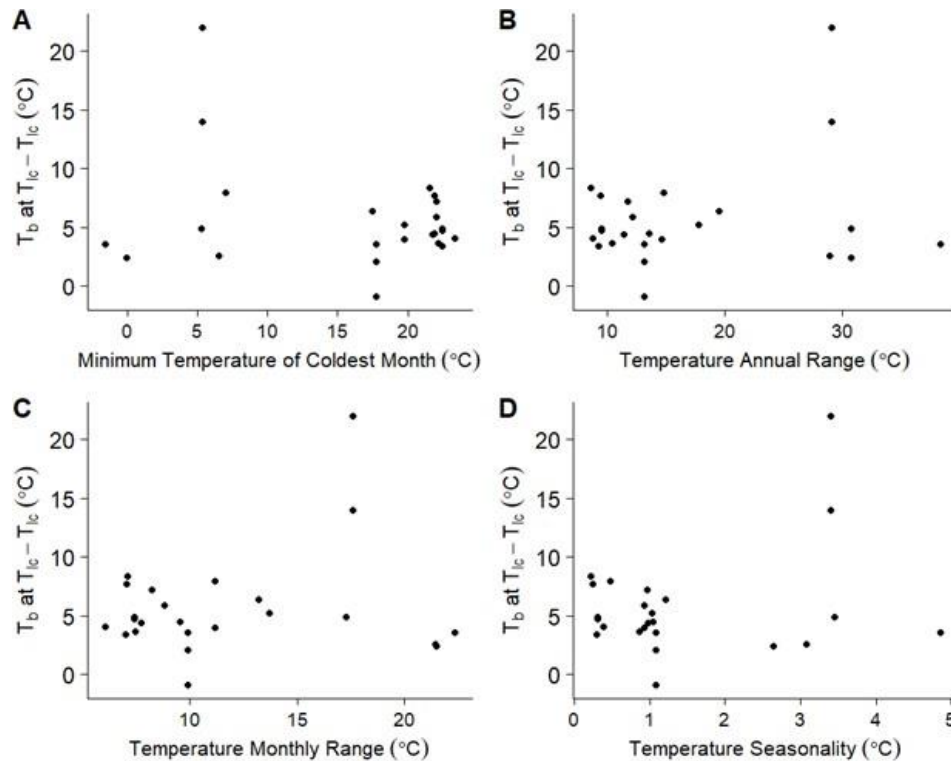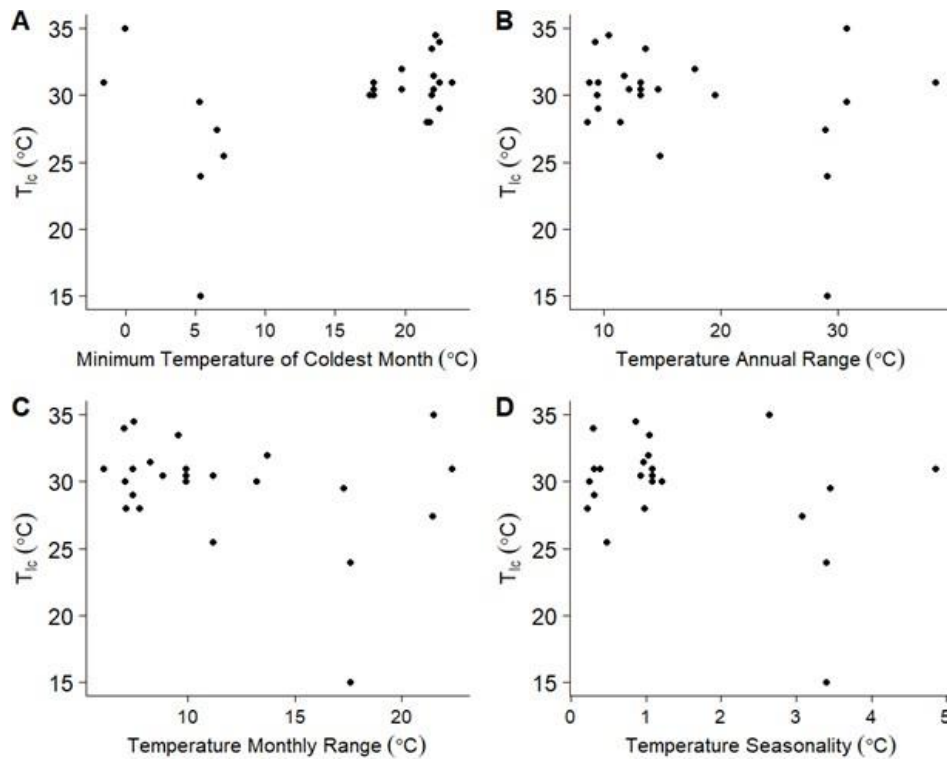
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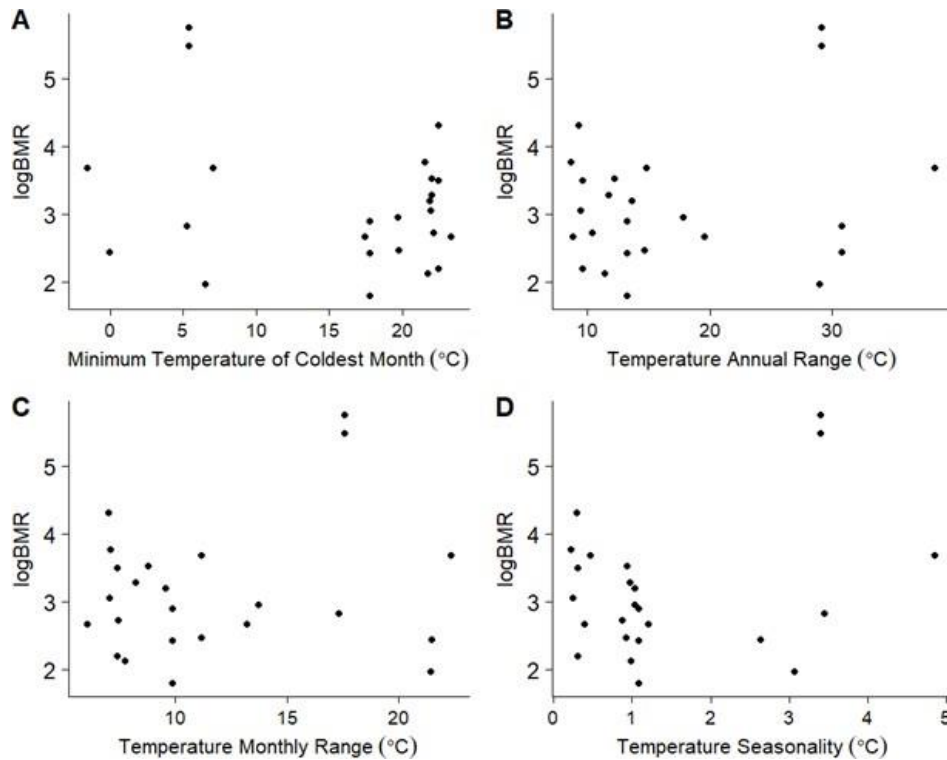
Figure 4.8: The minimum temperature of the coldest month of the year (A), the temperature annual range (B), the temperature monthly range (C), and the temperature seasonality (the standard deviation of the average monthly temperatures; D) have no effect on log-transformed BMR for 24 Chiropteran species.

White, 2018). Small mammals, however, are constrained in their ability to make these

behavioral and physiological adaptations to cooler temperatures. For example, as body size

decreases, the surface area to volume ratio increases, and with it so does the energetic cost

of maintaining T_b(Schmidt-Nielsen, 1984). Bats especially must contend with surface area

to volume ratio constraints at both thermal extremes; to avoid losing significant heat to the

environment via convective cooling from their highly vascularized wing membranes,

Brazilian free-tailed bats (*Tadaridabrasiliensis*) lower their body temperatures during

flight to reduce the temperature gradient between the bat and the environment, thus

reducing the amount of heat lost to the environment when ambient temperatures are cooler

than body temperatures (Reichard et al., 2010). Additionally, it has been hypothesized

that bats are constrained in dissipating sufficient heat during high daytime temperatures

because of exogenous heat from solar radiation, which may explain partially why bats remain nocturnal (Voigt and Lewanzik, 2011). Therefore, these restrictions on heat balance may mean that metabolic heat production may be one of the most important mechanisms to cope with ambient temperatures for smaller mammals, whereas other factors have a greater impact on larger animals (Naya, Naya and White, 2018). Consequently, we found a strong correlation between residual BMR and climate variables.

Our study further highlights a gap in available physiology literature. Although equatorial regions are the most biologically diverse places on earth (Burgin et al., 2018), disproportionately few physiological studies have been conducted in those regions (Lovegrove, 2000). Studies of thermoregulation across latitudes must contend with the literature bias against tropical species, as very few publications on thermoregulatory phenotypes exist for species outside the temperate zone. This imbalance may skew some meta-analyses that could be sensitive to the reduced sampling effort (Lovegrove, 2003; Boyles et al., 2013). Our study contained relatively few species, as a majority of publications we studied did not meet the requirements necessary to ensure uniformity of the variables. For instance, BMR should be collected from adult, non-reproductive, post-absorptive individuals at rest in their thermoneutral zone. Meeting these criteria in the field may be complicated, along with instrument limitations and low sample sizes. T_b measurements can also range in quality. Surgically implanted data loggers ensure accurate core T_b data, yet performing data logger surgeries are extremely invasive and not practical on large scales. Many of the studies investigated in this study had poor T_b data which had to be omitted, for example having small sample sizes or measurements outside of the thermoneutral zone. Finally, bats themselves are extremely heterothermic (Boyles et al., 2013), and do not always defend a body temperature during measurements, making it

extremely difficult to determine a normothermic T_b.

Chiroptera have a very broad range of diverse thermoregulatory phenotypes (from obligate hibernators to species that rarely, if ever, enter torpor) and as a clade have some of the most variable T_bs (Boyles et al., 2013). Their diverse range of thermoregulatory phenotypes is as diverse as the clade's diets, climates, and habitats. We aimed to test which of these variables, or interactions between the variables, predicted the thermoregulatory phenotypes such as upper or lower critical temperatures, the size of the range between those two temperatures, and the resting body temperature of the species. Additionally, as the only mammals capable of powered flight, bats do not face the same constraints as terrestrial locomotion and the relative immobility that accompanies small size. As the clade with the smallest migratory mammals, bats are thus able to avoid extreme seasonality other mammals of similar size must contend with. This anomaly separates them from other clades, where the assumption is that those smaller mammalian species must cope with climatic variables and therefore have behavioral and physiological adaptations to survive extreme conditions, whereas larger species may migrate (Lovegrove, 2000). For this reason, bats were often omitted from metabolic and thermoregulatory studies (Lovegrove, 2003). Future analyses should also investigate what strategies Chiropteran species use to cope with winters, as migration and hibernation both entail extreme physiological limits that may obscure any broad thermoregulatory patterns when not taken into account (i.e. long-distance flight and fat storage capacity).

4.6 Acknowledgments

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4.7 Supplementary Information

Supplementary table for bat chapter: Thermoregulatory variables and climate data for 24 Chiropteran species.

CHAPTER 5

THERMAL FLEXIBILITY VARIES ACROSS LATITUDES IN RODENTIA

5.1 Chapter Abstract

All mammals are endothermic and capable of maintaining stable body temperatures. However, because of the high energetic cost of defending high body temperatures, many mammals vary their body temperature in response to changes in ambient temperature to save energy, either via torpor and reducing their metabolic rate or by alternating their range of defended body temperatures. We hypothesize that tropical and subtropical species can effectively expand their thermoneutral zone by increasing their flexibility in normothermic body temperature regulation, resulting in greater thermolability and thus a greater range of inhabitable temperatures. To test this and other hypotheses on the evolution of mammalian thermoregulation, we assembled a high-quality database containing species' body temperature in relation to the upper and lower limits of the thermoneutral zone and metabolic rates at those temperatures. We found both higher body temperatures at the upper limits of the thermoneutral zone and the degree to which those body temperatures were defended above the upper thermal limits as the minimum and maximum latitudinal ranges of species increased. Additionally, the thermoneutral zone was wider for species with ranges at latitudes closer to the equator than to the poles.

5.2 Introduction

The climatic variability hypothesis (CVH) predicts that because species at higher latitudes or altitudes experience more fluctuation in climatic variables than lower-latitude species, they must have more flexible thermoregulatory phenotypes that allow them to tolerate such shifts in biotic factors (Janzen, 1967; Stevens, 1989; Ghalambor, 2006). Several studies have focused on the variability of metabolic rate across global distributions and climatic variables because of the importance of metabolism in all life processes (Brown

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et al., 2004; Williams et al., 2010; Chung et al., 2018). Metabolic rates set the pace of life; its importance transcends across the ecosystem, species, population, individual, organ-system, and cellular levels. Emerging patterns show that basal metabolic rate increases with increasing latitude; as seasonal rainfall and temperatures become less variable and more predictable, species tend to have higher metabolic rates (Lovegrove, 2003; Rezende, Bozinovic and Garland, 2004). Basal metabolic rate is also negatively correlated with environmental temperature, as annual average temperature decreases poleward (Lovegrove, 2003), but is significantly more variable in sub-tropical versus temperate species (McKechnie, Noakes and Smit, 2015). Additionally, other thermoregulatory variables are correlated with latitude: thermoregulatory scope (the difference between a species average body temperature (T_b) during activity and lowest T_b recorded) in heterotherms increases with latitude (Boyles et al., 2013), and high latitude species have greater range between maximum and basal metabolic rate than sub-tropical species (Naya et al., 2012).

Although metabolic rate is extremely important ecologically (i.e. Metabolic Theory of Ecology; Brown et al. 2004), thermoregulation and the rigidity with which it is maintained is also crucial in the life histories of endotherms (Grigg, Beard and Augee, 2004; Lovegrove, 2012). Interspecific comparisons of endothermic thermoregulation in relation to ambient temperature often use thermal profiles, also known as a Scholander-Irving / SI curve (Scholander et al., 1950). The thermal profile of a species is obtained by measuring metabolic rate across a range of ambient temperatures (Scholander et al., 1950). The thermoneutral zone (TNZ) is the range of ambient temperatures above the point where metabolic heat production increases, the lower critical temperature (T_{lc}) and below the point where EWL increases, the upper critical temperature (T_{uc}). Within the TNZ, minimal metabolism and water use are required to maintain homeothermic T_b (IUPS Thermal Commission, 2003; Withers et al., 2016; Mitchell et al., 2018). Basal metabolic rate is the metabolic rate within the TNZ, and should remain constant if measured in

non-reproductive, post-absorptive adults during their rest-phase (Porter and Kearney,

2009; Rezende and Bacigalupe, 2015). More recently, studies have added concurrent

measurements of Tb and evaporative water loss to more effectively delineate the change in

physiological state from the thermoneutrality to a state of thermoregulating, either by

dumping, producing, or storing heat. By measuring rates of water loss, researchers are able to

see increases in heat dissipation in the form of sweating, panting, and grooming (IUPS

Thermal Commission, 2003; Withers et al., 2016). In animals maintaining homeothermy, or

constant T_b, the T_b will fluctuate minimally with changing ambient temperatures. However

many species have daily cycles of T_b and display regional heterothermy, with different organs

and body parts being different temperatures (i.e. brown adipose tissue is often significantly

warmer than skin and body core (Lovegrove, Heldmaier and Knight, 1991). Homeothermy

requires an increase in metabolic heat production at cooler ambient temperatures, and heat

loss increases at higher ambient temperatures (Withers, 1992; Withers et al., 2016). To keep

the T_b lower than ambient at high temperatures, animals must increase their evaporative

water loss (EWL) through panting and sweating, and increase their thermal conductance (or

their inverse of insulation) to dissipate heat (IUPS Thermal Commission, 2003; Withers et al.,

2016; Mitchell et al., 2018). The classical SI models, as well as general calculations based on

the model, assume that species must be maintaining a constant T_b during the measurement

(see (Boyles et al., 2019) for a review). However, not all endotherms are homeotherms: some

instead fluctuate their T_b in response to changing ambient temperatures, adverse weather

patterns, or periods of low food availability, a strategy known as heterothermy (Audet and

Fenton, 1986; Stawski and Geiser, 2010; Ayala-Berdon et al., 2017; Nowack, Stawski and

Geiser, 2017; Reher and Dausmann, 2021).

In mammals, heterothermy is now generally believed to be the ancestral condition, from

which the more derived homeothermy evolved (Grigg, 2004; Grigg, Beard and Augee, 2004;

Lovegrove, 2012). Heterothermy has its evolutionary roots in the transition from ectothermy

to endothermy (Crompton, Taylor and Jagger, 1978; Grigg, 2004; Grigg, Beard and Augee,

2004; Lovegrove, 2012). Some species periodically lower their internal T_b to near-ambient temperatures and employ torpor, a controlled state of reduced T_b and metabolic rate (Bartholomew and Cade, 1957; Heldmaier and Ruf, 1992; Ortmann and Heldmaier, 1997; Ruf and Geiser, 2015). Torpor is the most efficient energy-saving mechanism for birds and mammals, especially when used during low food or water availability or inclement weather (Warnecke, Turner and Geiser, 2008; Warnecke and Geiser, 2009; Körtner and Geiser, 2011). In addition to torpor, some endotherms use less extreme forms of heterothermy, varying their T_b outside of torpor (thermolability). Thermally-labile species allow their T_b to track ambient temperature while still maintaining normal activity levels, reducing the need for EWL and metabolic heat production and saving energy for other uses like reproduction (Withers, Cooper and Larcombe, 2006; Angilletta et al., 2010; Canale and Henry, 2010; Levesque, Nowack and Stawski, 2016). Determining the thermoneutral zone of a heterothermic endotherm not maintaining constant T_b, either during torpor or when the animal is being thermally-labile, can be very difficult, as the T_b will track ambient temperature and show no clear inflection points in metabolic rate at either the lower or upper bound of the thermoneutral zone (Stephenson and Racey, 1994; Levesque and Lovegrove, 2014).

Contrary to the general belief that endotherms spend most of their lives below their thermoneutral zone (Humphries and Careau, 2011), tropical species and subtropical must contend with environmental temperatures either within or above their TNZ, and have thus adapted various strategies to cope with high temperatures (Levesque, Lobban and Lovegrove, 2014; Welman, Tuen and Lovegrove, 2017; Levesque, Tuen and Lovegrove, 2018; Reher and Dausmann, 2021). EWL via sweating or panting allows species to dump heat to the environment using the energy loss during evaporation to cool body surfaces (Withers, 1992; Tattersall et al., 2012). Some species modify their rate of heat exchange between themselves and their environment seasonally by varying their thermal conductance, or their amount of insulation as fur or a subcutaneous fat layer (Withers, 1992; Tattersall et al.,

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2012; Naya et al., 2013). Finally, some lower latitude species rely on thermolability and use facultative hyperthermia, allowing their T_b to increase beyond normothermic levels, tracking ambient temperatures (Hetem et al., 2010; Lovegrove et al., 2014; Gerson et al., 2019; Reher and Dausmann, 2021). By tolerating increases in T_b in such a way that the thermal differential between ambient and T_b is maintained at a minimum, metabolic heat can be passively dissipated to the environment. This reduction in the use of heat dissipation methods saves vast amounts of water, which is especially important for species in water constrained environments (Irene Tieleman and Williams, 1999; Gerson et al., 2019; McKechnie and Wolf, 2019).

In warm climates, especially where food or water may be limited, we would expect thermolability to be a common phenotype. In this study we sought to identify global patterns in the distribution of thermolability. We chose the Order Rodentia as the focus of our study because of their wide range of size, thermal phenotypes, cosmopolitan distribution, and the fact that they are the most specious order of Mammalia (Burgin et al., 2018; Read et al., 2018). We hypothesize that low- to mid-latitude species are more thermally-labile, fluctuating their resting, normothermic T_b to a wider degree, than temperate species. Additionally, we hypothesize that they should have wider limits of their thermoneutral zone than species in higher latitudes or in non-tropical regions. To test these hypotheses we assembled a comprehensive database containing all available data on rodent thermoregulatory physiology. We conducted a literature review and identified the upper and lower thermal critical limits of the TNZ, as well as T_b at those points. We quality-controlled data with appropriate assumptions of the biophysics of thermoregulatory physiology into our database, so that it can serve as a foundation for more accurate mechanistic models.

5.3 Methods

We assembled a comprehensive database containing all thermal profile studies on Rodentia that have been published to date. We started with the rodents from a published dataset of mammalian body temperatures (Clarke and O'Connor, 2014), and combined with databases of quality controlled basal metabolic rates (Genoud, Isler and Martin, 2017) and limits of thermoneutrality and evaporative water loss (Wolf et al., 2017; Thonis et al., 2020). For each study, we noted whether or not upper and lower limits of the thermoneutral zone had been measured accurately (see below), and compiled those values as well as the T_bs, metabolic rates, and rates of evaporative water loss (Supplementary table 1). To quality-control the data taken from these studies, we noted the sample sizes of each study and when studies were conducted on free-ranging versus captive studies. Additionally, for metabolic rate to be considered basal, the individuals must be non-reproductive, fasted adults during the rest period of their day within their thermoneutral zone (McNab, 1997). We also noted what time of the year studies were conducted and, when possible, prioritized those taken during summer months to standardize interspecies measurements. There are several contending methods of determining a species' upper limit of thermoneutrality: an increase in metabolic rate sensu (Wolf et al., 2017), or an increase in evaporative water loss (IUPS Thermal Commission, 2003). We noted which method was used, and which statistical test the authors of each study used to quantify their result. Range data consisting of each species' maximum and minimum latitude was taken from the IUCN Red List database (IUCN, 2022).

All statistical tests were performed using R studio (Version: 2022.02.3+492 "Prairie Trillium" Release), and Phylogenetic Generalized Least Squares tests (PGLS) were performed using the "pgls" function in the R package *caper* with maximum likelihood to estimate the value of phylogenetic signal (Orme et al., 2013). We tested the effects of species' latitudinal range and thermoregulatory variables using PGLS with the most up-to-date mammalian phylogeny from treeoflife.org trimmed to rodent species (Upham,

Esselstyn and Jetz, 2019). Specifically, we investigated metabolic rates and T_b at both upper and lower thermal limits of thermoneutrality as well as the limits themselves as functions of the absolute latitude of the midpoint of each species' range, the differential between the maximum and minimum latitudinal points, and the overall extent of the species' range.

5.4   Results
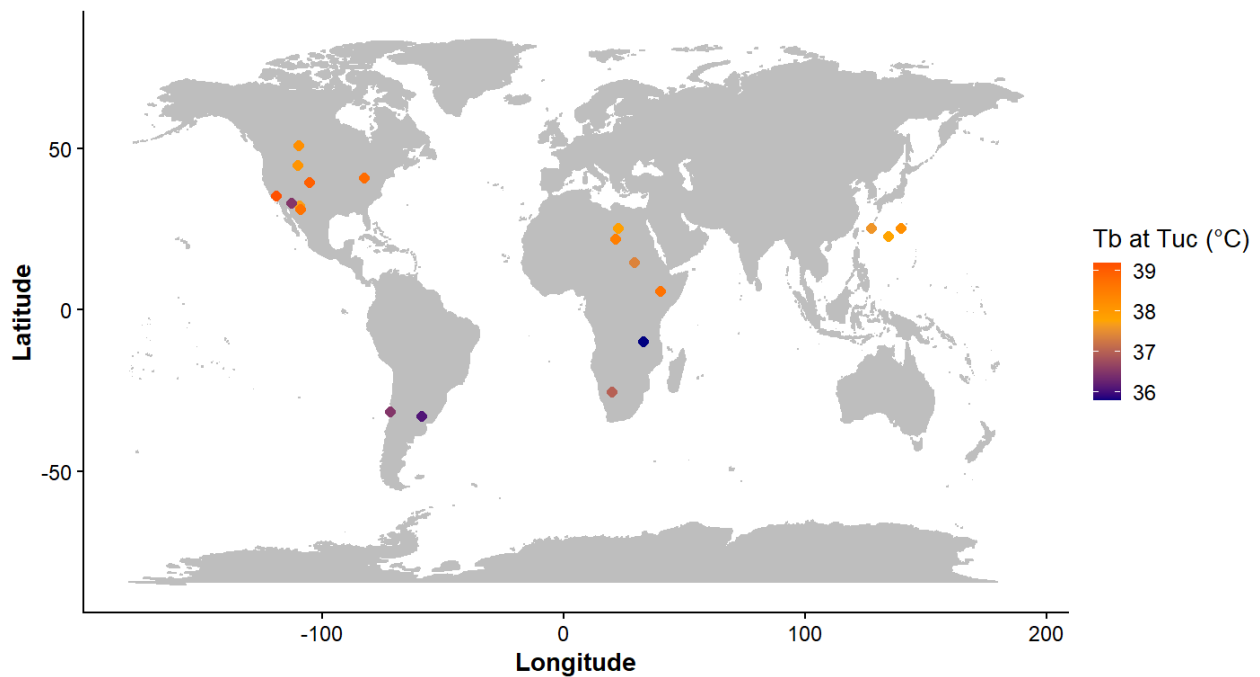
Figure 5.1: Midpoints of the ranges of 19 species of rodent, with colors depicting T_b at T_{uc}.

The combined datasets of (Clarke and O'Connor, 2014), (Genoud, Isler and Martin, 2017), and (Thonis et al., 2020) together contained 310 entries of T_b and/or metabolic rate for 216 rodent species. We investigated each of the studies and found 100 identified upper limits of thermoneutrality. Of those 100 upper thermal limits, 32 passed our quality control checks of having an adequate sample size and having tested at high enough ambient temperatures following (Wolf et al., 2017) and were included in further analyses. Only 23 of the 32 studies also had body temperatures at the upper limit of thermoneutrality, and 19 at

the lower limit, with 19 having T_b at both limits of the TNZ. The mass of rodent species in our final dataset ranged from the 12.6 g least gerbil (*Gerbillus pusillus*) to the
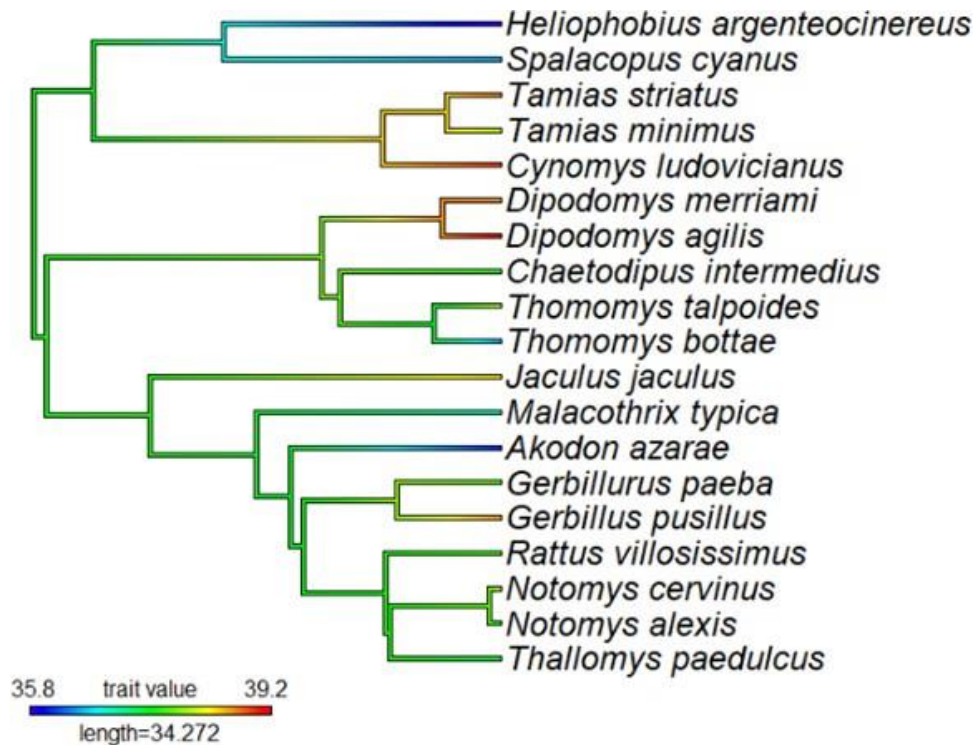
Figure 5.2: A continuous trait map showing the relationship between 19 rodent species with body temperature (T_b) at the lower limit of thermoneutrality (T_{uc}) in °C.

1112.3 g black-tailed prairie dog (*Cynomys ludovicianus*), with an average mass of 129.8 g. Of the 19 species in our final dataset, two were species endemic to the tropics (with ranges below the tropic of cancer and above the tropic of Capricorn), seven species were endemic to temperate zones, and the remaining 10 species had ranges crossing over into each zone (Figure 5.1). Specifically, six species were endemic to the Holarctic, one with its entire range below the tropic of Capricorn, and only three with entire ranges in the southern hemisphere. None of the final species had ranges over the equator, the species with the northernmost range was the least chipmunk (*Tamias minimus*; 66.55°), and the species with the southernmost range was the Coruro (*Spalacopus cyanus*). T_b at T_{uc} ranged from at T_b of 35.8°C at T_{uc} of 33.0°C in the silvery mole-rat (*Heliophobius argenteocinereus*) to a T_b of 39.2°C at a T_{uc} of 35.0°C in the agile kangaroo rat

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(*Dipodomys agilis*, 5.2). T_b at T_{uc} increased with increasing minimum and maximum range latitudes (p = 0.002, R2 = 0.45 and p = 0.0009, R2 = 0.49; Figure 5.3A,B), as did
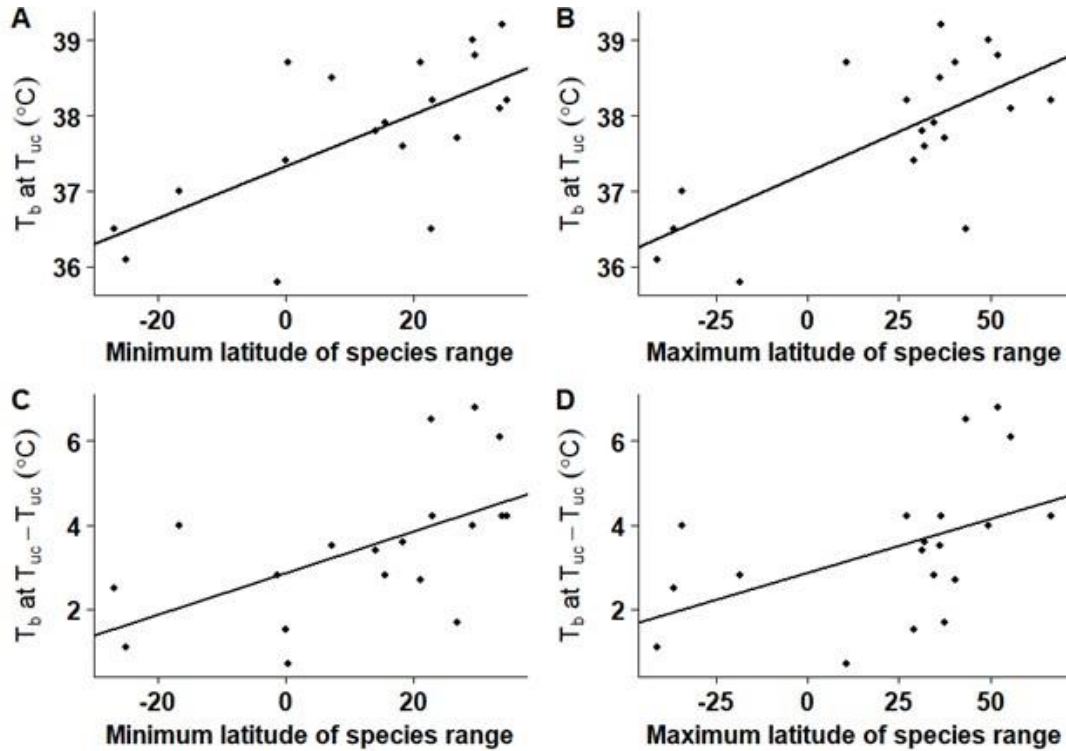
Figure 5.3: Body temperature (T_b) at the upper limit of thermoneutrality (T_{uc}) increases as the minimum (A) and maximum (B) latitude of species' range increase. The differential of T_b at T_{uc} and T_{uc} increases as the minimum (C) and maximum latitude of species' range increase.

the differential between T_b at T_{uc} and T_{uc} (p = 0.01, R2 = 0.40 and p = 0.03, R2 = 0.24; Figure 5.3C,D). Both T_b at T_{uc} and the differential between T_b at T_{uc} and T_{uc} were positively correlated to the midpoint of species' ranges (p = 0.0009, R2 = 0.49 and p = 0.02, R2 = 0.28; Figure 5.4A,B) and negatively correlated to species latitudinal range size (p = 0.03, R2 = 0.24). The distance from the equator to the nearest range limit was negatively correlated to the width of the TNZ (T_{uc} - T_{lc}; p = 0.04, R2 = 0.22; Figure 5.5A) and positively correlated with the differential between T_b at T_{uc} and T_{uc} (p = 0.02, R2 = 0.27. ; Figure 5.5B) and mass-controlled BMR (residuals of log-transformed BMR; p = 0.03, R2 = 0.24; Figure 5.5C). The width of TNZ was not correlated with any other

latitudinal extents of species' ranges (p > 0.05). T_{uc}, T_{lc}, T_b at T_{lc}, the differential
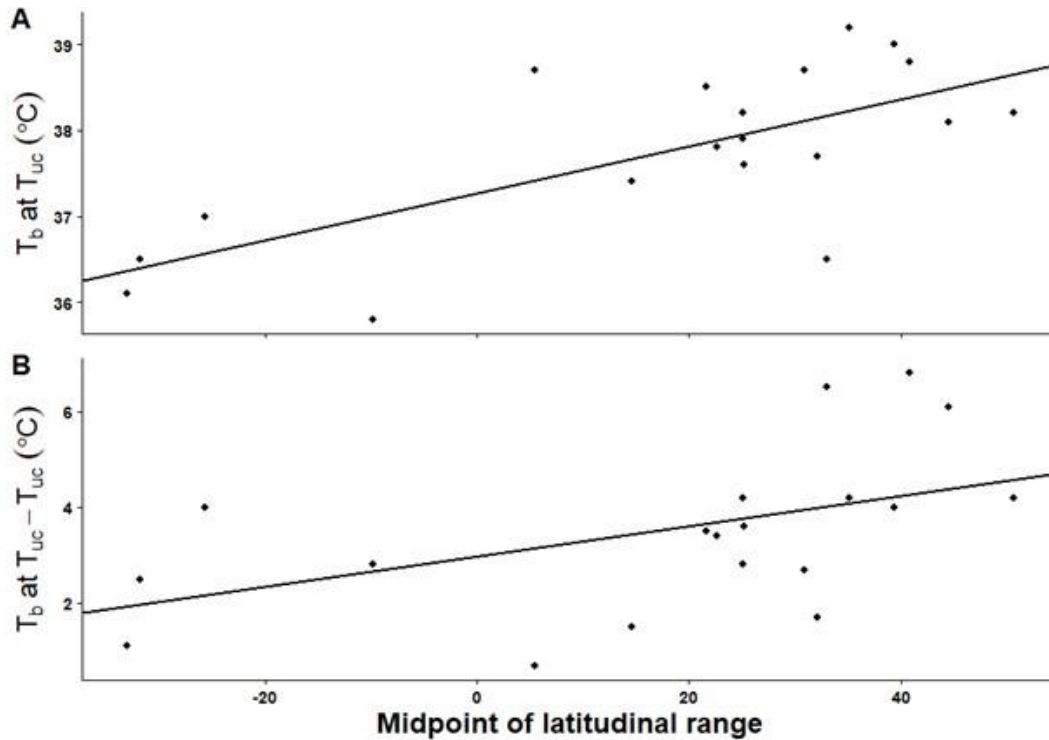
Figure 5.4: As the midpoint of the latitudinal range increases, both (A) body temperature (T_b) at the upper limit of thermoneutrality (T_{uc}), and the differential of T_b at T_{uc} and T_{uc} increase

between T_b at T_{lc} and T_{lc}, and the differential between T_b at T_{lc} and T_b at T_{uc} were not correlated with any latitudinal extents of species' ranges (p > 0.05).

5.5 Discussion

We hypothesized that rodent species in the tropics and subtropical regions should be more thermally labile and fluctuate T_b around the limits of thermoneutrality more than temperate species. We therefore expected species at lower latitudes to have higher upper limits of thermoneutrality than temperate species, but instead, of the 19 species with good quality data available, T_{uc} showed no relationship to either absolute or relative values of latitude. We also expected lower latitude species to have more flexible body temperatures at T_{uc}, but instead found that both the minimum and maximum latitude of species ranges had a significant, positive correlation to T_b at T_{uc} and the differential between T_b at T_{uc}

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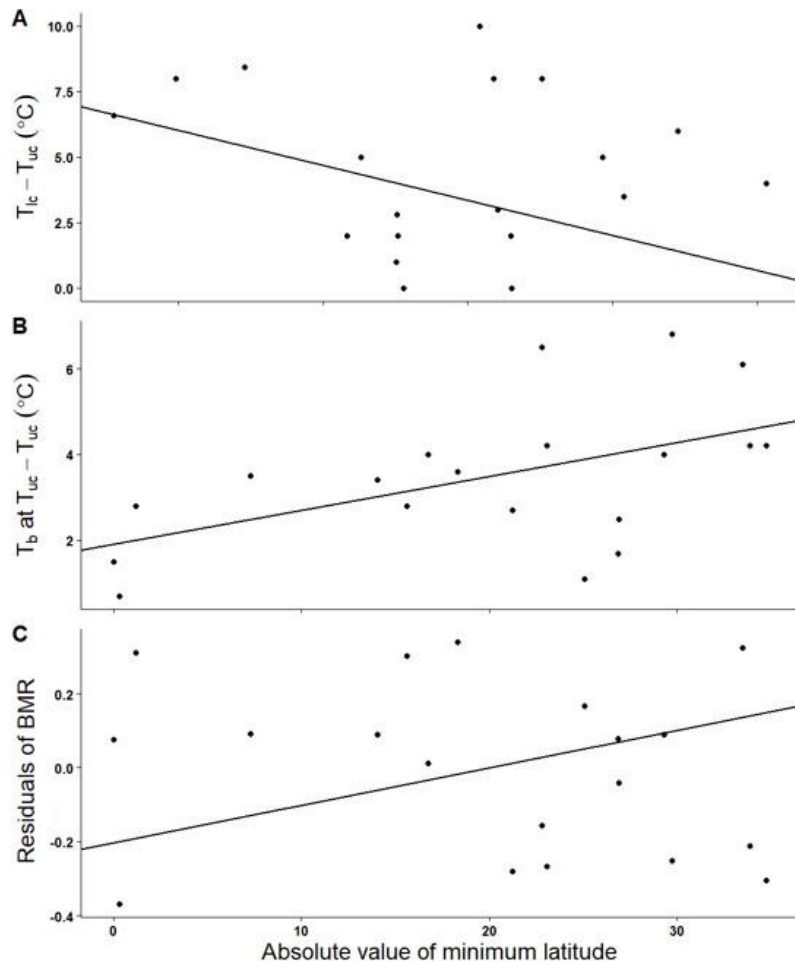
Figure 5.5: As the absolute value of the minimum latitude increases, (A) the width of the thermoneutral zone (TNZ; T_{uc} - T_{lc}) decreases, and (B) the differential between body temperature (T_b) at the upper limit of thermoneutrality (T_{uc}), and T_{uc}, and (C) the residuals of BMR increase.

and T_{uc} (Figure 5.3). All of the T_{uc} differentials were positive, showing that species

were maintaining T_b above T_{uc}, and the differential significantly increased as latitude

increased northward (Figure 5.3 & Figure 5.4). Our data supported the hypothesis that

tropical and subtropical species should have wider ranges of their thermoneutral zone

than species in higher latitudes (Figure 5.5A). As the minimum latitude of species'

ranges moved poleward, the size of the thermoneutral zone decreased. This supported

our hypothesis that tropical and subtropical species should have wider thermoneutral

zones because of their ability to fluctuate body temperature at the upper and lower

limits of thermoneutrality, and therefore push those boundaries to save energy and water. However, we did not see any relationship between the lower thermal limit and body temperature at that limit and latitude.

Higher latitude species maintained a significantly greater differential between T_{uc} and T_b at that limit of thermoneutrality than lower-latitude species (Figure 5.3C,D, Figure 5.4B and Figure 5.5B). This relationship between latitude and the T_{uc} differential can be partly explained by the higher T_b at T_{uc} in the higher-latitude species. T_{uc} also had a weak negative relationship with all metrics of latitudinal ranges (limits or breadths of the ranges, or midpoint values), although none of the relationships were statistically significant. The significantly higher T_{uc} differential at higher latitudes is likely a combination of slightly lower T_{uc}s and higher T_b at those points. Additionally, as the ranges moved further from the equator, both the T_{uc} differential, and the mass-controlled residuals of BMR increased significantly (Figure 5.5B, C). High latitude environments like the Nearctic and Palearctic have predictable seasonal food availability, low average annual temperatures, and low and predictable rainfall patterns, all of which lead to selection for the fast end of the pace-of-life continuum and its associated high BMRs and high body temperatures (Lovegrove, 2003; Rezende, Bozinovic and Garland, 2004; Lobban, Lovegrove and Rakotondravony, 2014). Thus the positive relationship we see in our data between BMR and absolute latitude (Figure 5.5C) supports the slow-fast metabolic continuum, where the lower the average annual temperatures and increased predictability of rainfall, the higher the metabolic rates and body temperatures of species in those environments (Lovegrove, 2003; Rezende, Bozinovic and Garland, 2004).

Several contending definitions for T_{uc} exist, either as the inflection point in metabolic rate, or the inflection point in evaporative water loss (IUPS Thermal Commission, 2003;

Withers et al., 2016; Mitchell et al., 2018), as increases in metabolic rate may be confounded by increased activity in individuals at the uncomfortably high ambient test temperatures. Notably, all of the studies in our database used an inflection point in metabolic rate to define T_{uc} because so few measured inflection points in EWL (Thonis et al., 2020). Quantifying the upper limit of thermoneutrality requires researchers to conduct metabolic measurements at high enough ambient temperatures to elicit a response in the form of an inflection point in either metabolic rate, or evaporative water loss (Wolf et al., 2017). Studies must therefore contain reliable data from at least two individuals, for at least two temperatures above the implied upper thermal limit, to conclusively delineate T_{uc} for a species (Wolf et al., 2017). Several studies from our original compiled database were

therefore removed because of insufficient data, or lack of high enough ambient temperature tests to quantify an upper thermal limit.

The findings from our study were highly variable, compounded by the paucity of data meeting our stringent requirements of an already-difficult-to-quantify thermoregulatory variable, T_{uc}. Future studies of thermoregulation at the thermal limits could be strengthened by the inclusion of other potentially explanatory variables: activity patterns

(nocturnality, diurnality, cathermal and crepuscular), diet (folivores, insectivores, granivorous, etc; Clarke, Rothery and Isaac, 2010), lifestyle (arboreal, fossorial, terrestrial, aquatic; Arends and McNab, 2001), and specific microclimate variability, (mean annual temperature, and seasonality of rainfall; Lovegrove, 2000, 2003). Although we were able to obtain insight on upper limits of thermoneutrality and thermolability at those limits, our study and its interpretations are limited by the low number of studies that passed our quality filters listed above. In particular, the lack of reliable body temperature measurements,

studies containing large enough sample sizes, and tests of high enough ambient

temperatures to elicit changes in physiological state indicative of upper thermal limits is

fundamental to determine thermolability of species and its global distribution. It is also

worth noting that the majority of quality-controlled data in our study were from the

Northern Hemisphere (15 out of 19), due to a biased sampling effort, even though the

species density is much higher in the equatorial regions (Burgin et al., 2018). The collection

of high-quality, thermoregulatory data from non-Holarctic species, especially free-ranging

data from small, tropical endotherms is pertinent as we investigate species resiliency to

changing ambient temperatures and the plasticity with which species are able to

thermoregulate.

CHAPTER 6
GENERAL CONCLUSION

Although endothermy allows species to decouple the body temperature from environmental temperatures, it does not imply that endothermic species maintain constant body temperatures. Instead, heterothermic species can fluctuate their temperatures during difficult periods, entering torpor to save vast amounts of energy (Geiser & Ruf, 1995). By allowing body temperature to track ambient temperature, the required elevated metabolic rate or evaporative water loss levels required to maintain that constant body temperature are no longer needed, saving both energy and water (Reher & Dausmann, 2021). Species can also use non-torpid heterothermy, during which activity levels are maintained, and body temperature is left to fluctuate with ambient temperatures (Levesque, Lobban, & Lovegrove, 2014; Treat et al., 2018). These increases in ambient temperatures above the thermoneutral zone, and reduced body temperatures below the thermoneutral zone essentially allow species to expand their limits of thermoneutrality. By retaining a less rigid body-temperature setpoint, species gain similar benefits as torpor, saving energy and water. For species living within, and even above, their thermoneutral zone, such as those living in the tropics and subtropics, thermolability is likely to be an adaptive strategy (Levesque, Nowack, & Stawski, 2016). Endothermy likely evolved warm, humid conditions, similar to the climate of today's tropics (Crompton, Taylor, & Jagger, 1978; Lovegrove, 2000, 2017). It is thought that a higher body temperature allowed previously diurnal mammals to invade the nocturnal niche, where they were able to avoid predation (Crompton et al., 1978; Kemp, 2006). Endothermy allowed species to decouple their body temperature from ambient temperatures, which allows them to be more active during the colder night hours (Crompton et al., 1978; Kemp, 2006).

I investigated a nocturnal, murid rodent, *Sundamys muelleri*, endemic to the biodiversity hotspot of Southeast Asia. Although this region contains the highest density of mammal species in the world (De Bruyn et al., 2014), disproportionately few thermoregulatory studies have been

conducted on its mammals. Studying a nocturnal rodent such as *S. muelleri* allowed me to both expand our knowledge of thermoregulation in nocturnal tropical mammals and test how they may thermoregulate in the hotter environmental temperatures predicted with climate change. To do so, I quantified the body temperature across a range of ambient temperatures, the lower limit of thermoneutrality and basal metabolic rate of *S. muelleri.* Overall, I found that *S. muelleri* falls closer to the homeotherm end of the heterothermic-homeothermic continuum. Basal metabolic rate of *S. muelleri* was comparable to similarly-sized mammals. I found that *S. muelleri* increased evaporative water loss and subcutaneous body temperature at ambient temperatures of 33°C, and while these inflection points may indicate an upper thermal limit, their metabolic rate showed no increase up to the highest tested ambient temperature (38.2°C). These findings, together with the fact that current average temperatures at the field site are significantly lower (Thonis, Ceballos, Tuen, Lovegrove, & Levesque, 2020), suggests that this species will likely tolerate future climatic changes.

I then chose to study a more heterothermic species, *Tenrec ecaudatus*, also endemic to the subtropics (where thermolability is likely to be advantageous). I sought to identify their limits of thermoneutrality, which I expected to be flexible depending on their range of thermolability. Tenrecs have one of the lowest mammalian body temperatures, and have been shown to defend constant body temperatures exclusively when pregnant or lactating (Levesque & Lovegrove, 2014). I found *Tenrec ecaudatus* to have a lower limit of thermoneutrality at 19.1 °C, indicated by an inflection point in metabolic rate, which occurred due to an increase in variability, rather than a clear increase as found in other species. In this extremely thermally-labile species, the definition of the thermoneutral zone may be better defined as the range of ambient temperatures in which variation in metabolic rate remains low, instead of the inflection points in metabolic rate. Notably, I found tenrecs to display a wide range of intraindividual and interindividual variability in both

metabolic rate and body temperature at ambient test temperatures, both above and below the thermoneutral zone, indicating a range of thermolability which may be adaptive to the hot conditions in which tenrecs live in the wild .

After conducting two species-specific studies on species falling at different points along the heterothermic-homeothermic continuum, I sought to look at how thermolability might be affected by climate. Using available data on Chiropteran thermoregulation, I tested the relationships between microclimate data and thermoregulatory patterns. Chiroptera, the second-most speciose clade of mammals (Burgin, Colella, Kahn, & Upham, 2018), are often omitted from large-scale studies because of its diverse range of unique thermoregulatory phenotypes. I quantified the relationship between environmental temperatures and thermolability variables, yet even after controlling for phylogeny, we found no relationship between thermoregulatory variables of body temperature and lower thermal limits and associated climatic variables. The unique thermoregulatory adaptations in Chiroptera can give us insights into the physiology and evolution of thermolability.

Finally, to see a more clear, global pattern of the distribution of thermolability, I investigated thermolability in Rodentia, the most diverse and specious Mammalian clade (Burgin et al., 2018). I focused on thermolability at the upper and lower limits of thermoneutrality in Rodentia, and found that body temperatures at the upper limits of thermoneutrality and the differential between the upper limit and the body temperature at that limit increase with the latitudinal extents of species' ranges. However, the thermoneutral zone was wider for species with ranges at latitudes closer to the equator than the poles. Lower latitude species have lower temperatures at upper thermal limits and maintain their body temperatures closer to those limits. With my experiments, I sought to expand our knowledge of thermolability using a multiscale approach. I investigated a potentially thermally-flexible, tropical, nocturnal species (*Sundamys*

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muelleri), tested current thermoregulatory definitions at the extremes (*Tenrec ecaudatus*) and tried to understand the relationship between the global distribution of thermal phenotypes and climates and latitude in Chiroptera and Rodentia, respectively. Although my research confirmed some of the current understanding of thermoregulation in endotherms, it brought to light the need for more research on tropical and subtropical species, and the limitation of existing tools at our disposal for a more comprehensive understanding of the physiological advantages of thermolability. Future directions include increasing the number of thermoregulatory studies in understudied, biodiverse regions, a more rigorous, uniform quantification of thermoregulatory physiology in natural settings, and the use of evaporative water loss measurements to more accurately quantify thermoregulation at hotter ambient temperatures. Through these future steps, we will gain even better insight on the path that led to the evolution of endothermy, the adaptive advantages of thermolability and the intricacies of thermoregulation at high environmental temperatures, which in turn will elucidate a still debated part of our evolutionary history with implications for biomedical sciences and conservation.

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APPENDIX

TABLES

Table A.1: Chapter 4 - Thermoregulatory variables and climate data for 24 Chiropteran species.

Species	Tb at Tlc (°C)	Tlc (°C)	Tb at Tlc - Tlc	BMR	Body mass (g)	Tmin of coldest month (°C)	Tmax of warmest month (°C)	Range of annual T (°C)	Range of monthly T(°C)	Temperature Seasonality
Choeroniscus godmani	37.2	32	5.2	19.1	10.1	19.7	37.5	17.8	13.7	1
Dobsonia minor	37.4	34	3.4	74.4	73.7	22.5	31.7	9.2	7	0.3
Erophylla bombifrons	34.1	30.5	3.6	18.1	16.4	17.7	30.9	13.2	9.9	1.1
Eumops perotis	34.6	31	3.6	39.8	56	-1.6	36.8	38.4	22.3	4.9
Glossophaga longirostris	38.7	31.5	7.2	26.5	13.5	22	33.7	11.7	8.2	1
Hipposideros cervinus	35.75	31	4.75	8.96	8.3	22.5	32	9.5	7.4	0.3
Hipposideros diadema	33.9	29	4.9	33	37.2	22.5	32	9.5	7.4	0.3
Hipposideros maggietaylorae	37.7	30	7.7	21.1	18.2	21.9	31.3	9.4	7	0.2
Leptonycteris curasoae	36.4	30.5	5.9	34.1	24	22	34.2	12.2	8.8	0.9
Macroglossus minimus	35.1	31	4.1	14.4	16.4	23.3	32.1	8.7	6	0.4
Melonycteris melanops	36.4	28	8.4	43.2	53.3	21.5	30.1	8.6	7.1	0.2
Monophyllus redmani	32.1	30	2.1	11.3	8.8	17.7	30.9	13.2	9.9	1.1
Mormoops megalophylla	38	33.5	4.5	24.4	16.5	21.9	35.5	13.6	9.5	1
Natalus tumidirostris	32.4	28	4.4	8.32	5.4	21.7	33.2	11.4	7.7	1
Nyctophilus geoffroyi	37.4	35	2.4	11.4	8	-0.1	30.7	30.7	21.5	2.6
Peropteryx macrotis	34.5	30.5	4	11.78	5.1	19.8	34.4	14.6	11.2	0.9
Pteronotus davyi	38.2	34.5	3.7	15.3	9.4	22.1	32.5	10.4	7.4	0.9
Pteronotus quadridens	30.1	31	-0.9	6	4.8	17.7	30.9	13.2	9.9	1.1
Pteropus poliocephalus	37	15	22	316.9	598	5.4	34.5	29.1	17.6	3.4
Pteropus scapulatus	38	24	14	242.5	362	5.4	34.5	29.1	17.6	3.4
Rhinolophus capensis	30	27.4	2.6	7.14	11.9	6.5	35.5	28.9	21.4	3.1
Saccopteryx bilineata	36.4	30	6.4	14.5	7.8	17.4	37	19.5	13.2	1.2
Sturnira erythromos	33.5	25.5	8	39.9	15.9	7	21.8	14.8	11.2	0.5
Syconycteris australis	34.4	29.5	4.9	16.95	16.3	5.3	36	30.8	17.3	3.5

Table A.2: Chapter 5 - Thermoregulatory variables and latitudinal range data for 19 Rodentia species.

Species	Tlc (°C)	Tb at Tlc (°C)	Tb at Tlc - Tlc (°C)	Tuc (°C)	Tb at Tuc (°C)	Tb at Tuc - Tuc (°C)	TNZ breadth (°C)	Tb at Tuc - Tb at Tlc (°C)	BMR	Mass (g)	Midpoint of latitudinal range	Range of latitude
Gerbillus pusillus	31.4	34.4	3	38	38.7	0.7	6.6	35.7	13.48	12.6	5.515	10.41
Chaetodipus intermedius	33	36.3	3.3	36	37.7	1.7	3	34.4	15.6	14.6	32.085	10.51
Cynomys ludovicianus	30	36.5	6.5	35	39	4	5	32.5	426	1112.3	39.33	20.08
Dipodomys agilis	27	37.6	10.6	35	39.2	4.2	8	28.6	63.6	60.6	35.13125	2.505656
Tamias minimus	30	36.9	6.9	34	38.2	4.2	4	31.3	51.3	33.5	50.645	31.81
Tamias striatus	28.5	37.9	9.4	32	38.8	6.8	3.5	29.4	90	87.4	40.8	22.2
Thomomys talpoides	26	36.2	10.2	32	38.1	6.1	6	27.9	102.6	82.6	44.535	22.13
Dipodomys merriami	26	37	11	36	38.7	2.7	10	27.7	41.64	34.7	30.84255	19.242918
Gerbillurus paeba	32.3	37	4.7	35.1	37.9	2.8	2.8	33.2	27.71	31.13	25.1	19
Jaculus jaculus	33	37.6	4.6	35	38.5	3.5	2	33.9	91.6	74.5	21.655	28.75
Notomys alexis	32	37.7	5.7	34	37.6	3.6	2	31.9	40.2	33	25.195	13.73
Notomys cervinus	33	38.2	5.2	34	38.2	4.2	1	33	41.72	34.2	25.085	4.03
Rattus villosissimus	29.4	35.9	6.5	34.4	37.8	3.4	5	31.3	107	184.4	22.635	17.17
Thallomys paedulcus	27.46	36.6	9.14	35.89	37.4	1.51	8.43	28.26	68.5	124.7	14.575	29.15
Thomomys bottae	28	36.1	8.1	30	36.5	6.5	2	28.4	120.1	143	32.985	20.35
Heliophobius argenteocinereus	25	34.7	9.7	33	35.8	2.8	8	26.1	154	223	-9.853436	17.303348
Akodon azarae	35	36.1	1.1	35	36.1	1.1	0	35	38.7	22.5	-33.03967	15.97181
Spalacopus cyanus	26	36.3	10.3	34	36.5	2.5	8	26.2	101.3	136	-31.79763	9.804666
Malacothrix typica	33	37	4	33	37	4	0	33	20.62	21.7	-25.58199	17.665614

BIOGRAPHY OF THE AUTHOR

Ana M. Breit was born and raised in Wisconsin Rapids, Wisconsin and a graduate of Pacelli High School. In 2015 she graduated from the University of Wisconsin Stevens Point with a Bachelor of Science in Biology and Wildlife Ecology Research and Management and a minor in Economics. She pursued her Master's of Science in Bioscience, Technology, and Public Policy from the University of Winnipeg where she studied Pathogen transmission and thermoregulation in the endangered little brown bat (*Myotis lucifugus*) with Dr. Craig K.R. Willis. She is the co-author on five peer-reviewed publications, 10 popular science articles, and one technical report. Ana Breit is the member of the International Hibernation Society (IHS), the Society of Experimental Biology (SEB), the Society of Integrative and Comparative Biology (SICB), and the Genomes to Phenomes to Populations Research Coordination Network (National Science Foundation). She is a candidate for the Doctorate of Philosophy degree in Ecology and Environmental Science from The University of Maine in May 2023.