

Doctoral thesis

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Rune Sørås

# Energy management of heterothermic bats at northern latitudes

Understanding the physiological flexibility of bats and how this enables them to live in the northern edge of their distribution

**NTNU**  
Norwegian University of Science and Technology  
Thesis for the Degree of  
Philosophiae Doctor  
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## Acknowledgments

One is taught many things during the length of an education and PhD, but writing Acknowledgements that appropriately describes the assistance and gratitude one has had seems to fall outside the scope of the curriculum. Hence, I will take full literary freedom in this quest. So please apologize for my (to the ungifted reader) odd sentences, internal humor, and boatload of questionable poetry.

At first, I would like to thank the people whom to date has played important roles in my bat quest so far. Hence, in chronological order: First, I would like to thank Geir A. Sonerud, whom a decade ago gave me the opportunity to fuff around in the forest at night, only trailing the directional beeps provided by some elusive Boreal owls, which went along with their nightly parental duties. Second, I would like to thank Katrine Eldegard for giving me the opportunity to expand on this peculiar owl expertise, and instead trail after even more elusive bats at night. Third, I would like to thank Jeroen van der Kooij for being the local bat expert who welcomed me into his backyard, and shared bat facts and stories dating back to (almost) before I was born. You have been an important inspiration, collaborator, and friend during the 6 years I have worked with bats, and we are not done quite yet (I have 1000+ wingphotos you need to look through!!). This gratitude of course also includes his warm, hospitable and ever-welcoming family, whom has accepted me frequently roaming in the backyard every summer. Fourth, I would like to thank Joakim Siljedal and Kristian Kristiansen for coping with their completely underqualified field coordinator in the first summer we worked together. It was fun roaming the deep forests of Nittedal at night with both of you. Fifth, I would like to thank Clare Stawski for offering me the position I am now about to complete. I really do appreciate the faith you had in me, when you said it would be easier to teach me animal physiology, than it would be to teach an animal physiologist how to catch bats etc. I would also like to thank both Clare and Katrine for giving me the opportunity to venture to Europe in their stead to mingle with a multitude of other collaborative bat nerds. Sixth, I would like to thank Helene M. Hannestad and Håvard A. Hald for assistance in the field and the occasional nerdy bat discussion. Seventh, I would like to thank Claus Bech and Karoline H. Skåra, for assisting me along the way in working with the data, and teaching me some rad stuff in animal physiology. And finally, I would like to thank Mari A. Fjellidal for managing to cope with me for three field seasons in a row, as well as countless hours in a shared office. I was initially frightened like a little boy when I was told I would need to work closely with another PhD student for four years. Luckily, we got along very well, managed to get some intensive field work done, gathered some new fun data on elusive species, learned from each other, and maintained sanity.

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responsibility for that happening). I would also like to thank Cassandra, Anne, Myranda, Laura, Ragnhild, Hamish, Francois and Wouter for frequently spanning their wings.

Imposter syndrome is a well known phenomenon in the life of a PhD candidate. Fortunately, while studying a generally understudied group of animals, I have rarely needed to express the phrase “I don’t know”, as I more often can say; “we don’t know”. And if I at any point felt uncertain, I could think for a few seconds on the general content that is presented by the dimwits in the group-chat of the “Pre-Dr\_Archenie\_Consultation\_Meme\_Group.png”, and reckon: I am not that bad.

And on a random scatter of thanks, I give massive thanks to Jeroen van der Kooij and Keith Redford for letting me use their astounding photos of bats in flight, both in this thesis, and previous presentations and posters. Thanks to landowners in both Nittedal and Trondheim for welcoming us to ramble around in their backyards and putting up nets to catch those odd animals as the sun was setting. Thanks to Joy O’Keefe and Reed Crawford for letting me come visit and work with them in the US, exchanging experiences, attending a big bat conference with a group of people, and seeing some American bats (and birds!).

As the duration of my PhD also contained two years with on-and-off Covid regulations, it is obligatory that I pay tribute to the 3-digit number of small birds that have lived in symbiosis with my my garden birdfeeder and wallet. These were eventually accompanied by the occasional squirrel and hungry badgers. Despite draining me of a 5-digit number of Norwegian kroners in 2020 to 2022, I am grateful for your willingness to pose effortlessly in front of my camera and maintained my sanity when I was told to limit my exposure to humans. Alongside the elusive animals, there is a multitude of friends outside of work I would like to thank, with Viljar, Caroline, Lukas, Eldrun, Øyvind, Lina, Martin, Bård, Thomas, and Thomas playing important roles.

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As obvious as it may seem, I am grateful for the love and support I have received from my family. Although it has been going on for 9 years, I am still not used to the (debatable) superhero-look I get from my nephew and niece. Also, I have no words for the unrestrained support I have received from my parents Guri and Kåre, who has always supported me, whichever odd and uncommon career path I have chosen. Hence, as I put my heel to my toe, and balance of on some undefined career path that appears in my periphery, I know I will have your support, for that I am enormously grateful.

At last, I would like to try, despite its obvious impossibility, to put words to my gratitude to Lisa. It is remarkably difficult to describe the meaning you have had to me, as you gradually developed from being a random colleague, to becoming my unpaid therapist, and eventually partner. The absence of stress leading up to me finishing this painting of a panic attack, can to a phenomenal extent be designated to your presence, alongside your fellow trash panda. I am fine now. Thank you.





## Abstract

Understanding how bats manage their energy budgets under different thermal conditions is an important component in understanding the limits of distribution and their ability to cope with environmental changes, such as ongoing climate change. In particular, as multiple species of bats are predicted to increase their distribution northward, this thesis aimed to estimate energy consumption (i.e., metabolic rate) in bats currently inhabiting high latitudes in the northern hemisphere. Metabolic rate was estimated using indirect calorimetry, while bats were exposed to an increasing temperature profile during daytime. This was supplemented with reviewing previously published papers for comparison.

Interestingly, energy consumption in bats at high northern latitudes is affected by a multitude of factors. For instance, the extent to which torpor is used can be highly dependent on energy availability, as bats with higher body mass delay torpor entry, and perform more costly arousals at lower ambient temperatures to achieve normothermic body temperatures. Despite being exposed to near-freezing temperatures, increased metabolic rate, indicating a propensity to prevent body temperature from passing past a critical level, was only evident within half the bats in brown long-eared bat (*Plecotus auritus*), and none in northern bat (*Eptesicus nilssonii*). Length of time needed to enter torpor, can also differ between species, primarily as a result of differences in whole-animal metabolic rate, and thermal effects on cooling.

To date, basal metabolic rate has only been estimated in 3.4% of all species of the Vespertilionidae family. This limited dataset contains too few data points to perform analysis on how basal metabolic rate differs between sexes, season, and reproductive state at a grander scale. Accordingly, body mass is the main predictor describing variation in basal metabolic rate between species. Similarly, on a global scale, the minimum torpor metabolic rate is fairly universal across different habitats and species. Minimum torpor metabolic rates are first and foremost affected by ambient temperature. However, some species of bats inhabiting more arid climate zones present minimum torpor metabolic rates at higher ambient temperatures, thus indicating a need to defend a higher body temperature.

In its totality, this thesis provides further insight into the physiological flexibility of bats living at high latitudes in the northern hemisphere. With an opportunistic use of torpor to manage their energy budgets, and a particularly wide thermoneutral zone, bats can sustain long periods of limited food supply. On a global scale, energy consumption in bats has been largely overlooked, as work so far has been limited to a few species, often focused around small geographic areas. Thus, to better predict, manage, and conserve species in the face of climate change, more work is needed to understand the physiological and behavioral responses of bats to different environmental conditions in bats.



## Sammendrag

Forståelse av hvordan flaggermus klarer å forvalte eget energi budsjett under forskjellige klimatiske forhold er en svært viktig komponent i vår forståelse av hva som begrenser dere utbredelse og evne til å tåle miljøforandringer. Slik som pågående klimaendringer. Denne avhandlingen har et mål om å estimere energi forbruket til flaggermus som forekommer på høye breddegrader, ettersom flere arter av flaggermus er predikert til å utvide sine utbredelser i en nordlig retning. Metabolisme ble estimert ved å måle oksygenmengde i luften hvor flaggermus ble eksponert for en økende temperaturprofil gjennom dagen. I tillegg ble resultatene sammenlignet med eksisterende vitenskapelig litteratur som omhandler metabolismen til flaggermus.

Energiforbruket til flaggermus blir påvirket av flere faktorer. For eksempel vil flaggermusenes energitilgang i form av kroppsmasse påvirke hvor mye de går i korttidsdvale. Flaggermus med større kroppsmasse utsetter når de går inn i korttidsdvale, samtidig som de bruker mer energi på å forlate korttidsdvale ved en lavere temperatur. Selv om de ble utsatt for temperaturer ned mot frysepunktet, var det kun halvparten av individene av brunlangøre (*Plecotus auritus*) som økte metabolismen for å forsvare kroppstemperaturen fra å synke forbi et kritisk punkt. Samtidig som ingen individer av nordflaggermus (*Eptesicus nilssonii*) gjorde det. Hvor lang tid flaggermusene bruker på å senke metabolismen og gå inn i korttidsdvale kan også være forskjellig mellom arter, som en effekt av individets fulle metabolisme, og temperatur effekter på nedkjøling.

Per dags dato har basalmetabolisme, eller hvilestoffskifte, kun blitt estimert hos 3.4% av alle artene i Vespertilionidae familien. Dette begrensede datasettet består av for få datapunkter til å kunne gjøre analyser på hvordan basalmetabolisme varierer mellom kjønn, sesonger, og reproduksjonsstadiet på en større skala. Kroppsmasse er den beste prediktoren for å beskrive variasjon i basalmetabolisme mellom arter. På samme måte er laveste metabolisme i korttidsdvale svært universell mellom forskjellige habitat og arter. Det er først og fremst påvirket av omgivelsestemperatur. Noen arter som forekommer i tørre klimasoner har derimot lavest metabolisme ved høyere temperaturer. Dette indikerer at de har et behov for å forsvare en høyere kroppstemperatur.

Totalt sett bidrar denne avhandlingen med ytterligere innsikt inn i den fysiologiske fleksibiliteten flaggermus som forekommer ved høye breddegrader innehar. Med en opportunistisk bruk av dvale for å administrere energibruken, og en bred termonøytral sone kan flaggermus lengre perioder på lite eller ingen mat. På en global skala har energiforbruk hos flaggermus blitt i stor grad oversett, ettersom arbeidet har vært begrenset til noen få arter i mindre geografiske områder. Dermed er mer arbeid nødvendig for å bedre forstå hvordan flaggermus responderer, både fysiologisk og atferdsmessig, på miljømessige utfordringer. På den måten kan vi bedre predikere, forvalte, og bevare arter i møte med pågående klimaendringer.



# Table of Contents

<b>Abstract</b> .....	7
<b>Sammendrag</b> .....	9
<b>List of abbreviations</b> .....	13
<b>Introduction</b> .....	15
Plasticity in heterotherms .....	15
Heterothermy as a solution to seasonal problems .....	16
Heterothermy in bats.....	16
Knowledge gaps and the advent of climate change .....	17
Scope of this thesis.....	18
<b>List of papers</b> .....	23
<b>Author contributions</b> .....	23
<b>Aims</b> .....	25
<b>Thesis summary</b> .....	27
<b>Methods summary</b> .....	27
Study area.....	27
Field methods.....	27
Respirometry.....	27
Statistical analysis in brief .....	29
<b>Results and Discussion</b> .....	31
<b>Conclusive remarks and future prospects</b> .....	35
Metabolism at rest – in the context of roost selection.....	35
The paradox of latitude .....	36
The dos and don'ts of torpor use.....	37
Coping with a changing world.....	38
<b>References</b> .....	41
<b>Paper I</b> - State dependence of arousal from torpor in brown long-eared bats ( <i>Plecotus auritus</i> )	
<b>Paper II</b> - High latitude northern bats ( <i>Eptesicus nilssonii</i> ) reveal adaptations to both high and low temperatures	
<b>Paper III</b> - Defining torpor entries of bats based on rate of change in metabolic rate per minute	
<b>Paper IV</b> - Energetics of whiskered bats in comparison to other bats of the family Vespertilionidae	
<b>Paper V</b> – Universality of torpor expression in bats	



## List of abbreviations

$T_a$	Ambient temperature
$T_{crit}$	Critical temperature in torpor
$T_{lc}$	Lower critical temperature
$T_{uc}$	Upper critical temperature
$T_{set}$	Set temperature
TMR	Torpor metabolic rate
RMR	Resting metabolic rate
BMR	Basal metabolic rate
TNZ	Thermoneutral zone
$\dot{V}O_2$	Oxygen consumption
$\dot{V}CO_2$	Carbondioxide production



## Introduction

Understanding how endothermic animals manage their energy budgets at different temperatures is of paramount importance in order to better conserve biodiversity in a changing environment. As global temperatures continue to increase, particularly in the northern hemisphere (Rantanen et al. 2022), how endotherms respond to environmental change; be it extreme heat (McKechnie and Wolf 2019), extreme weather (Nowack et al. 2015), or milder winters (Reusch et al. 2023), is knowledge that is, to a large extent missing. Thus, understanding the phenotypic plasticity of animals when faced with a changing environment, and how animals may cope physiologically and/or behaviorally to alleviate this can aid in our ability to predict, conserve, and manage species at risk of extinction.

One physiological trait which is imperative for the survival of many endotherms is the ability to enter what is known as steady state torpor (see Geiser 2021). By selectively choosing to abandon normothermia and inhibit metabolic rate, heterothermic animals can minimize energy expenditure by decreasing heart rate (Geiser et al. 2014; Currie et al. 2022) and lower body temperature ( $T_b$ ) to be similar or slightly higher than the ambient temperature ( $T_a$ ), and thereby thermoconform (Boyer and Barnes 1999; Geiser 2004). As metabolic rates in normothermic animals (i.e., maintaining a stable  $T_b$ ) scales positively with increasing body mass, larger animals have a larger whole-animal metabolic rate. However, as smaller endotherms have a larger body surface to mass ratio, they need to compensate for a greater heat loss (Hill et al. 2016). Hence, mass-specific metabolic rate increases with decreasing size (Fernández-Verdejo et al. 2019). Accordingly, many small endotherms are heterothermic and selectively enter torpor when access to food is scarce.

### Plasticity in heterotherms

Although an animal in steady state torpor can save vast amounts of energy as opposed to being normothermic (Willis et al. 2005b; Geiser 2021), a single torpor bout is always preceded by a torpor entry, in which metabolism is substantially reduced (Geiser 1988; Bucher and Chappell 1992; Boyer and Barnes 1999; Geiser et al. 2014; Currie et al. 2022; Fjellidal et al. 2023), and followed by a costly arousal, in which metabolism increases abruptly to regain a normothermic  $T_b$  (Humphries et al. 2003; Heldmaier et al. 2004; Pretzlaff et al. 2021). When entering torpor, a heterotherm downregulates metabolism (Geiser 1988), reduces heart rate (Boyer and Barnes 1999; Currie et al. 2014; 2018), ventilation frequency (Bucher and Chappell 1992; Elvert and Heldmaier 2005), and eventually  $T_b$  (Nicol and Andersen 2007; Wolf et al. 2020). While this decrease can be largely explained by temperature in many heterotherms (Geiser 1988), metabolic decreases in torpid animals differs from animals showing a hypothermic response, as the decrease in metabolic rate precedes the decrease in  $T_b$  in animals entering torpor (Geiser et al. 2014). In addition, this relationship is further aggravated between heterotherms able to undergo multiday torpor (i.e., hibernators), as opposed to heterotherms which are distinguished by only shorter bouts of torpor (i.e., daily heterotherms, Currie et al. 2022), as metabolic inhibition in addition to temperature effects play a larger role in hibernators (Geiser 1988). A trait which allows for hibernators to decrease metabolism quicker (Currie et al. 2022), and reach a lower metabolic rate than daily heterotherms (Ruf and Geiser 2015).

As steady state torpor generally is a metabolic strategy heterotherms use when available foraging conditions are too limited to sustain a normothermic  $T_b$ , be it daily (Pretzlaff et al. 2010; Czenze et al. 2017) or seasonally (Karpovich et al. 2009; Ben-Hamo et al. 2013), animals frequently need to arouse from torpor when food is sufficiently available. Arousing from torpor is the most energy demanding phase of a torpor bout (Thomas et al. 1990), and the cost increases with body mass (Geiser 1988), as larger animals would need more time for  $T_b$  to reach normothermia. Thus, hibernation by extensive metabolic reduction in torpor, where  $T_b$  is decreased by more than  $10^{\circ}\text{C}$ , is mainly restricted to small mammals weighing on average less than 100 g (Geiser 2004).

The cost of a single arousal is also highly affected by the difference between the torpid  $T_b$  an animal is arousing from, and the normothermic  $T_b$  at which a full arousal ends (Karpovich et al. 2009; Pretzlaff et al. 2021). Accordingly, heterothermic animals arouse more frequently when exposed to warmer  $T_a$  (Nowack et al. 2019), and passive rewarming is often used to cut the cost of an arousal (Turbill et al. 2008; Currie et al. 2015), especially in daily heterotherms. Unfortunately, as arousal is the most expensive part of torpor bouts (Thomas et al. 1990), the physiology of torpor entries has been largely overlooked in many metabolic rate studies.

### **Heterothermy as a solution to seasonal problems**

The extraordinary ability to downscale metabolism to save energy has allowed heterothermic animals to colonize ecosystems which otherwise would not be suitable, especially at northern latitudes. For instance, animals living at high latitudes in the northern hemisphere need to cope with a seasonal climate, which is characterized by long, dark, and cold winters, in which food availability is limited or absent. As well as a short growing season in which food is potentially abundant, but the environmental cycle on a day to day basis is characterized by very short nights. In order to survive winter at high latitudes, endotherms either migrate to avoid prolonged exposure to low  $T_a$  (Åkesson et al. 2012; Tøttrup et al. 2012), hibernate for prolonged periods (Boyer and Barnes 1999; Geiser 2021; McGuire et al. 2022), resist it by consuming enough food to stay normothermic (Mosbacher et al. 2016; Andreasson et al. 2020), or do a trade-off between different strategies (Lehnert et al. 2018; Auteri 2022). Which strategy works best for an organism varies substantially based on size, mobility, food source, and the seasonal variability of food.

### **Heterothermy in bats**

One group of endotherms, which has to a great extent specialized in utilizing heterothermy to deal with energetic constraints are bats (Chiroptera). As a particularly rich order of mammals, consisting of 1456 species (Simmons and Cirranello 2022), some species have the ability to reduce metabolism by a 1000-fold between normothermic resting metabolic rate (RMR) and minimum torpid metabolic rate (TMR, Willis et al. 2005b), yet also increase metabolic rate extensively during flight (Thomas and Suthers 1972). The ability to enter torpor, as well as their ability to perform powered flight, has equipped bats with two essential traits which has allowed them to colonize every continent apart from Antarctica (Wilson and Mittermeier 2019). In the northern hemisphere in particular, bats face a long cold winter by either hibernating at a local scale (Jonasson and Willis 2012; Rydell et al. 2018; McGuire et al. 2022),

or a combination of migration and torpor at a wider geographic scale (Cryan and Wolf 2003; Voigt et al. 2016; Lehnert et al. 2018).

Similarly, as bats at higher latitudes in the northern hemisphere primarily rely on invertebrate prey (Rydell 1989; Robinson 1990), food availability is very limited when  $T_a$  drops below 10–14°C (Speakman et al. 2000; Mas et al. 2022), and the overall length of the hibernation season can thus extend to more than 9 months (Frafjord 2021; Hranac et al. 2021). Additionally, as bats are strictly nocturnal (Speakman et al. 2000), available foraging time in summer decreases with increasing latitude (Michaelsen et al. 2013; Boyles et al. 2016) as complete darkness is limited or non-existent for parts of the summer. Bats can alleviate the problem of relatively light summer-nights by foraging in habitats which are darker due to topography (Siivonen and Wermundsen 2008; Michaelsen et al. 2011) or cluttered habitat (Michaelsen et al. 2018). However, the limited time available to consume enough energy can still limit survival, and in particular reproductive opportunities for small nocturnal heterotherms. Hence, bats frequently use torpor on a day-to-day basis to moderate energy expenditure during long days in summer (Boyles et al. 2016).

### **Knowledge gaps and the advent of climate change**

Despite being the second most species rich order of mammals, the biology of bats has received relatively little attention. For instance, ecosystem services provided by bats has only recently been empirically tested and quantified (William-Guillén et al. 2008; Boyles et al. 2011; Maas et al. 2013; Puig-Montserrat et al. 2015; Beilke and O’Keefe 2022), and more than half of all bat species have unknown population trends (Frick et al. 2020). Of all bat species, 16% are listed as threatened species. While 18% are classified as data deficient (see Festa et al. 2023). Bat abundance can for example be affected by habitat loss and degradation (Russo and Ancillotto 2015; López-Baucells et al. 2021; Hunnink et al. 2022), wind turbines (Voigt et al. 2016), and transmission of diseases (such as white-nose syndrome, Cheng et al. 2021). However, more recently, global climate change is becoming a more well-known threat to bat populations (Sherwin et al. 2013; Festa et al. 2023), with more frequently occurring mortality events following heatwaves (McKechnie and Wolf 2019; McKechnie et al. 2021).

With the advent of global climate change, understanding phenotypic plasticity in physiological traits can provide a better understanding of how bats can cope with, and adapt to these changes. In order to facilitate heat loss, endothermic animals will increase metabolism when faced with higher  $T_a$  (Scholander et al. 1950; McKechnie and Wolf 2019). At which  $T_a$  this occurs, is defined as the upper critical temperature ( $T_{uc}$ , see Box 1), and has only rarely been quantified in bats (Webb et al. 1992; Cryan and Wolf 2003; Willis et al. 2005a; Genoud and Christie 2011; Czenze et al. 2020; 2022a). With the outstanding flexibility in metabolic rate that bats show, some species also enter torpor at higher  $T_a$  (Reher and Dausmann 2021), presumably to counteract the need to evaporate heat. While others show a linear increase in metabolism above the lower critical temperature ( $T_{lc}$ , Marom et al. 2006). Hence, some species of bats indicate a thermoneutral point (Box 1; see Nichelmann 1983) instead of a thermoneutral zone (TNZ, Scholander et al. 1950), similar to what has been found in some small mammals (Skop et al. 2020) and some species of birds (Nichelmann 1983; Andreasson et al. 2020).

As a predictive response to climate change, and in an attempt to aid in the conservation of bats on larger geographic scales, a multitude of studies have recently tried to predict bat species adaptation to global climate change (Humphries et al. 2003; Rebelo et al. 2010; Khaliq et al. 2014; Buckley et al. 2018; Novella-Fernandez et al. 2021), generally predicting a northward expansion of bats. Although these studies are well-intended, they are largely performed without including details on the physiological traits of bats, or have included an oversimplified interpretation of physiological parameters based on the Scholander-Irving model (see McKechnie et al. 2017; Boyles et al. 2019). Additionally, all studies to date fail to include the restraining effect that lack of darkness can put on nocturnal animals. Hence, for future species distribution models to have a stronger and more accurate predictive effect, a wider understanding of the phenotypic plasticity of bats is necessary, particularly in the northern hemisphere. As the physiological traits of bats has rarely been quantified (Ruf and Geiser 2015; Festa et al. 2023; Froidevaux, Toshkova et al. in review), more research is needed to accommodate our understanding of climate change effects on bats.

### **Scope of this thesis**

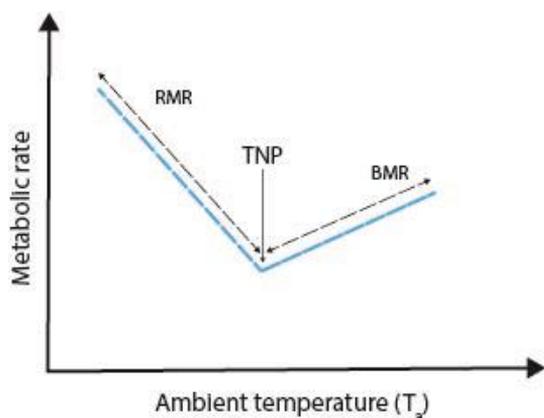
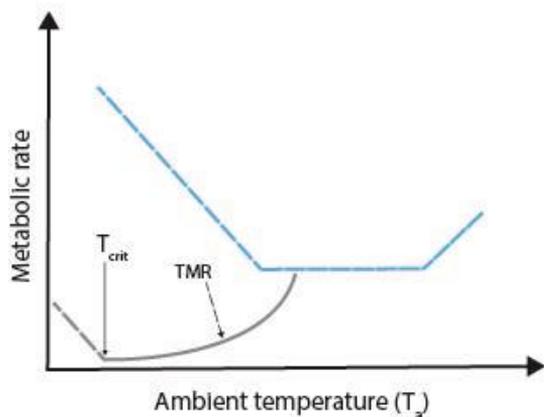
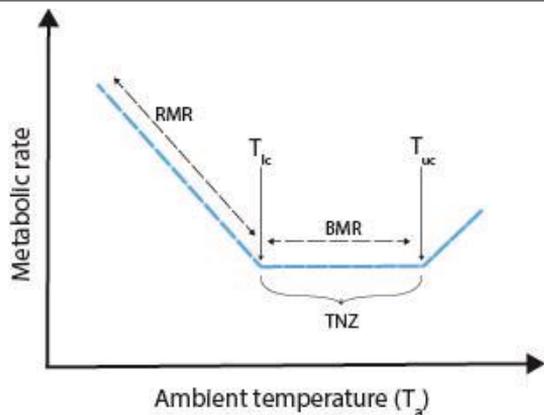
In its totality, this study aims to provide further knowledge about the physiological traits of both previously documented and formerly undocumented species of bats (see Box 2) present in the northern hemisphere. As consumption of energy requires oxygen ( $O_2$ ) and produces carbondioxide ( $CO_2$ ), estimating oxygen consumption ( $\dot{V}O_2$ ) and carbon dioxide production ( $\dot{V}CO_2$ ) through indirect calorimetry (Lighton 2018) is a commonly used proxy for energy consumption. Hence, I measured metabolic rate by exposing individual bats to an increasing  $T_a$  profile from 0 to 40°C. Accordingly, I aim to provide new insight into the metabolic rate of brown long-eared bats (*Plecotus auritus*) at a higher northern latitude than previously measured (Paper I), describe the physiological breadth of torpid and normothermic northern bats (*Eptesicus nilssonii*) at two separate locations (Paper II), utilize a newly developed method for estimating and describing torpor entries from metabolic rate studies (Paper III), compare the normothermic metabolic rate of whiskered bats (*Myotis mystacinus*) to other bats in the Vesperilionidae family (Paper IV), and review the existing literature on minimum torpid metabolic rates in bats (Paper V).

## Box 1: Alternative models of metabolic theory

**The Scholander-Irving model** has been the hallmark of thinking in physiology since it was first published in 1950 (Scholander et al. 1950). It postulates that RMR increases with decreasing  $T_a$  below the  $T_{lc}$ . The  $T_{lc}$  and angle of RMR would be lower in endotherms with a high insulation. At  $T_a$  above  $T_{lc}$ , an animal would not need to increase metabolism to maintain a stable  $T_b$ , which is the minimum energy an animal will need to expend to maintain homeostasis (i.e., BMR). If exposed to  $T_a$  above the  $T_{uc}$ , an animal will need to increase metabolism to dissipate heat.

**The exponential curve of thermoconforming heterotherms** was not described in the Scholander-Irving model, and bats were merely described as poikilotherms (Scholander et al. 1950). Later studies have shown how the metabolic rate in thermoconforming animals increases exponentially with increasing  $T_a$  above a  $T_{crit}$ . Below  $T_{crit}$  thermoregulating TMR increases linearly in parallel with RMR to defend a lowered  $T_b$ , assuming an equal insulation and/or surface area. Interestingly, the ability to maintain torpor below BMR at  $T_a$  above  $T_{lc}$  differs between species.

**The biological optimum temperature (or thermoneutral point)** was first presented by Nichelmann (1983). It postulates that the relationship between metabolic rate and  $T_a$  can be represented by a parabola, where the bottom is characterized as the biological optimum temperature (BOT). Additionally, this BOT is not a fixed value, and is influenced by multiple factors such as circadian rhythm, acclimatization, insulation, and humidity.



## Box 2: Species

The **Brown long-eared bat** (*Plecotus auritus*) is a medium-sized (6-9 g) vespertilionid bat, which exclusively eats invertebrate prey (Rydell 1989; Robinson 1990), and has evolved extreme longevity (Wilkinson and Adams 2019). Habitat selection is primarily confined to cluttered forest habitats (Entwistle et al. 1996; Wermundsen and Siivonen 2008), orchards (Ashrafi et al. 2013), and high forest stands (Ancillotto et al. 2022). Consequently, it is negatively affected by forest fragmentation (Ekman and de Jong 1996). Maternity roosts are primarily situated in larger buildings, such as churches (Ashrafi et al. 2013; Rydell et al. 2017; 2021) and farm buildings (Entwistle et al. 1997). In the current study area, *P. auritus* may also use rock crevices individually (K. Eldegard, unpublished data) and tree cavities individually or in groups (M. Fjellidal, unpublished data). As *P. auritus* is a particularly light-averse species (Rydell 1992), characterized by short broad wings and a relatively slow flight speed (McLean and Speakman 2000), its vulnerability to light pollution has resulted in strong population declines or roost abandonment in Scandinavia (Rydell et al. 2017).

The physiology of *P. auritus* has previously been studied in two separate populations in Scotland (Speakman et al. 1991; Webb et al. 1992; McLean and Speakman 2000) and Germany (Becker et al. 2012; 2013a; Otto et al. 2015). In summary, *P. auritus* are rather unaffected by external stimuli when in torpor, but energy expenditure when in torpor increases with  $T_a$  (Speakman et al. 1991). Additionally, *P. auritus* have a thermoneutral zone between 34.5 and 39°C (Webb et al. 1992), basal metabolic rate does not differ between pregnant and post-lactating females (Becker et al. 2012), and torpor utilization is minimized in late pregnancy (Otto et al. 2015).

The **Northern bat** (*Eptesicus nilssonii*) is a medium-sized (9 - 13 g) vespertilionid bat, which is the northern most occurring bat species globally, with maternity colonies reaching as far north as 69°N (Speakman et al. 2000), as well as a continent-wide distribution (López-Baucells and Burgin 2019). As an aerial-hawking predator (Rydell 1993), *E. nilssonii* is less light averse and frequently forages insects under street lamps (Rydell 1992). Habitat use includes areas related to water and forest edges (Siivonen and Wermundsen 2008), as well as mature forests (Vasko et al. 2020), and the utilization of each can be affected by extended daylight periods (Frafjord 2013a). Populations of *E. nilssonii* in Scandinavia has recently been found to decline both in winter hibernacula (Rydell et al. 2018) and summer (Frafjord 2013b; Rydell et al. 2020).

Unlike *P. auritus*, the physiology of *E. nilssonii* has received fairly little attention. Information on metabolic rate is limited to a single study which measured torpid *E. nilssonii* in Yakutia, Russia (Anufriev and Revin 2006).

The **Whiskered bat** (*Myotis mystacinus*) is a small (4-7 g) vespertilionid bat, which is present throughout most of Europe (Wilson and Mittermeier 2019). *M. mystacinus* frequently forages in forested habitats (Wermundsen and Siivonen 2008), and roost in buildings (Kurek et al. 2020) or trees (Buckley et al. 2013; McKay 2020). As the echolocation calls of *M. mystacinus* are indistinguishable from those of Brandt's bat (*Myotis brandtii*) and other species of the *Myotis* genera, and the anatomy is particularly similar to *M. brandtii* and Alcathe bat (*Myotis alcatheae*, Wilson and Mittermeier 2019), little species specific information is available from population trends based on hibernacula counts and acoustic studies.



Top left: Brown long-eared bat (*Plecotus auritus*).  
Middle left: Whiskered bat (*Myotis mystacinus*).  
Bottom left: *Myotis* spp. in hibernation  
Right: Northern bat (*Eptesicus nilssonii*).





## List of papers

- I) **Sørås R**, Fjellidal MA, Bech C, van der Kooij J, Skåra KH, Eldegard K, Stawski C (2022) State dependence of arousal from torpor in brown long-eared bats (*Plecotus auritus*). *Journal of Comparative Physiology B* 192:815-827. <https://doi.org/10.1007/s00360-022-01451-8>
- II) **Sørås R**, Fjellidal MA, Bech C, van der Kooij J, Eldegard K, Stawski C (in Review) High latitude northern bats (*Eptesicus nilssonii*) reveal adaptations to both high and low temperatures. *Submitted to Journal of Experimental Biology*
- III) **Sørås R**, Fjellidal MA, Eldegard K, Stawski C. Defining torpor entries of bats based on rate of change in metabolic rate per minute. *Manuscript*
- IV) Skåra KH, Bech C, Fjellidal MA, van der Kooij J, **Sørås R**, Stawski C (2021) Energetics of whiskered bats in comparison to other bats of the family Vespertilionidae. *Biology Open* 10:bio058640. <https://doi.org/10.1242/bio.058640>
- V) Fjellidal MA, **Sørås R**, Stawski C (2022) Universality of torpor expression in bats. *Physiological and Biochemical Zoology* 95:326-339. <https://doi.org/10.1086/720273>

### Author contributions

**Paper I:** RS and CS framed the idea of the experiment. RS, MAF, and JK collected the data. RS and KHS performed the preliminary analysis of the data. RS wrote the initial draft. CS acquired the funding for the project. CS, KE, CB, and JK provided additional resources. All authors participated in the writing and revision of the manuscript.

**Paper II:** RS and CS framed the idea of the experiment. RS, MAF, and JK collected the data. RS performed the preliminary analysis of the data. RS wrote the initial draft. CS acquired the funding for the project. CS, KE, CB, and JK provided additional resources. All authors participated in the writing and revision of the manuscript.

**Paper III:** RS and CS framed the idea of the experiment. RS and MAF collected the data. MAF developed the initial code used for the analysis. RS performed the preliminary analysis of the data. RS wrote the initial draft. CS and KE provided additional resources. All authors participated in the writing and revision of the manuscript.

**Paper IV:** Conceptualization: KHS, CS. Methodology: KHS, CB, RS, CS. Formal analysis: KHS, CB, MAF, RS, CS. Investigation: KHS, RS, CS. Resources: CB, JK, CS. Data curation: KHS, CS. Writing – original draft: KHS. Writing – review & editing: KHS, CB, MAF, JK, RS,

CS. Visualization: KHS, CB, CS. Supervision: CB, CS. Project administration: CS. Funding acquisition: CS.

**Paper V:** CS and MAF conceived the study; MAF and **RS** conducted fieldwork, and all authors contributed to the collection of published data from the literature; MAF carried out the data analyses; MAF wrote the manuscript with input from CS and **RS**.

## Aims

Bats are expected to expand their ranges northward in response to climate change. Hence, the overall aim of this thesis was to study the physiological traits of bats living close to the northern edge of their distribution. Specifically, I aimed to quantify the energetic cost of staying normothermic over a wide range of  $T_a$ . Yet also estimate the energetic benefits of utilizing torpor, and which conditions may facilitate torpor use. Additionally, I aimed to facilitate further investigation into quantifying torpor entries, as these are generally overlooked in previous studies.

**Paper I** aims to understand the plasticity of torpor usage in *P. auritus*, and how this may differ with an individual's current energetic state, and quantify the energetic cost of staying normothermic. It also highlights the limiting effect of long days and how this is related to body mass.

**Paper II** aims to highlight the metabolic rate and resilience to both high and low  $T_a$  in *E. nilssonii*, which has previously been predicted to be especially threatened by increasing global temperatures.

**Paper III** aims to utilize a newly developed method to quantify the start, end, and rates of decrease in metabolic rate during torpor entries, as these are often an overlooked segment of a torpor bout.

**Paper IV** aims to quantify the metabolic rate of *M. mystacinus*, and compare its normothermic metabolism to other species of the Vespertilionidae family.

**Paper V** aims to summarize available data on torpid metabolic rate collected in summer on a global scale, and investigate potential divergences in relation to latitude or habitat.



## Thesis summary

### Methods summary

#### Study area

This study was performed in two different locations at two different latitudes in Norway. The southern study area was located in Nittedal municipality, Viken County (60°4'23''N, 10°52'20''E). It is characterized by a valley running from north to south, in which the main river Nitelva runs through the center of the valley, and is primarily surrounded by deciduous forest, farmland, and marshland, while coniferous forests cover the slopes of the valley and the surrounding hills. Settlement density is higher on the west side of the valley, and increases in a southward direction. The local bat fauna consists of *P. auritus*, *E. nilssonii*, *M. mystacinus*, Brandt's bat (*Myotis brandtii*), Daubenton's bat (*Myotis daubentonii*), as well as the occasional soprano pipistrelle (*Pipistrellus pygmaeus*), common noctule (*Nyctalus noctula*), and parti-coloured bat (*Vespertilio murinus*). All bats used in this study were captured just east of Rotnes, a small town populated by ~7000 inhabitants.

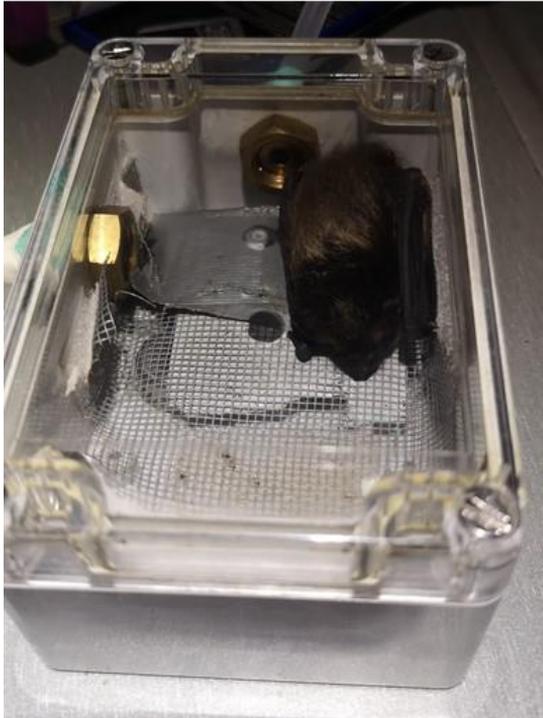
The northern study area was located in Trondheim municipality, Trøndelag County (63°25'49''N, 10°23'42''E), with one additional location in Orkland municipality, Trøndelag County (63°10'24''N, 9°28'34''E). Trondheim municipality is characterized by a river running from south to north, primarily surrounded by farmland, urban areas interspersed with multiple parks, and coniferous forests on surrounding hills. The local bat fauna primarily consists of *E. nilssonii* and *M. brandtii*. Bats captured in this study area were captured in backyards in suburban areas in the city of Trondheim (~200 000 inhabitants), as well as surrounding farmland. One additional *E. nilssonii* was captured in open coniferous forest in Orkland municipality.

#### Field methods

Bats were captured using mistnets when exiting known roosts, along important flyways, while foraging, or when visiting a location frequently revisited during the night, presumably for information transfer (Furmankiewicz and Jones 2021). Following removal from the net, standard morphometric measurements (body mass (g), forearm length (mm), sex, reproductive state), and individual wing photos for individual identification (see Amelon et al. 2017) were collected. Bats which were not reproductively active were brought back to one of two flight cages. In Nittedal municipality, bats were transported to an outdoor flight cage (see Paper I). In Trøndelag county, bats were transported to an indoor flight cage (see Paper II).

#### Respirometry

Energy consumption was estimated by indirect calorimetry by placing bats in an airtight chamber (325 mL) which was supplied with 315 mL air min<sup>-1</sup> when the bat was euthermic, and 101 – 248 mL min<sup>-1</sup> if the bat was torpid. We exposed bats to a stepwise increase of set temperatures ( $T_{set}$ ), as we wanted to estimate  $\dot{V}O_2$  and  $\dot{V}CO_2$  in both torpid and normothermic bats, as well as provide a better understanding of their usage of torpor. In paper I, II, III, IV and V, we exposed bats to 0, 5, 10, 15, 20, 25, 28, 31, 34, and 37°C. The bats were first put into



**Top left:** Five *P. auritus* in captured. **Bottom left:** *E. nilssonii* in respirometry chamber. **Top right:** Foreram measurement. **Middle right:** Weight measurement. **Bottom right:** Wing photo.

the chamber at night at a  $T_{\text{set}}$  of 5°C (in Paper I, II and III) or 10°C (in Paper IV), in order to facilitate torpor entry. The following morning,  $T_{\text{set}}$  was reduced to 0°C (in Paper I, II and III) or 5°C (in Paper IV). After which the  $T_{\text{set}}$  was increased stagewise every hour. Each experimental hour started with a 15-minute baseline in which the sample air passed through an identical, empty chamber.

Additionally, in order to specifically estimate the  $\dot{V}O_2$  and  $\dot{V}CO_2$  of normothermic *E. nilssonii*, a subset of individuals were fed up to five mealworms (*Tenebrio molitor*) just prior to being put in the chamber at a  $T_{\text{set}}$  of 15°C. Thereafter,  $T_{\text{set}}$  was increased every hour to an increasing  $T_a$  profile consisting of 21, 26, 30, 33, 36, 38 and 40°C. Bats were weighed prior to entering the chamber, and immediately after extraction.

The air was supplied by a pump (Eheim 100, EHEIM GmbH & Co., Deizisau, Germany), which passed through Drierite before and after passing through the two chambers. Flow rate was controlled by a flowmeter prior to entering the chambers. Eventually, a subset of air was pumped into a FOXBOX analyzer (Sable Systems International, Las Vegas, NV, USA). This first analyzed the fractional content of O<sub>2</sub>, followed by CO<sub>2</sub> (Fig. 1). The data was stored in minute-mean measurements in Expedata (Sable Systems International, Las Vegas, NV, USA), which was attached to the FOXBOX.

We calculated  $\dot{V}O_2$  and  $\dot{V}CO_2$  using equation 10.5 and 10.6 in Lighton (2018), respectively. Within each  $T_{\text{set}}$ , measurements spanning over at least 5 minutes when metabolism was stable were selected for further analysis. Using the runMean function in the TTR package (Ulrich 2021) in R (version 4.1.3), we calculated the lowest 5-minute mean within each  $T_{\text{set}}$ , as well as the  $T_a$  in the chamber at the same time.

Most metabolic rate studies tend to assume a linear decrease in mass loss within each experimental run. However, as bats in this study spent the majority of time in torpor, in which energy consumption, and thus mass loss, is minimal, we calculated body mass at any given time using the equation:

$$M_{\text{cur}} = M_{\text{prev}} - (\dot{V}O_{2\text{cur}}) / (\dot{V}O_{2\text{tot}}) * M_t$$

where  $M_{\text{cur}}$  is the calculated mass in any given minute,  $M_{\text{prev}}$  is the calculated mass in the previous minute,  $\dot{V}O_{2\text{cur}}$  is the O<sub>2</sub> consumption in the current minute,  $\dot{V}O_{2\text{tot}}$  is the total calculated O<sub>2</sub> consumption over the entire experiment, and  $M_t$  is the total loss of body mass over the entire experiment.

Statistical analysis in brief

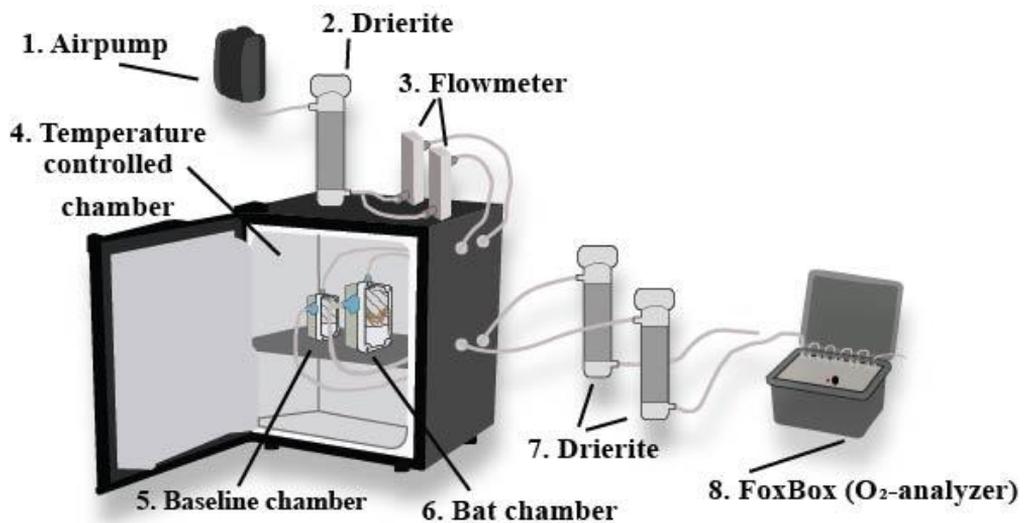
In papers I, II and IV we fitted linear mixed effects models to estimate the effect of different variables ( $T_a$ , season, sex, body mass) on the whole-animal metabolic rate of *P. auritus*, *E. nilssonii* and *M. mystacinus*, respectively, while accounting for individual differences by adding BatID as a random effect. In order to provide an estimate of  $T_{\text{lc}}$ , we performed broken stick regression on all data points from normothermic bats in Papers I and II. To quantify TMR in thermoconforming bats, we fitted exponential functions to all data points from thermoconforming bats in Papers I, II and V.

In paper I, we also fitted a linear mixed effects model to investigate the determinants of variation in body mass between all *P. auritus* captured in the southern study area.

In paper III, we used the newly presented method of Fjellidal et al. (2023), in which we aimed to define different sections of torpor entries from metabolic rate measurements. Additionally, we fitted exponential functions on entries to estimate per minute decreases in metabolic rate throughout the length of entries.

In paper IV, we also reviewed available literature and fitted a linear regression with log-transformed BMR as a response variable, and log-transformed body mass as an explanatory variable to assess differences between vespertilionid bats.

**Figure 1:** An overview of the experimental setup used in this study. (1) Outside air was supplied from an airpump before (2) passing through Drierite which rid the air of moisture before (3) passing through a flowmeter, after which it entered the (5) baseline- and (6) bat chamber. The air then passed through one of two respective tubes of (7) Drierite, before finally entering the (8) FoxBox.



## Results and Discussion

**In paper I**, we studied the metabolic rate of both normothermic and torpid *P. auritus* over a wide temperature scale and investigated how their metabolic strategy was dependent on an individual's energetic state. Utilization of torpor is a highly flexible trait in many animals (Matheson et al. 2010; Zervanos et al. 2013; Bieber et al. 2014), and can be considered crucial for surviving harsh seasonal conditions (Humphries et al. 2002; Karpovich et al. 2009), or detrimental and extreme weather (Stawski et al. 2015; Fjellidal et al. 2021). However, as torpor utilization is not cost-free (Nowack et al. 2017; Boyles et al. 2020), and limits fetal development (Dzal and Brigham 2013), the cognitive decision of torpor entry or arousal should be considered a trade-off between costs and benefits relative to energy availability. Therefore, we predicted that timing of torpor entry and arousal, and the extent to which bats would thermoregulate at low  $T_a$ , would be dependent on available energy (i.e., body mass).

As predicted, bats with a higher body mass delayed torpor entry for longer, and aroused at lower  $T_a$  than lighter bats. Thus supporting the assumption that bats with a larger energy reserve exert more resources to maintain normothermia. This is similar to what has previously been shown in little brown bats (*Myotis lucifugus*), where individuals with a higher food intake delayed torpor entry (Matheson et al. 2010). While the bats with a higher energy availability aroused at lower  $T_a$ , the bats with a smaller body mass delayed arousal until  $T_a$  exceeded their  $T_{lc}$ . Hence, by arousing later, they limited energy expenditure both by decreasing the cost of arousal, and limiting the amount of time spent normothermic. Thus losing less body mass over the length of the experiment.

Interestingly, the  $T_{lc}$  at 29.7°C we found is 4.8°C lower than what has previously been published for *P. auritus* (Webb et al. 1992), and northern populations may thus have a higher insulation (Scholander et al. 1950). Unfortunately, we were unable to estimate  $T_{uc}$  in this population, which has previously been estimated at 39.0°C (Webb et al. 1992), as we did not expose *P. auritus* to high enough  $T_a$ . BMR was not affected by neither body mass, nor  $T_a$ , and thus fitted the traditional Scholander-Irving model (see Box 1).

Whether or not bats that thermoregulated had higher energy reserves was inconclusive, as body mass was only marginally higher in the bats which increased metabolism at low  $T_a$ . The ten bats which did, increased their metabolism below an average critical temperature ( $T_{crit}$ ) of 6.7°C, but this point of increase differed between individuals. In contradiction to previous studies (Currie et al. 2018; see Geiser 2021), the thermoregulatory curve in torpid bats was more shallow than for normothermic bats. In the context of this study, it could potentially be due to increased insulation, where bats only defend  $T_b$  in internal parts of their body, or decreased surface area as *P. auritus* hibernate with their ears tucked alongside their body, but extend them when normothermic.

In summary, we present how *P. auritus* utilize torpor in relation to available energy reserves, as a trade-off between the costs of torpor and benefits of being normothermic. Accordingly, the cognitive choice of entering, maintaining, or leaving torpor provides heterotherms with a

valuable trait to combat the negative effects of climate change. A trait which seem to be more behaviorally flexible than previously assumed.

**In paper II**, we investigated the metabolic rate of both normothermic and torpid *E. nilssonii* at a wide range of  $T_a$ . *Eptesicus nilssonii* are the northernmost occurring bats globally, but is currently declining in at least parts of their range (Rydell et al. 2018; 2020), and predicted to be particularly vulnerable to climate change (Rebelo et al. 2010; Sherwin et al. 2013). To aid in the conservation of the species, we wanted to quantify the previously undocumented physiological traits of *E. nilssonii*. Given their continent-wide distribution in Eurasia (López-Baucells and Burgin 2019), tolerance of relatively light conditions (Frafjord 2021), and particularly short active season (Frafjord 2021; Hranac et al. 2021), we expected them to show a wide thermal tolerance in congruence with Janzen's theory (Janzen 1967).

To specifically study energy consumption in normothermic bats, we facilitated normothermia by feeding individual *E. nilssonii* up to five mealworms just prior to the start of experiment. Despite this, 12 out of 16 bats entered torpor shortly after being put in the respirometry chamber at  $T_a$  above 15°C.

Despite being exposed to  $T_a$  between 1.9 and 39.3°C, *E. nilssonii* did not show any increased  $\dot{V}O_2$  at low  $T_a$ , nor any increased  $VO_2$  at high  $T_a$  to dissipate heat. Thus indicating a better tolerance to higher  $T_a$  than other northern hemisphere bats (Czenze et al. 2022a). In addition to the indication of a higher  $T_{uc}$ , *E. nilssonii* had a particularly low  $T_{lc}$  at 27.6°C, only equaled by big brown bats (*Eptesicus fuscus*), with an estimated  $T_{lc}$  at 26.7°C (Willis et al. 2005a). Hence, our results show that *E. nilssonii* close to the northern edge of their range have a fairly wide thermal tolerance. Interestingly, the  $\dot{V}CO_2$  indicated a higher  $T_{lc}$  at 29.5°C, presumably caused by an increased release of  $CO_2$  following arousal in two individual bats, as a result of  $CO_2$  retention following torpor bouts which lasted for more than 10 h, thus leading to a particularly high respiratory exchange ratio (> 1.00).

The lack of increased metabolism at low  $T_a$  supports the idea that *E. nilssonii* can survive particularly cold  $T_a$  during hibernation without defending a higher  $T_b$ , which may be particularly important as *E. nilssonii* can overwinter at hibernacula where temperatures decrease to -5.3°C (Masing and Lutsar 2007).

Although *E. nilssonii* have been predicted to be particularly vulnerable to climate change, both as a result of increasing temperatures and increased competition (Rebelo et al. 2010; Rydell et al. 2020; Novella-Fernandez et al. 2021), our results show how the species, at least in the western part of its range, is more suited to cope with increased temperatures than previously assumed. Similarly, even though increased temperatures may facilitate northward range expansion from more southern species, bats expanding northwards will need to cope with a stricter daily cycle with shorter nights, and a limited availability of time for foraging compared to their current distribution.

In summary, we report on a particularly wide thermal tolerance in *E. nilssonii*, presumably as an adaptation to a particularly varied and seasonal climate over its wide distributional range. This underpins how a wider understanding of the physiological flexibility of a species is particularly important to improve the predictive power of future distribution models.

**In paper III**, we highlight the need for a greater exploration into the characteristics of torpor entries in heterotherms, and present a new method for which to calculate the start and end of torpor entries in heterothermic animals. As arousal is generally considered the most energetically costly part of a torpor bout (Thomas et al. 1990), the physiological traits of a torpor entry, and how this may differ depending on environmental conditions and species has received little attention. For small heterotherms, which frequently enter torpor, both on a day to day basis, and with reoccurring bouts of euthermia throughout hibernation, a more rapid entrance into torpor could serve to further enhance the energetic savings of a torpor bout. In order to motivate future work to incorporate estimation of torpor entry traits, we present a new method initially proposed for  $T_b$  data, which uses the rate of change between consecutive data points to define the start and end of a torpor entry (Fjellidal et al. 2023).

Using this method, we show that torpor entry length is primarily determined by the difference in metabolism at the onset and end of the entry. Hence, the smaller *P. auritus*, which has a higher whole-animal resting metabolic rate, needs a longer timespan to enter torpor compared to *E. nilssonii*. Surprisingly, due to a shorter decrease rate in metabolism at higher  $T_a$ , torpor entry length does not decrease with  $T_a$ . This elongated torpor entry time may be due to the bats potential ability to slow their own rate of cooling (Matheson et al. 2010), or due to a limiting effect of enzyme inhibition at higher  $T_a$  (van Breukelen and Martin 2001).

By fitting exponential functions to each torpor entry, we found that individual bats decrease metabolism by on average 9.1% per minute (range: 5 – 16% per minute). Unfortunately, with the limited sample size presented here, we were unable to determine what factors could cause this variation in metabolic decrease during torpor entry.

In summary, as the physiology of torpor entries is still largely unknown (see Heldmaier and Elvert 2004), and decrease in metabolism may be cognitively affected (Matheson et al. 2010), as well as affected by thermodynamic effects (van Breukelen and Martin 2001), we provide researchers with a new tool to estimate physiological traits of torpor entries in a standardized way.

**In paper IV**, we present novel data on the euthermic metabolism of *M. mystacinus*, and compare this to the BMR of other Vespertilionid bats for which this has been quantified. As it has been postulated that BMR should increase with latitude to meet the increasing energetic demands, particularly in small mammals (Lovegrove 2003), we hypothesized that mass-specific BMR would be higher in bats living in colder environments to enable the cost of living for a particularly small species of bats with an average body mass of 4.4 g ( $N = 7$ ).

In contrast to what we hypothesized, *M. mystacinus* had a BMR which was 99% of that predicted for a vespertilionid bat. Hence, BMR was not higher than other species of the Vespertilionidae family. Despite being the most species-rich family of bats, consisting of 698 species (Simmons and Cirranello 2022), BMR estimates were only available for 24 species. This limitation in available data also restrained the possibility to test for differences between sexes, season, reproductive state or captivity. We found no relationship between residual BMR and mean environmental temperature, but this does not reflect the scope of environmental variation bats may be exposed to at different latitudes (see Addio-Bediako et al. 2000).

The low BMR in *M. mystacinus* and other vespertilionid bats may be an adaptation to limit heat production in a cold environment, and essentially shift the thermoregulatory curve down (Stawski et al. 2017). Additionally, given their limited energy availability on a day to day basis, bats of this size can be expected to utilize torpor frequently at northern latitudes, as exemplified by the few data points of euthermic *M. mystacinus* below  $T_{lc}$  at 33.1°C in this study.

In summary, vespertilionid bats seem to have evolved low BMR and frequent use of torpor to cope with environmental and thermal challenges at high latitudes. Although we found no effect of latitude on metabolism in the present study, we emphasize that this only contained measurements of 3.4% of all species in the family of Vespertilionidae, and these involve little to no geographical variation within any species.

**In paper V**, we review the minimum measured TMR available from bats globally, alongside information on torpor patterns based on skin temperature data from free-ranging bats. As recent studies show how torpor in bats is applied globally (Stawski et al. 2014; Reher and Dausmann 2021; McGuire et al. 2022), we aimed to summarize and compare TMR in different climatic zones, emphasizing how torpor is not only a trait for coping with cold temperatures and low energy availability, but also a strategy used for coping with extreme heat and drought.

We obtained average TMR from 41 different species, which ranged between 0.014 mL O<sub>2</sub> g<sup>-1</sup> h<sup>-1</sup> and 1.01 mL O<sub>2</sub> g<sup>-1</sup> h<sup>-1</sup> globally. This variation was primarily explained by variation in  $T_a$ , as minimum average TMR increased with increasing  $T_a$ , and was not explained by differing climatic zones. Although minimum TMR tended to be higher in bats inhabiting the desert climate zone, all climatic zones from which we found data had bats which showed minimum TMR below 0.15 mL O<sub>2</sub> g<sup>-1</sup> h<sup>-1</sup>, which is indicative of hibernation (Ruf and Geiser 2015).

By also presenting detailed torpor metabolic measurements of two different species inhabiting two different hemispheres (*Nyctophilus bifax* and *P. auritus*), we show how these species equally increase torpor metabolic rate with increasing  $T_a$  when thermoconforming. This general lack of variation may be caused by microhabitat selection when in torpor (McGuire et al. 2022), despite large macroclimate differences.

In summary, our review highlights the general similarities in the physiology of torpor on a wide geographic scale in bats. Minimum TMR is primarily a result of  $T_a$  at which torpor occurs, and how TMR increases with  $T_a$  when thermoconforming is remarkably similar between different species of bats in different hemispheres. This review also provides support for the hypothesis that heterothermy is an ancestral trait. Hence, homeothermy has rather evolved in species where the benefits of torpor have diminished.

## Conclusive remarks and future prospects

This thesis had the main goal of quantifying energy management in species of bats at high latitudes in the northern hemisphere, further increasing our understanding of the physiology of bats, with a particular aim at understanding the flexibility in energy consumption in heterothermic bats. By facilitating torpor usage in *P. auritus*, and thereafter exposing them to an increasing  $T_a$ , we show how individual bats with a higher energy availability (i.e., body mass) both delay torpor entry and perform more costly arousals at lower  $T_a$ . This exemplifies how individual condition can help tailor the behavioral choices a bat makes when faced with differing environmental conditions, whether it be to limit the costs related to torpor usage, or benefit from the advantages of being euthermic (Paper I). Other studies have provided results that show differing behavioral decisions in other bats, thus adding some nuance to the interpretation of decision-making that bats undergo. For example, Stawski and Geiser (2010) showed how Australian eastern long-eared bats (*Nyctophilus bifax*) in better condition used torpor more frequently, presumably to avoid predation, and facilitate longevity. Fjelldal et al. (2021), working on an extended dataset of the same species, presented indications that bats in worse condition were more active under poor weather conditions, thus abandoning torpor to avoid critically low energy reserves.

When exposing *E. nilssonii* to a similar  $T_a$  exposure as *P. auritus*, a similar preference for normothermia was not evident. Despite being fed prior to entering the chamber at a  $T_{set}$  of 15°C, 12 out of 16 bats entered torpor within one to two hours. Additionally, five out of seven *E. nilssonii* did not arouse at  $T_a$  below  $T_{lc}$ , despite having a relatively high body mass. As all *P. auritus* were measured in June and July, while *E. nilssonii* were measured between June and mid-September, the willingness to delay arousal and remain in torpor for individual *E. nilssonii* may thus be a preparation for hibernation. This possibility to tailor their energy consumption, be it based on energy availability or season, is a crucial trait allowing for the survival of bats globally (Paper II).

## Metabolism at rest – in the context of roost selection

Being mammals with a low body mass, energy consumption in bats is best estimated using flow-through respirometry, in which the animal is kept under strictly controlled environmental conditions (Lighton and Halsey 2011). Accordingly, animals are kept in a confined space for an extended amount of time, while exposed to a range of temperatures in a particularly dry environment. These strict experimental conditions tamper the ecological relevance of metabolic rate measurements (Levesque et al. 2016), but facilitate scientific comparison within and between species at a wider geographical scale (Hulbert and Else 2003).

As the energy consumption and torpor utilization is highly affected by  $T_a$ , roost selection in bats can be an important part of the total daily energy expenditure of a bat, particularly for bats at higher latitudes. For instance, as a response to environmental temperature, and the seasonal needs of reproductive investments, female Indiana bats (*Myotis sodalis*) use warmer, more sun exposed roosts (particularly bat boxes), presumably to facilitate normothermia when pregnant, and more shaded roosts as a trade-off between normothermia and torpor when lactating (Bergeson et al. 2021). Although sun exposed bat boxes may promote normothermia in bats,

increasing global temperatures have been suggested to severely increase the risk of overheating for bats, particularly in warmer climates (Crawford and O’Keefe 2021).

Within the study areas described here, daily air temperatures rarely promote normothermia in *E. nilssonii*, *P. auritus*, and *M. mystacinus* as shaded roosts will rarely increase to within thermoneutrality at 27.6, 29.7, and 33.1°C, respectively (Paper I, II, IV). Thus, bats in these study areas can to a greater extent be expected to choose more sun exposed roosts, particularly during the reproductive season. Accordingly, *P. auritus* in Scandinavia has a well-known preference for old wooden churches (Rydell et al. 2017; 2020; Siljedal 2018) and barns (Kristiansen 2018) in summer, which can allow for relocation within the roost if risk of overheating occurs.

Being a smaller bat with a higher  $T_{lc}$ , *M. mystacinus* seem to prefer roosting within segments of the roofs at smaller houses (Siljedal 2018; McKay 2020), which allow for greater sun exposure, but less possibilities for microhabitat adjustments in risk of overheating. With a high surface-to-mass ratio, maintaining normothermia below  $T_{lc}$  is unavoidably costly for *M. mystacinus*, as indicated by the few normothermic datapoints below  $T_{lc}$  presented here. A similar avoidance of normothermia at  $T_a$  below  $T_{lc}$  has previously been reported for other small bats (Speakman and Thomas 2003). Thus, quantification of torpor usage below  $T_{lc}$  and a further understanding of how this species may cope with increased  $T_a$ , estimation of  $T_{uc}$ , can prove beneficial in predicting how this and similar species may cope with a changing climate.

### **The paradox of latitude**

In small mammals, latitude is considered an important predictor of BMR, with a positive relationship between increased latitude and BMR (Lovegrove 2003). Similar relationships have not been found in bats, neither globally for the whole of the taxa (Speakman and Thomas 2003), nor within the Vespertilionidae family (Paper IV). Speakman and Thomas (2003) postulated that this lack of effect in bats was due to a selection of thermally stable roosts, and the excessive production of heat while foraging at night (Thomas and Suthers 1972; Norberg and Rayner 1987).

Despite the lack of any known latitudinal differences in BMR between the few species in which it has been studied (Paper IV), little is known about the variation in BMR within species at a wide geographical scale. To date, the BMR of *P. auritus* has been studied in three populations in the western part of its range; Norway (Paper I), Scotland (Webb et al. 1992; McLean and Speakman 2000), and Germany (Becker et al. 2013a), revealing a higher mass-specific BMR in Norway (60°N; 1.83 mL O<sub>2</sub> g<sup>-1</sup> h<sup>-1</sup>) than in Scotland (58°N; 1.29 mL O<sub>2</sub> g<sup>-1</sup> h<sup>-1</sup>) and Germany (50°N; 1.19 mL O<sub>2</sub> g<sup>-1</sup> h<sup>-1</sup>), and a higher  $T_{lc}$  in Norway (29.7°C) than Scotland (34.5°C). Consequently, it seems *P. auritus* in Norway are adapted to staying normothermic at lower  $T_a$  than more southern conspecifics, but more data on a wider geographical scale is needed to confirm this.

Interestingly, latitude is commonly used as a proxy for environmental conditions, with high latitudes being associated with low mean and extreme  $T_a$ , and longer winters (i.e., hibernation periods in this context). However, the simplicity of this assumption is challenged by latitudinal effects in the different hemispheres, and the environmental effects of ocean currents, as large

ocean areas buffer seasonal variation in winter temperatures in the southern hemisphere (Addo-Bediako et al. 2000), and the heating effect of the Gulf Stream ocean current provide western Europe, and particularly Scandinavia, with high annual temperature relative to its latitude (Caesar et al. 2018; Praetorius 2018). The environmental effects of the latter is likely crucial as to why *E. nilssonii* occurs at higher latitude in Scandinavia, as opposed to interior Eurasia (see López-Baucells and Burgin 2019). Hence, variation in BMR in relation to latitude, or lack thereof, should be considered with caution.

Janzen (1967) hypothesized that animals living in the tropics, particularly ectotherms, would be adapted to a narrower thermal niche as they were more specifically adapted to a very stable environment, while temperate animals are adapted to a seasonal climate. Building on this, Pollock et al. (2020) recently showed how temperate birds had higher heat tolerance limits and a higher  $T_{uc}$  than tropical birds. Hence, the particularly wide TNZ in *E. nilssonii*, as well as the lowered  $T_{lc}$  in Norwegian *P. auritus* can be considered indications of similar heightened phenotypic plasticity as an adaptation to lower environmental temperatures and prolonged winters. Accordingly, future studies should investigate phenotypic plasticity within species at a wider spatial scale than what has been done to date to further investigate the relevance of Janzen's theory (Janzen 1967) on heterothermic bats, as this thermal tolerance may be crucial in their ability to cope with the ongoing climate change.

### **The dos and don'ts of torpor use**

Living in habitats close to the northern edge of a species' distribution range, individual bats roosting will frequently be exposed to temperatures which present them with an issue to which they have two possible solutions; either increase energy consumption to defend a normothermic  $T_b$ , or decrease energy consumption and enter torpor, thereby decreasing  $T_b$ . Staying normothermic is undoubtedly necessary to perform essential bodily functions, such as digestion (Turbill et al. 2008), production of milk (Kurta et al. 1989), and sleep (Humphries et al. 2003). However, particularly in areas with less time available for foraging, and  $T_a$ s which fall outside thermoneutrality, maintaining normothermia will be especially challenging for insectivorous bats. Thus, torpor is used frequently to avoid depletion of energy storage.

The universality of torpor expression (Paper V), shows how the minimum TMR in bats is fairly similar in different climate zones, and is primarily determined by the  $T_a$  at which a bat exhibits its minimal TMR before increasing metabolism to defend a higher  $T_b$ . The  $T_a$  at which an animal increases TMR is often referred to as a critical temperature, which is primarily conceived as species specific (Geiser 2021), or may differ between populations of the same species (Stawski and Geiser 2011). Alternatively, the individual condition of a bat may affect its willingness to exert energy during parts of the year, as thermoregulation at low  $T_a$  differed between individual *P. auritus* (Paper I). This may have profound effects on energy consumption in bats throughout hibernation, both in terms of microhabitat selection, but also reproductive success the following year, as condition upon departure from hibernacula consequently can affect reproductive success the following season (Jonasson and Willis 2012).

In particular for species hibernating for prolonged periods in the northern hemisphere, even at subzero temperatures (Webb et al. 1996), the critical temperature in torpor should ideally be low. In contrast, bats which are adapted to warmer climate zones, deserts, steppes, and

subtropical habitats (see Paper V), may only use torpor on a day to day basis, and thus defend  $T_b$  at a higher  $T_a$  (Ruf and Geiser 2015). With its particularly long hibernation season, *E. nilssonii* does not show any increase in metabolism down to 1.1°C, and is particularly well adapted to living in the extreme north (Paper II).

### **Coping with a changing world**

With the current trajectory of global change, with increasing global temperatures (Rantanen et al. 2022), increase in heatwaves (McKechnie and Wolf 2019), or habitat loss (Frick et al. 2020) following an increasing human population, understanding bats ability to adapt, both locally and globally, needs further attention (see Festa et al. 2023). Bats at high latitudes in the northern hemisphere are characterized by a slow pace of life, with long periods of inactivity (Humphries et al. 2002; Hranac et al. 2021), slow reproductive rates (Turbill et al. 2011; Becker et al. 2013b), high survival rates (Boyles and Brack 2009), and extreme longevity (Podlutzky et al. 2005; Foley et al. 2018; Wilkinson and Adams 2019). Recently, studies have unveiled how bats adapt to this both morphologically, as Bechstein's bat (*Myotis bechsteinii*) have been shown to produce larger females with a shorter lifespan in recent years (Mundinger et al. 2022), and behaviorally, as Natterer's bats (*Myotis nattereri*) may depart from hibernacula earlier, but face a lower survival rate in doing so (Reusch et al. 2023).

Undoubtedly, heterothermy in bats is a prerequisite for their ability to adapt to climate change. Even though temperature niche has been suggested to be of less importance in explaining the population trends of bats (Bowler et al. 2015), this suggestion inadvertently relies on a small subset of bat species (see Festa et al. 2023). For instance, many bats belonging to the suborder Yinpterochiroptera, such as Wahlberg's epauletted fruit bat (*Epomorphus wahlbergi*), face mass die-offs when environmental temperature increases above ~42° C (McKechnie et al. 2021). In contrast, bats in the suborder Yangochiroptera, both in the northern (Czenze et al. 2022a) and southern hemisphere (Czenze et al. 2020; 2022b), can sustain  $T_b$  above ~44°C. Czenze et al. (2022b) suggested that the heat tolerance in the six species they studied had co-evolved with roost preference. Interestingly, only one of these species showed a stable BMR which was not affected by  $T_a$  between 28 and 42°C. While the remaining five showed a linear increase in RMR with increasing  $T_a$  between 28 and 34°C (Czenze et al. 2022b). In contrast, Czenze et al. (2022a) showed how BMR remains unaffected by  $T_a$  up to 34 to 37°C, after which RMR increased with  $T_a$  in four species. Thus, the bats in the southern hemisphere may have evolved a narrower TNZ, as suggested by Janzen's theory (see Janzen 1967), or show indications of a biologically optimum temperature (see Nichelmann 1983), while bats in the northern hemisphere are more in line with the Scholander-Irving model (Scholander et al. 1950). Interestingly, the lowered  $T_{lc}$  in *P. auritus*, and the particularly wide TNZ shown in *E. nilssonii*, are indicative as an adaptation to a highly varied thermal niche, thus providing further support for Janzen's theory.

Species distribution models have become a popular tool for estimating global effects of climate change on animal populations, but their reliability is particularly dependent on intricate knowledge of the animals behavior and physiological traits. For bats in particular, understanding the physiological flexibility within species, their tolerance to high  $T_a$ , variability in torpor usage, and its effects on roost selection will to a great extent improve the predictive

power of these models. Accordingly, as presented in this thesis, understanding how *P. auritus* tailor their energy consumption to meet their current needs (Paper I), the particularly wide TNZ of the world's northernmost occurring bat species (Paper II), how the low resting metabolic rate of *E. nilssonii* allow for more efficient torpor entries (Paper III), and the cost of normothermia in the small *M. mystacinus* (Paper IV), provide further insight into the phenotypic plasticity of these bats living close to the northern edge of their distribution. Additionally, reviewing energy consumption in both normothermic (Paper IV) and thermoconforming (Paper V) bats reveals a generally similar physiology within the relatively few species that have been studied to date (but see Festa et al. 2023). Hence, to augment the survivability and future existence of bats in an ever-changing world, more research should be focused on comprehending the physiology of bats, both among and within species, and how this is crucial for their future existence.



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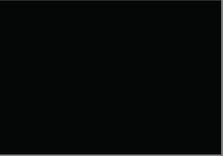
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# Paper I







# State dependence of arousal from torpor in brown long-eared bats (*Plecotus auritus*)

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## Abstract

To cope with periods of low food availability and unsuitable environmental conditions (e.g., short photoperiod or challenging weather), many heterothermic mammals can readily go into torpor to save energy. However, torpor also entails several potential costs, and quantitative energetics can, therefore, be influenced by the individual state, such as available energy reserves. We studied the thermal energetics of brown long-eared bats (*Plecotus auritus*) in the northern part of its distributional range, including torpor entry, thermoregulatory ability during torpor and how they responded metabolically to an increasing ambient temperature ( $T_a$ ) during arousal from torpor. Torpor entry occurred later in bats with higher body mass ( $M_b$ ). During torpor, only 10 out of 21 bats increased oxygen consumption ( $VO_2$ ) to a greater extent above the mean torpor metabolic rates (TMR) when exposed to low  $T_a$ . The slope of the torpid thermoregulatory curve was shallower than that of resting metabolic rate (RMR) during normothermic conditions, indicating a higher thermal insulation during torpor. During exposure to an increasing  $T_a$ , all bats increased metabolic rate exponentially, but the bats with higher  $M_b$  aroused at a lower  $T_a$  than those with lower  $M_b$ . In bats with low  $M_b$ , arousal was postponed to an  $T_a$  above the lower critical temperature of the thermoneutral zone. Our results demonstrate that physiological traits, which are often considered fixed, can be more flexible than previously assumed and vary with individual state. Thus, future studies of thermal physiology should to a greater extent take individual state-dependent effects into account.

**Keywords** Respirometry · Torpor · Vespertilionidae · Chiroptera · Temperature · Body mass

## Abbreviations

$T_b$  Body temperature  
 $T_a$  Ambient temperature

TMR Torpid metabolic rate  
TNZ Thermoneutral zone  
 $M_b$  Body mass  
 $VO_2$  Oxygen consumption  
 $T_{set}$  Set temperature  
BMR Basal metabolic rate  
RMR Resting metabolic rate  
 $T_{lc}$  Lower critical temperature

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## Introduction

Many endothermic animals have a remarkable capacity to alter their metabolic rate (MR) and heart rate, and thereby body temperature (Schmidt-Nielsen 1997; Currie et al. 2014). By lowering their energy consumption to the bare minimum for survival, some heterothermic endotherms can reduce their MR by up to ~1000-fold compared to their active metabolism (Willis et al. 2005) when entering the energy saving state of torpor (Geiser 2021). The large

potential energy savings gained from employing short-term torpor or hibernation during inclement conditions have been found to enhance individual survival probability and even reduce the risk of species extinctions (Geiser and Turbill 2009; Liow et al. 2009). Torpor is primarily present and most pronounced in small mammals such as rodents (Buck and Barnes 2000; Zervanos et al. 2010; Pretzlaff et al. 2021), marsupials (Franco et al. 2012), and bats (Dunbar and Tomasi 2006; Jonasson and Willis 2012; McGuire et al. 2021), as well as some birds (Wolf et al. 2020; Geiser 2021). As an adaptation to various conditions, the use of torpor is prevalent during both summer and winter, and in both the Southern and Northern hemispheres (Stawski et al. 2014; Ruf and Geiser 2015; Boyles et al. 2016; Geiser 2021; McGuire et al. 2021; Reher and Dausmann 2021).

For small mammals with a large surface area to volume ratio, obtaining enough energy to maintain a stable body temperature ( $T_b$ ) is particularly challenging if they depend on a limited and seasonal food source (Buck and Barnes 2000), and employing torpor is a widely used strategy to cope with these challenges. At higher northern latitudes, the need for employing torpor on a day-to-day basis is further emphasized in nocturnal animals, as the length of summer night decreases with increasing latitude (Michaelsen et al. 2011). This means that nocturnal animals at high latitudes will need to build up sufficient energy reserves on a daily basis to compensate for the long days, and thereby long time between foraging bouts. Thus, the ability to enter torpor for shorter periods of time may be an essential strategy to both survive and reproduce during summers at high latitudes.

As torpor is not cost-free (Humphries et al. 2003; Boyles et al. 2020), there is a trade-off between the costs of maintaining torpor and the benefits of staying euthermic. Costs related to torpor include sleep deprivation (Humphries et al. 2003), memory loss (Millesi et al. 2001), and risk of predation (Estok et al. 2010; Haarsma and Kaal 2016), although the severity of the latter has been questioned (Turbill et al. 2011). In contrast, the benefits of maintaining a high  $T_b$  during daytime in summer include digestion of previously consumed food (Turbill et al. 2008) and allow for the development of fetus and lactation, which will be greatly delayed or reduced during torpor (Kurta et al. 1989; Dzal and Brigham 2013; Stawski et al. 2014).

It has recently been suggested that torpor is a more flexible trait than previously assumed (Reher et al. 2022). For example, torpor entry is often delayed (Matheson et al. 2010), and torpor duration decreased (Geiser and Broome 1993) in recently fed animals. Insectivorous bats (Chiroptera) at higher latitudes have a short reproductive season (Frafjord 2021), and rely on a food source which varies seasonally and occurs irregularly (Selås et al. 2013). Given their small body size, loss of heat is a major challenge when bats are faced with  $T_a$ s below the thermoneutral zone

(TNZ) (Bartels et al. 1998). Their limited potential for fat storage means that a substantial reduction of metabolic rate is the only possible option for non-migratory species when faced with longer periods of food shortage during winter at northern latitudes (Wermundsen and Siivonen 2010). Nevertheless, when and to which extent bats utilize torpor differs between species and environmental conditions (Stawski and Geiser 2010; Boyles et al. 2017), such as extreme heat (Reher and Dausmann 2021), unpredictable weather (Downs et al. 2012), or less suitable foraging conditions (Geiser et al. 2018).

As torpid bats may thermoregulate to some extent for short relocations within roost despite having a low  $T_b$  (Bartonička et al. 2017; Mayberry et al. 2017), it is reasonable to assume that there is an individual state component in studies investigating metabolic rate in bats that is often overlooked. Hence, as the cost of arousal increases with lower  $T_a$  (Wojciechowski et al. 2007), only bats with a higher energy reserve or better food availability can be expected to arouse at lower temperatures to counteract costs related to torpor (see Landes et al. 2020). When energy reserves are at a particularly low level, bats may not be able to arouse at low  $T_a$  as energetic reserves are insufficient to fuel arousal.

The brown long-eared bat (*Plecotus auritus*) is a medium sized (6–9 g) insectivorous Vespertilionid bat distributed widely across the Western Palearctic region (Wilson and Mittermeier 2019). Because the amount and availability of their prey is drastically reduced during the winter season, individuals must reduce their energy consumption to be able to hibernate through the winter. Towards the northern range of its distribution, *P. auritus* may also benefit from using torpor on a day-to-day basis throughout the year as night length, and thereby foraging opportunities, decrease with increasing latitude in summer (Michaelsen et al. 2011).

Some studies measuring metabolic rate have already been conducted within two populations of *P. auritus* in the western margin of its distribution range (Speakman et al. 1991; Webb et al. 1992; McLean and Speakman 2000; Becker et al. 2012, 2013). As *P. auritus* at high latitude are likely to enter short-term torpor on a day-to-day basis, understanding the cost and timing of arousal can improve our understanding of how bats survive in the northern hemisphere. Hence, in the present study, we experimentally studied this by inducing torpor in *P. auritus* and studied the physiology of torpor and the arousal from torpor. We predicted that individuals with more energy reserves should arouse earlier to reduce torpor-related costs, while those with low energy reserves would remain torpid for a longer period of time. We also predicted that *P. auritus* with larger energy reserves would utilize more energy to thermoregulate during torpor to reduce the costs and risks associated with deep torpor at low  $T_a$ .

## Materials and methods

Bats exiting both potential and known roosts, or commuting along important flyways, were captured using mist-nets in Nittedal, Norway (60° 4' 23" N, 10° 52' 20" E) in June and July from 2019 to 2021. Upon capture, bats were immediately put into individual cloth bags, before measuring  $M_b$  to the nearest 0.1 g (Aweigh MB-50), forearm length to the nearest 0.1 mm (RS PRO 150 mm Digital Caliper 0.03 mm), while sex and reproductive state were determined.  $M_b$  was used as a proxy for energy reserves. Additionally, the wings of all captured bats were photographed using a standard DSLR camera with predetermined settings (1/160 s,  $f_{16}$ , ISO 100), and an external flash providing back light to use the wing membrane for individual identification (see Amelon et al. 2017). Female bats that showed signs of reproduction (i.e., palpated abdomen or signs of lactation) were released after the individual morphometric measures. All males for which MR was measured were captured between June 1st and July 20th and did not show any signs of spermatogenesis.

After examination in the field, bats ( $N=22$ ) which were not reproductively active or not born the same year (i.e., closed epiphyseal gap) were brought back to an outdoor flight cage (2.5 m  $\times$  5 m  $\times$  2 m), which was equipped with bat boxes for roosting. Two of the four walls consisted of mesh netting, giving the cage an open air supply and a natural variation in light and environmental temperature. All bats were adult and consisted of 9 females and 13 males. On eight occasions, a single bat was brought back. Whereas, on some occasions, two ( $N=5$ ) or four ( $N=1$ ) bats were brought back at the same time. On one occasion, four bats were brought back in groups of two on two consecutive days. The following day, one bat was used for the experiment, while the other(s) were handfed *Tenebrio molitor* twice a day and given water ad libitum. Bats were always handfed before 21:00, so that they would be post-absorptive when the experiment started. When multiple bats were brought back the same day, females were always measured the following day, while males were measured on the 2nd day. Each bat was released at the capture site after sunset the same day the measurement had been performed. Thus, bats were held in captivity for 1 ( $N=13$ ), 2 ( $N=5$ ), 3 ( $N=3$ ) and 4 days ( $N=1$ ).

We measured metabolic rate indirectly as  $\dot{V}O_2$  using open-flow respirometry. Bats were placed in a sealed chamber (325 ml) which was backlit from 03:30 to maintain a normal circadian rhythm, and placed inside a temperature-controlled cabinet. The closed chamber was connected to a pump (Eheim 100, EHEIM GmbH & Co., Deizisau, Germany), which supplied air from outside. Air was dried of humidity using Drierite before and

after passing through the chambers, before finally entering a FOXBOX analyser (Sable Systems International, Las Vegas, NV, USA), which analyzed both  $\dot{V}O_2$  and carbon dioxide production. The sample air also passed through an identical but empty chamber, which was used to perform baseline measurements for 15 min every hour (i.e., each set  $T_a$  consisted of 15 min of baseline measurements followed by 45 min of bat measurements). The analyser was zeroed at the onset of each field season using a 100% stock nitrogen. Additionally, the analyser was span-calibrated to 20.95%  $O_2$  in the middle of the first baseline at the onset of the experiment, as well as the prolonged baseline when  $T_{set}$  was reduced to 0 °C the following morning. Data recorded by the analyser were logged and stored in the software Expedata (Sable Systems International, Las Vegas, NV, USA) every 1 min.

To measure  $T_a$ , an iButton (model DS1923-F5, Dallas Semiconductor Inc., Dallas, TX, USA) was placed in the bottom of each chamber. The iButtons recorded temperature every minute to the nearest 0.001 °C. As other models of iButtons have been shown to emit ultrasound (Willis et al. 2009), we checked for this prior to the field season using a heterodyne bat detector (Model D200, Petterson Elektronik AB, Uppsala, Sweden) and observed no indication of ultrasound noise. Additionally, iButtons were calibrated in a water bath against a precision thermometer and revealed little difference between iButtons, similar to what was reported by Davidson et al. (2003). The upper half of the chamber was covered with mesh netting, on which the bat could roost. When placed inside the chamber, the cabinet was set to 5 °C to motivate the bat to enter torpor. Bats were placed in the chamber at differing times throughout the night (mean  $\pm$  SD, 160.5  $\pm$  70.3 min after sunset). Upon arrival to the flight cage, one bat was immediately placed into the chamber and thus not handfed or supplied with water before the experiment. But digestive state presumably differed as time of capture differed between bats. At approximately 09:00, the following morning, the set temperature ( $T_{set}$ ) was reduced to 0 °C. Thereafter, the  $T_{set}$  was increased by 5 °C every hour until it reached 25 °C, after which we increased it by 3 °C per h until it reached 37 °C. For one measurement, technical issues in the recording computer meant we could only record data at  $T_a > 26$  °C for one bat. The lowest mean  $T_a$  at which MR was measured was 0.99  $\pm$  1.28 °C ( $\pm$  SD,  $N=21$ ). The highest mean  $T_a$  was 36.14  $\pm$  1.40 °C ( $\pm$  SD,  $N=22$ ). The total length of each experiment lasted on average for 16.0  $\pm$  1.4 h ( $\pm$  SD,  $N=22$ ).

Incurrent flow rate was set at 315 mL min<sup>-1</sup> when the bat was placed in the chamber. At approximately 09:00, flow rate was reduced, as all bats were in torpor, and kept between 101 and 248 mL min<sup>-1</sup> while the bat was torpid. To determine when a bat had exited torpor, we routinely observed the  $O_2$  measurements in the software Expedata, as well as

observing via a camera in the temperature-controlled cabinet. As soon as the bat exited torpor, flow rate was increased to 315 mL min<sup>-1</sup>.

To calculate  $\dot{V}O_2$ , we selected a series of stable values over at least five consecutive measurements (i.e., at least 5 min) at each  $T_{set}$ . Using the software *R* (version 4.0.2), the lowest 5 min mean within this selection was extracted using the `runMean()` function in the *TTR* package (Ulrich 2021) for further analysis. Overall, the  $T_a$  generally fluctuated slightly within each hour. At  $T_{set}$  of 0 °C, the  $T_a$  fluctuated above the  $T_{set}$ , whereas between  $T_{set}$  of 5 and 37 °C the  $T_a$  fluctuated slightly below the  $T_{set}$ . At the lowest  $T_{set}$ , we selected at least five consecutive measurements when the  $T_a$  was lowest to calculate to which extent the bats showed active thermoregulation (Table S1).

We corrected for drift and calculated  $\dot{V}O_2$  using Eq. (10.5) in Lighton (2018),

$$\dot{V}O_2 = FR_i [(F_iO_2 - F_eO_2) - F_eO_2 (F_eCO_2 - F_iCO_2)] / (1 - F_eO_2)$$

where  $FR_i$  is the incurrent air flow rate,  $F_iO_2$  is the fractional content of incurrent oxygen,  $F_eO_2$  is the fractional content of excurrent oxygen,  $F_iCO_2$  is the fractional content of incurrent carbon dioxide, while  $F_eCO_2$  is the fractional content of excurrent carbon dioxide.

Bats were weighed ( $\pm 0.1$  g) immediately before being placed in the chamber, and again immediately after the metabolic trial. As  $\dot{V}O_2$  is very low during torpor, mass loss is correspondingly very low during torpor. Therefore, to be able to calculate a more precise value of mass-specific MR, we calculated body mass at any given time based on the equation:

$$M_{cur} = M_{prev} - (\dot{V}O_{2cur}) / (\dot{V}O_{2tot}) * M_t$$

where  $M_{cur}$  is the calculated mass at each minute,  $M_{prev}$  is the calculated mass in the previous minute,  $\dot{V}O_{2cur}$  is the  $O_2$  consumption in the current minute,  $\dot{V}O_{2tot}$  is the total calculated  $O_2$  consumption over the entire experiment, and  $M_t$  is the total loss of  $M_b$  over the entire experiment. During baseline and periods where the flow rate was too high and the oxygen analyzer therefore had problems picking up the minor  $\dot{V}O_2$  of torpid bats, we calculated the mean  $\dot{V}O_2$  of the 5 min before and after this period and assigned it to these periods as the  $\dot{V}O_2$  for use in the calculations of  $M_{cur}$ .

As we investigated the  $\dot{V}O_2$  measurements of each individual visually, it was evident that 45% of the bats ( $N=10$ ) maintained torpor at higher temperatures (i.e.,  $> 30$  °C), as MR increased exponentially with increasing  $T_a$ . These were considered thermoconforming within the TNZ (referred to as Group 1). The remaining bats ( $N=12$ ) aroused at lower  $T_a$ . The latter group was defined as euthermic for the rest of their experiment as they did not reenter torpor at higher  $T_a$  (referred to as Group 2). All stable measurements at a lower

$T_a$  before arousal were considered TMR. As previous studies have shown that bats thermoregulate below 1.8–6.7 °C (Willis et al. 2005; Stawski and Geiser 2011; Currie et al. 2018), an increase in  $\dot{V}O_2$  consumption with decreasing  $T_a$  at low ambient temperatures was defined as active thermoregulation, as they actively increased heat production.

We chose to analyse these two groups separately, as the latter group showed more stable readings over multiple temperatures within the TNZ. To quantify the arousal events, we defined an arousal as the point in time when  $\dot{V}O_2$  in torpid bats increased substantially. The  $T_a$  measured at the same minute as metabolic rate started to increase was used as the  $T_a$  of the start of the arousal. The length of the arousal in minutes was quantified from the start of the increase to the time at which mass-specific  $\dot{V}O_2$  peaked or stabilized. Although bats are known to overshoot their  $\dot{V}O_2$  relative to the RMR during arousals at the same  $T_a$  (Turbill et al. 2008), the decrease phase of the arousal was not included in the analyses as it was often interrupted by baseline measurements. We used the difference in  $\dot{V}O_2$  between the minute at which the  $\dot{V}O_2$  peaked and the last measurement before the increase in  $\dot{V}O_2$  started as a proxy for  $\dot{V}O_2$  during the arousal (Figures S1 and S2).

## Statistical analyses

### Body mass

All analyses were performed using *R* (version 4.0.2). Results are presented in the language of evidence as suggested by Muff et al. (2022).

To analyze factors influencing changes in  $M_b$  upon capture we fitted a linear model with  $M_b$  as a response variable, and days after June 1st, time after sunset (minutes), and sex as fixed effects, assuming a Gaussian error distribution. Two males were measured two times in different years, but since the recaptures were a year apart, and  $M_b$  differed highly between captures for both individuals, we treated the measurements as independent. To see if there were any differences in  $M_b$  between the bats which did not exit torpor prior to the TNZ (Group 1) and those that did (Group 2), we performed linear regression analyses with  $M_b$  at the start of the experiment as a response variable, and group as a fixed effect. Additionally, to investigate if  $M_b$  at the onset of the experiment affected the mass loss and the  $M_b$  at the end of the experiment, we fitted simple linear regressions with  $M_b$  at the end of the experiment as the response variable, and  $M_b$  at the onset of the experiment and Group as fixed effects.

### Metabolic rate

As the timing of torpor entry differed between bats, we fitted a linear regression model using the `lm()` function with time

spent before torpor entry in minutes as the response variable, and  $M_b$  at the onset of the experiment as the explanatory variable. To estimate how metabolic rate differs with increasing  $T_a$  in the bats which aroused at lower  $T_a$ , we fitted a linear regression model using the `lm()` function with  $VO_2$  as a response variable, and  $T_a$  as an explanatory variable. Thereafter, we used Davies test, using the `davies.test()` function in the `segmented` package (Muggeo 2008) to check for the presence of a significant inflection point in the relationship between  $VO_2$  and  $T_a$ . If an inflection point was identified, we performed a broken stick regression using the `segmented()` function in the `segmented` package (Muggeo 2008) to identify at which  $T_a$  the relationship with  $VO_2$  changed. To check if the `segmented()` function provided a better fit than the initial `lm()` function, we performed ANOVA analysis on both functions. After identifying a potential inflection point, we defined all measurements at  $T_a$  below the inflection point as RMR and all measurements at  $T_a$  above the inflection point as BMR.

The RMR was estimated using a linear mixed-effects model using the `lmer()` function (Bates et al. 2015) with  $VO_2$  below the inflection point as a response variable, and  $T_a$  and  $M_b$  as a fixed effects. Individual bat ID was added as a random effect. A similar analysis was performed on BMR with  $VO_2$  above the inflection point as a response variable. We fitted an exponential growth curve to the TMR data for each group using the `nls()` function in *R* and performed a linear mixed model with minimum TMR of each individual as the response variable and days after June 1st, sex, and group as fixed effects.

### Active thermoregulation

To estimate the slope of active thermoregulation during torpor at low  $T_a$ , we performed a linear mixed-effects model with  $VO_2$  as the response variable, and  $T_a$  at which the measurement was taken as a fixed effect. Individual ID was added as a random effect. This analysis consisted of a subset of individuals, as some individuals did not show any clear increase in  $VO_2$  at low  $T_a$  ( $N=8$ ), while in some cases the difference in  $T_a$  between two means were too big to give a reliable estimate ( $N=3$ ).

### Arousal

To better understand the physiology and timing of arousals, we performed four separate analyses. First, we performed a simple *t* test to compare the  $VO_2$  during arousal between the two groups. Second, to estimate arousal costs with decreasing  $T_a$ , we fitted a simple linear regression with  $VO_2$  during arousal as the response variable, and  $T_a$  as an explanatory variable. Third, we performed a simple *t* test to compare the number of minutes needed to arouse between the two groups. Fourth, we

fitted a simple linear regression with the  $T_a$  at which arousal occurred as a response variable and  $M_b$  at the onset of the experiment as an explanatory variable to investigate if body condition affected arousal. This was also repeated with estimated  $M_b$  at the timing of arousal as an explanatory variable. We performed simple linear regressions as all bats in our study only aroused once per experimental run, and only completed one torpor bout.

## Results

### Body mass

Average  $M_b$  upon capture was  $7.82 \pm 0.96$  g (range 6.6–10.7 g,  $N=22$ ), while forearm length averaged  $39.4 \pm 1.3$  mm (range 36.7–43.2 mm). There was strong evidence that  $M_b$  increased with time after sunset ( $M_b$   $0.009$  g  $\text{min}^{-1} \pm \text{SE } 0.003$ ,  $p=0.004$ ), but no evidence that it increased with days after June 1st ( $p=0.86$ ) or forearm length ( $p=0.40$ ), and there was no difference between sexes ( $p=0.48$ ). In contrast, the average  $M_b$  for all *P. auritus* ( $N=90$ ,  $n=156$ ) captured in the study area between 2017 and 2021 was  $8.30 \pm 1.31$  g ( $N=156$ ), which is higher than for the bats included in the present study ( $t_{33,3} = -2.08$ ,  $p=0.045$ ). In addition to a higher  $M_b$  with time after sunset, there was also very strong evidence that  $M_b$  was positively related to forearm length, and that there was a difference between sexes (Table 1, Fig. 1). Because females have an earlier reproductive period and had greater  $M_b$  than males (females:  $8.62 \pm 1.32$  g,  $N=112$ ; males:  $7.49 \pm 0.91$  g,  $N=44$ ,  $t_{113,4} = 6.1$ ,  $p < 0.0001$ ), and as females had longer forearms (females:  $39.6 \pm 1.2$  mm,  $N=112$ ; males:  $38.9 \pm 1.4$  mm,  $N=44$ ,  $t_{64,0} = 3.2$ ,  $p=0.002$ ) we fitted linear mixed-effects models for each sex separately (Table 1).

At the onset of the experiment, there was strong evidence that average  $M_b$  for the bats that aroused below  $T_{lc}$  ( $8.29 \pm 1.04$  g,  $N=12$ , Group 2) was higher ( $p=0.009$ ) than for the bats that aroused above  $T_{lc}$  ( $7.25 \pm 0.53$  g,  $N=10$ , Group 1, Fig. 2a). There was strong evidence that  $M_b$  at the end of the experiment increased with higher  $M_b$  at the onset of the experiment ( $g = 1.73 + 0.67 \times g$ ,  $r^2 = 0.87$ ,  $p < 0.005$ , Fig. 2b), but did not differ between the bats which aroused from torpor prior to entering the TNZ and those that did not ( $p=0.88$ ).  $M_b$  showed a noticeable decrease at the onset of the experiment, followed by a long period in which the  $M_b$  remained stable during torpor, until it decreased gradually at higher  $T_a$  (Figures S1 and S2).

### Metabolic rate

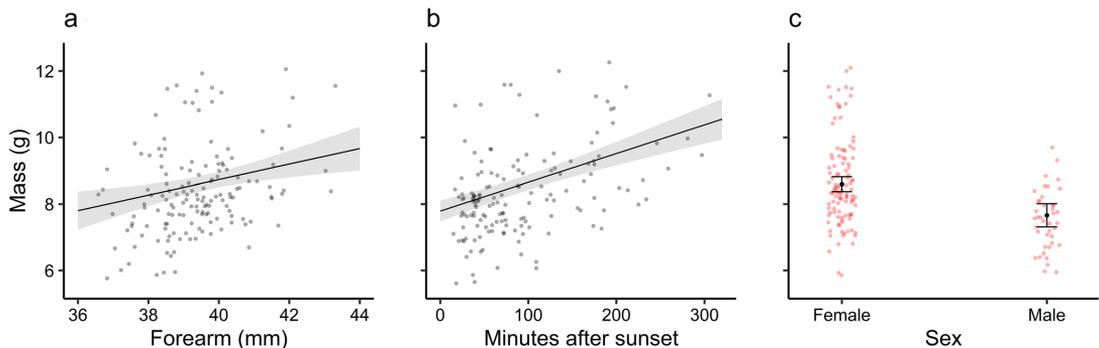
Bats entered torpor after  $102.8 \pm 85.3$  min ( $\pm \text{SD}$ ,  $N=19$ ) into the experiment. Torpor entry occurred later in bats that weighed more at the onset of the experiment

**Table 1** Fitted models and model statistics for modelling body mass (g) as a function of days after June 1st, forearm length (mm); time after sunset (minutes); and sex (male or female) in brown long-eared bats (*Plecotus auritus*)

Explanatory variable	Model (group)	Estimates	SSQ	F	df	$r^2$	p	$\sigma^2$	$\tau_{00}$
Intercept	M1 (all)	-1.267	68.654	0.190		0.487	0.663	0.90	0.17
Days after June 1st		-0.003	0.834	0.930	140.6		0.337		
Forearm length		0.233	9.132	10.184	74.3		<b>0.002</b>		
Time after sunset		0.009	41.974	46.809	135.0		<b>&lt;0.001</b>		
Sex		-0.933	16.713	18.639	69.1		<b>&lt;0.001</b>		
Intercept	M2 (Females)	-2.646	40.564	0.433		0.437	0.514	1.01	0.29
Days after June 1st		-0.005	1.774	1.753	104.0		0.188		
Forearm length		0.271	7.257	7.170	43.3		<b>0.010</b>		
Time after sunset		0.009	31.533	31.153	85.9		<b>&lt;0.0001</b>		
Intercept	M3 (Males)	-0.233	10.107	-0.005		0.469	0.946	0.46	0.06
Days after June 1st		0.001	0.072	0.157	31.4		0.694		
Forearm length		0.178	1.875	4.117	20.4		0.056		
Time after sunset		0.008	8.159	17.913	33.3		<b>0.0002</b>		

P values of explanatory variables which had a strong effect on body mass are presented in bold

Results from linear mixed models with continuous response assuming Gaussian error distribution. M1 show fitted model for both sexes combined. M2 and M3 show fitted models and associated model statistic for females and males, respectively

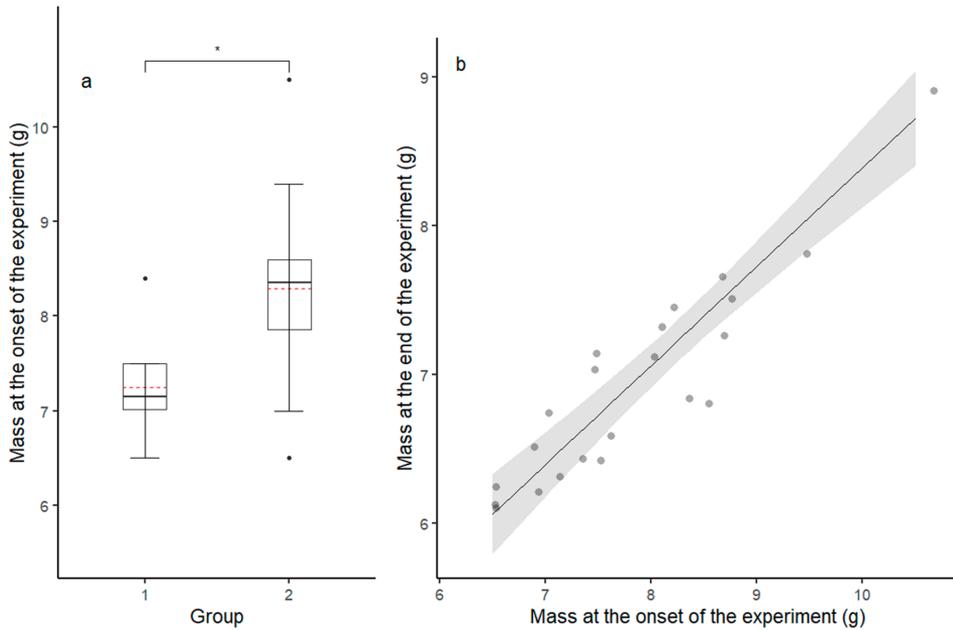


**Fig. 1** Fixed effects influencing body mass (g) in captured *P. auritus* ( $N=89$ ,  $n=157$ ) in Nittedal, Norway between 2017 and 2021. Body mass increased with **a** forearm length (mm), **b** time after sunset (min), and **c** was higher in females than in males. In **a** and **b** solid

lines show estimated relationships and shaded polygons show 95% confidence limits. In **c** the black dots show the estimated average and the whiskers show the 95% CIs. Dots show measured values

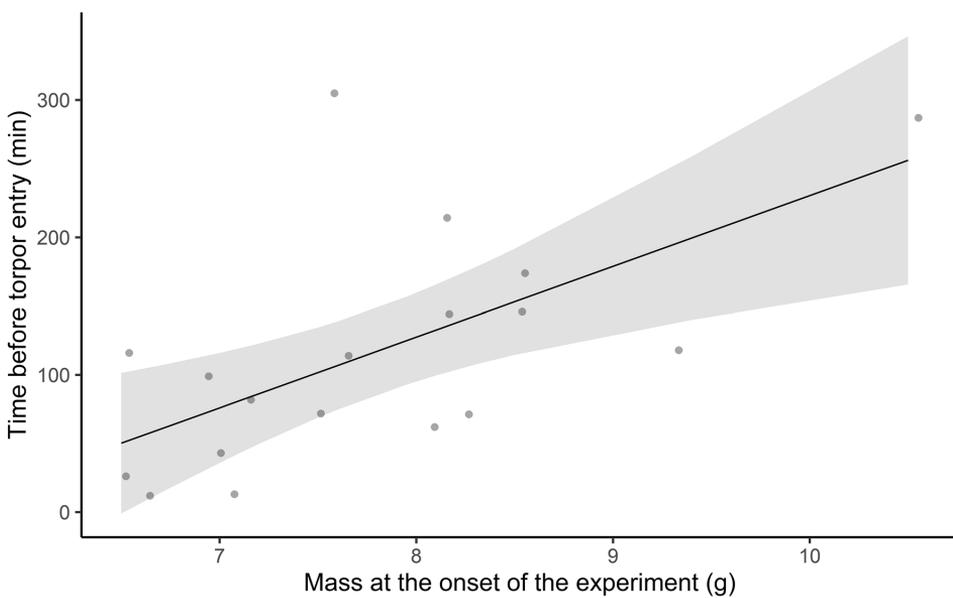
(minutes =  $-284.2 + 51.5 \times g$ ,  $r^2=0.36$ ,  $p=0.005$ ,  $N=19$ , Fig. 3). Broken stick regression revealed that  $VO_2$  decreased until  $29.7^\circ\text{C}$  and provided a better fit than the linear model ( $F=42.5$  vs  $F=23.2$ , respectively). RMR increased with decreasing temperature ( $\text{mL O}_2 \text{ h}^{-1}$ :  $119.1 - 2.94 \times T_a$ ,  $p=0.002$ ,  $N=8$ ,  $n=12$ ), but was not affected by  $M_b$  ( $p=0.658$ ). We, therefore, removed  $M_b$  from the model and repeated the analysis ( $\text{mL O}_2 \text{ h}^{-1}$ :  $104.9 - 2.9 \times T_a$ ,  $p=0.002$ ,  $N=8$ ,  $n=12$ , Fig. 4a). BMR was affected by neither  $M_b$  ( $p=0.403$ ), nor  $T_a$  ( $p=0.549$ ), and was estimated at  $15.5 \pm 3.4 \text{ mL O}_2 \text{ h}^{-1}$  ( $N=11$ ,  $n=24$ ). TNZ was between  $29.7$  and at least up to  $36.4^\circ\text{C}$ . Four individual bats measured had an increased MR at  $36.4 \pm 0.6^\circ\text{C}$  (range  $35.7\text{--}37.0^\circ\text{C}$ ).

During torpor,  $VO_2$  increased exponentially with increasing temperatures for bats that exited torpor before the TNZ was reached ( $\text{mL O}_2 \text{ h}^{-1} = 0.127 \times 1.148^{T_a}$ ,  $r^2=0.65$ ,  $N=12$ ,  $n=43$ , Fig. 4a). A similar relationship was found for bats that remained torpid within the TNZ ( $\text{mL O}_2 \text{ h}^{-1} = 0.141 \times 1.151^{T_a}$ ,  $r^2=0.86$ ,  $N=10$ ,  $n=86$ , Fig. 4b). The minimum measured TMR ( $0.644 \pm 0.493 \text{ O}_2 \text{ h}^{-1}$ ,  $N=21$ ) occurred at  $10.1 \pm 5.2^\circ\text{C}$  ( $N=21$ ) but varied between individuals (range  $0.3\text{--}18.4^\circ\text{C}$ ). The minimum measured TMR per individual was not related to days after June 1st, nor did it differ between bats that aroused before the  $T_{lc}$  and those that did not, or between males and females ( $p=0.756$ ).

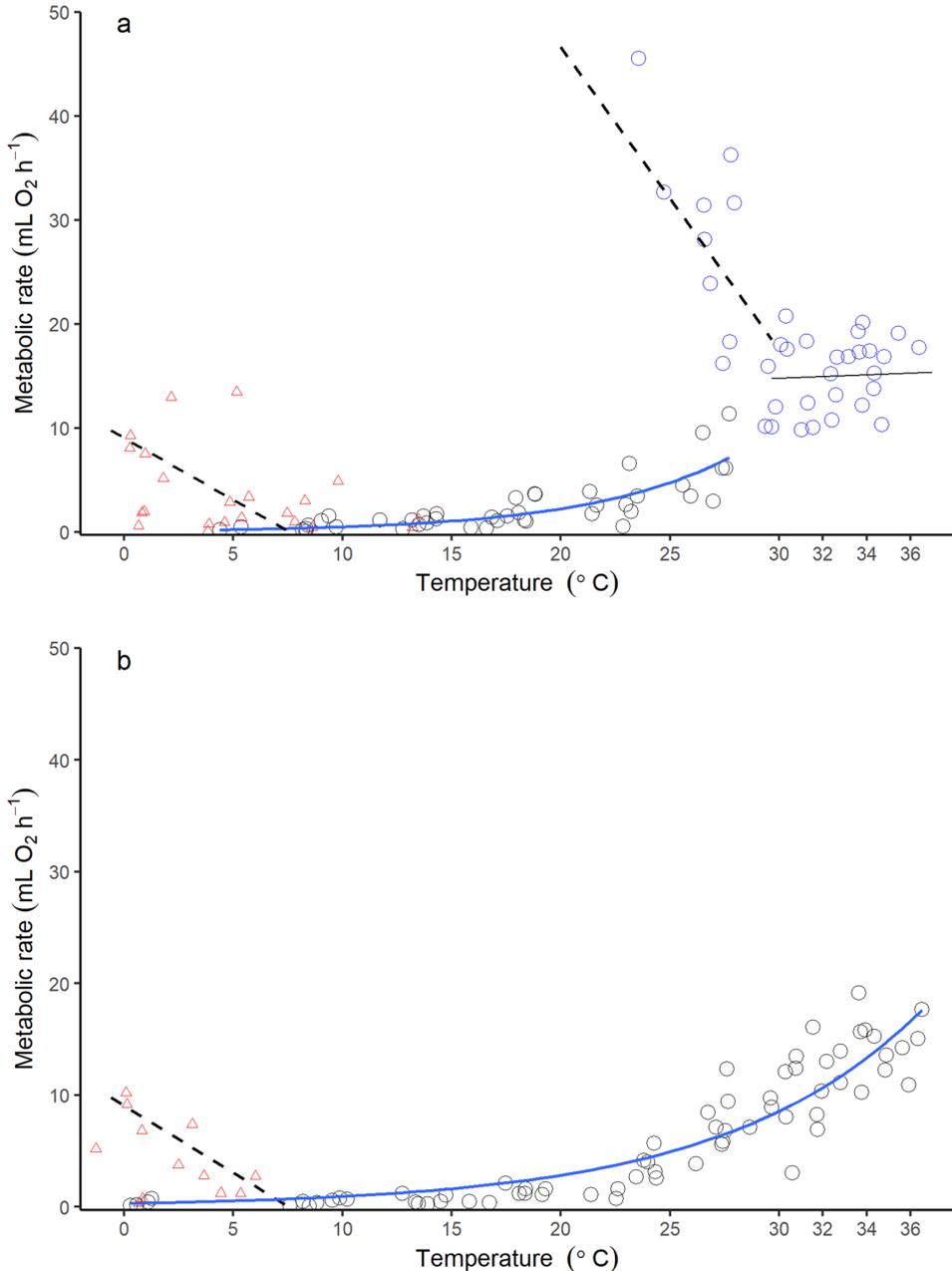


**Fig. 2** Body mass (g) at **a** the onset of the experiment was higher in bats that exited torpor before the estimated lower critical temperature ( $T_{lc}$ ) of the TNZ (Group 2), than those that did not (Group 1).

Body mass (g) at **b** the end of the experiment was higher in bats with a higher body mass at the onset of the experiment ( $g = 1.73 + 0.67 \times g$ ,  $r^2 = 0.87$ ,  $p < 0.005$ )



**Fig. 3** Bats with a higher body mass (g) spent more time exploring the new environment before entering torpor. Solid lines show estimated relationships, shaded polygons show 95% confidence limits and dots show measured values



**Fig. 4** Metabolic rate ( $\text{mL O}_2 \text{ h}^{-1}$ ) as a function of ambient temperature ( $T_a$  °C). Metabolic rate of bats which **a** exited torpor before the TNZ, and bats which **b** remained torpid at temperatures above  $T_{lc}$ . The dotted line in **a** shows the increase in metabolism with decreasing  $T_a$  ( $2.91 \text{ mL O}_2 \text{ h}^{-1} \text{ } ^\circ\text{C}^{-1}$ ,  $N=8$ ,  $n=12$ ) for euthermic bats (RMR). While the dotted line present in both **a** and **b** at  $T_a$  below  $6.73$  °C shows the increase in metabolism with decreasing  $T_a$  ( $1.30 \text{ mL O}_2 \text{ h}^{-1} \text{ } ^\circ\text{C}^{-1}$ ,  $N=10$ ,  $n=21$ ). Blue circles show measure-

ments of included in broken stick regression for RMR and BMR ( $15.5 \pm 3.4 \text{ mL O}_2 \text{ h}^{-1}$ ,  $N=11$ ,  $n=24$ ), indicated by the black line which also shows the range of the TNZ ( $29.67$ – $36.4$  °C). Black circles in both plots show measurements of TMR. Blue line in both plots indicate the exponential growth curve of TMR with increasing  $T_a$  (**a**),  $0.127 \times 1.148^{T_a}$  and **b**  $0.141 \times 1.151^{T_a}$ . Red triangles show plots where bats showed active thermoregulation (colour figure online)

## Active thermoregulation during torpor

When exposed to  $T_{a,s}$  below  $6.7^\circ\text{C}$ , as indicated by the intercept of the thermoregulatory curve of thermoregulating bats and the exponential curve of TMR, bats which showed active thermoregulation increased TMR by  $1.30\text{ mL O}_2\text{ h}^{-1}\text{ }^\circ\text{C}^{-1}$  ( $N=10, n=21$ ; Fig. 4). Average  $M_b$  of the bats that increased  $\text{VO}_2$  at low  $T_a$  was marginally higher ( $8.34 \pm 1.06\text{ g}$ ,  $N=10$ ) at the onset of the experiment than the average  $M_b$  of those that did not increase  $\text{VO}_2$  ( $7.46 \pm 0.81$ ,  $N=8$ ,  $t_{16,0}=1.99$ ,  $p=0.064$ ).

## Arousal

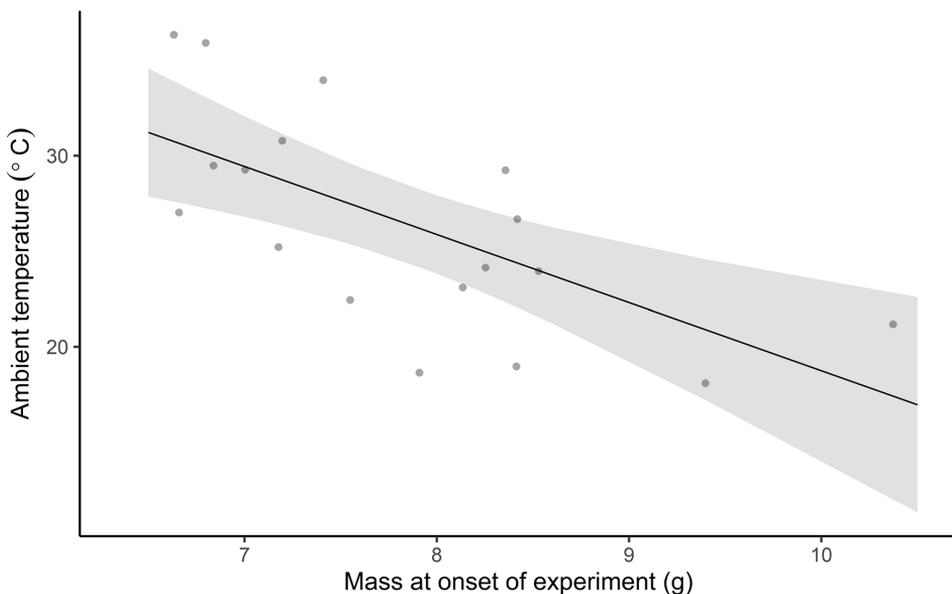
Bats that exited torpor below the  $T_{lc}$  aroused at  $22.9 \pm 3.5^\circ\text{C}$  ( $N=11$ ), while bats that remained torpid into the TNZ aroused at  $31.9 \pm 3.7^\circ\text{C}$  ( $N=7$ ). In four cases, arousal was either not apparent, or occurred during baseline measurements. There was strong evidence for a higher  $\text{VO}_2$  during arousal ( $t_{11,24}=3.96$ ,  $p=0.002$ ) in the bats that aroused at a lower temperature ( $73.78 \pm 40.99\text{ mL O}_2\text{ h}^{-1}$ ), compared to those that aroused later ( $23.44 \pm 7.97\text{ mL O}_2\text{ h}^{-1}$ ), and increased with decreasing  $T_a$  ( $\text{mL O}_2\text{ h}^{-1}=198.38 - 5.46 \times T_a$ ,  $p < 0.001$ ). The number of minutes the bats needed to arouse did not differ between the bats which aroused

at  $T_a < 29.7^\circ\text{C}$ , and those that aroused at  $T_a > 29.7^\circ\text{C}$  ( $t_{11,09} = -1.39$ ,  $p=0.19$ ). Bats with higher  $M_b$  at the onset of the experiment aroused at lower ambient temperatures ( $T_a = 54.38 - 3.56 \times g$ ,  $p=0.003$ , Fig. 5b), but also had a higher estimated  $M_b$  when arousal occurred ( $T_a = 58.08 - 4.42 \times g$ ,  $p=0.004$ ), despite having delayed torpor entry for longer at the onset of the experiment.

## Discussion

By studying how *P. auritus* close to the northern range of its distribution respond physiologically to different temperature conditions, we found that individual state influenced quantitative energetics in this species. As predicted, individual bats with a higher  $M_b$  aroused from torpor at lower  $T_a$ , while individuals with a lower  $M_b$  postponed arousal to higher  $T_a$ . Although we observed substantial active thermoregulation at low temperatures in almost half of the measured bats, the relationship between increased  $\text{VO}_2$  at low  $T_a$  and  $M_b$  was unclear, with only a marginal difference in  $M_b$ .

$M_b$  at time of capture increased as expected with time after sunset. As bats at high latitudes are subject to short nights for foraging during summer (Frafjord 2013; Michaelsen et al. 2018), they need to build up a large energy reserve on an almost daily basis to survive and reproduce. At the same time, they stay in the roost for



**Fig. 5** The ambient temperature ( $T_a$  °C) at which bats aroused decreased ( $y=58.08 - 4.42x$ ,  $p=0.004$ ,  $N=18$ ) with increasing body mass (g) at the onset of the experiment

approximately 20 h each day. Thus, depending on the amount of time during the day spent torpid, they generally leave the roost with a relatively low  $M_b$  as they will have consumed a large portion of their gut fill.

TMR was similar between bats that aroused before the  $T_{lc}$ , and those that did not. TMR increased exponentially with increasing  $T_a$ , and is similar to that of equally sized vespertilionid bats in the southern hemisphere (Geiser and Brigham 2000; Turbill et al. 2008; Stawski and Geiser 2011). Thus, further supporting the notion that torpor in subtropical vespertilionid bats does not differ from temperate vespertilionid bats (Stawski and Geiser 2011; Fjellidal et al. 2022), as torpor is a mechanism to cope with energy limitation at low  $T_a$  regardless of the habitat.

Interestingly, only half of the bats in the present study showed signs of active thermoregulation during torpor at low  $T_a$ , despite all 21 bats being exposed to  $T_a$  below the estimated critical  $T_a$  of 6.7 °C. However, bats which showed active thermoregulation only had a marginally higher  $M_b$  at the onset of the experiment compared to those that did not. In a study conducted by Currie et al. (2018) where both RMR and the torpor thermoregulation curve of bats was estimated, the metabolic rate of both curves had an equal increase. A trend which is similar to what is found in other animals that employ torpor (see Geiser 2021). In contrast, in our study, active thermoregulation at low  $T_a$  was with a much shallower increase in TMR than what was observed in resting bats (i.e., RMR).

In the literature, the critical  $T_a$  is often referred to as a species or population specific temperature threshold, which bats have evolved as long-term adaptations to their respective environmental conditions (Stawski and Geiser 2011). However, as the critical  $T_a$  estimated in the present study is higher than the  $T_a$  in which *P. auritus* regularly hibernate at this latitude (Wermundsen and Siivonen 2010), and considerably higher than the lowest measured  $T_b$  of -2 °C (Eisentraut 1956), this indicates that individual bats may choose the level of thermoregulation to some extent at different  $T_a$  to avoid torpor-related costs. A lower increase in  $VO_2$  when thermoregulating at low  $T_a$  may be related to a higher level of thermal insulation. As the length of fur coat is equal during the individual experiments here, the assumed difference in insulation could be due to a greater peripheral part of the body being kept cold, and  $T_b$  is only defended in vital parts of the body. Due to this increase in insulation, bats will only need to increase  $VO_2$  to a lesser extent to prevent tissue damage to vital organs. It is also worth noting that our measurements were performed during summer, as bats may defend a higher  $T_b$  at this time of year as opposed to during the hibernation season. But the question as to why some bats choose to thermoregulate and some do not, remains unanswered.

It is becoming more and more clear that  $M_b$  not only affects metabolism but can also affect the metabolic strategy of the animal. As observed here, bats with higher  $M_b$  readily spent more energy to delay torpor. All bats in our study showed similar TMR when thermoconforming but differed in level of exploration at the onset of the experiment, active thermoregulation at lower  $T_a$ , and timing of arousal. Bats with a higher  $M_b$  at the onset of the experiment aroused at lower  $T_a$ , and lost more  $M_b$  during the experiment. This loss in  $M_b$  was also related to these bats delaying torpor for a longer period at the onset of the experiment. Similarly, recently fed *Myotis lucifugus* delay torpor entry independent of temperature (Matheson et al. 2010). Similar behavioural responses have been seen in edible dormice (*Glis glis*), where heavier animals aroused more frequently and stayed euthermic for longer (Bieber et al. 2014), and woodchucks (*Marmota monax*), which defended a higher  $T_b$  when more energy was available (Zervanos et al. 2013).

The delay of torpor in recently fed bats, along with an earlier arousal, is presumably related to a trade-off between different costs and benefits of torpor. Although there are potential costs of maintaining torpor for longer time periods, such as sleep deprivation (Humphries et al. 2003), predation (Estok et al. 2010; Haarsma and Kaal 2016), and buildup of waste materials (Thomas and Geiser 1997; Ben-Hamo et al. 2013; Landes et al. 2020), the physiological benefits include water and energy conservation. Thus, on a day-to-day basis during the active season of the year, the physiological benefits of limiting energy consumption and water loss is likely to outweigh the costs.

As bats in our study area, and at similar latitudes, will daily spend up to 20 h in roosts without access to water (i.e., barns or trees), the risk of dehydration is potentially a contributing reason to remain in torpor during daytime. As water consumption prior to the experiment was not controlled for,  $M_b$  could also be affected by hydration level in individual bats. This effect may be exacerbated in typical studies of metabolic rate, as dry air will lead to an increased evaporative water loss.

Another potential explanation for the earlier arousal can be that heavier bats exert the reserve energy to allow for restorative activities, such as sleep (Humphries et al. 2003), protein synthesis (Heldmaier et al. 2004; Landes et al. 2020), or even digestion of previously consumed food (Turbill et al. 2008). As all bats left fecal droppings in the chamber during the experiment, some digestion must have occurred. It is, however, unknown whether this occurred prior to torpor entry, following arousal, or both. Thus, reinitiating digestion and being able to move away from potential threats in an unfamiliar environment may explain why bats with larger  $M_b$  opted for leaving torpor at an earlier stage. Additionally, in a wider context, it may be beneficial for individual bats to arouse earlier to allow

for social interactions, such as grooming (Chaverri et al. 2018) and information transfer (Gager 2018). Thus, the optimal timing of arousal from torpor is probably a trade-off between the physiological benefits of torpor and the ecological costs of missing out on the benefits of euthermia. Accordingly, the condition of an individual bat should affect its decision-making under these opposing pressures.

In summary, the present study highlights how metabolism of individual bats is affected by their condition, as bats with a larger energy reserve readily arouse at lower  $T_{as}$ , possibly to counteract the negative effects of torpor. Similarly, active thermoregulation occurred in only half of all measured bats and at  $T_{as}$  higher than what they normally experience during hibernation. Additionally, the increase in metabolic rate during active thermoregulation when torpid has a shallower slope compared to that of normothermic bats resting at similar temperatures below the TNZ. This indicates that the bats altered their thermal conductance by an increased insulation. Essentially, when and to which extent individual bats actively increase heat production may be behaviorally flexible, and not physiologically fixed.

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**Author contributions** RS and CS framed the idea of the experiment. RS, MAF, and JK collected the data. RS and KHS performed the preliminary analysis of the data. RS wrote the initial draft. CS acquired the funding for the project. CS, KE, CB and JK provided additional resources. All authors participated in the writing and revision of the manuscript.

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**Data availability** The data collected and analysed during the current study are available from the corresponding author on reasonable request.

## Declarations

**Conflict of interest** The authors declare that they have no conflict of interest in the authorship of this article. Use of product or corporation names is for descriptive purposes only and implies no endorsement by any author or affiliation.

**Ethics approval** Permits for the capture and handling of *P. auritus* were granted by the Norwegian Food Safety Authority (FOTS ID 23284) and the Norwegian Environment Agency (ref. 2018/4899).

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## State dependence of arousal from torpor in Brown long-eared bats (*Plecotus auritus*)

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## Supplementary information

**Figure S1** Example of one male *Plecotus auritus* which exited torpor prior the TNZ. Top panel shows mass-specific metabolic rate ( $\dot{V}O_2$ , mL O<sub>2</sub> h<sup>-1</sup> g<sup>-1</sup>), with each color indicating each  $T_{set}$ . Start and peak of arousal is indicated by red arrows.  $\dot{V}O_2$  from when the bat was placed in the chamber and the experiment started has been excluded. The middle panel shows estimated mass loss over the course of the experiment. The lower panel shows the variation in  $T_a$  over the course of the experiment, with each  $T_{set}$  illustrated with the same colors as in the upper panel.

**Figure S2** Example of one male *Plecotus auritus* that exited torpor after entering the TNZ. Top panel shows mass-specific metabolic rate ( $\dot{V}O_2$ , mL O<sub>2</sub> h<sup>-1</sup> g<sup>-1</sup>), with each color indicating each  $T_{set}$ . Start and peak of arousal are indicated by red arrows.  $\dot{V}O_2$  from when the bat was placed in the chamber and the experiment started has been excluded. The middle panel shows estimated mass loss over the course of the experiment. The lower panel shows the variation in  $T_a$  over the course of the experiment, with each  $T_{set}$  illustrated with the same colors as in the upper panel.

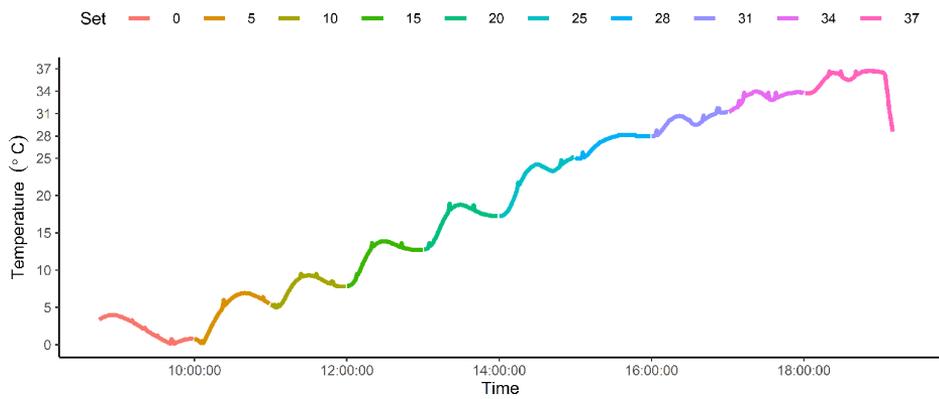
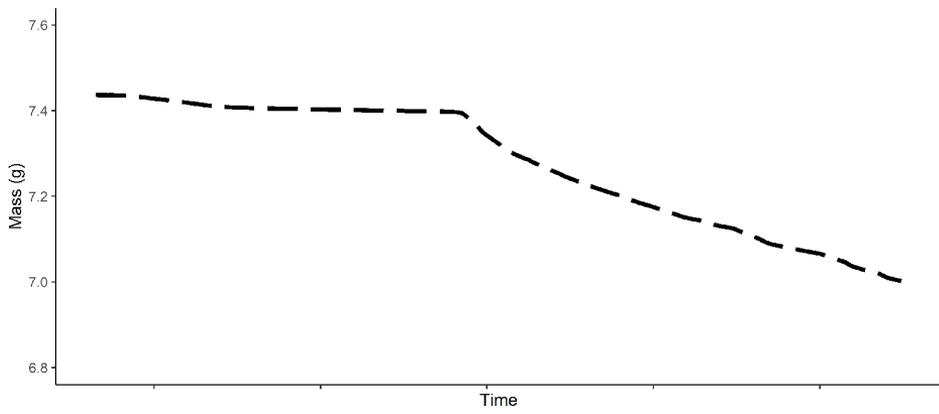
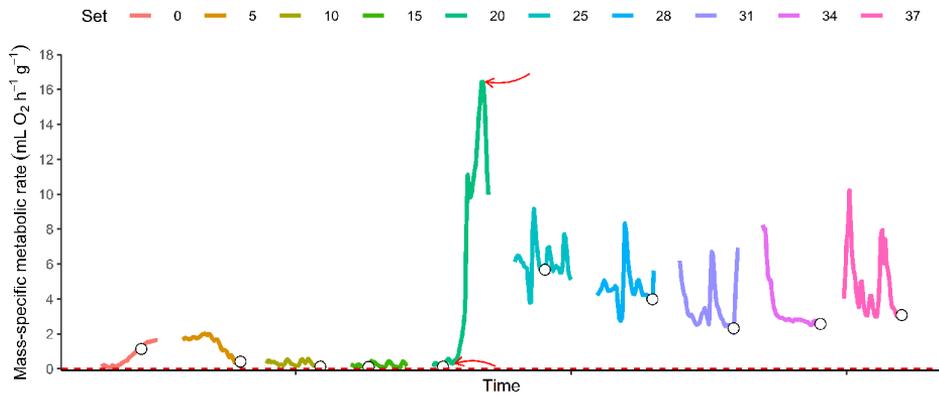


Figure S1

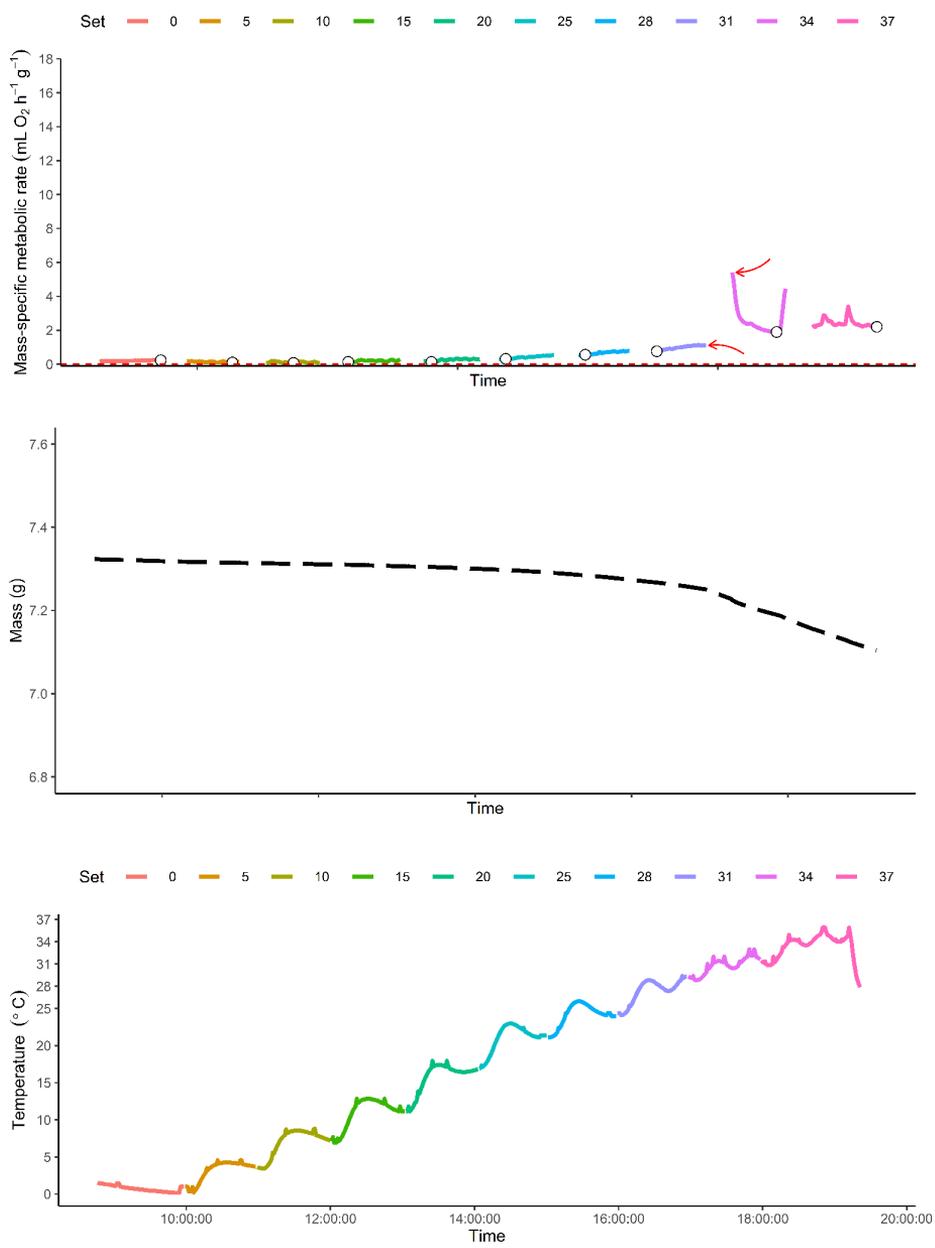


Figure S2

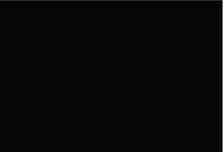
Table S1 Summary information of mean  $\pm$  SD torpor metabolic rate (TMR), thermoregulation of torpid bats (TMR<sub>th</sub>), resting metabolic rate (RMR), basal metabolic rate (BMR), and ambient temperature ( $T_a$ ) at each Set temperature ( $T_{set}$ ). Number of samples ( $N$ ) at each  $T_{set}$  is also included.

$T_{set}$	0°C	5°C	10°C	15°C	20°C	25°C	28°C	31°C	34°C	37°C
TMR	0.36 $\pm$ 0.42	0.17 $\pm$ 0.11	0.11 $\pm$ 0.13	0.11 $\pm$ 0.05	0.18 $\pm$ 0.11	0.44 $\pm$ 0.37	0.78 $\pm$ 0.36	1.20 $\pm$ 0.44		
$T_a$	0.85 $\pm$ 1.47	4.15 $\pm$ 1.89	8.75 $\pm$ 0.69	13.6 $\pm$ 0.76	17.8 $\pm$ 1.0	23.0 $\pm$ 1.0	26.7 $\pm$ 0.97	29.5 $\pm$ 1.43		
$N$	6	15	18	16	19	17	13	10		
TMR <sub>th</sub>	0.89 $\pm$ 0.39	0.86 $\pm$ 0.63								
$T_a$	1.09 $\pm$ 0.87	5.44 $\pm$ 0.39								
$N$	9	2								
RMR						4.84 $\pm$ 1.18	3.17 $\pm$ 0.90			
$T_a$						24.2 $\pm$ 0.82	27.3 $\pm$ 0.60			
$N$						2	7			
BMR								1.61 $\pm$ 0.60	1.75 $\pm$ 0.46	1.90 $\pm$ 0.24
$T_a$								29.7 $\pm$ 1.27	32.7 $\pm$ 0.95	35.1 $\pm$ 0.94
$N$								11	21	15

# Paper II



Sørås, Rune; Fjelldal, Mari Aas; Bech Claus; van der Kooij, Jeroen; Eldegard Katrine; Stawski, Clare. High latitude northern bats (*Eptesicus nilssonii*) reveal adaptations to both high and low temperatures. Submitted to Journal of Experimental Biology.



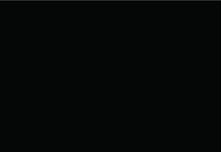
This paper is in review for publication and is therefore not included.



# Paper III



Sørås, Rune; Fjellidal, Mari Aas; Eldegard, Katrine; Stawski, Clare.  
Defining torpor entries of bats based on rate of change in metabolic rate  
per minute. Manuscript.

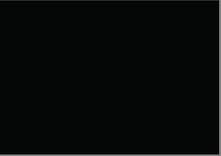


This paper is not yet published and is therefore not included.



# Paper IV





## RESEARCH ARTICLE

# Energetics of whiskered bats in comparison to other bats of the family Vespertilionidae

Karoline H. Skåra<sup>1,\*</sup>, Claus Bech<sup>1</sup>, Mari Aas Fjellidal<sup>1</sup>, Jeroen van der Kooij<sup>2</sup>, Rune Sørås<sup>1</sup> and Clare Stawski<sup>1,‡</sup>

## ABSTRACT

Bats inhabit a variety of climate types, ranging from tropical to temperate zones, and environmental differences may therefore affect the basal metabolic rate (BMR) of bats from different populations. In the present study, we provide novel data on the energetics of whiskered bats (*Myotis mystacinus*), which is the smallest species within Chiroptera measured to date. We investigated the thermoregulatory strategies of *M. mystacinus* close to the northern limits of this species' distribution range and compared these data to other vespertilionid bats living in different climates. As mammals living in colder areas experience elevated thermoregulatory costs, often leading to an increase in BMR, we hypothesised that BMR of this northern population of whiskered bats would be higher than that of bats from climates with warm environmental temperatures. From a systematic literature search we obtained BMR estimates ( $N=47$ ) from 24 species within Vespertilionidae. Our metabolic measurements of *M. mystacinus* in Norway (body mass of 4.4 g; BMR of 1.48 ml  $O_2$   $g^{-1}$   $h^{-1}$ ) were not different from other vespertilionid bats, based on the allometric equation obtained from the systematic literature search. Further, there was no effect of environmental temperature on BMR within Vespertilionidae. How these tiny bats adapt metabolically to high latitude living is thus still an open question. Bats do have a suite of physiological strategies used to cope with the varying climates which they inhabit, and one possible factor could be that instead of adjusting BMR they could express more torpor.

This article has an associated First Person interview with the first author of the paper.

**KEY WORDS:** Allometric scaling, BMR, Chiroptera, Insectivorous, *Myotis mystacinus*, Thermoregulation

## INTRODUCTION

The daily energetic challenges experienced by animals readily affect how they function at the individual, population, and species levels (Garland and Adolph, 1991). While energetically expensive, sustaining a high body temperature is often beneficial for endotherms as it allows for behavioural adjustments such as faster

movement and the ability to stay active in cold temperatures. All endotherms display a range of temperatures where they do not expend additional energy to maintain a high body temperature, known as the thermoneutral zone. Energy expenditure, or metabolic rate, within the thermoneutral zone is termed basal metabolic rate (BMR) and represents the minimum amount of energy needed to maintain homeostasis (Hill et al., 2016; Withers et al., 2016). As variation in BMR reflects the habitat or ecosystem the animal lives in, BMR is often used as a standard energetic parameter in ecological studies (Garland and Adolph, 1991; White and Kearney, 2013).

Basal metabolic rate varies greatly in mammals, with body mass explaining 95.5% of the overall variation (McNab, 2008; Withers et al., 2016). Body mass thus explains most of the variation in BMR, with the remaining variation being due to a number of factors, such as food habits, climate and habitat (McNab, 2008). It has been suggested that BMR, after removing the effect of body mass, should increase with latitude to meet the energetic demands of inhabiting colder areas (Lovegrove, 2003). An increase in BMR with increasing latitude, and consequently a decreasing environmental temperature, has indeed been found in both mammals (Clarke et al., 2010; Lovegrove, 2003; Raichlen et al., 2010) and birds (Hails, 1983; Tieleman et al., 2002; Weathers, 1979; Williams and Tieleman, 2000). It has also been reported in studies of single mammalian groups, such as canids (Careau et al., 2007) and rodents (MacMillen and Garland, 1989; Rezende et al., 2004; Speakman, 1999). As an increase in BMR with increasing latitude has been found in birds and other mammals, this trend could also be expected for bats. Yet, Cruz-Neto and Jones (2006) and Speakman and Thomas (2003) investigated the effect of latitude on BMR in bats in general, and neither found a significant effect of latitude. As most bat species are found in the tropics (Barclay and Harder, 2003), it has been suggested that environmental temperature could be a better predictor of BMR in bats than latitude (Speakman and Thomas, 2003). However, Willis et al. (2005b) found no effect of mean environmental temperature on BMR among different populations of bats. Nevertheless, heat loss in cold environments is inevitable, and adaptations to inhabit cold environments and living at high latitudes should thus have evolved in bats. As bats inhabit a variety of climate zones, different species and populations may have adapted a variety of metabolic traits to survive the environmental conditions encountered. Belonging to the extremely diverse and species-rich order of Chiroptera, bats are found on all continents except Antarctica, with breeding populations of the northernmost species found at latitudes of 69°N, north of the Arctic circle (Rydell, 1992; Rydell et al., 1994).

Vespertilionidae is the largest family within Chiroptera, making up around one-third of all bat species (Barclay and Harder, 2003). The body size of vespertilionid bats is in general small, varying from 2 to 91 g, and almost all species are insectivorous (Moratelli et al., 2019). The BMR of vespertilionid bats is lower than expected from their body mass compared to other bat species (McNab, 1980b) and

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to mammals in general, being 53–93% of that predicted for mammals of the same size (Hosken and Withers, 1997). One of the smallest bats within the family Vespertilionidae is the whiskered bat (*Myotis mystacinus*), weighing between 4 and 7 g. The species is observed throughout most of Europe, with the northernmost observation at 64°N (Dietz and Kiefer, 2016; Rydell et al., 1994). Small insectivorous bats at northern latitudes, such as *M. mystacinus*, encounter seasonally cold temperatures and low prey abundance, which impose great energetic costs (Speakman et al., 2000). Due to long winters and short summer nights, the seasonal and daily active periods are limited, which also imposes a constraint on energy acquisition. The thermal challenges associated with these constraints may impact the thermoregulatory curve and BMR of *M. mystacinus*.

Therefore, the aim of the present study was twofold: (1) to measure the BMR and establish the thermoregulatory curve of the previously unstudied *M. mystacinus* inhabiting a seasonally cold environment at their northern distribution limits in Norway, and (2) to investigate whether the BMR of *M. mystacinus* reveal a higher BMR compared to other vespertilionid bats in order to cope with the energetic challenges encountered in a seasonally cold environment. We hypothesised that environmental temperature would affect BMR of *M. mystacinus*, enabling the species to adapt to various climates, including high latitude living. Additionally, we predicted that BMR will be higher in vespertilionid bats living in climates with colder environmental temperatures at high latitudes compared to warmer climates at low latitudes. Knowledge on the thermoregulatory challenges and adaptations encountered by small mammals, such as bats, to a range of environmental conditions is vital for understanding how they may or may not cope with future energetic challenges.

## RESULTS

### Thermoregulation in *M. mystacinus*

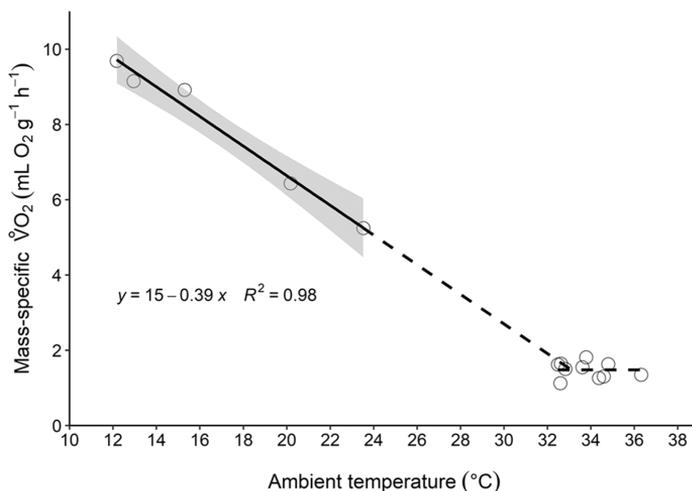
At low ambient temperatures there was a significant negative effect of ambient temperature on the resting  $\dot{V}O_2$  of *M. mystacinus* ( $f_{1,3}=162.2$ ,  $P=0.001$ , Fig. 1 and Table S1), with ambient temperature explaining 98% of the variation in  $\dot{V}O_2$ . The  $\dot{V}O_2$  increased by  $0.39\pm 0.03$  ml  $O_2$   $g^{-1}$   $h^{-1}$  per 1°C decrease in ambient temperature (body mass= $4.9\pm 0.2$  g,  $N_{\text{individuals}}=4$ ,  $N_{\text{measurements}}=5$ ).

The linear relationship between ambient temperature and  $\dot{V}O_2$  at low ambient temperature is given by the equation  $y=14.51-0.39x$  (Table S1). The slope of this regression approximates thermal conductance ( $0.39$  ml  $O_2$   $g^{-1}$   $h^{-1}$   $^{\circ}C^{-1}$ ; body mass= $4.9\pm 0.2$  g,  $N_{\text{individuals}}=4$ ,  $N_{\text{measurements}}=5$ ), and extrapolating the line to the x-axis provided an estimate of body temperature of  $37.2^{\circ}C$ . The mean BMR was  $1.48\pm 0.07$  ml  $O_2$   $g^{-1}$   $h^{-1}$  (body mass= $4.4\pm 0.1$  g,  $N_{\text{individuals}}=6$ ,  $N_{\text{measurements}}=10$ ), illustrated by the horizontal dashed line in Fig. 1. The inflection point between the two dashed lines indicates a lower critical temperature of  $33.1^{\circ}C$ .

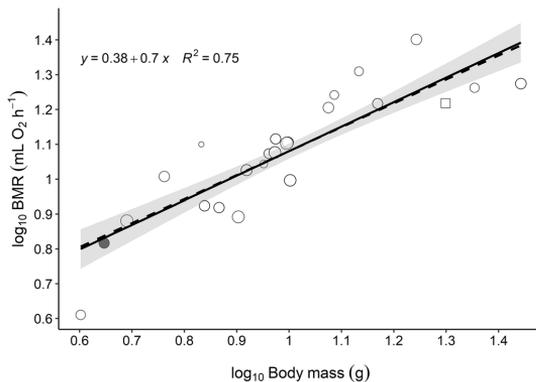
### BMR in Vespertilionidae

As expected, the BMR of bats within Vespertilionidae was significantly correlated to body mass ( $f_{1,44}=129.0$ ,  $P<0.001$ , Fig. 2 and Table S1), with body mass explaining 75% of the variation in BMR. This did not include our own BMR measurement of *M. mystacinus*. The equation of the linear relationship between  $\log_{10}$  body mass and  $\log_{10}$  BMR was  $y=0.38+0.70x$  (Table S1). The slope of this equation, i.e. the allometric scaling exponent, was  $0.71\pm 0.01$ . A weighted linear regression was also calculated, in which each estimate was weighted by their precision. The precision was given as the inverse of the variation, calculated as the mass-specific standard error (SE) (Fig. S4). One of the estimates included in the analysis was a measure of only one individual, and as a result had no measure of variation. This estimate was therefore not included in the weighted regression (dashed line, Fig. 2), but it was included in the non-weighted regression (solid line, Fig. 2) as it did not significantly affect the scaling exponent. There was no significant difference between the weighted and the non-weighted regressions, thus possible variation due to methodology did not affect the results.

The observed BMR for *M. mystacinus* was, based on the data for Vespertilionidae obtained in the present study, 99% of the predicted BMR for a vespertilionid bat of 4.4 g (Fig. 2). Using the allometric equation for mammals in general from McNab (2008), the mean BMR for vespertilionid bats included in the present study had a BMR 65% of that predicted for a mammal weighing 11.3 g. Using the same equation, the BMR of *M. mystacinus* was 64% of that predicted for a mammal weighing 4.4 g. As body mass had a strong effect on BMR, residuals from the relationship between BMR and



**Fig. 1. The thermoregulatory curve obtained for *M. mystacinus*.** The metabolic rate is given as mass-specific  $\dot{V}O_2$  (ml  $O_2$   $g^{-1}$   $h^{-1}$ ) as a function of ambient temperature ( $^{\circ}C$ ). Each circle represents one  $\dot{V}O_2$  measurement ( $N_{\text{individuals}}=7$ ,  $N_{\text{measurements}}=15$ ). The solid line represents the linear regression for resting metabolic rate, with the equation  $y=14.51-0.39x$  ( $f_{1,3}=162$ ,  $P=0.001$ , Table S1). The grey area shows the 95% confidence interval for the regression line. The decreasing dashed line is an extrapolation of the linear regression, whereas the horizontal dashed line is a visual representation of the mean BMR.



**Fig. 2. The relationship between  $\log_{10}$  body mass (g) and  $\log_{10}$  BMR ( $\text{ml O}_2 \text{ h}^{-1}$ ) in vespertilionid bats.** The size of the data points represents the precision of the BMR estimate, calculated as the inverse of the standard error (SE) corrected for body mass. The larger the circle, the more precise is the estimate. The solid line and the equation show the linear relationship between body mass and BMR, excluding the BMR estimate of *M. mystacinus* measured in the present study. This estimate is instead illustrated with the filled circle. The non-weighted allometric equation excluding the BMR of *M. mystacinus* was  $y=0.38+0.7x$  ( $f_{1,44}=129.0$ ,  $P<0.001$ , Table S1), whereas including the BMR of *M. mystacinus* it was  $y=0.37+0.71x$  ( $f_{1,45}=146.6$ ,  $P<0.001$ , Table S1). The dashed line shows the same relationship as the solid line but calculated using weighted means ( $y=0.35+0.73x$ ,  $f_{1,43}=85.2$ ,  $P<0.001$ , Table S1). The grey area shows the 95% confidence interval for the non-weighted regression. The square shape represents a BMR estimate of one individual, which prevented the calculation of precision.

body mass were used to compare the BMR independently of body mass. Based on visual inspection, the variance in residual BMR had no obvious relations to differences in sex, time of measurement during the year, reproductive state, or whether kept in captivity or not (Fig. 3). Enough detailed information was not available to test these effects statistically (Fig. S1). Hence, more information is needed to take the possible effects from sex, season, reproductivity and captivity into account.

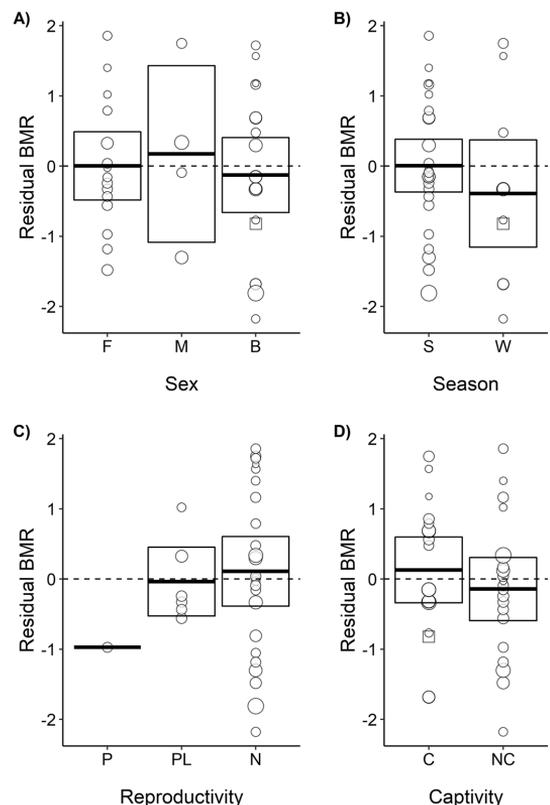
To analyse the variation in BMR within Vespertilionidae along a temperature gradient, we also included our own BMR estimate of *M. mystacinus* in the allometric equation used to calculate residual BMR. The equation of this allometric relationship was  $0.37+0.71x$  ( $f_{1,45}=146.6$ ,  $P<0.001$ , Table S1). The BMR estimates included in the present study were represented by populations inhabiting areas ranging from  $42^\circ \text{S}$  to  $60^\circ \text{N}$  (Fig. S2). The measured environmental temperature collected from these locations ranged from a mean of  $5.0\pm 0.3^\circ \text{C}$  to a mean of  $21.1\pm 1.0^\circ \text{C}$ , representing a wide environmental gradient. However, there was no significant relationship between residual BMR and mean environmental temperature ( $f_{1,45}=0.020$ ,  $P=0.66$ , Fig. 4 and Table S1), even though there was a significant negative relationship between mean environmental temperature and latitude (Fig. S3). The relationship did not change if the southern or northern estimates were excluded.

## DISCUSSION

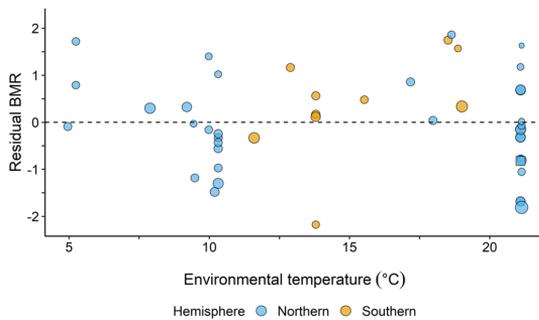
The present study provides novel data on the thermal energetics of *M. mystacinus* living at high latitudes in Norway, including BMR of the smallest bat species and northernmost bat population ever measured. In addition, we provide information on the variation of BMR within vespertilionid bats along a latitudinal and

environmental temperature gradient. Our results contribute to an increased understanding of the energetic costs faced by bats in thermally challenging environments. In contrast to our hypothesis, the population of *M. mystacinus* in the present study revealed a BMR that was not higher than expected from the allometric equation obtained from vespertilionid bats. This was unexpected as a higher BMR was predicted to compensate for increased heat loss to deal with the cold environmental temperature associated with high latitude living. Further, there was no effect of environmental temperature on the BMR in vespertilionid bats that have been studied to date. It is likely that *M. mystacinus* employ other thermoregulatory strategies at their northern range limit to deal with the harsh winter conditions.

The estimated lower critical temperature of  $33.1^\circ \text{C}$  for *M. mystacinus* was within the range of what has been measured for other bat species in general, ranging between  $25.0$  and  $34.7^\circ \text{C}$  (Soriano et al., 2002), and also for that of vespertilionid bat species, ranging between  $30.5$  and  $37.7^\circ \text{C}$  (Genoud, 1993; Genoud and Christie, 2011; Kolokotronis et al., 2010; McNab, 2008; Marom et al., 2006; Muñoz-García et al., 2012; Stawski and Geiser, 2011; Willis et al., 2005a,b). In addition, Fristoe et al. (2015) presented



**Fig. 3. Residual BMR of bats in different groups.** Effect of sex [females (F), males (M), both (B)], season [summer (S), winter (W)], reproductivity [pregnant (P), post-lactating (PL), non-reproductive (N)] and captivity [captive (C), non-captive (NC)]. Measurements of BMR where this information was not available are not included here but shown in Fig. S1.



**Fig. 4. Residual BMR as a function of mean environmental temperature for species from both the southern and northern hemispheres.** Residual BMR was calculated from the relationship between body mass and BMR from Fig. 2. A residual variation of 0.0 is illustrated by the dashed line. The size of the data points represent the precision of the estimate, calculated as the inverse of the variance corrected for body mass. The larger the circle, the more precise is the estimate. The square shape represents a BMR estimate of one individual, which prevented the calculation of precision.

data on lower critical temperature of 41 bat species. Using these data, we obtained an allometric equation relating lower critical temperature with body mass within Chiroptera [lower critical temperature ( $^{\circ}\text{C}$ ) =  $32.650 \times \text{body mass (g)}^{-0.0439}$ ]. As a result of this, we found that the lower critical temperature of *M. mystacinus* was 109% (body mass =  $4.7 \pm 0.2 \text{ g}$ ,  $N_{\text{individuals}}=7$ ,  $N_{\text{measurements}}=15$ ) of the predicted lower critical temperature of bat species within Chiroptera of the same body mass. A steep increase in metabolic heat production was found below this lower critical temperature as ambient temperature declined. This was expected as the body mass of *M. mystacinus* is small, and thermal conductance is generally high for small mammals (Withers et al., 2016). Using the same data from Fristoe et al. (2015) as mentioned above, we obtained an allometric equation relating thermal conductance with body mass within Chiroptera [thermal conductance ( $\text{ml O}_2 \text{ g}^{-1} \text{ h}^{-1} \text{ }^{\circ}\text{C}^{-1}$ ) =  $0.9307 \times \text{body mass (g)}^{0.5481}$ ]. Compared to other bat species, the population of *M. mystacinus* measured in the present study had a low thermal conductance, being 88% of that expected for a bat of similar body mass. In addition, the calculated body temperature of  $37.2^{\circ}\text{C}$  for this population was close to a probable body temperature for vespertilionid bats, which has been found to be  $>32^{\circ}\text{C}$  in eutheria (Currie et al., 2015; Geiser and Brigham, 2000; Genoud, 1993; Hosken and Withers, 1997, 1999; Withers et al., 2016). Therefore, the low thermal conductance could indicate that this northern population may have evolved good insulation mechanisms to maintain a high body temperature while maintaining the same BMR as bats from warmer climates; thereby avoiding increased energy requirements.

The BMR of *M. mystacinus* was 99% of that predicted for a vespertilionid bat weighing  $4.4 \text{ g}$ , which indicates that no specific adaptation in BMR has evolved for this specific population to inhabit the cold temperatures at a latitude of  $60^{\circ}\text{N}$  compared to other bat species of Vespertilionidae. Even though the BMR of this population of *M. mystacinus* fitted the allometric relationship well, it is possible that the BMR of southern populations would not as the distribution of this species ranges from  $35^{\circ}\text{N}$  to  $64^{\circ}\text{N}$  (Moratelli et al., 2019). However, no prior studies have investigated the thermal strategies of *M. mystacinus*. Therefore, it remains unknown how the BMR calculated in the present study compares to that of other populations.

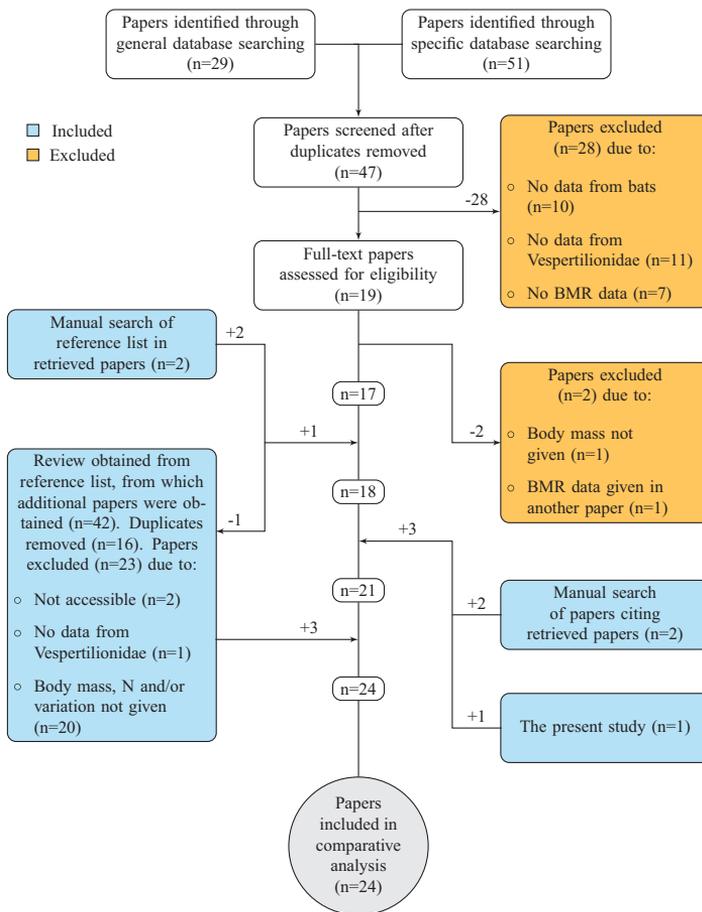
Importantly, our data are also a vital contribution as no studies have measured the BMR of *M. mystacinus* nor of bats residing at high, northern latitudes, such as in Norway. Using the allometric equation obtained from McNab (2008), the vespertilionid bats from the present study displayed a BMR that was 65%, and *M. mystacinus* a BMR that was 64%, of that predicted for mammals of the same size.

The low BMR in vespertilionid bats compared to other mammals could have evolved to sustain the high energetic requirements encountered by all these species, such as that required by flying or inhabiting thermally challenging environments. In theory, flight by bats increases the metabolic rate 17 times above BMR, so the energetic costs are high, which would also increase body temperature (Withers et al., 2016). Hence, in addition to reducing the overall energetic costs, low BMR could also be a trade-off to avoid increased heat production and overheating during flight. Even though low BMR is characteristic of Vespertilionidae, mass-independent variation in BMR was still present within the family, and this variation remains to be explained.

No effect of environmental temperature on BMR was found in vespertilionid bats, which could indicate that other adaptations to high latitude living have evolved. Bats residing in areas with low environmental temperatures should have to compensate for increased heat loss, for example by revealing higher BMR compared to bats residing in warmer areas. Increasing BMR would enhance cold tolerance, but it would also require even more energy in an already energy scarce environment. If BMR was lowered, on the other hand, it could shift the whole thermoregulatory curve down, reducing the energy requirements even at low temperatures (Stawski et al., 2017; Withers et al., 2016). A decrease in environmental temperature is paralleled by a decrease in available resources, including insects, which could potentially result in a lower BMR in bats inhabiting cold temperatures (Dunbar and Brigham, 2010; Lovegrove, 2000). However, no effect of environmental temperature on BMR was found among the vespertilionid bats included in the present study.

Aside from increasing BMR, there are other potential strategies that bats could employ to deal with the cold. Bats residing in cold environments could increase torpor use, a controlled reduction in metabolic rate and body temperature to reduce thermoregulatory costs, to effectively deal with the cold temperatures. Most bat species studied to date employ torpor, even in warm tropical climates, suggesting it is a vital energy management strategy in bats (Stawski et al., 2014). Interestingly, Cooper and Geiser (2008) found that low BMR was associated with increased use of torpor within rodents, but not within bats. They suggested that bats with high BMR may in fact enter torpor more frequently than bats with low BMR, as their energetic costs are higher and need to be compensated for. Instead, a low BMR could be a result of lower energy availability (Cooper and Geiser, 2008). In addition to the already mentioned strategies, some vespertilionid species are also able to migrate to warmer climates, and escaping the cold may be less energetically costly than coping with it (Fleming and Eby, 2003). Hence, it would be interesting to include information on torpor use and migration patterns in future comparative analyses of BMR among species of Vespertilionidae. However, little detailed information is currently available on these parameters (Moratelli et al., 2019).

The BMR of *M. mystacinus* in Norway, inhabiting a cold environment at a high latitude, was not high compared to other species of bats. In addition, this population did not differ from bats in general in relation to other thermoregulatory parameters, such as lower critical temperature and body temperature. Hence, other adaptations to high latitude living may have evolved for this



**Fig. 5. Flow chart of the systematic literature search.** The search conducted in Web of Science combined inclusion criteria to screen through the abstracts of all relevant papers, and exclusion criteria to carefully evaluate each included paper.

population. One such adaptation could be increased insulation, for example of fur or fat, resulting in low thermal conductance, as was observed in the present study. Bat species in Vespertilionidae revealed on average low BMR compared to other mammals of the same size. Vespertilionid bats therefore appear to have evolved characteristic traits allowing conservative energy use, such as low BMR and torpor. This is consistent with the high energetic costs associated with for example flying and inhabiting energy-scarce environments. However, as BMR is a trait measured under strict conditions, data selection is an important aspect of a comparative analysis of the variation in BMR. Analysing a poor data set can affect the results considerably, despite including a large sample size. Consequently, data selection should be done with caution (Boyles et al., 2019; Genoud et al., 2018). Energetics studies should include detailed information on methodology and the individuals used to better account for differences between the species included in any future comparative analyses. For bats in particular, the effects of torpor use and roost temperature on BMR are fruitful avenues of research. This is especially interesting to understand how a northern population like *M. mystacinus* is able to cope with the environmental conditions encountered at a high latitude, as no adaptation of BMR was revealed in the present study.

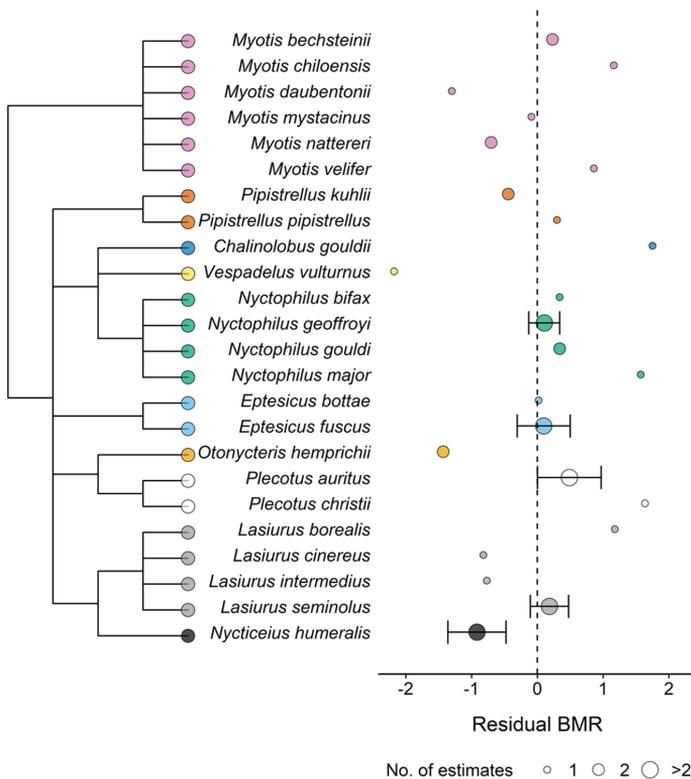
## MATERIALS AND METHODS

Ethics approval to undertake this project was granted by the Norwegian Food Safety Authority (FOTS ID 15944) and the Norwegian Environment Agency (ref. 2018/4899).

### Thermoregulation and BMR in *M. mystacinus*

Male *M. mystacinus* were captured using mist nets during summer 2018 (July to August,  $N=5$ ) and summer 2019 (June to July,  $N=2$ ) in Nittedal, Norway ( $60^{\circ}4'23''N$ ,  $10^{\circ}52'20''E$ ), weighing  $4.7 \pm 0.2$  g ( $N=7$ ) at capture. Bats were transported to a field laboratory immediately after capture, and metabolic measurements were initiated within 2 h after capture. They had no access to food or water before or during measurements and were hence post-absorptive during metabolic measurements. Mealworms and water were provided immediately after the measurements were completed, and the bats were released the following night. Bats were weighed to the nearest 0.1 g before and after measurements.

Oxygen consumption ( $\dot{V}O_2$ , ml  $O_2$   $h^{-1}$ ) of the bats was measured using open-flow respirometry. Bats were placed in a respirometry chamber inside a temperature-controlled Pelt Cabinet (Sable Systems International Inc., Las Vegas, NV, USA), which was regulated using a Pelt-5 Temperature Controller (Sable Systems International Inc., Las Vegas, NV, USA). The bats were measured at  $10^{\circ}C$  until 9 a.m. the morning following capture. The temperature inside the temperature-controlled cabinet was subsequently increased in  $5^{\circ}C$  increments every hour until the temperature had reached



**Fig. 6. The residual BMR of the study species included in the comparative analysis.** Residual BMR calculated from the relationship between body mass and BMR in Fig. 2. The phylogeny is shown to the left to visualise the lack of phylogenetic influence on residual BMR. A residual variation of 0 is illustrated by the dashed line. Mean residual BMR was calculated for species with multiple estimates of BMR, shown by increased circle size. Error bars (SE) are presented for means of three or more species.

25°C, after which it was increased in 2°C increments until the temperature had reached 37°C. Each temperature was maintained for at least one hour before increasing to the next temperature, which was enough to ensure that the temperature was stable for at least 30 min.

A FOXBOX analyser (Sable Systems International, Las Vegas, NV, USA) was used for oxygen analysis. The analyser was calibrated using 100% stock nitrogen prior to measurements each year at the university laboratory of Norwegian University of Science and Technology (NTNU) in Trondheim, Norway. During metabolic measurements two pumps were used to push ambient air through two channels, one leading to the respirometry chamber and the other to an empty chamber used for baseline measurements. Both channels led the air through a flow-controller prior to entering the chambers, subsequently through the chambers, and at last to the oxygen analyser. Both the incurrent and excurrent air was scrubbed using silica gel and Drierite prior to entering the chambers and the oxygen analyser. The respirometry chamber which was placed inside the temperature-controlled cabinet had a volume of 325 ml, and hessian fabric was glued to the back of the respirometry chamber allowing bats to hang in a resting position. The oxygen content of air was sampled every minute with a flow rate of 315 ml min<sup>-1</sup>. Baseline measurements of the ambient air were recorded for 15 min at the start of every temperature increase to correct for possible drift in the analyser. The temperature inside the respirometry chamber was recorded with thermocron iButtons (Dallas Semiconductor Inc., Dallas, TX, USA), and the temperature data from these were used in further analyses.

The  $\dot{V}O_2$  at each set temperature was calculated as the lowest 10-min period of  $\dot{V}O_2$  using a 10-min running mean. To ensure that the measurements included in the running mean were obtained from stable  $\dot{V}O_2$  periods, only mean values with an SE below 0.1 ml O<sub>2</sub> h<sup>-1</sup> were used. Since the system had a high flow-to-volume ratio, there was no need to use

instantaneous  $\dot{V}O_2$  measurements (Bartholomew et al., 1981). The  $\dot{V}O_2$  was calculated using Eqn 10.2 from Lighton (2008),

$$\dot{V}O_2 = \frac{FR_i(F_iO_2 - F_eO_2)}{[1 - F_eO_2(1 - RQ)]},$$

where  $FR_i$  is the incurrent air flow rate,  $F_iO_2$  is the fractional content of incurrent oxygen,  $F_eO_2$  is the fractional content of excurrent oxygen, and  $RQ$  is the respiratory quotient, i.e.  $\dot{V}CO_2/\dot{V}O_2$ . As  $\dot{V}CO_2$  was not measured, a  $RQ$  of 0.8 was assumed. As  $RQ$  varies from 0.7 to 1.0, using a  $RQ$  of 0.8 will produce a relatively small error in  $\dot{V}O_2$ , from -3 to 5% (Lighton, 2008). Mass-specific  $\dot{V}O_2$  (ml O<sub>2</sub> g<sup>-1</sup> h<sup>-1</sup>) was calculated assuming a linear reduction in body mass over time, using the body mass recorded before and after initiation of the metabolic measurements. All  $\dot{V}O_2$  measurements were converted to standard temperature and pressure conditions.

Statistical analyses were performed using the R 3.6.3 software environment (R Core Team, 2020). Metabolic data are given as mass-specific  $\dot{V}O_2$  (ml O<sub>2</sub> g<sup>-1</sup> h<sup>-1</sup>) ± SE. Basal metabolic rate was defined as the mean value of  $\dot{V}O_2$  measurements within the thermoneutral zone. However, because *M. mystacinus* is a previously unmeasured species and hence the temperature range for the thermoneutral zone is unknown, the thermoneutral zone was identified based on visual observation and a flattening of the curve at higher ambient temperatures. Resting values of metabolic rate were obtained at ambient temperatures below the flat part of the curve, often referred to as the resting metabolic rate. Using a linear regression of resting metabolic rate against ambient temperature and the calculated BMR, the lower critical temperature was estimated as the inflection point between the two lines. Extrapolation of the linear regression of resting metabolic rate at low ambient temperatures to the x-axis provided an estimate of body temperature, and the

slope was considered a measure of the thermal conductance (McNab, 1980a). Raw data are available in Supplementary File 1.

### BMR in Vespertilionidae

A systematic literature search was conducted to obtain data for the comparative analysis of BMR in vespertilionid bats. Inclusion criteria were used to screen papers obtained from the search, whereas exclusion criteria were used to assess the relevant papers for eligibility. The inclusion criteria were (1) a measure of BMR, (2) vespertilionid bats and (3) insectivorous species. The exclusion criteria were (1) a lack of body mass measure and (2) a lack of variation measure for BMR.

The literature was searched with Web of Science using two search terms, one general and one specific. The general search term was used to search through the topic of each paper, i.e., only title and abstract, and contained the following string: "basal metabo\*" OR "BMR" OR "resting metabo\*" OR "RMR" AND "bat\*" AND "insect\*" OR "vespe\*" in topic. The specific search term was used to search through entire papers and contained the following string: "basal metabo\*" OR "BMR" AND "Bat\*" AND "genus" in all fields. The word "Genus" was replaced with each of the 27 genera in Vespertilionidae. This resulted in 27 separate searches using the latter search string. The papers obtained from both the general and the specific search terms were evaluated with the inclusion and exclusion criteria, yielding 17 papers to the comparative analysis.

In addition, we went through the reference lists of these 17 papers, and also through the papers that had cited the specific papers. This exercise yielded another six papers with BMR data. Papers were accessed through the electronic collections of the library at NTNU during the period 07.11.2019 to 14.01.2020. A detailed flow chart of the systematic literature search is illustrated in Fig. 5.

The BMR estimate, number of individuals ( $N$ ) used to measure BMR, and the corresponding body mass were extracted from each paper, as well as a measure of variation for both BMR and body mass. Our own respiratory results on *M. mystacinus* were combined with the data from the systematic literature search, creating a final database of 24 species, comprising 47 estimates of BMR. Because more than one estimate of BMR was sometimes retrieved from multiple populations of a single species, we calculated mean BMR for these species. Consequently, this resulted in a total of 24 estimates of BMR used in the statistical analyses. The mean body mass of the bats included was  $11.3 \pm 0.8$  g.

To ensure that the BMR estimates were comparable, the methodology was carefully evaluated in each paper. The BMR estimates that were included were measured using open-flow respirometry in resting, post-absorptive and non-reproductive adults at an ambient temperature within their thermoneutral zone. Where possible based on the information provided in the papers, estimates were classified according to sex, length of captivity, reproductive state, which season the BMR was measured in, and the coordinates where the study was conducted (Fig. 3). However, none of these categories could be used in the quantitative analyses, as all the relevant information was not reported in most papers (Fig. S1). Thus, the categories were not well represented, and were instead used to visualise possible effects graphically.

Measures of BMR were converted to absolute BMR for the analyses, expressed as oxygen consumption per hour ( $\text{ml O}_2 \text{ h}^{-1}$ ). Estimates given in watts were converted using a conversion factor of  $20.1 \text{ J ml}^{-1}$ , assuming a respiratory quotient of 0.8 (Withers et al., 2016), unless a conversion factor was stated in the original paper. If BMR was given as a median value with range as a measure of variation, it was converted to mean and variance using the method of Hozo et al. (2005). All measures of variation were converted to SE. Body mass and BMR were log-transformed, and standardised residual BMR was calculated from a linear regression of BMR against body mass. Two allometric equations are reported, one equation excluding our own BMR measurement of *M. mystacinus* to compare this estimate to other bats in Vespertilionidae, and one equation including this estimate to analyse the effect of environmental temperature on BMR within Vespertilionidae. All estimates in the comparative analysis are given as means  $\pm$  SE.

Linear models were fitted to the data using the R 3.6.3 software environment (R Core Team, 2020). To determine the effects of mean

environmental temperature on BMR between populations of vespertilionid bats, we collected data on environmental temperature from each latitude where BMR measurements were obtained. For the species where we calculated mean BMR based on multiple populations, we also calculated mean environmental temperature. The natural environmental temperature data were obtained from the website World Weather Online (2020), and the means  $\pm$  SE used in the analyses were calculated from January 2009 to December 2019 for the given coordinates obtained from each paper. Data from earlier years were not available. Even though some of the BMR estimates were retrieved from papers older than 2009, a mean of the environmental temperature from 2009 to 2019 was considered representative as a proxy for the different climate types in the given areas. Sufficient information was not provided in the original papers to use seasonal or monthly temperature means, and thus long term mean temperature was considered the best option. Information on latitude was available from most published studies. Where the specific latitude was not reported, we used the location reported in the study to obtain an estimate of the latitude.

Multiple environmental factors are found to correlate with BMR in mammals, such as environmental productivity and rainfall parameters (Lovegrove, 2003; Mueller and Diamond, 2001; Withers et al., 2016). It is therefore possible that other environmental factors could be better predictors for BMR than environmental temperature. However, many environmental factors are correlated with each other and we were unable to include more factors due to low sample size and consequently low statistical power. Using latitude information obtained from the studies included in the present analysis, we found that environmental temperature significantly increased with decreasing latitude (Fig. S3). This indicates that environmental temperature captures the environmental variance experienced by vespertilionid bats at different latitudes. Because limited information is available regarding the details of the phylogenetic relationships within Vespertilionidae, we could not obtain a phylogenetic tree of high accuracy. Therefore, we did not statistically test for a phylogenetic signal on BMR among the species. However, a lack of phylogenetic influence on residual BMR is visualised among the species included in the present analysis (Fig. 6).

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### Competing interests

The authors declare no competing or financial interests.

### Author contributions

Conceptualization: K.H.S., C.S.; Methodology: K.H.S., C.B., R.S., C.S.; Formal analysis: K.H.S., C.B., M.A.F., R.S., C.S.; Investigation: K.H.S., R.S., C.S.; Resources: C.B., J.V.D.K., C.S.; Data curation: K.H.S., C.S.; Writing - original draft: K.H.S.; Writing - review & editing: K.H.S., C.B., M.A.F., J.V.D.K., R.S., C.S.; Visualization: K.H.S., C.B., C.S.; Supervision: C.B., C.S.; Project administration: C.S.; Funding acquisition: C.S.

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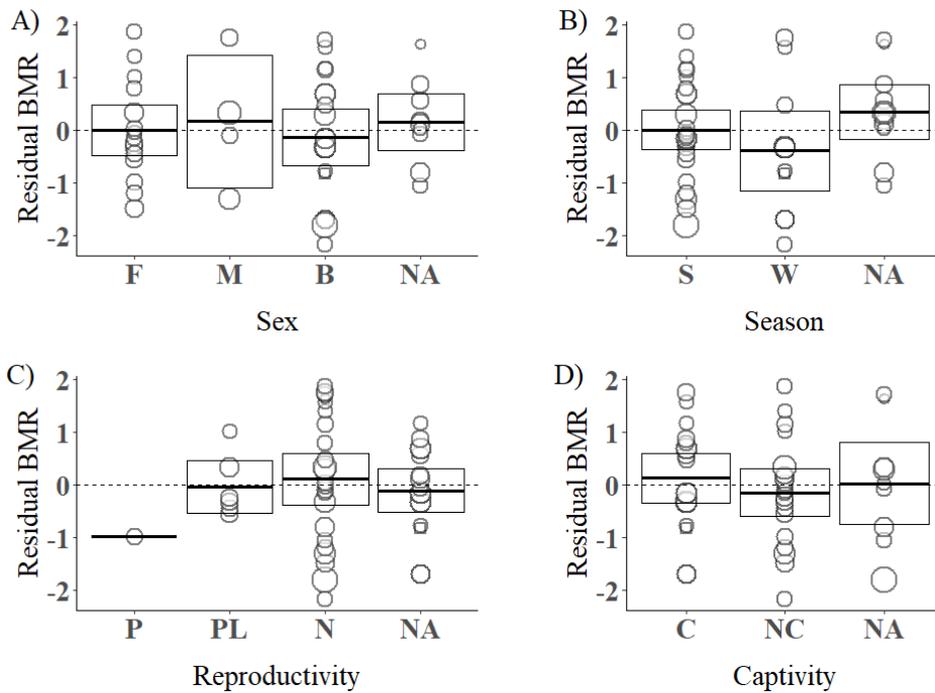
### Data availability

Raw data are available in the supplementary material.

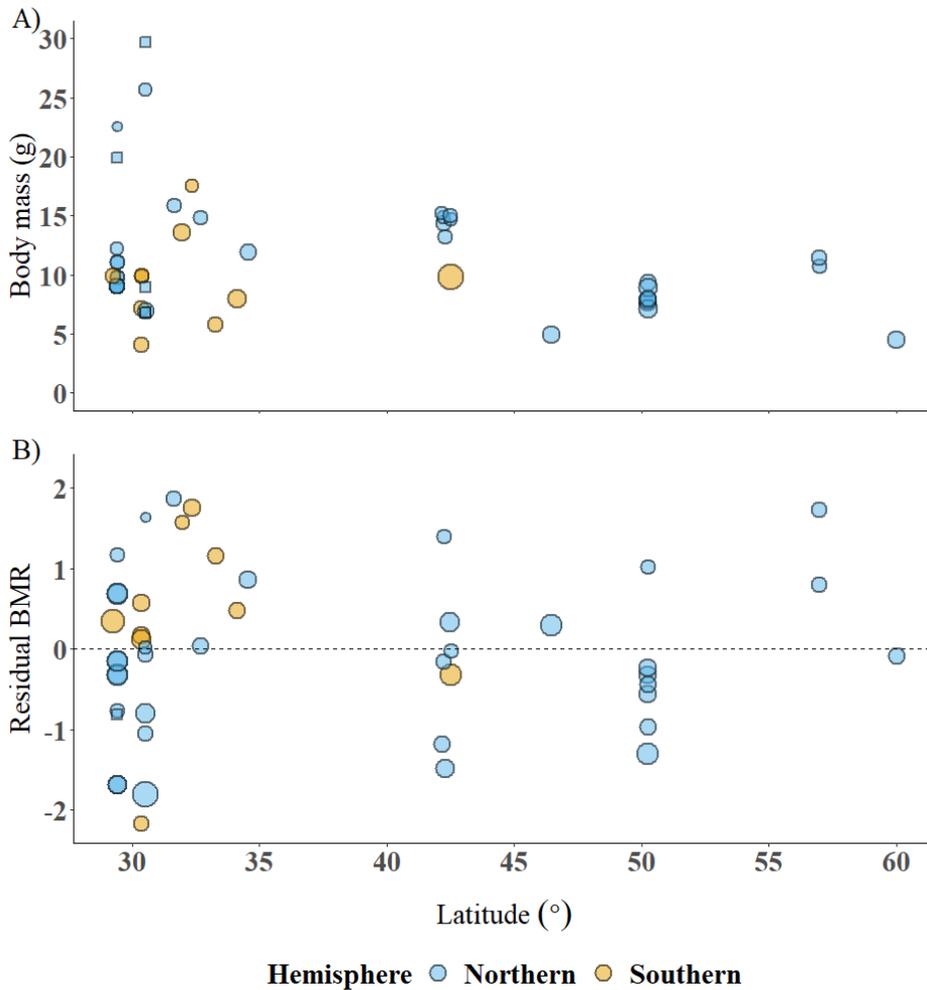
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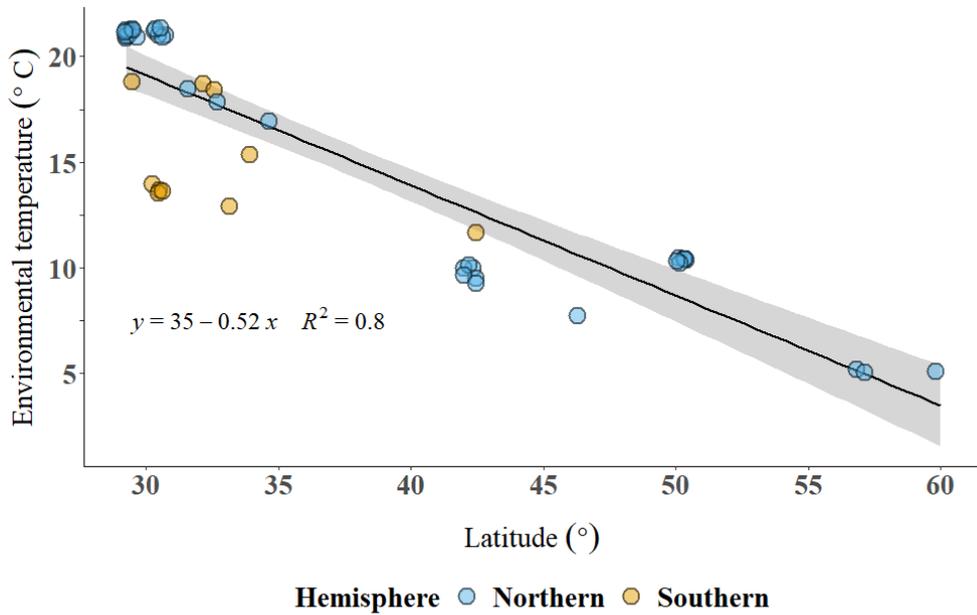
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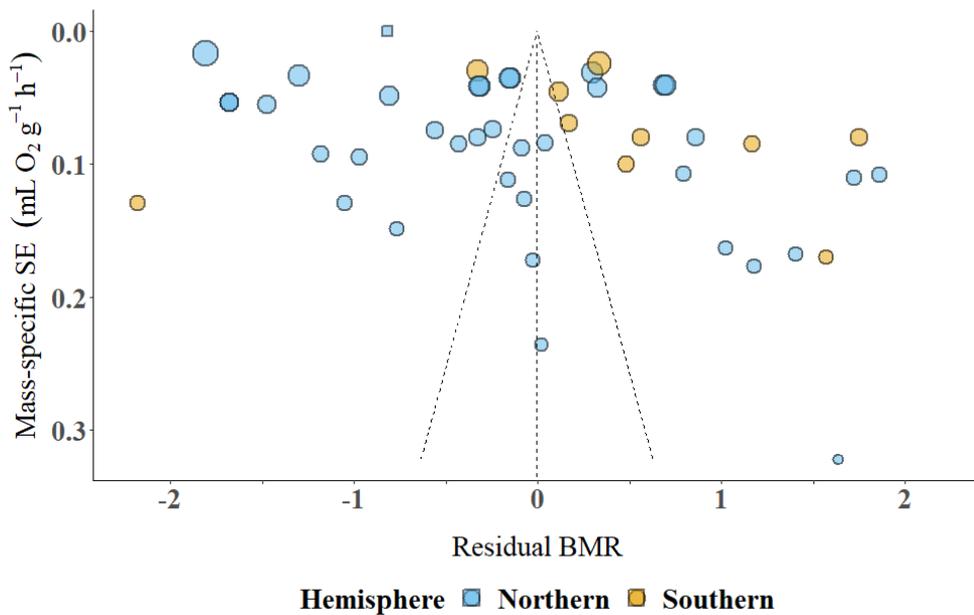
**Fig. S1. Residual BMR of bats in different groups.** Effect of sex (F=females; M=males; B=both), season (S=summer; W=winter), reproductivity (P=pregnant; PL=post-lactating; N=non-reproductive) and captivity (C=captive; NC=non-captive). Measurements of BMR where this information was not available are also shown (NA=not available).



**Fig. S2. Body mass (A) and residual BMR (B) as function of latitude.** Residual BMR was calculated from the relationship between body mass and BMR in Fig. 3. A residual variation of 0.0 is illustrated by the dashed line. The size of the data point represents the precision of the estimate, calculated as the inverse of the variance corrected for body mass. The larger the circle, the more precise is the estimate. Square shapes represent A) estimates where measures of variation were not obtained, and B) a BMR estimate of one individual, which prevented the calculation of precision.



**Fig. S3.** Average environmental temperature (°C) in the areas of which bats included in the present analysis were measured as a function of latitude (°). The regression equation for this relationship was  $y = 34.73 - 0.39x$  ( $f_{1,45} = 180$ ,  $P < 0.001$ , Table S1), and the grey area shows the 95% confidence interval for the regression. The temperatures represent yearly average environmental temperature from 2009-2019.



**Fig. S4.** Mass-specific standard error (SE) (mL O<sub>2</sub> g<sup>-1</sup> h<sup>-1</sup>) of residual BMR used to calculate the precision of the included BMR estimates. Estimates with low mass-specific SE, and therefore estimated with high precision, are represented with increased circle size. The mean residual BMR (0.0057 mL O<sub>2</sub> g<sup>-1</sup> h<sup>-1</sup>) for the species included in the analysis and 95% confidence intervals are illustrated by the dashed lines.

**Table S1.** Summary statistics from the analyses conducted in the present study (SE = standard errors (SE), CI = confidence intervals, N = number of observations, DF = degrees of freedom). Model 1) is from our own metabolic measurements of *Myotis mystacinus*, whereas models 2–6) are from the comparative analysis of BMR within Vespertilionidae.

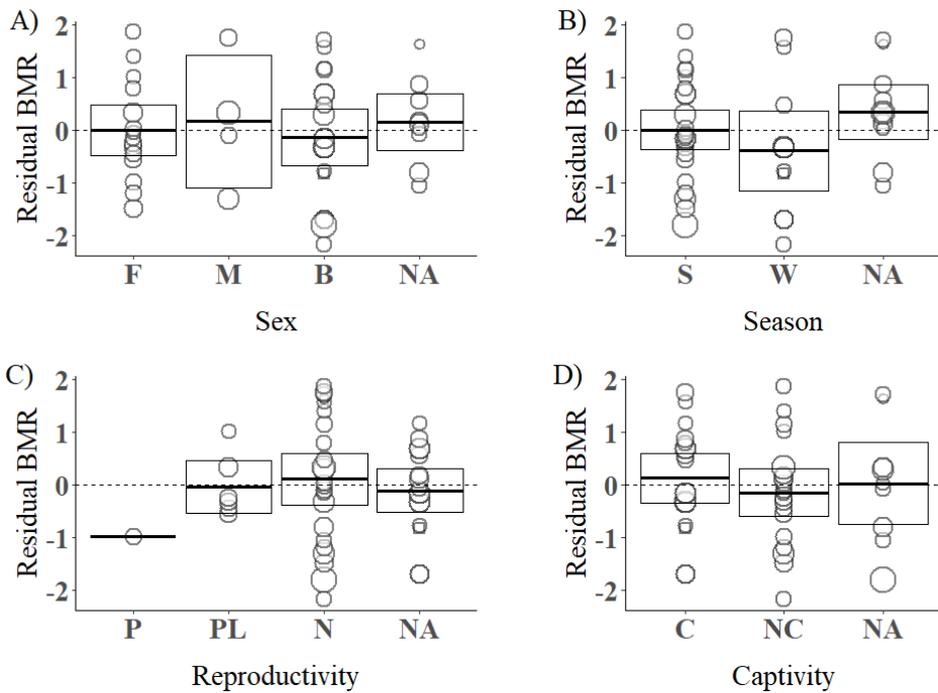
<i>Coefficient</i>	<i>Coefficient</i>	<i>SE</i>	<i>t-value</i>	<i>P-value</i>	<i>95% CI</i>
<b>Model 1)</b>	Response: Mass-specific VO <sub>2</sub> (mL O <sub>2</sub> g <sup>-1</sup> h <sup>-1</sup> )				
<i>Intercept</i>	14.51	0.54	27.02	<b>&lt;0.001</b>	[12.80, 16.21]
<i>Temperature</i>	-0.39	0.031	-12.73	<b>0.001</b>	[-0.49, -0.30]
<i>N</i>	5				
<i>Residual SE</i>	0.30 on 3 DF				
<i>Multiple R<sup>2</sup> / adjusted R<sup>2</sup></i>	0.98 / 0.98				
<i>F-statistics</i>	162.2 on 1 and 3 DF, P-value: <b>0.001</b>				
<b>Model 2)</b>	Response: Log BMR, excl. <i>Myotis mystacinus</i>				
<i>Intercept</i>	0.38	0.064	5.84	<b>&lt;0.001</b>	[0.25, 0.51]
<i>Log body mass</i>	0.70	0.062	11.36	<b>&lt;0.001</b>	[0.58, 0.83]
<i>N</i>	46				
<i>Residual SE</i>	0.073 on 44 DF				
<i>Multiple R<sup>2</sup> / adjusted R<sup>2</sup></i>	0.75 / 0.74				
<i>F-statistics</i>	129.0 on 1 and 44 DF, P-value: <b>&lt;0.001</b>				
<b>Model 3)</b>	Response: Weighted log BMR, excl. <i>Myotis mystacinus</i>				
<i>Intercept</i>	0.35	0.081	4.36	<b>&lt;0.001</b>	[0.19, 0.52]
<i>Temperature</i>	0.73	0.079	9.22	<b>&lt;0.001</b>	[0.57, 0.88]
<i>N</i>	45				
<i>Residual SE</i>	0.091 on 43 DF				
<i>Multiple R<sup>2</sup> / adjusted R<sup>2</sup></i>	0.66 / 0.66				
<i>F-statistics</i>	85.1 on 1 and 43 DF, P-value: <b>&lt;0.001</b>				
<b>Model 4)</b>	Response: Log BMR, incl. <i>Myotis mystacinus</i>				
<i>Intercept</i>	0.37	0.060	6.17	<b>&lt;0.001</b>	[0.25, 0.49]
<i>Temperature</i>	0.71	0.059	12.12	<b>&lt;0.001</b>	[0.59, 0.83]

<i>N</i>	47
<i>Residual SE</i>	0.072 on 45 DF
<i>Multiple R<sup>2</sup> / adjusted R<sup>2</sup></i>	0.77 / 0.76
<i>F-statistics</i>	146.6 on 1 and 45 DF, P-value: <b>&lt;0.001</b>

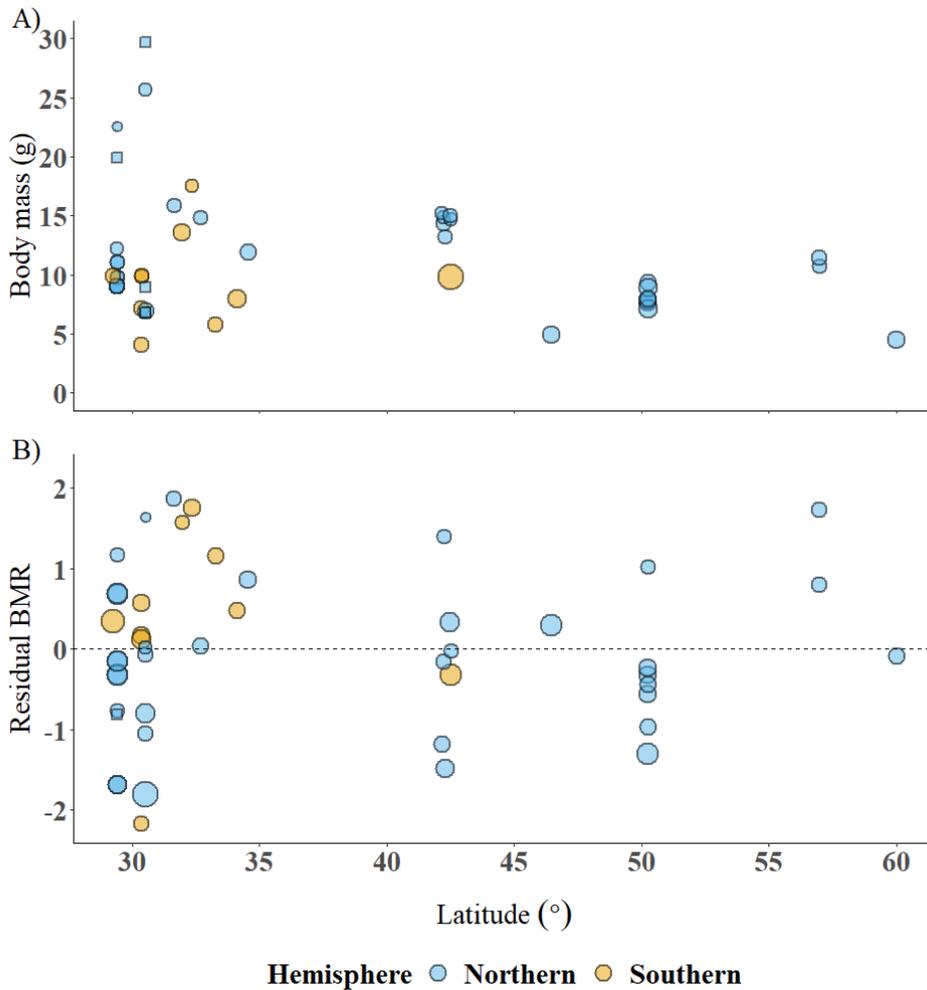
<b>Model 5)</b>	Response: Environmental temperature (°C)				
<i>Intercept</i>	34.73	1.50	23.23	<b>&lt;0.001</b>	[31.72, 37.74]
<i>Temperature</i>	-0.52	0.039	-13.41	<b>&lt;0.001</b>	[-0.60, -0.44]
<i>N</i>	47				
<i>Residual SE</i>	2.48 on 45 DF				
<i>R<sup>2</sup> / R<sup>2</sup> adjusted</i>	0.80 / 0.80				
<i>F-statistics</i>	179.8 on 1 and 45 DF, P-value: <b>&lt;0.001</b>				
<b>Model 6)</b>	Response: Residual BMR				
<i>Intercept</i>	0.18	0.45	0.41	0.68	[-0.72, 1.09]
<i>Temperature</i>	-0.012	0.028	-0.45	0.66	[-0.068, 0.043]
<i>N</i>	47				
<i>Residual SE</i>	1.03 on 45 DF				
<i>Multiple R<sup>2</sup> / adjusted R<sup>2</sup></i>	0.0045 / -0.018				
<i>F-statistics</i>	0.20 on 1 and 45 DF, P-value: 0.66				

**Table S2**

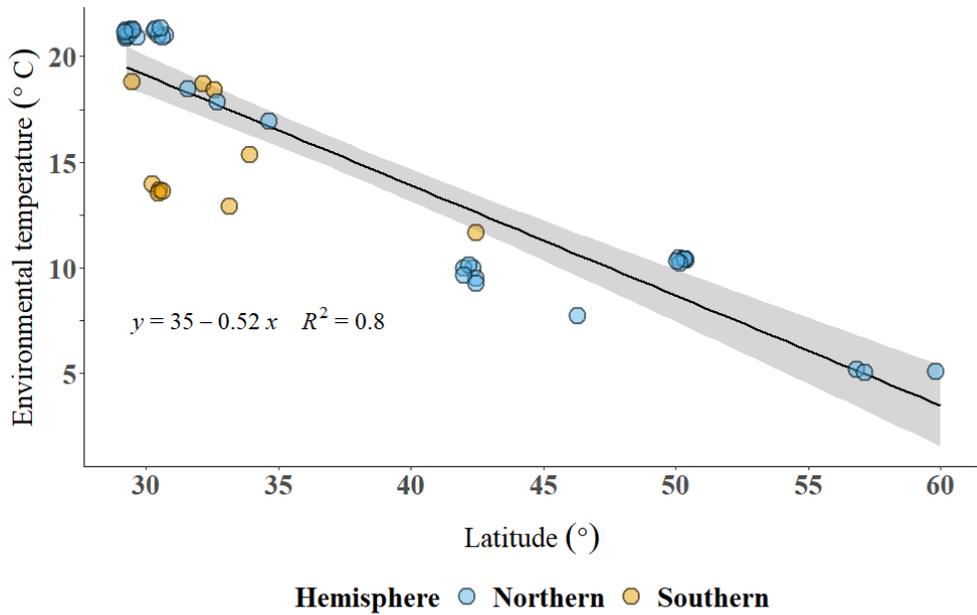
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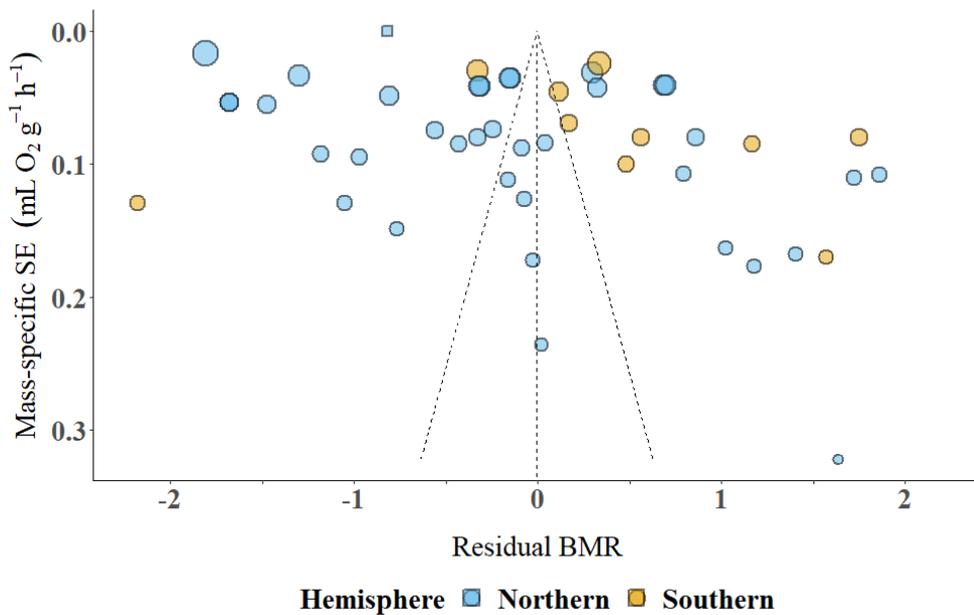
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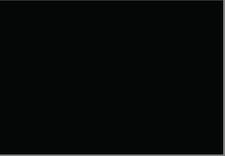
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<i>F-statistics</i>	0.20 on 1 and 45 DF, P-value: 0.66				

**Table S2**

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# Paper V





## Universality of Torpor Expression in Bats\*

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### ABSTRACT

Although heterothermy is employed by species at a global level within the order of Chiroptera (bats), the possibility of torpor being expressed in bat species inhabiting warmer climate zones has been explored only in the past couple decades. Recent studies suggest that the benefit of expressing torpor is not limited to saving energy during cold exposure or food shortage but may be just as important for saving water during heat waves. Thus, even if the physiological challenges faced by bats may depend on the habitat they live in, species expressing torpor should be found in any climate zone where employing torpor may yield benefits and increase their survival probability. Here, we summarize available data on torpor metabolic rates and daily skin temperature patterns of bats across climate zones, emphasizing similarities found in the data. We also present data that we have collected from a southern subtropical species (*Nyctophilus bifax*) and a northern subarctic species (*Plecotus auritus*) to illustrate specific examples of torpor expressions in two bat species living in highly different environments. Our findings highlight that torpor metabolic rates and skin temperature patterns of bats outside of the hibernation season can be universal across vastly different habitats, although arid environments indicate potential divergence in mean minimum torpor metabolic rates compared with measurements of populations inhabiting other climate zones.

**Keywords:** ancestral trait, Chiroptera, heterothermy, radiotelemetry, skin temperature, thermoconforming, thermoregulation, torpor metabolic rate.

\*This paper was submitted in response to the Focused Collection call for papers "Time-Out for Survival: Hibernation and Daily Torpor in Field and Lab Studies."

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### Introduction

Torpor, a controlled and reversible reduction of active thermoregulation and metabolic rate (MR), is a globally applied energy-saving strategy within the order of Chiroptera (Stawski et al. 2014). Despite early assumptions of torpor being employed only by temperate bat species to avoid the cold and food-deprived winter period, torpor and hibernation have over the past decades been observed in individuals from all climate zones and throughout all seasons (Geiser and Stawski 2011; Stawski et al. 2014; Levin et al. 2015; Reher and Dausmann 2021). The wide phylogenetic and geographical distribution of bats employing torpor suggests that this physiological strategy is an ancestral trait that has persisted throughout the evolution of bats, likely because heterothermy reduces the risk of mammalian extinctions (Wojciechowski et al. 2007; Geiser and Turbill 2009).

Shifting between thermoregulation (i.e., controlling body temperature [ $T_b$ ] via increases in MR) and thermoconforming (i.e., abandoning regulation of  $T_b$  by reducing MR) can be highly energetically favorable in bats facing physiologically challenging environments (Geiser 2004). Torpor can function as a survival strategy during cold or warm temperature exposures, droughts, migration, and food shortages (e.g., Coburn and Geiser 1998; Speakman and Thomas 2003; McGuire et al. 2014; Reher and Dausmann 2021). As bats are found on all continents except Antarctica, species across climate zones are adapted to living in highly different environments, from subarctic habitats with strong seasonality to arid and hot desert landscapes. However, recent findings suggest that active selection of preferred microclimates in roosts results in similar hibernation physiology for bat populations across various macroclimates (McGuire et al. 2021). At the same time, roost type selection within populations has been found to alter thermoregulatory behavior and torpor use in bats (Bergeson et al. 2021). Therefore, roost types and microclimate selection might be stronger predictors of heterothermic responses than macroclimatic variation, although climate zones and habitat types are likely to affect which roost types and microclimates are available.

Throughout Chiroptera in all habitats torpor strategies are found to not only save energy or water during inclement conditions but to avoid predators and to save current fat reserves by remaining torpid during the foraging period (Stawski and Geiser

2010; Fjellidal et al. 2021). However, the use of heterothermy in bats is perhaps most explored in relation to cold exposure in hibernating species (Ruf and Geiser 2015). Winter is challenging because of low temperatures and reductions in food supply, particularly in temperate regions in which many bats display typical hibernation patterns. While winter can also be challenging in warmer regions, torpor use there is often more opportunistic and takes advantage of warmer periods (e.g., Czenze et al. 2017; Reher et al. 2018). However, summer also provides energetic challenges, such as heat waves and unpredictable storms that prevent foraging bouts, to small bats. Yet it is not known how similar torpor expression is in bats across latitudes and habitats outside of the hibernation season.

Our aim is to summarize the data available on insectivorous bats for torpor metabolic rates (TMRs) to assess torpor energetics throughout the year and on skin temperature ( $T_{skin}$ ) patterns outside of the hibernation season (thus, excluding winter studies) in species adapted to different climate types worldwide. TMR is a commonly reported trait, while daily  $T_{skin}$  patterns in bats, including W-shaped torpor bouts (bats expressing torpor in the morning and in the evening), one-bout torpor patterns (bats expressing one torpor bout per day), and full-day torpor expressions, have been previously described in only a few studies (see, e.g., Turbill et al. 2003a, 2003b). In addition to the literature review, we present TMR data and torpor  $T_{skin}$  patterns that we have collected from one southern subtropical bat species (*Nyctophilus bifax*) and one northern subarctic bat species (*Plecotus auritus*) as specific examples of torpor expression in bats from two highly different climate zones. As many bats appear capable of both short and long torpor bouts outside of the hibernation season (thus, we are not using the terminology of “hibernation”) and energetic bottlenecks are likely to occur in all habitat types, we present the following two hypotheses: (1) TMR will be similar among populations and species and (2) torpor patterns displayed by bats are similar in different climatic zones.

## Methods

Permits to undertake research on Australian eastern long-eared bats (*Nyctophilus bifax*) were approved by the University of New England Animal Ethics Committee (AEC08/046 and AEC09/058) and New South Wales National Parks and Wildlife Service (no. S12448). Ethics approvals for the capture and handling of Norwegian brown long-eared bats (*Plecotus auritus*) were granted by the Norwegian Food Safety Authority (FOTS ID 23284) and the Norwegian Environment Agency (ref. 2018/4899).

Individuals of *N. bifax* were captured at a subtropical location in Australia at Iluka Nature Reserve (29°24'S, 153°22'E) in 2008 and 2009 (for details, see Stawski and Geiser 2011), while individuals of *P. auritus* were captured at a subarctic location in Nittedal, Norway (60°03'N, 10°52'E), during the summer of 2019 and 2020. Mist nets were used for capture at both locations, and the bats were usually kept for 1–2 d in captivity (maximum 3 d) during MR measurements. While in captivity, bats were hand-fed until saturation two times per day, while water was available ad lib. They were never fed later than 2100 hours

when MR measurements were scheduled for the next day to ensure that bats were postabsorptive during the experiment. After measurements the bats were released at the capture site with transmitters attached. Captured pregnant and lactating females were not brought back for MR measurements but were released the same evening with transmitters at the capture site.

## Torpor Metabolic Rates

The rate of oxygen consumption ( $\text{mL O}_2 \text{ g}^{-1} \text{ h}^{-1}$ ) in torpid thermoconforming individuals was measured in *N. bifax* ( $N_{ind} = 9$ ) and in *P. auritus* ( $N_{ind} = 15$ ) using open-flow respirometry. TMR for *N. bifax* was measured by placing the bats in cylindrical, clear Perspex tubes (160 mL) lined with mesh to allow the bats to roost during measurements. The ambient temperature ( $T_a$ ) was lowered in 5°C increments between 30°C and 10°C followed by 2°C increments when  $T_a < 10^\circ\text{C}$  (for further details on the experimental setup, see Stawski and Geiser (2011)). TMR of *P. auritus* was measured by placing each individual in square chambers (325 mL) lined with mesh for a minimum of 5 h at 5°C to allow the bats to enter torpor before lowering the  $T_a$  to 0°C and then increasing the  $T_a$  in 5°C increments between 0°C and 25°C followed by 3°C increments when  $T_a > 25^\circ\text{C}$ . Metabolic rates were measured at each set temperature for at least 1 h, and the exact temperature in the chamber was logged every 10 min (*N. bifax*) or every 1 min (*P. auritus*) during the experiment using temperature-sensitive data loggers placed inside the chamber (0.5°C, DS1921G Thermochron iButtons, Maxim Integrated Products, Sunnyvale, CA). Oxygen consumption was measured every 3 min (*N. bifax*) or every 1 min (*P. auritus*) with a FoxBox (Sable Systems International, Las Vegas, NV), which was calibrated using 100% stock nitrogen before the field season. For *P. auritus*, both  $\text{O}_2$  consumption and  $\text{CO}_2$  production were measured. We therefore calculated TMR using equation (10.5) in Lighton (2018):

$$\dot{V}_{\text{O}_2} = \frac{\text{FR}_i[(F_i\text{O}_2 - F_e\text{O}_2) - F_e\text{O}_2(F_e\text{CO}_2 - F_i\text{CO}_2)]}{1 - F_e\text{O}_2},$$

where  $\text{FR}_i$  is the incurrent air flow rate,  $F_i\text{O}_2$  is the fractional content of incurrent oxygen,  $F_e\text{O}_2$  is the fractional content of excurrent  $\text{O}_2$ ,  $F_i\text{CO}_2$  is the fractional content of incurrent  $\text{CO}_2$ , and  $F_e\text{CO}_2$  is the fractional content of excurrent  $\text{CO}_2$ . The bats were measured at flow rates of 65–200  $\text{mL O}_2 \text{ min}^{-1}$  (*N. bifax*) and 185–315  $\text{mL O}_2 \text{ min}^{-1}$  (*P. auritus*). The large ranges in flow rates were due to downregulation of the air flow when the bats entered torpor and upregulation when bats aroused. Exponential models of TMR as a function of  $T_a$  in thermoconforming torpid individuals were fitted using the nls function in the statistical software R (ver. 4.0.3).

We collected information on TMR for as many bat species as we could find studies on in the primary literature, but we considered data only from insectivorous species to avoid dietary effects on MRs. Only TMR data reported as a mean minimum value were included. Climate zones for each study were determined using the climate zone world map available through <https://www.arcgis.com>, which operates with 11 different world climate

zones: tropical rain forest, tropical savanna/monsoon, desert, steppe, humid subtropical, marine west coast, Mediterranean, humid continental, subarctic, tundra, and ice cap.

The mean minimum TMR should reflect the lowest point of energy expenditure in the thermoregulatory curve for each studied population. As such, the temperature at which the minimum TMR is measured should be above the critical point of thermoregulation but not high enough to passively increase physiological processes (see, e.g., fig. 1.9 in Geiser 2021). To test whether temperature impacted the reported minimum TMR, we fitted a mixed model testing the effect of  $T_a$  on the log-transformed lowest mean TMR using the lmer function from the lmerTest package (Kuznetsova et al. 2017), with climate zone as a random effect. The proportion of variation explained by the random effect was calculated using the get\_variance function from the insight package (Lüdtke et al. 2019).

When comparing physiological traits between species and higher taxa, phylogenetic relationships should be considered (Garland et al. 2005). Unfortunately, owing to a small sample size, uneven numbers of study populations per species, and lack of information regarding branch lengths and polytomies, we could not perform proper phylogenetic statistical analyses. Instead, we plotted the scaled residual TMR from the mixed linear model against a simplified phylogenetic tree using the ape package (Paradis and Schliep 2019) for visualization.

#### Torpor Skin Temperature Patterns

We used collected  $T_{\text{skin}}$  data from *N. bifax* and *P. auritus* to identify different torpor  $T_{\text{skin}}$  patterns commonly expressed by free-ranging insectivorous bats during a bat day. Data were collected by attaching temperature-sensitive transmitters (~0.5 g, LB-2NT, Holohil Systems, Carp, Ontario, and ~0.5 g, PIP31, Lotek Wireless, Dorset, UK) to the dorsal region using a skin adhesive after trimming a small patch of fur to access the skin. Afterward, the bats were released and tracked using radio-telemetry to find their day roosts and install data loggers that registered pulse intervals from the transmitters every 10 min. These pulse intervals could afterward be converted to  $T_{\text{skin}}$  measurements according to calibration of the transmitters before attachment (calibrated in water baths from 0°C to 45°C with 5°C increments).  $T_{\text{skin}}$  data from 19 *N. bifax* were collected during the austral spring and summer in 2008 (see Stawski 2010; Stawski and Geiser 2010), and data from 32 *P. auritus* were collected during the Norwegian summer in 2019 and 2020. The data collection period for each individual ended when the transmitter fell off the tagged bat, a period that ranged from 1.5 to 19 d for *P. auritus* and from 1 to 12 d for *N. bifax*, with the median being 5 d for both species. Torpor for *N. bifax* was defined as  $T_{\text{skin}}$  measurements <28°C as described in Stawski (2010), while for *P. auritus* we used the following equation developed by Willis (2007) to calculate a torpor onset value:

$$T_{\text{onset}} - 1 \text{ SE} = 0.041 \times \text{body mass} + 0.040 \times T_a + 31.083.$$

From our data, we calculated the species-specific  $T_b$  torpor onset value to be 32.1°C. However, as  $T_b - T_{\text{skin}}$  measurements usually

are <2°C for small mammals (Audet and Thomas 1996; Barclay et al. 1996), we extracted 2°C from our mean species-specific torpor onset value and used 30.1°C as the  $T_{\text{skin}}$  torpor onset value for *P. auritus*.

To assess the use of the different torpor  $T_{\text{skin}}$  patterns in bat species across climate zones, we gathered data from radiotelemetry studies using temperature-sensitive transmitters on free-ranging insectivorous bats outside of the hibernation season (thus, winter studies are not included). We also excluded studies if torpor was not observed at all during the data collection period, as we are interested in comparing torpor patterns. Populations were divided into climate zones using the ArcGIS climate zone world map, as described above.

## Results and Discussion

### Torpor Metabolic Rates

For the subtropical population of *Nyctophilus bifax* the mean minimum TMR was measured at  $T_a$  between 6.5°C and 11.4°C with an  $O_2$  consumption of  $0.048 \pm 0.006 \text{ mL } O_2 \text{ g}^{-1} \text{ h}^{-1}$  ( $N_{\text{ind}} = 9$ ,  $T_a = 8.9^\circ\text{C} \pm 0.5^\circ\text{C}$ ). Mean minimum TMR for the subarctic population of *Plecotus auritus* was measured at  $T_a$  between 5.3°C and 9.7°C with an  $O_2$  consumption of  $0.052 \pm 0.021 \text{ mL } O_2 \text{ g}^{-1} \text{ h}^{-1}$  ( $N_{\text{ind}} = 10$ ,  $T_a = 7.2^\circ\text{C} \pm 1.9^\circ\text{C}$ ). TMR in thermoconforming individuals of both species increased exponentially with increasing  $T_a$  (*N. bifax*:  $\text{TMR} [\text{mL } O_2 \text{ g}^{-1} \text{ h}^{-1}] = 0.0144 \times 1.148^{T_a}$ ,  $r^2 = 0.92$ ,  $N_{\text{ind}} = 9$ ; *P. auritus*:  $\text{TMR} [\text{mL } O_2 \text{ g}^{-1} \text{ h}^{-1}] = 0.0198 \times 1.137^{T_a}$ ,  $r^2 = 0.81$ ,  $N_{\text{ind}} = 15$ ; fig. 1).

Searching the primary literature, we obtained a total of 41 average TMR values (including the two values presented above) from species inhabiting nine different climate zones: tropical savanna/monsoon ( $N = 1$ ), desert ( $N = 5$ ), steppe ( $N = 3$ ), humid subtropical ( $N = 7$ ), marine west coast ( $N = 6$ ), Mediterranean ( $N = 5$ ), humid continental ( $N = 9$ ), subarctic ( $N = 3$ ), and tundra ( $N = 2$ ). The mean minimum TMR ranged between  $0.014 \text{ mL } O_2 \text{ g}^{-1} \text{ h}^{-1}$  and  $1.01 \text{ mL } O_2 \text{ g}^{-1} \text{ h}^{-1}$  (table 1). Of the 41 bat populations measured, 29 expressed a TMR <  $0.1 \text{ mL } O_2 \text{ g}^{-1} \text{ h}^{-1}$ , and only six of the studies had a TMR >  $0.31 \text{ mL } O_2 \text{ g}^{-1} \text{ h}^{-1}$ , of which three belonged to bat populations inhabiting desert habitats (fig. 2A).

Summarizing available torpor data from bat populations at a global level revealed that all nine of the climate zones that we obtained TMR data from were represented with at least one bat population expressing  $\text{TMR} \leq 0.15 \text{ mL } O_2 \text{ g}^{-1} \text{ h}^{-1}$ . The apparent general similarities between the TMR across species and climate zones can be compared with the results presented in the recent study by McGuire et al. (2021). The authors measured TMR in bat populations across a wide geographical range in North America encompassing varying climate types but found no effect of the locations or  $T_a$  on the TMR within species. The authors suggested that the lack of geographical variation could be due to the bats selecting similar microclimates for roosting even if the macroclimates largely differ. Such behavioral microhabitat selection may also apply across bat species and climate zones and could be the reason for the physiological similarities displayed by species that employ torpor in this species-rich mammal group.

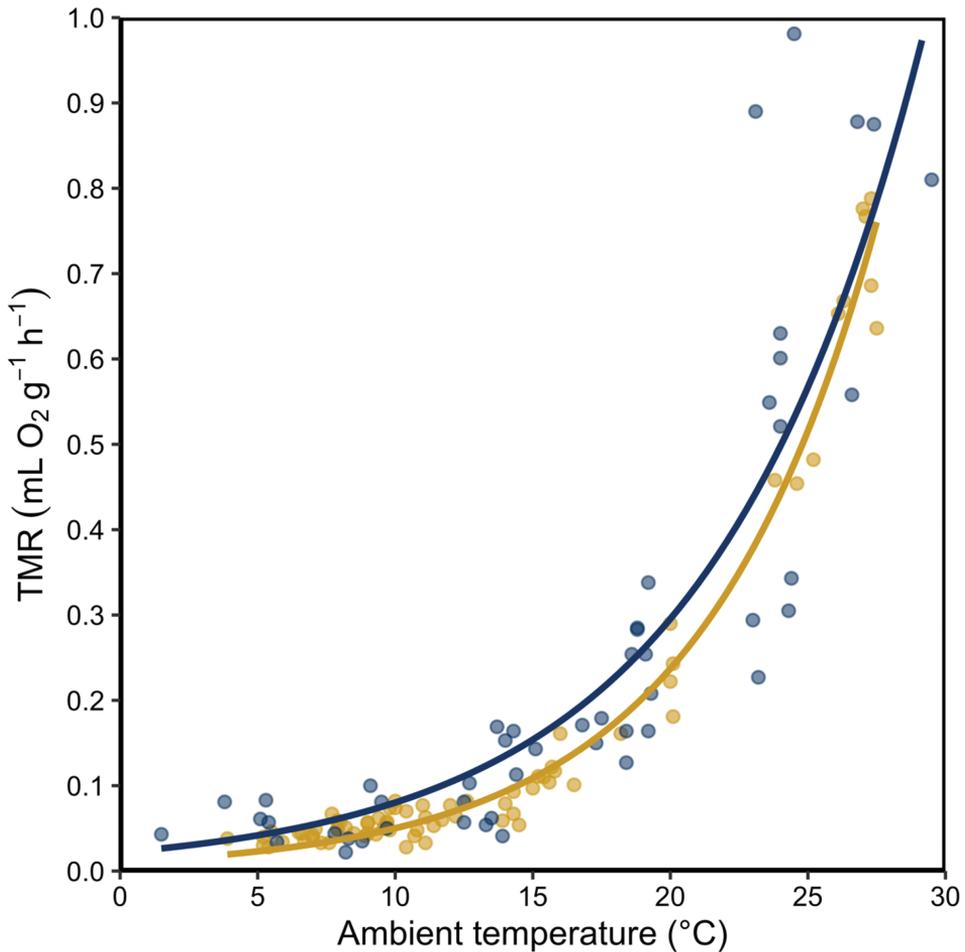


Figure 1. Torpor metabolic rates (TMRs) in two bat species. TMR as a function of ambient temperature ( $T_a$ ) in thermoconforming *Plecotus auritus* (blue) and *Nyctophilus bifax* (yellow). TMR decreased exponentially with  $T_a$  following the equations  $\text{TMR (mL O}_2 \text{ g}^{-1} \text{ h}^{-1}) = 0.0198 \times 1.137^{T_a}$  for *P. auritus* and  $\text{TMR (mL O}_2 \text{ g}^{-1} \text{ h}^{-1}) = 0.0144 \times 1.148^{T_a}$  for *N. bifax*.

However, we cannot argue that TMR appears entirely independent of climate zones, as three of the four highest values belonged to species inhabiting desert environments and none of the five desert populations had a TMR below  $0.15 \text{ mL O}_2 \text{ g}^{-1} \text{ h}^{-1}$  (fig. 2A). This was further explored in the fitted mixed effects model, which revealed strong evidence that  $T_a$  positively affected the log-transformed mean minimum TMR ( $0.031 \pm 0.0096$ ;  $P < 0.01$ ;  $N_{\text{pop}} = 41$ ). Excluding the five desert populations from the model changed the climate zone random effect from explaining 23.1% of the total variation in TMR to 0%, while the  $T_a$  effect only slightly increased ( $0.034 \pm 0.0071$ ;  $P < 0.001$ ;

$N_{\text{pop}} = 36$ ). This indicates that no additional variation in TMR could be explained by populations inhabiting any other climate zone than the desert habitat. A visualization of the log-transformed TMR values against  $T_a$  (from table 1) can be found in figure S1.

Interestingly, the wide range of temperatures (from  $2.0^\circ\text{C}$  to  $27.2^\circ\text{C}$ ) yielding the lowest TMR across populations and species indicates variation in the torpor thermoregulatory curves. Although the overall shape of the curves may be similar, with increased TMR below the critical temperature for thermoregulation and increased TMR with further increasing temperatures

Table 1: Mean minimum torpor metabolic rate (TMR) for bat populations across climate zones

Species	Climate zone	$M_b \pm SD$ (g)	$N_{ind}$	TMR ( $\text{mL O}_2 \text{ g}^{-1} \text{ h}^{-1}$ )	$T_a$ ( $^{\circ}\text{C}$ )	Reference(s)
<b>Hipposideridae:</b>						
<i>Hipposideros terasensis</i>	Humid subtropical	53.1 $\pm$ 2.0	5	.046 $\pm$ .003	14.6 $\pm$ .3	Liu and Karasov 2012
<i>Triaenops menamena</i>	Steppe	10.5 $\pm$ .8	11	.055 $\pm$ .030	26.6 [7.4–35.7]	S. Remmers, M. Schoroth, H. Rabarison, K. H. Dausmann, and S. Reher, unpublished data
<b>Miniopteridae:</b>						
<i>Miniopterus schreibersii</i>	Humid subtropical	10.2 $\pm$ 1.0	76	.31 $\pm$ .16	<24 <sup>a</sup>	Brown 1999
<b>Molossidae:</b>						
<i>Tadarida brasiliensis</i>	Humid subtropical	9.9 $\pm$ .6	11	.061 $\pm$ .016	10.0 <sup>a</sup>	Herreid 1963
<i>Tadarida teniotis</i>	Tundra	34.9 $\pm$ 4.7	4	.062 $\pm$ .02	8.75 <sup>a</sup>	Arletta et al. 2000
<b>Phyllostomidae:</b>						
<i>Macronycteris commersoni</i>	Steppe	53.7 $\pm$ 13.3	5	.034 $\pm$ .025	27.2 <sup>a</sup>	Reher et al. 2018
<b>Rhinopomatidae:</b>						
<i>Rhinopoma cystops</i>	Mediterranean		5	.16 $\pm$ .04	20 <sup>a</sup>	Levin et al. 2015
<i>Rhinopoma microphyllum</i>	Mediterranean		5	.087 $\pm$ .01	20 <sup>a</sup>	Levin et al. 2015
<b>Vespertilionidae:</b>						
<i>Barbastella barbastellus</i>	Marine west coast	7 <sup>a</sup>	4	.04 <sup>a</sup>	3.5 <sup>a</sup>	Pohl 1961
<i>Chalinobobus gouldii</i>	Marine west coast	14.3 $\pm$ 2.6	6	.02 $\pm$ .01	2.1 $\pm$ .3	Currie et al. 2018
<i>C. gouldii</i>	Mediterranean	17.5 <sup>a</sup>	10	.10 $\pm$ .02	10 <sup>a</sup>	Hosken and Withers 1997
<i>Corynorhinus townsendii</i>	Humid continental	9.6 $\pm$ 1.0	26	.052 $\pm$ .089	8 <sup>a</sup>	McGuire et al. 2021
<i>C. townsendii</i>	Humid continental	10.2 $\pm$ 1.0	31	.052 $\pm$ .065	10 <sup>a</sup>	McGuire et al. 2021
<i>C. townsendii</i>	Humid continental	10.3 $\pm$ 1.2	57	.057 $\pm$ .075	5 <sup>a</sup>	McGuire et al. 2021
<i>C. townsendii</i>	Humid continental	10.6 $\pm$ 1.4	26	.061 $\pm$ .087	8 <sup>a</sup>	McGuire et al. 2021
<i>C. townsendii</i>	Mediterranean	10.8 $\pm$ .9	12	.043 $\pm$ .045	8 <sup>a</sup>	McGuire et al. 2021
<i>Eptesicus bottae</i>	Desert	9.0 <sup>a</sup>	6	.39 $\pm$ .30	15 <sup>a</sup>	Muñoz-García et al. 2016
<i>Eptesicus fuscus</i>	Humid continental	14.7 $\pm$ 1.3	4	.028 $\pm$ .012	3.5 $\pm$ 1.2	Willis et al. 2005 <sup>a</sup>

<i>Lasionycteris noctivagans</i>	Humid subtropical	10.5 (±.3)	3	.035 <sup>a</sup>	5.0 ± 1.0	Dunbar 2007
<i>Lasius borealis</i>	Humid subtropical	11	9	.035	5 <sup>a</sup>	Dunbar and Tomasi 2006
<i>Lasius cinereus</i>	Steppe	25–40	23	.38 ± .09	10–20	Cryan and Wolf 2003
<i>Myotis lucifugus</i>	Humid continental	5.2 <sup>a</sup>	21	.022 <sup>a</sup>	2.0 <sup>a</sup>	Hock 1951
<i>M. lucifugus</i>	Humid continental	6.3	11	.02 <sup>a</sup>	5.0 <sup>a</sup>	Thomas et al. 1990
<i>M. lucifugus</i>	Humid continental	7.7 ± .9	39	.051 ± .070	2.0 <sup>a</sup>	McGuire et al. 2021
<i>M. lucifugus</i>	Subarctic	10.0 ± 1.0	20	.050 ± .058	5.0 <sup>a</sup>	McGuire et al. 2021
<i>M. lucifugus</i>	Subarctic	9.4 ± 1.0	40	.030 ± .021	5.0 <sup>a</sup>	McGuire et al. 2021
<i>M. myotis</i>	Marine west coast	24.0 <sup>a</sup>	14	.02 <sup>a</sup>	3.5 <sup>a</sup>	Pohl 1961
<i>M. myotis</i>	Humid continental	25.4 ± .4	4	.18 ± .016	16 <sup>a</sup>	Wojciechowski et al. 2007
<i>Myotis velifer</i>	Humid subtropical	11.9 (±.2)	8	.02 (±.003)	20.0 <sup>a</sup>	Riedesel and Williams 1976
<i>Nyctalus noctula</i>	Tundra	28.9 ± 1.8	3	.036 ± .004	3.75 <sup>a</sup>	Arlettaz et al. 2000
<i>Nyctophilus bifax</i>	Humid subtropical	9.8 ± 1.1	9	.048 ± .006	8.9 ± .5	This study (see also Stawski and Geiser 2011)
<i>N. bifax</i>	Tropical savanna	9.8 ± 1.1	8	.046 ± .007	9.0 ± .8	Stawski and Geiser 2011
<i>Nyctophilus geoffroyi</i>	Marine west coast	7.1 ± .8	17	.037 ± .014	5.9 ± 2.7	Geiser and Brigham 2000
<i>N. geoffroyi</i>	Mediterranean	8.0 (±.1)	7	.26 ± .11	14.5 <sup>a</sup>	Hosken and Withers 1999
<i>Nyctophilus gouldi</i>	Marine west coast	10 ± 1.1	6	.052 ± .01	8.9 ± 1.4	Geiser and Brigham 2000
<i>Otonycteris hemprichii</i>	Desert	29.7 <sup>a</sup>	6	.15 ± .44	15 <sup>a</sup>	Muñoz-Garcia et al. 2016
<i>O. hemprichii</i>	Desert	25.4 ± 2.1	6	.209 <sup>a</sup>	20–23	Marom et al. 2006
<i>Pipistrellus kuhlii</i>	Desert	6.8 <sup>a</sup>	9	.60 ± .82	10 <sup>a</sup>	Muñoz-Garcia et al. 2016
<i>Plecotus auritus</i>	Subarctic	8.3 ± .8	5	.098 ± .065	8.5 ± .7	This study
<i>Plecotus christii</i>	Desert	6.8 <sup>a</sup>	7	.85 ± 1.50	10 <sup>a</sup>	Muñoz-Garcia et al. 2016
<i>Vespadelus vulturnus</i>	Marine west coast	4.0 ± .7	5	.014 ± .0006	4.6 ± .4	Willis et al. 2005b

Note. Values in parenthesis indicate reported SE values instead of SD, and values in square brackets indicate range values. The value from the study by Marom et al. (2006) is rereported from Nowack et al. (2020).  $M_n$  = body mass;  $T_a$  = ambient temperature.

<sup>a</sup>SD not reported.

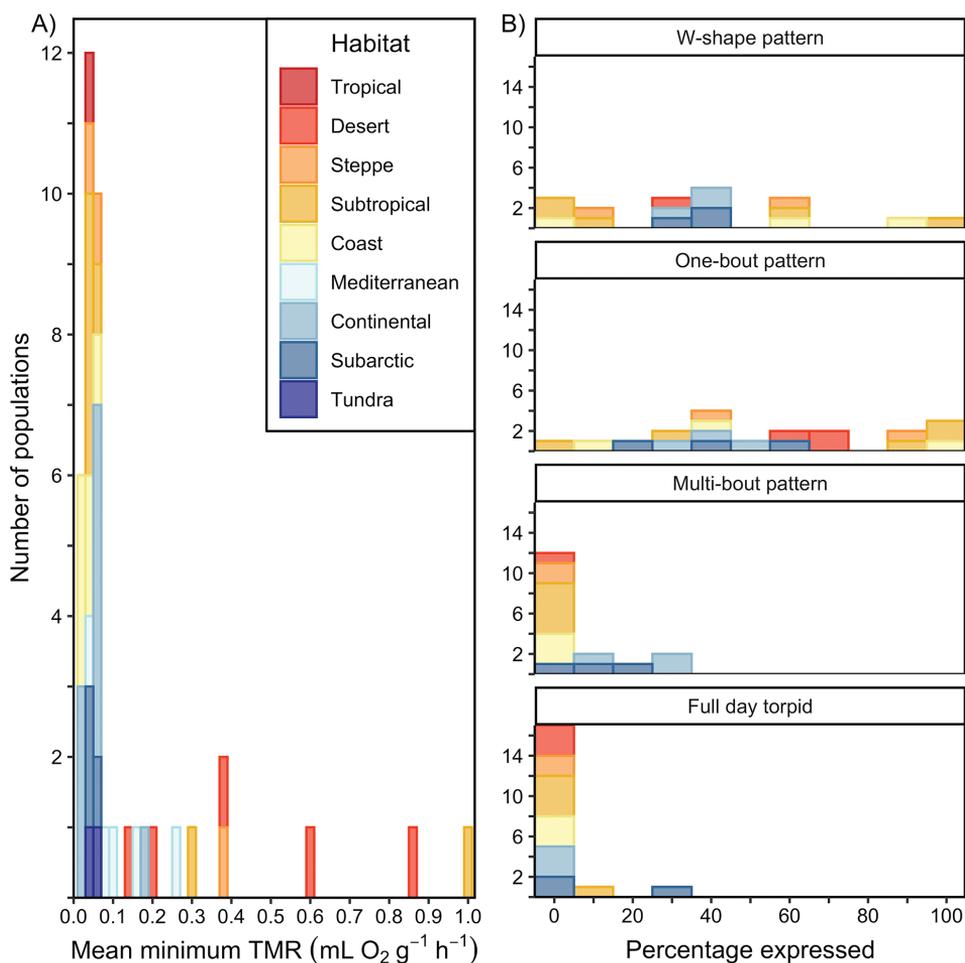


Figure 2. Distribution of torpor expression data collected from bat populations inhabiting different environments. Habitats for each population have been determined using the climate zone world map available through <https://www.arcgis.com>. Some of the habitat names have been shortened for figure presentation purposes. A, Mean minimum torpid metabolic rate (TMR; mL O<sub>2</sub> g<sup>-1</sup> h<sup>-1</sup>) measured in thermoconforming bats. B, Percentage of bat days where bats expressed torpor skin temperature cycles corresponding to each of the four torpor patterns identified, with number of populations on the y-axis. Only values from studies where it was possible to obtain exact percentage values (table 2) were included.

(see, e.g., fig. 1.9 in Geiser 2021), the critical  $T_a$  for thermoregulation seems to differ between populations and species. As a result, the lowest mean TMR is recorded at various  $T_a$ 's, with generally higher TMRs at higher  $T_a$ 's, likely as a result of temperature effects on physiological processes leading to a passive increase in oxygen consumption. However, above 10°C the data is highly scattered and includes data points with large standard deviations, which might suggest that the reported mean minimum TMR includes

high individual variations and might not be fully representative. Four of the five desert populations had a standard deviation between 77% and 176% of the mean minimum TMR, while the last population did not report a standard deviation. Also, the two steppe populations at  $T_a > 25^\circ\text{C}$  should be cautiously interpreted, as they were field studies and thus TMR was not tested across a given temperature range; however, the low reported TMRs at such high  $T_a$ 's are still interesting to compare with other species and

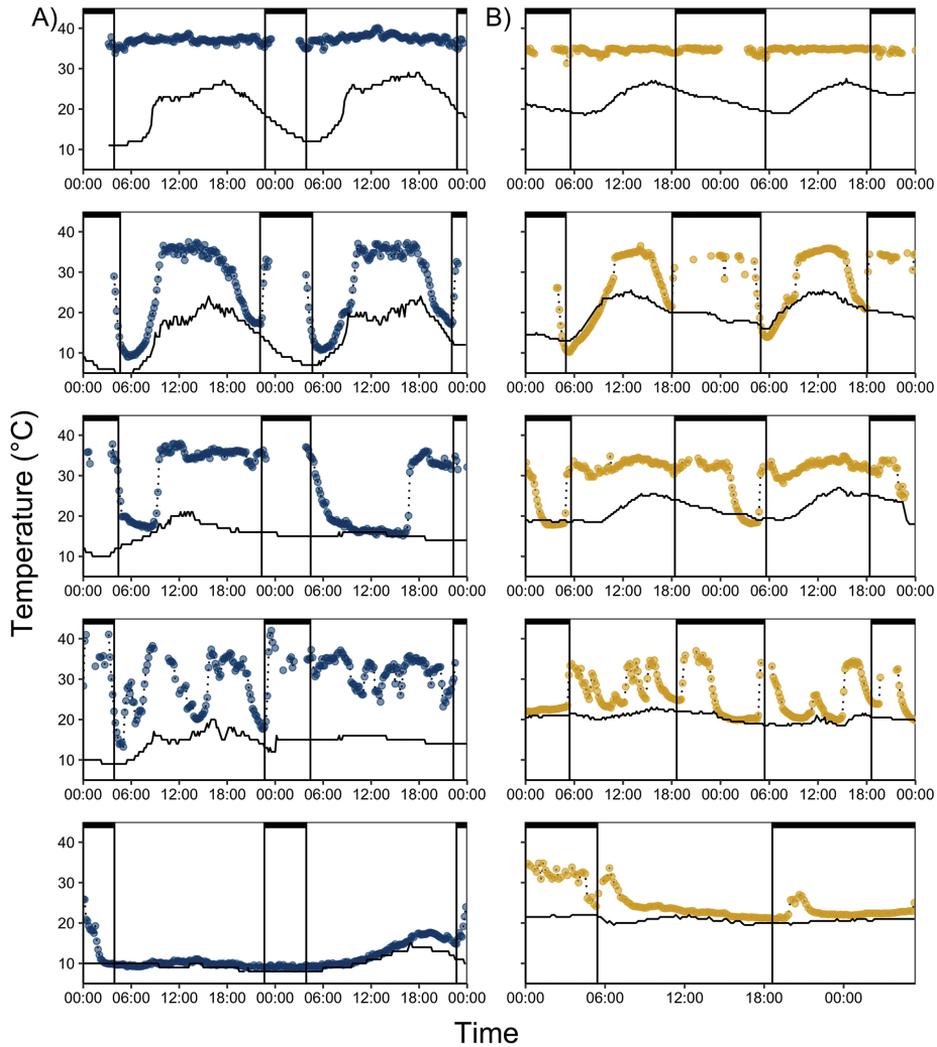


Figure 3. Torpor skin temperature ( $T_{\text{skin}}$ ) patterns. Examples of  $T_{\text{skin}}$  (upper traces) and ambient temperature (lower traces) across 2 d for the cold temperate northern species *Plecotus auritus* (A) and the warm subtropical southern species *Nyctophilus bifax* (B), showing five distinct patterns. Row 1: normothermic. Row 2: W-shaped pattern with two torpor bouts per day. Row 3: one-bout torpor pattern, with one torpor bout per day. Row 4: multibout torpor pattern, with  $>2$  torpor bouts per day. Row 5: full-day torpid pattern, which also included staying torpid throughout the night. Bars indicate time between sunset and sunrise. Missing data points during this period are caused by bats being out of range of the data loggers while foraging. Note that row 4 in A consists of 24-h observations of two separate individuals, as there were no continuous 48-h observations of this torpor pattern in *P. auritus*. Note also the different x-axis in row 5 in B.

populations, as these bats could be more capable of larger energy savings at higher temperatures, potentially as local adaptations to their environment.

To examine whether the harsh environment of the desert or steppe climate zones results in higher or lower  $O_2$  uptake in torpid thermoconforming bats than in bats inhabiting other habitats,

Table 2: Torpor patterns from radiotelemetry studies on free-ranging bats across climate zones

Species	Climate zone	Roost type	$N_{ind}$	Normo-thermic (d)	Torpor (d)	Type 1 (W shape; d)	Type 2 (one bout; d)	Type 3 (multibout; d)	Type 4 (full day; d)	Reference(s)
<b>Molossidae:</b>										
<i>Mops condylurus</i> <sup>a</sup>	Steppe	Buildings	≥16	22	19	2 (10.5%)	17 (89.5%)	0	0	Vivier and Van Der Merwe 2007 <sup>b</sup> Bondarenco et al. 2013
<i>Mormopterus</i> sp.	Desert	Trees	8	18	38	10 (26.3%)	28 (73.7%)	0	0	
<b>Vespertilionidae:</b>										
<i>Antrozous pallidus</i>	Subarctic	Rock crevices	8	1	54	19 (35.2%)	34 (63.0%)	1 (1.8%)	0	Rambaldini and Brigham 2008 Turbill 2006 <sup>b</sup>
<i>Chalinolobus morio</i>	Marine west coast	Trees	6	2	15	0	15 (100%)	0	0	
<i>Corynorhinus rafinesquii</i>	Humid subtropical	Trees, rock crevices, caves, buildings	46	198	183	16 (8.7%)	167 (91.3%)	0	0	Johnson and Lacki 2014
<i>Eptesicus fuscus</i>	Steppe	Rock crevices	24	24	136	Shown	Shown			Lausen and Barclay 2003 Rintoul and Brigham 2014 <sup>b</sup> Fjellidal et al., unpublished data Ossa et al. 2020
<i>E. fuscus</i>	Humid continental	Trees and buildings	27	36	67	17 (25.4%)	27 (40.3%)	23 (34.3%)	0	
<i>Eptesicus nilssonii</i>	Subarctic	Buildings	6	4	32	13 (40.6%)	6 (18.8%)	2 (6.2%)	11 (34.4%)	
<i>Histiotus magellanicus</i>	Tundra	Trees	8			Shown	Shown			
<i>Lasurus borealis</i>	Humid subtropical	Trees	17	58	49	Shown	Shown		Shown	Monarchino and Johnson 2021 Klug and Barclay 2013
<i>Lasurus cinereus</i>	Humid continental	Trees	15	5	184	Shown	Shown		Shown	Dietz and Hörig 2011 Otto et al. 2015
<i>Myotis bechsteinii</i>	Marine west coast	Trees	12			Shown	Shown		Shown	
<i>M. bechsteinii</i>	Marine west coast	Trees				Shown	Shown			
<i>Myotis chiloensis</i>	Tundra	Trees	4			Shown	Shown			Ossa et al. 2020
<i>Myotis daubentonii</i>	Marine west coast	Trees	11			Shown	Shown			Dietz and Kalko 2006
<i>M. daubentonii</i>	Marine west coast	Trees	11			Shown	Shown		Shown	Dietz and Hörig 2011
<i>Myotis evotis</i>	Steppe	Rock crevices	17	0	52	Shown	Shown			Chruszcz and Barclay 2002

Species	Subarctic	Rock crevices	20	8	85	Shown	Mentioned	0	Solick and Barclay 2006
<i>Myotis lucifugus</i>	Humid continental	Bat boxes	36	21	131	55 (42%)	39 (29.8%)	37 (28.2%)	Bestler and Broders 2019 <sup>b</sup>
<i>M. lucifugus</i>	Subarctic	Buildings	10			Shown	Shown	0	Boyles et al. 2016
<i>Myotis natterii</i>	Marine west coast	Trees							Otto et al. 2015
<i>Myotis sodalis</i>	Humid continental	Trees and bat boxes	58	45		21 (36.2%)	30 (51.7%)	7 (12.1%)	Bergeson et al. 2021 <sup>b</sup>
<i>Myotis thysanodes</i>	Steppe	Rock crevices and trees	7	0	27	16 (59.3%)	11 (40.7%)	0	Alston et al. 2021
<i>Nyctophilus bifax</i>	Humid subtropical	Trees	19	17	71	41 (57.7%)	22 (31.0%)	2 (2.8%)	This study (see also Stawski 2010; Stawski and Geiser 2010)
<i>Nyctophilus geoffroyi</i>	Marine west coast	Trees	7	0	39	23 (59.0%)	15 (38.5%)	0	Turbill et al. 2003a
<i>Nyctophilus gouldi</i>	Marine west coast	Trees	4	1	15	13 (86.7%)	2 (13.3%)	0	Turbill 2006a
<i>Plecotus auritus</i>	Subarctic	Buildings and trees	29	22	132	40 (30.3%)	58 (43.9%)	32 (24.3%)	This study
<i>P. auritus</i>	Marine west coast	Trees							Otto et al. 2015
<i>Scotophilus dinganii</i>	Humid subtropical	Buildings	6	0	10	0	10 (100%)	0	Jacobs et al. 2007
<i>Scotophilus milanganii</i>	Humid subtropical	Trees	2	0	6	0	6 (100%)	0	Jacobs et al. 2007
<i>Scotorepens badstoni</i>	Desert	Trees	5	10	24	Merged with type 3 (total 9 d)	Merged with type 1 (total 9 d)	0	Bondarenco et al. 2016
<i>Scotorepens greyii</i>	Desert	Trees	10	10	50	Merged with type 3 (total 14 d)	Merged with type 1 (total 14 d)	0	Bondarenco et al. 2016
<i>Vespadelus pumilus</i>	Humid subtropical	Trees	3	0	4	4 (100%)	0	0	Turbill et al. 2003b

<sup>a</sup>Studies performed on individuals in captivity.

<sup>b</sup>Authors were contacted for additional information beyond that published in their article and included in this table.

more data from such populations are needed. Mammals capable of prolonged torpor bouts (i.e., hibernators) typically display  $\text{TMR} \leq 0.1 \text{ mL O}_2 \text{ g}^{-1} \text{ h}^{-1}$ , whereas those that employ only daily torpor display  $\text{TMR} > 0.1 \text{ mL O}_2 \text{ g}^{-1} \text{ h}^{-1}$  (Ruf and Geiser 2015). Therefore, while most bats studied to date are likely able to undertake prolonged torpor bouts, arid zone bats are perhaps more often daily heterotherms. However, it is known that some arid zone species, such as *Rhinopoma microphyllum* and *Rhinopoma cystops*, do hibernate (Levin et al. 2015), suggesting that they may just display higher TMRs. Hot torpor in bats (see, e.g., Reher and Dausmann 2021) is a relatively new phenomenon, but it is an interesting new development within heterothermy studies and may lead to more torpor studies being undertaken on bats inhabiting warmer climates. Visualizing the scaled residuals of log-transformed TMR after accounting for the temperature effect showed no apparent phylogenetic clustering (fig. S2), although statistical analyses are needed to draw conclusions.

#### Torpor Skin Temperature Patterns

Some torpor patterns outside of the hibernation season have already been identified in previous studies (e.g., Turbill et al. 2003a, 2003b). Here, we present a full list of patterns currently available in the literature and identified in our own collected data on *N. bifax* and *P. auritus* (fig. 3).

*Normothermic.* Bats did not employ torpor across 24 h.

*W-shaped torpor pattern.* Bats expressed two torpor bouts during the day, commonly one in the morning after returning to the roost and one in the afternoon before arousing at sunset to forage.

*One-bout torpor pattern.* Bats expressed a single torpor bout during the day, commonly in the morning.

*Multibout torpor pattern.* Bats employed torpor at multiple occasions (>2) during the day.

*Full-day torpid pattern.* Bats either entered torpor in the morning or were already torpid by the beginning of the day and stayed torpid for the full day and following night.

The  $T_{\text{skin}}$  patterns do not take into account length or depth of the torpor bouts and thus compare only the number of torpor bouts expressed during a bat day. Excluding winter season studies and studies reporting no heterothermy during the data collection period resulted in torpor  $T_{\text{skin}}$  patterns obtained from a total of 33 populations of free-ranging bats (including *N. bifax* and *P. auritus* presented in this study). The populations were from seven different climate zones: desert ( $N = 3$ ), steppe ( $N = 4$ ), humid subtropical ( $N = 6$ ), marine west coast ( $N = 9$ ), humid continental ( $N = 4$ ), subarctic ( $N = 5$ ), and tundra ( $N = 2$ ). We were not always able to obtain exact values for the number of bat days with expression of different torpor patterns, but we have included studies that either showed one or more of these patterns in their figures or mentioned any of the patterns in the text (table 2). The W-shaped and one-bout torpor patterns were the most common heterothermy patterns observed during a bat day and were observed in at least one population from each of the seven climate zones (table 2; fig. 2B). The multibout and full-day torpor patterns were also reported for several species, although

these were never the most common torpor pattern expressed in any population studied.

Torpor expression in bats may vary with environmental factors like temperature, light, and weather, and the use of heterothermy has been found to be heavily state dependent, especially regarding an individual's reproductive state (Stawski et al. 2014). The telemetry studies we are presenting  $T_{\text{skin}}$  data from are from males and females alike and include data from nonreproductive individuals as well as from pregnant, lactating, and postlactating females. This indicates that reproductive individuals may show torpor pattern cycles similar to those of nonreproductive individuals. However, we have not included information on the depth or length of torpor bouts expressed, which have been found to be highly variable between reproductive states (e.g., Lausen and Barclay 2003; Johnson and Lacki 2014; Besler and Broders 2019). Furthermore, bats actively choose roost types and select microclimates that fit their current preferences based on individual state or weather conditions to help optimize energetic benefits (Sedgely 2001; Lausen and Barclay 2003; Lourenço and Palmeirim 2004).

Although different climate zones and habitat types are likely to influence the microclimates of roosts available for local bat populations, torpid bats might be buffered against some of the largest microclimate differences by their active choice of roosting conditions, thus expressing similar patterns of  $T_{\text{skin}}$  cycles. We did not, however, detect any clear distributions in  $T_{\text{skin}}$  torpor patterns for different roost types (fig. S3), which might be due to potential large variations in microclimates even within the same roost. It is possible that investigations of depth or length of daily torpor and use of passive versus active arousals would reveal the effects of different roost types (Doty et al. 2016; Bergeson et al. 2021), but types of torpor  $T_{\text{skin}}$  patterns alone seem to be similar across roost types and climate zones. Even though torpor depth and length vary with reproductive state,  $T_a$ , and other environmental factors, and therefore likely between habitats, we argue that when torpor is expressed, specific patterns can be recognized independently of reproductive state or climate zones, likely driven by microclimate conditions experienced in the roost.

#### Conclusions

Our data on torpor energetics and behavior expressed in two bat species inhabiting highly different environments on opposite sides of the globe are examples of how heterothermy may be seen as a similar physiological response across bat species and climate zones. Regardless of local weather conditions, the available data reveal that heterothermic bats around the world reduce MRs to similar levels during torpor, although the populations vary in the temperature for observed mean minimum TMR, possibly indicating variations in the critical temperature for thermoregulation during torpor. Furthermore, desert and steppe populations may seem to have respectively higher and lower TMRs compared with the  $T_a$  they were measured at, but more data from such populations are needed to draw conclusions. Daily  $T_{\text{skin}}$  fluctuations outside of the hibernation season revealed no apparent differences between climate zones in number of torpor bouts

expressed, but depth and length of torpor bouts between populations should be further assessed. The observed similarities in torpor physiology might be driven by careful selection of roosts and microclimates at the individual level, even when macroclimatic conditions across geographical locations vary drastically. Although similarities in traits across species and higher taxa may evolve through convergence, we believe that this review lends further support to the hypothesis that heterothermy in bats, and likely all mammals, is an ancestral state and has been lost only in those species where it no longer provides any benefits.

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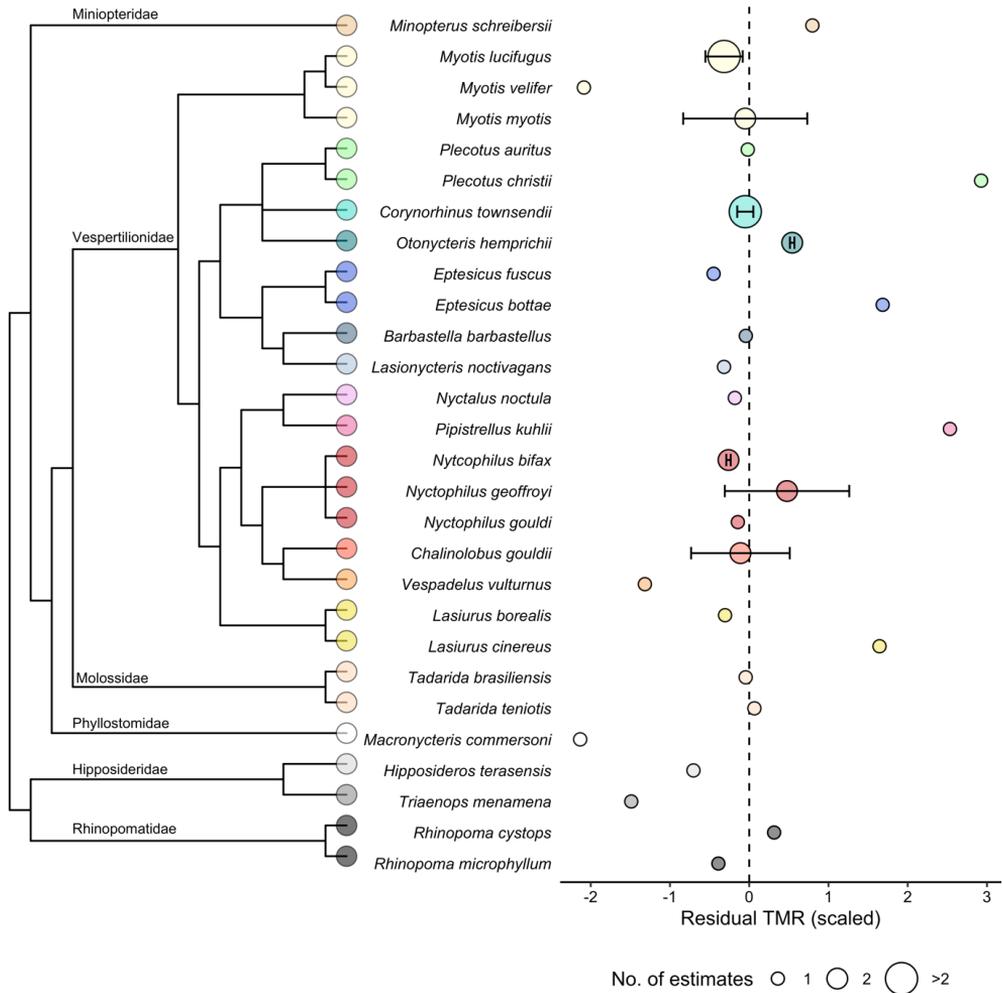
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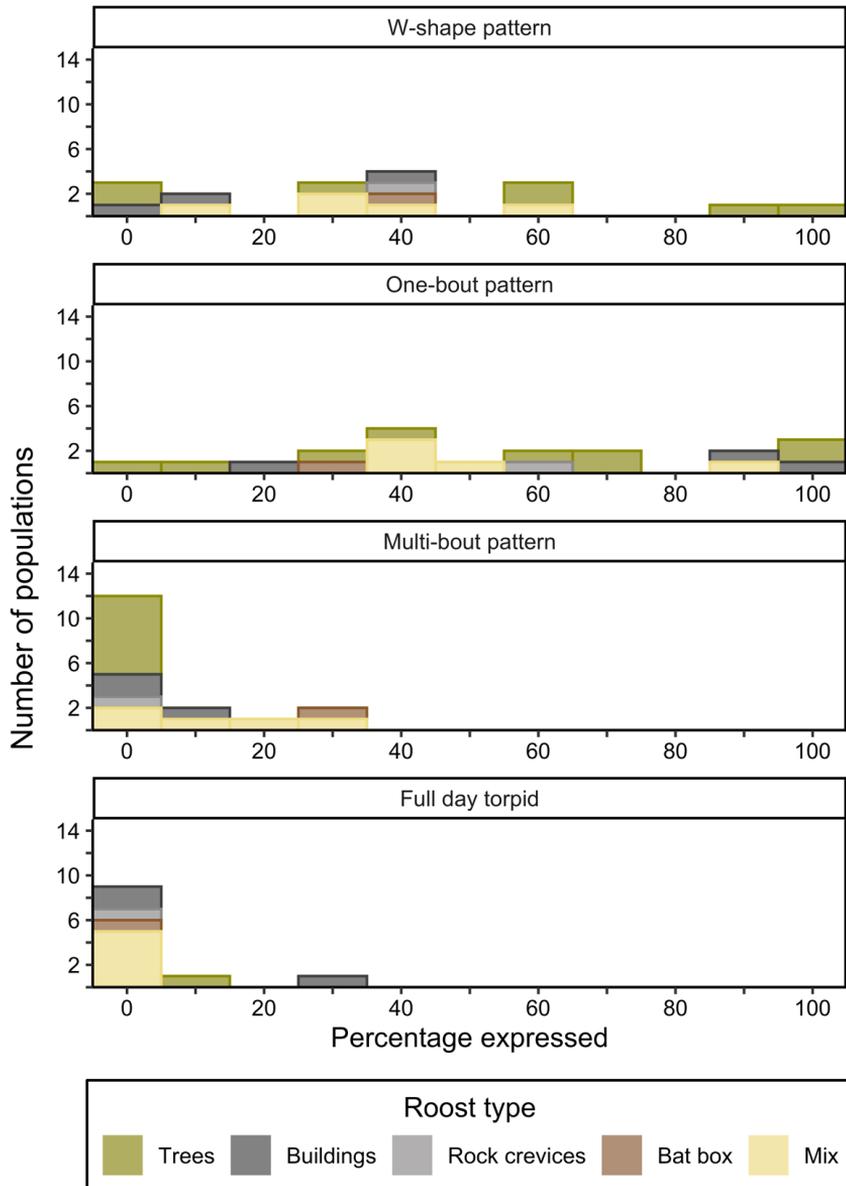


## Supplementary Materials 2: Residual TMR in phylogenetic tree



**Figure S2:** A simplified phylogenetic tree showing the distribution of the residual scaled TMR after accounting for the temperature effect. Species within the same family share colour.

### Supplementary Materials 3: Roost types and $T_{skin}$ patterns



**Figure S3:** Distribution of torpor expression data collected from bat populations using different roost types shown as the percentage of bat-days where bats expressed torpor  $T_{skin}$  cycles corresponding to each of the four torpor patterns identified. Only values from studies where it was possible to obtain exact percentage values (Table 2) were included in the figure. The studies reported data from bats using one of four different roost types (trees, buildings, rock crevices, bat boxes) or of bats using multiple (>2) roost types (shown as “Mix” in the figure) during the data collection.

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