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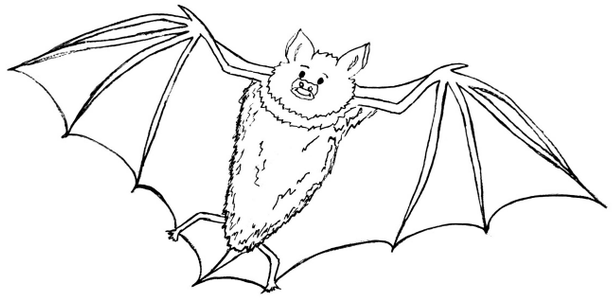

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Variation in basal metabolic rate within bats of the family Vespertilionidae

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Master's thesis in biology
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Abstract

Many physiological traits, such as metabolic rate, are thought to vary with climate and latitude. However, little information is available on the thermoregulation of bats at high latitudes. Therefore, I investigated the thermoregulatory strategies of whiskered bats (*Myotis mystacinus*) from the family Vespertilionidae close to this species' northern distribution range. The rate of oxygen consumption (VO_2) increased with decreasing ambient temperature (T_a) below a lower critical temperature (T_{LC}) of around 33.1°C, a T_{LC} which was in the range of that found for other bats. The thermal conductance was low compared to other mammals, which indicated that effective thermal insulation mechanisms have evolved. A high cold-induced metabolic scope was reached at 12°C, revealing that other heat-producing or energy-saving mechanisms than shivering may need to be initiated at lower temperatures to avoid hypothermia, such as activity or the use of torpor. The basal metabolic rate (BMR) of *M. mystacinus* from a population in Norway at 60°N was 1.48 mL O_2 g^{-1} h^{-1}, which was 99% of what was predicted for a vespertilionid bat weighing 4.4 g, and 64% of what was predicted for a mammal weighing 4.4 g.

A low BMR is often associated with warm environmental temperatures, whereas mammals living in colder areas experience elevated thermoregulatory costs leading to an increase in BMR. This is especially apparent in small mammals, such as bats, due to their high surface area to volume ratio resulting in high rates of heat loss, high mass-specific BMR and therefore high energetic costs. Bats inhabit a variety of climate types, ranging from tropical to temperate zones, and the environmental temperature differences may influence BMR of bats from different populations. In the present study, I conducted a comparative analysis to investigate how BMR varies in vespertilionid bats living in different climates. I hypothesised that BMR would differ between species, predicting that BMR would be higher in climates with cold environmental temperatures compared to warmer climates. From a systematic literature search I obtained BMR estimates (N = 47) of 21 species within Vespertilionidae, which also included my own metabolic measurements of *M. mystacinus* in Norway. A decrease in mean environmental temperature was apparent with increasing latitude, but I found no effect of neither latitude nor environmental temperature on BMR. Hence, these data do not support the prediction that BMR increases with latitude and with decreasing environmental temperature in this particular group of bats. How these bats adapt metabolically to high latitude living is thus still an open question. One possible factor could be that instead of adjusting BMR, they could express a more frequent use of temporal heterothermy.

Sammendrag

Mange fysiologiske variabler, slik som metabolsk rate, varierer med klima og breddegrad. Det finnes imidlertid lite informasjon om termoregulering i flaggermus ved høye breddegrader. Derfor undersøkte jeg de termoregulerende responsene til en populasjon med skjeggflaggermus (*Myotis mystacinus*) fra Glattnesefamilien nær denne artens nordre distribusjonsgrense. Oksygenopptaksraten (VO_2) økte med synkende omgivelsestemperatur (T_a) under en temperaturgrense (T_{LC}) på rundt 33.1°C, en T_{LC} som er i samme område som for andre flaggermus. Termisk konduktivitet var lav sammenlignet med andre pattedyr, noe som indikerte at effektive mekanismer for varmeisolasjon har blitt utviklet. Et stort omfang av kuldeindusert metabolsk rate ble funnet ved 12°C, som kan bety at andre varmeproduserende eller energisparende mekanismer enn skjelving må finne sted ved temperaturer under dette, som for eksempel aktivitet eller torpor. Det ble målt en basal metabolsk rate (BMR) på 1.48 mL O_2 g^{-1} h^{-1}, som var 99% av det som var forventet for en glattneseflaggermus som veier 4.4 g, og 64% av det som var forventet for et pattedyr som veier 4.4 g.

En lav BMR assosieres ofte med varme omgivelsestemperaturer, mens pattedyr som lever i kaldere omgivelser ofte har høye energikostnader som fører til en økning i BMR. Dette er mest tydelig i små pattedyr, slik som flaggermus, på grunn av deres høye volum-overflateforhold og dermed store varmetap, høy massespesifikk BMR og av den grunn store energikostnader. Flaggermus lever i ulike typer klima, alt fra tropiske til tempererte strøk, og variasjoner i omgivelsestemperatur kan dermed påvirke BMR hos flaggermus fra forskjellige områder. I denne studien utførte jeg en komparativ analyse for å forstå hvordan BMR varierer i glattneseflaggermus fra ulike klima. Min hypotese var at BMR ville være forskjellig mellom artene, og jeg antok at BMR ville være høyere i klima med kalde gjennomsnittstemperaturer sammenlignet med varmere klima. Fra et systematisk litteratursøk hentet jeg mål på BMR (N = 47) fra 21 arter i Glattnesefamilien, som også inkluderte mine egne metabolske målinger av *M. mystacinus* i Norge. Det ble funnet en nedgang i gjennomsnittlig omgivelsestemperatur med økende breddegrad, men jeg fant ingen effekt av verken breddegrad eller omgivelseststemperatur på BMR. Det er derfor fortsatt uvisst hvordan disse flaggermusene har tilpasset seg å leve i kalde omgivelser langt nord. I stedet for å regulere BMR, er det mulig at de uttrykker en hyppigere bruk av temporær heterotermi.

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Abbreviations

BMR	=	basal metabolic rate
C	=	conductance
I	=	insulation
F'_eO_2	=	fractional concentration of excurrent oxygen
F_iO_2	=	fractional consentration of incurrent oxygen
FR_i	=	incurrent mass flow rate
RMR	=	resting metabolic rate
RQ	=	respiratory quotient
SDA	=	specific dynamic action
STP	=	standard temperature and pressure
T_a	=	ambient temperature
T_b	=	body temperature
T_{LC}	=	lower critical temperature
TNZ	=	thermoneutral zone
T_{UC}	=	upper critical temperature
VO_2	=	rate of oxygen consumption

Introduction

Energy plays a vital role in animal functioning, as it is the fuel that drives physiological processes. It is essential for body maintenance and for all functions of the animal, such as reproduction and development. The energy budget of an animal is determined by its energy gain, storage and loss (Porter and Gates, 1969). Energy is gained by eating and drinking, it is stored in tissue, and lost in excreta, external work or as metabolic heat. The rate of energy consumption and energy turnover is known as metabolic rate, and measures how much energy is needed for different processes. High metabolic rates enable the allocation of energy to processes beyond general maintenance, such as increased activity and growth, which may be an advantageous investment in many conditions. The energetic costs associated with elevated metabolic rates are however high, especially in thermally challenging environments (Hill et al., 2016).

Temperature has a great effect on the rates of metabolic processes, and accordingly affects animal functioning. Maintaining a high body temperature (T_b) facilitates for example rapid nerve conduction, rapid muscle contraction and rapid nutrient absorption, which enables processes such as increased tissue building (Montgomery and MacDonald, 1990; Hill et al., 2016). Elevated T_b can be achieved by increasing heat production through metabolic processes. Even though this can be advantageous, it is costly in some conditions. Ambient temperature (T_a) readily affects the T_b of animals, and inhabiting areas with extreme or variable T_a may therefore impose energetic challenges. Since T_b is normally higher than T_a, heat is lost to the environment. This heat loss must be counterbalanced to avoid hypothermia. However, if T_b is lower than T_a, heat is gained from the environment and this excess heat must be disposed of to avoid hyperthermia. Both avenues are critical to the animal and must be overcome in thermally challenging environments (Hill et al., 2016; Porter and Gates, 1969).

Animals inhabit environments with a range of different thermal conditions. A number of strategies have evolved to meet the thermal challenges encountered in the given environment. The T_b can be regulated behaviourally to some extent, for example by basking in the sun, changing posture, or by huddling together (Hill et al., 2016). For some animals, known as ectotherms, behavioural regulation is the main strategy to affect T_b. Behaviourally sustaining a high T_b in varying environmental temperatures is challenging, and many ectotherms are therefore thermoconformers, allowing T_b to vary with the external environment (Stevenson, 1985). This strategy effectively avoids the energetic costs associated with high metabolic rates (Hill et al., 2016).

While the metabolic rate of ectotherms is generally low, endotherms use internal heat production through elevated metabolic rates to increase T_b. Sustaining a high T_b is often beneficial as it allows for example fast movement, being nocturnal and staying active in cold temperatures. Many endotherms are also homeothermic, as they thermoregulate to maintain a stable, elevated T_b irrespective of variations in T_a. Endotherms, such as birds and mammals, are therefore known as thermoregulators. Mammals generally regulate T_b between 31 and 38°C (Withers et al., 2016). However, most mammals are also heterothermic to some degree, as both diurnal and seasonal variation in T_b are often ob-

served (Levesque et al., 2016). Elevated T_b through thermoregulatory mechanisms include adjusting insulation with fur, feathers and fat, optimizing morphological structures such as countercurrent heat exchangers, regulating evaporative heat loss by for example sweating and panting, and elevated rates of metabolic processes. The latter can be achieved by for example increased level of activity, food processing and shivering, which consequently increases the internal heat production (Withers et al., 2016).

Direct measurement of internal heat production is the most accurate measure of the metabolic rate (Lighton, 2008). However, a more common approach is to measure the rate of oxygen consumption (VO_2) as a proxy for metabolic rate, a method known as indirect calorimetry (Kaiyala and Ramsay, 2011; Lighton, 2008). The thermal relations of an animal are shown in equation 1.1,

$$\text{Metabolic rate} = \text{C} * (\text{T}_b - \text{T}_a) \tag{1.1}$$

where C is the thermal conductance representing how readily heat is lost to the environment, and the difference between T_b and T_a is the driving force for dry heat loss, that is heat loss through conduction, convection and radiation (Hill et al., 2016). The inverse of C is a measure of the animal's thermal insulation (I) (Aschoff, 1981). Thus, the metabolic rate equals the animal's rate of heat loss, which is readily affected by changes in T_a (Hill et al., 2016).

The relationship between metabolic rate and T_a can be illustrated by a thermoregulatory curve (figure 1.1). The range of T_a where changes in heat loss are counterbalanced through mechanisms such as vasoregulation and regulation of piloerection and fat, that is the insulative layer, is known as the thermoneutral zone (TNZ) (Withers et al., 2016). The metabolic rate measured in the TNZ is known as the basal metabolic rate (BMR), which represents the minimum amount of energy needed to maintain homeostasis, *i.e.* a stable milieu interior and thus a stable T_b. To ensure measurements of true BMR, it has to be measured in the absence of external activity, in an adult animal that is in its TNZ during the resting phase of the daily cycle, and that is post-absorptive and non-reproductive. Any changes in these factors mean that BMR is no longer measured, such as if the animal is being active, in a postprandial phase or if T_a is outside TNZ (Hill et al., 2016; Withers et al., 2016).

The metabolic rate measured below and above the TNZ of an endotherm at rest is known as resting metabolic rate (RMR). At temperatures below the TNZ, the gradient between T_a and T_b increases further, and heat loss must be compensated for by increasing RMR. Thus, as T_a decreases, RMR increases linearly. The slope of this linear regression is a measure of C. If the regression is extrapolated to the x-axis, it intercepts the x-axis at a temperature which is normally close to T_b of a homeothermic endotherm. The inflection point between RMR at T_a below the TNZ and BMR is known as the lower critical temperature (T_{LC}), whereas the inflection point between BMR and RMR at T_a above the TNZ is known as the upper critical temperature (T_{UC}). At T_a above T_{UC}, excess heat is disposed of mainly by using evaporative heat loss, including mechanisms such as sweating and panting. These are energetically expensive mechanisms, and are achieved by increasing RMR. Consequently, encountering environmental temperatures outside TNZ can influence the energetic requirements of an endotherm considerably (Hill et al., 2016; Withers et al., 2016).

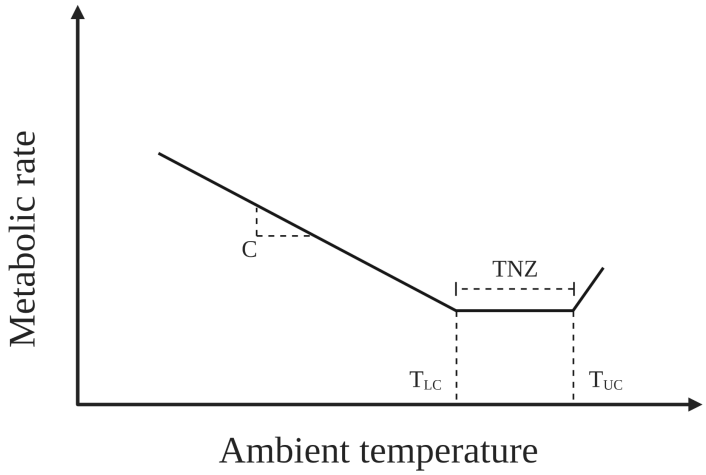

Figure 1.1: The thermoregulatory curve showing the thermoneutral zone (TNZ), lower critical temperature (T_{LC}), upper critical temperature (T_{UC}) and thermal conductance (C) of a homeothermic endotherm.

The energetic challenges experienced by animals readily affect functioning on the individual level, population level and species level (Garland Jr and Adolph, 1991). The environmental conditions are thought to have a great impact on BMR, and can accordingly influence animal performance (Lovegrove, 2000; Scholander et al., 1950). Hence, BMR is often used as a standard energetic parameter in ecological studies, as variation in BMR often reflects the habitat or ecosystem the animal lives in (Garland Jr and Adolph, 1991; White and Kearney, 2013).

The BMR varies greatly in mammals, with body mass explaining 95.9% of the variation (McNab, 2008; Withers et al., 2016). Large mammals have a higher rate of absolute energy turnover compared to small mammals, and accordingly BMR increases with body mass. However, BMR does not scale proportionally with body mass, as volume increases more rapidly than surface area does when size increases (Schmidt-Nielsen, 1984). The scaling relationship between body mass and BMR on logarithmic scales provides a linear relationship, from which the slope represents the interspecific scaling exponent. In figure 1.2, obtained from McNab (2008), the scaling exponent was found to be 0.72 for mammals.

A number of explanations have been proposed for the scaling relationship between body mass and BMR. One such explanation is the distribution network geometry theory, predicting a scaling exponent of 0.75 based on the distribution of oxygen and nutrients to tissues, which is determined by the fractally branching networks of blood vessels (West et al., 1997). The metabolic level boundaries (MLB) hypothesis predicts a variation of the scaling exponent between 0.67 and 1.0, as BMR is thought to be constrained by both surface-area and volume-related effects depending on the level of metabolism. The activity level of an animal determines the constraint of BMR, and hence the scaling exponent should also vary between different levels of metabolism (Glazier, 2005). The heat dissipation limits (HDL) hypothesis predicts that BMR is not constrained by energy acquisition, as is the case in the distribution network geometry theory, but by the ability to dissipate

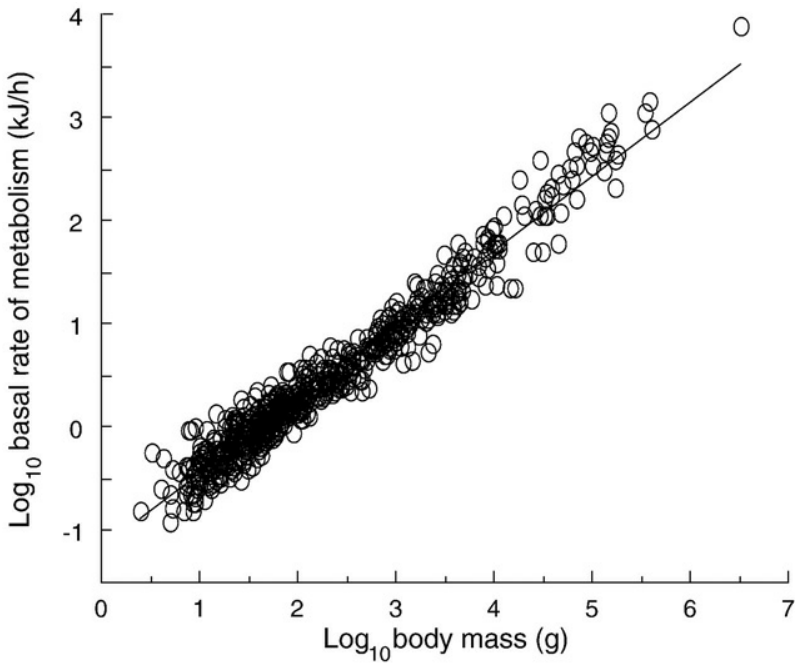

Figure 1.2: The metabolic allometry of basal metabolic rate in mammals. The allometric equation of the relationship in mL O_2 h^{-1} was BMR = 3.50 $*$ body mass$^{0.72}$, and the scaling exponent was thus 0.72. Figure obtained from McNab (2008).

heat. According to this theory, the scaling exponent for maximum capacity to dissipate heat is predicted to be between 0.47 and 0.50. The scope to increase metabolism decreases with body mass, determining the scaling relationship of BMR (Speakman and Król, 2010).

Regardless of which explanation to the metabolic exponent is correct, great variation is found in both the scaling exponent and the level of BMR in mammals (Glazier, 2005). Therefore, investigating environmental factors that could explain the remaining variation in BMR is an interesting area of research. It has for example been found that both body mass and BMR are affected by high latitude living (Raichlen et al., 2010). Because a large animal has a low surface area to volume ratio, heat is more easily retained in the body compared to a small animal (Schmidt-Nielsen, 1984). A large body also consists of more metabolically active tissue, and it is thus thought that large mammals are better suited to inhabit cold environments. Because the environmental temperature generally decreases with latitude, it has been suggested that body mass should increase with latitude, known as Bergmann's rule (Withers et al., 2016).

After removing the effects of body mass on BMR, it has been suggested that BMR should also increase with latitude to meet the energetic demands of inhabiting colder areas (Lovegrove, 2003). An increase in BMR with increasing latitude and decreasing environmental temperature has 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 found in studies of single mammalian groups, such as canids (Careau et al., 2007) and rodents (MacMillen and Garland Jr, 1989; Rezende et al., 2004; Speakman, 1999). Thus, a possible adaptation to inhabiting cold environments at high latitudes could be increased BMR.

Small mammals exposed to cold conditions face great thermoregulatory challenges, and therefore also face the need to sustain high metabolic rates and a constant allocation of energy. This requires increased food consumption, which may be difficult when temperatures are cold and food abundance low. Several strategies have thus evolved in small mammals to cope with these thermal challenges. One strategy is avoidance by migrating to warmer climates during cold seasons. However, this option is only available to birds and a limited number of mammals (Withers et al., 2016).

Most mammals do not have the possibility of migrating, and instead avoid the cold by huddling and hiding in warm locations. However, for some small mammals the energetic costs encountered in cold environments may be so great that strict homeothermy is abandoned. This strategy of reducing the metabolic rate and T_b is known as torpor, and effectively avoids the high metabolic rate associated with maintaining a high T_b in cold temperatures. Species that frequently use torpor are known as torpidators, and applies to potentially 40% of extant mammalian species (Geiser and Turbill, 2009; Withers et al., 2016). The T_b often fluctuates with ambient temperature during a torpor bout, and therefore torpidators are heterothermic. A number of mammalian groups, such as bats, are capable of entering torpor when conditions are unfavourable (Ruf and Geiser, 2015; Withers et al., 2016).

Bats belong to the mammalian order Chiroptera, which is a diverse group of mammals. They differ in many physiological traits, such as in body size, morphology and reproduction, but also in ecological traits such as roosting, foraging and flight behaviour. For example, some male bats mate in autumn before hibernation, whereas some mate in spring after hibernation (Hill and Smith, 1984). Many bats are heterothermic endotherms, using torpor both seasonally and daily. For example, many bats hibernate during winter, while some bat species avoid bad winter conditions and migrate instead (Dietz and Kiefer, 2016; Fleming, 2019). These strategies are most common in bats from temperate areas, but are also found in tropical species (Fleming, 2019; Stawski et al., 2014). Distinctive diets have also evolved, with species feeding on for example insects, fruits, nectar and fish. All these strategies impose different energetic challenges, and may thus reflect the variation in BMR within bats (Speakman and Thomas, 2003).

The BMR within bats has been found to increase with body mass with a scaling exponent of 0.74 (Cooper and Geiser, 2008; Withers et al., 2016). However, the variation in BMR when the effect of body mass is removed remains unexplained. As an increase in BMR with increasing latitude has been found in birds and other mammals, as mentioned previously, this trend could also be expected for bats. However, both Cruz-Neto (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. Speakman and Thomas (2003) suggested that more studies from temperate areas must be included to fully represent a latitudinal gradient.

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 for 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 at high latitudes should thus have evolved in bats.

Torpor use is a strategy that allows the individual to avoid unfavourable conditions, such as cold temperatures. Consequently, bats can effectively save energy for days or seasons when energy availability is low (Cooper and Geiser, 2008). It has therefore been suggested that bats can avoid having to sustain a high BMR in cold conditions by frequent use of torpor (McNab, 1983). However, 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). However, it can be problematic to separate latitudinal or temperature effects on thermoregulatory strategies from resource availability, as a decrease in environmental temperature with increasing latitude is paralleled by a decrease in available resources (Dunbar and Brigham, 2010; Lovegrove, 2000).

Most insectivorous species within the order of Chiroptera belong to the family Vespertilionidae. 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, 1980) and to mammals in general, varying between 53-93% of that predicted for mammals of the same size (Hosken and Withers, 1997).

One of the smallest bats within 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). Whiskered bats in Norway encounter seasonally cold temperatures and low prey abundance, which may impose great energetic costs. Due to long winters and short summer nights, these bats have limited active periods, which also imposes a constraint on energy acquisition. The thermal challenges associated with these constraints may impact the level of BMR. Investigating the BMR of a Northern population of whiskered bats close to its northernmost distribution limit is thus interesting, and could provide additional information to how vespertilionid bats cope with cold temperatures.

Hypothesis and predictions. The aim of the present study is to investigate the BMR of vespertilionid bats inhabiting a variety of environments and thus encountering different thermal challenges. The aims are twofold: 1) to provide the thermoregulatory curve and BMR of *M. mystacinus* inhabiting a seasonally cold environment in Norway, and 2) investigating the latitudinal and temperature effects on the BMR of species within Vespertilionidae. The second part is achieved by conducting a systematic literature search and comparative analysis of BMR in vespertilionid bats based on published data on BMR. I hypothesise that BMR in *M. mystacinus* will be low compared to other bats in Vespertilionidae as an adaptation to high latitude living. I also hypothesise that BMR within insectivorous vespertilionid bats will differ between species, with the prediction that BMR will be higher in climates with colder environmental temperatures at high latitudes compared to warmer climates at low latitudes.

Materials and methods

2.1 Metabolic rates of *M. mystacinus*

2.1.1 Animal capture and handling

Male *M. mystacinus* were captured using mist nets during summer 2018 (July-August, N = 7) and summer 2019 (June-July, N = 3) in Nittedal, Norway (60°4'23"N, 10°52'20"E). Metabolic measurements were conducted on all ten bats, weighing on average 4.7 ± 0.2 g at capture. The bats were not reproductive as mating occurs during fall for most vespertilionid bats (Park et al., 1998), although three individuals were possible juveniles. Bats were captured between 21:30 and 04:30, during which the mean ambient temperature at the capture sites was 13.6 ± 4.6°C.

The bats were transported to a field laboratory immediately after capture, and metabolic measurements were initiated within 2 hours after capture. They had no access to food or water before or during measurements. The bats were considered post-absorptive as they were kept in the respirometry chamber for a minimum of four hours at the same temperature. Four hours is considered long enough to avoid any effects of standard dynamic action (SDA) in vespertilionid bats (McLean and Speakman, 2000; Webb et al., 1993), and only measurements after this were used in the analysis. Mealworms and water were provided immediately after measurements were completed, and the bats were released the following night. Bats were weighed to the nearest 0.1 g before and after measurements.

2.1.2 Respirometry

Oxygen consumption (mL O_2 h^{-1}) was measured using open-flow respirometry. Immediately upon arrival at the field laboratory, the bats were placed in a respirometry chamber inside a temperature-controlled cabinet. They were measured at 10°C until 9 a.m. the morning following the capture. The temperature inside the temperature-controlled cabinet was subsequently increased in 5°C increments every hour until the temperature had reached 25°C, after which it was increased in 2°C increments until the temperature had reached 35°C. One bat was also measured at 37°C for 30 minutes. The chamber temperature was kept stable for at least 30 minutes before being increased to the next temperature.

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. 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 lead the air through a flow-controller prior to entering the chambers, subsequently through the chambers, and at last to the oxygen analyser (figure 2.1). Baseline measurements of the ambient air were recorded for 15 minutes at the start of every temperature increase to correct for possible

drift in the analyser. The temperature inside the temperature-controlled Pelt Cabinet (Sable Systems International Inc., Las Vegas, NV, USA) was regulated using a Pelt-5 Temperature Controller (Sable Systems International Inc., Las Vegas, NV, USA). The temperature and humidity inside the respirometry chamber were recorded with thermochron iButtons (Dallas Semiconductor Inc., Dallas, TX, USA), and the temperature data from these were used in the further analysis.

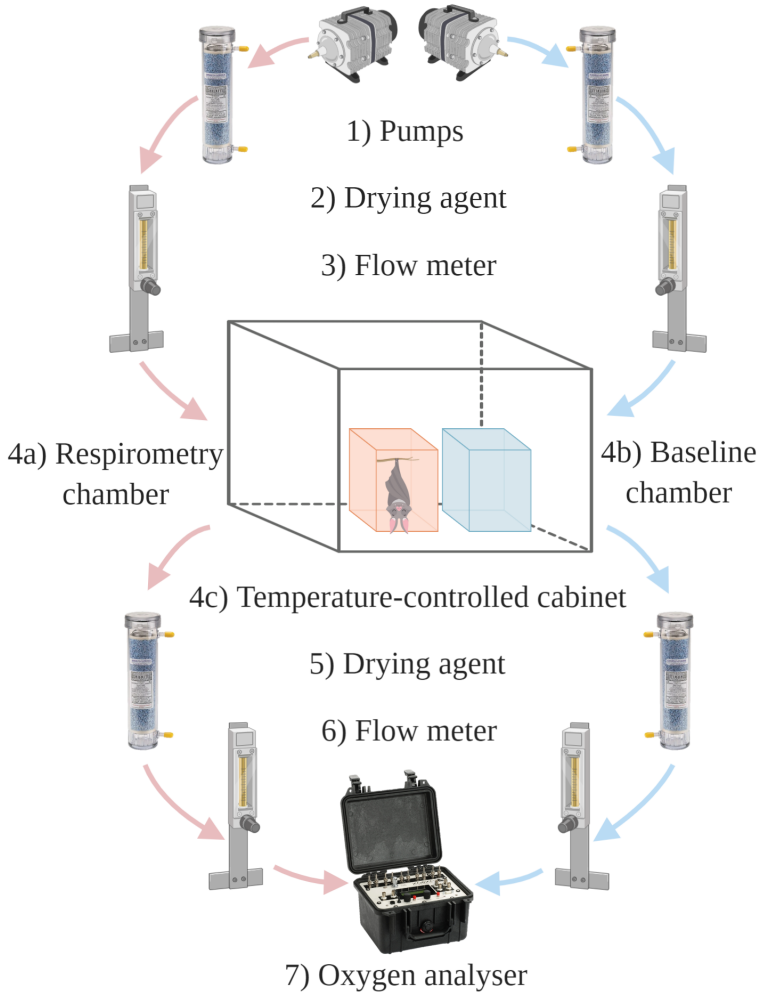

Figure 2.1: The steps of the metabolic setup used in the present study. 1) Pumps push outside air in two channels 2) through a drying agent to scrub the air of CO_2 and H_2O. Subsequently, 3) air is pushed through a flow meter to regulate flow rate before entering the 4a) respirometry or 4b) baseline chamber inside the 4c) temperature-controlled cabinet. 5) Air is then pushed through a drying agent to scrub the air yet again and finally, 6) through a flow meter before 7) entering the oxygen analyser.

Both the incurrent and excurrent air was scrubbed of H_2O and CO_2 prior to entering the chambers and the oxygen analyser, respectively. Silica gel was used as a chemical scrubber in 2018 and Drierite in 2019. The respirometry chamber which was placed inside the temperature-controlled cabinet had a volume of 325 mL, and fabric hessian 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^{-1}. In

fall 2019 I tested the flow meter to ensure that accurate flow rates were recorded during measurements. This was done by recording the time it took 1 L of water to be replaced by air in a tube at a set flow rate.

Torpor measurements were identified as low oxygen consumption succeeding a rapidly decrease. After manually removing torpor measurements from euthermic measurements, VO_2 at each set temperature was calculated as the lowest 10-minute period of VO_2 using a running mean. The lowest mean with a SE below 0.1 mL O_2 h^{-1} was used to ensure a stable period and to remove any measurements where bats were entering torpor. The SE of the VO_2 measurements and of the T_a within the respirometry chamber are shown in figure A2 A) and B) in appendix, respectively. Since the system had a high flow-to-volume ratio, there was no need of using instantaneous VO_2 measurements (Bartholomew et al., 1981). VO_2 was calculated using equation 2.1, retrieved from Lighton (2008),

$$VO_2 = \frac{FR_i(F_iO_2 - F'_eO_2)}{[1 - F'_eO_2(1 - RQ)]} \tag{2.1}$$

where FR_i is the incurrent mass flow rate scrubbed of CO_2 and H_2O, F_iO_2 is the fractional concentration of incurrent oxygen, F'_eO_2 is the fractional concentration of excurrent oxygen, and RQ is the respiratory quotient, *i.e.* VCO_2/VO_2. As VCO_2 was not measured, a RQ of 0.8 was assumed. As RQ varies from 0.7 to 1.0, using an RQ of 0.8 will produce a relatively small error in VO_2, from -3 to 5% (Lighton, 2008). Mass-specific VO_2 (mL O_2 g^{-1} h^{-1}) was calculated assuming a linear reduction in weight over time, using the body mass recorded before and after initiation of the metabolic measurements. All VO_2 measurements were converted to standard temperature and pressure (STP). An example of the data obtained from one bat is shown in figure A1 in appendix.

2.1.3 Statistics

The statistical analysis was performed using the R 3.6.3 software environment (R Core Team, 2020). Metabolic data are given as mass-specific VO_2 (mL O_2 g^{-1} h^{-1}) ± standard error (SE). Using Student's t-tests, no significant differences were found between neither years nor between possible juveniles and adults, and the groups were therefore further analysed together. As the bats were torpid during most of the time measured at low T_a, few data points were obtained at these temperatures. This prevented the estimation of T_{LC} with for example two-phase regression (Ryan, 2007). Instead, BMR was defined as the ten VO_2 observations measured at the highest T_a values within TNZ. Inclusion of more than ten measurements did not provide a BMR statistically different from the original BMR measured at the ten highest T_a values. This ensured that the estimated BMR was in fact measurements made within TNZ. The T_{LC} was defined as the inflection point between RMR and BMR. The number of individuals (N) and number of measurements (n) used to calculate BMR and RMR are given in the text.

2.2 Comparative analysis of BMR in Vespertilionidae

2.2.1 Systematic literature search and data selection

In order to obtain data for the comparative analysis of BMR in Vespertilionid bats, a systematic literature search was conducted. 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 the following search strings: *"Basal metabo*" OR "BMR" OR "Resting metabo*" OR "RMR" AND "Bat*" AND "Insect*" OR "Vespe*"* in *Topic*, and *"Basal metabo*" OR "BMR" AND "Bat*" AND "Genus"* in *All fields*. The first search string was used to search through the topic of each paper, *i.e.* only title and abstract. The latter search string was used to search through the entire papers, where the word "Genus" in the search was replaced with each of the 27 genera in Vespertilionidae. This resulted in 27 separate searches using the latter search string. The papers from all these searches were evaluated with the inclusion and exclusion criteria, yielding 17 papers to the comparative analysis.

In addition I went through the reference lists of these 17 papers, and also through the papers that had cited the specific paper. This exercise yielded another six papers with BMR data. I also included my own measurement of BMR from the present study. A total of 24 papers were thus provided for the comparative analysis. 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 figure 2.2.

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. Data from the papers were combined with my own respirometry results on *M. mystacinus* creating a final database of 21 species, comprising 47 estimates of BMR. The mean body mass of the bats included was 11.3 ± 0.8 g. Even though more than one estimate of BMR was sometimes retrieved from a single study, which violates the assumption of independence, it was considered the best option in order to increase the sample size.

To ensure that the BMR estimates obtained were comparable, the methodology was carefully evaluated in each paper. The BMR estimates included were measured using open-flow respirometry in resting, post-absorptive and non-reproductive adults at an ambient temperature within their TNZ. As female bats are often either pregnant, lactating or post-lactating during their active period of the year, especially in temperate areas where the active season is short, they were not removed as it would affect the sample size of this study dramatically. Females were instead examined in detail in the analysis.

All estimates were classified according to gender, length of captivity, reproductive state, which season the BMR was measured, and at which coordinates the study was conducted, based on the information provided in the papers. However, none of these categories could be used in the quantitative analysis, as all the relevant information was not reported in most papers. Thus, the categories were not well represented, and were instead used to

visualise possible effects graphically. The data where information was not provided or was unclear were assigned to the category 'NA'. Data and sources are presented in table A1, table A2 and table A3 in appendix.

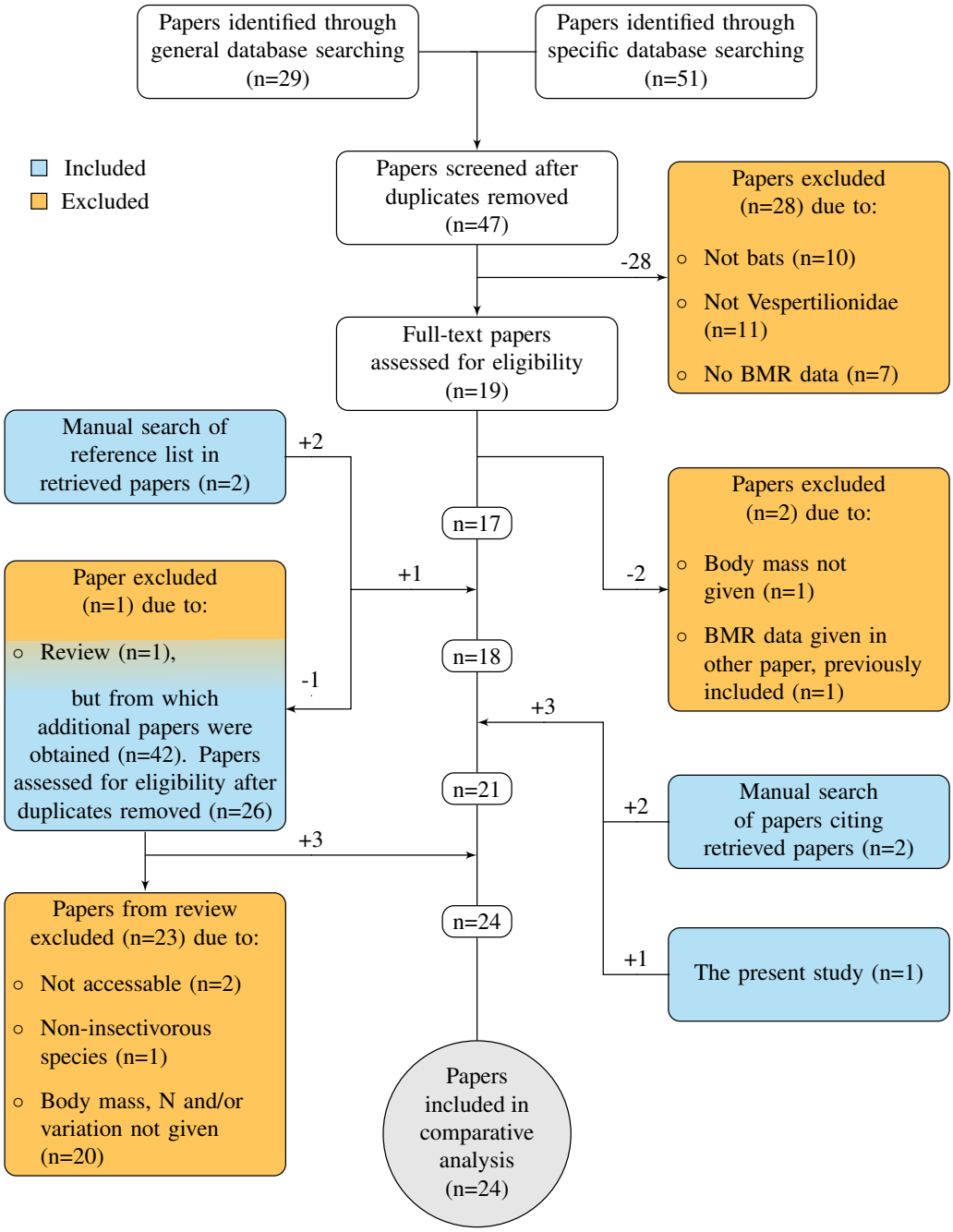

Figure 2.2: Flow chart of the systematic literature search conducted in the comparative analysis of BMR in Vespertilionid bats. The search conducted in Web of Science combined inclusion criteria to screen through the abstracts of all relevant papers, and exclusion criteria to carefully evaluate each included paper. This yielded in total 24 papers to the comparative analysis in the present study.

2.2.2 Statistics

The measures of BMR were converted to absolute BMR for the analysis, measured as oxygen consumption per hour (mL O_2 h^{-1}). Estimates given in watts were converted using a conversion factor of 20.09 J mL^{-1}, assuming a respiratory quotient of 0.85 (McLean and Speakman, 2000), unless a conversion factor was stated in the original paper. If BMR was given in median with range as a measure of variation, it was converted to mean and variance using the method of Hozo et al. (2005). Measures of variation in all papers 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. All estimates in the comparative analysis are given as means ± SE.

The statistical analysis was performed using R 3.6.3 software environment (R Core Team, 2020). The effect of taxonomy was established using the phylogenetic information of Vespertilionidae provided by Moratelli et al. (2019) and the R package 'ape' (Paradis and Schliep, 2018). Phylogenetic generalized least squares (PGLS) from the R package 'caper' (Orme et al., 2018) was used to determine whether a phylogenetic signal was present in the BMR of Vespertilionid bats. A value of 0 indicates no phylogenetic signal, whereas a value of 1 indicates a strong phylogenetic signal. Mixed effect models from the R package 'lme4' (Bates et al., 2015) were used to determine the effects of latitude and mean ambient temperature on BMR between populations. Species were included as a random factor as some of the species were represented by multiple estimates that were treated as independent replicates. The BMR estimates were also grouped by authors, and this grouping was included as a random factor to account for the non-independence between BMR estimates from papers of the same authors or scientific groups.

The natural environmental temperature data were obtained from the website World Weather Online (2020), and the means used in the analysis 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 ambient 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.

Results

3.1 Metabolic rates of *M. mystacinus*

There was a significant negative effect of T_a on the resting VO_2 of *M. mystacinus* at low T_a (P = 0.01, figure 3.1), with T_a explaining 98% of the variation in VO_2. The VO_2 increased with 0.39 ± 0.01 mL O_2 g^{-1} h^{-1} per °C decrease in T_a, and the estimated thermal conductance was thus 0.39 mL O_2 g^{-1} h^{-1} $°C^{-1}$ (body mass = 4.9 ± 0.1 g, N = 4, n = 5). According to the allometric equation of thermal conductance in Aschoff (1981), the thermal conductance of *M. mystacinus* obtained from the present study was 87% of that expected for a 4.9 g mammal. The linear relationship between T_a and VO_2 at low T_a is given by the solid line and the equation $y = 14.51 - 0.39x$ (table A5 in appendix). When this line is extrapolated to the x-axis, illustrated by the decreasing dashed line, it intercepts the x-axis at an estimated T_b of 36.9°C. The mean BMR was 1.48 ± 0.07 mL O_2 g^{-1} h^{-1} (body mass = 4.4 ± 0.1 g, N = 6, n = 10), illustrated by the horizontal dashed line. The inflection point between the two dashed lines indicated a T_{LC} around 33.1°C. The highest measurement of resting VO_2 was 9.7 mL O_2 g^{-1} h^{-1}, and was recorded at 12.2°C. This gave a minimum cold-induced metabolic scope of 6.6 for this population of *M. mystacinus*.

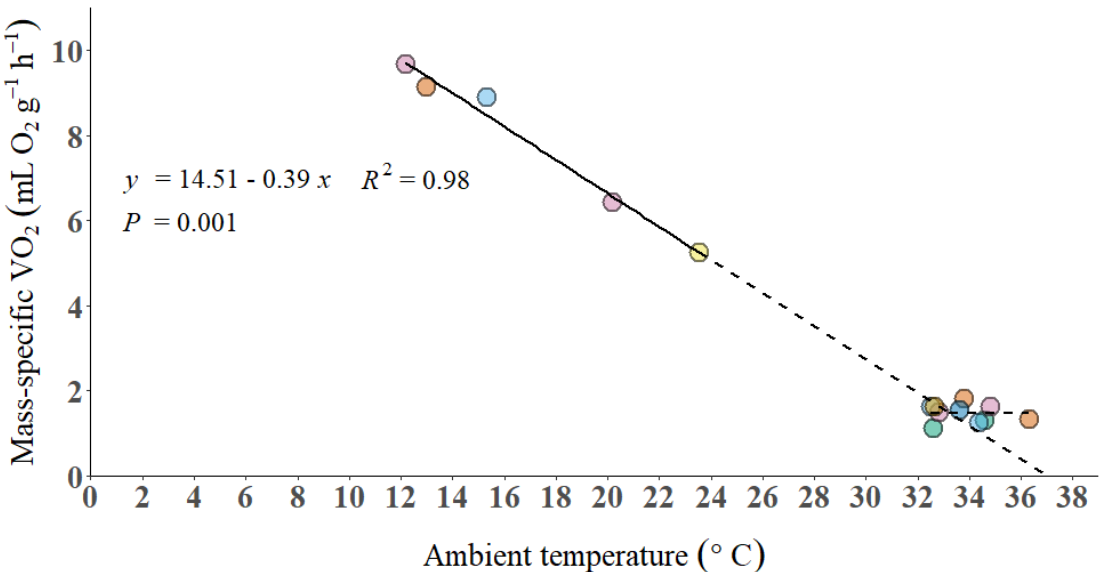

Figure 3.1: The thermoregulatory curve obtained for *M. mystacinus* in the present study, with mass-specific VO_2 (mL O_2 g^{-1} h^{-1}) as a function of T_a (°C) in the respirometry chamber. Each colour of the circles represents one individual.

3.2 Comparative analysis of BMR in Vespertilionidae

As expected, the BMR of bats within Vespertilionidae was significantly correlated to body mass ($P < 0.001$, figure 3.2), with body mass explaining 69% of the variation in BMR. The equation of the linear relationship between $\log_{10}$ body mass and $\log_{10}$ BMR was $y = 0.36 + 0.71x$ (table A5 in appendix). The slope of this equation, that is the scaling exponent, was 0.71 ± 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 (figure A4 in appendix). 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), but it was in the non-weighted regression (solid line) as it did not significantly affect the scaling exponent. There was no significant difference between the weighted and the non-weighted regressions, and thus possible variation due to methodology did not affect the results.

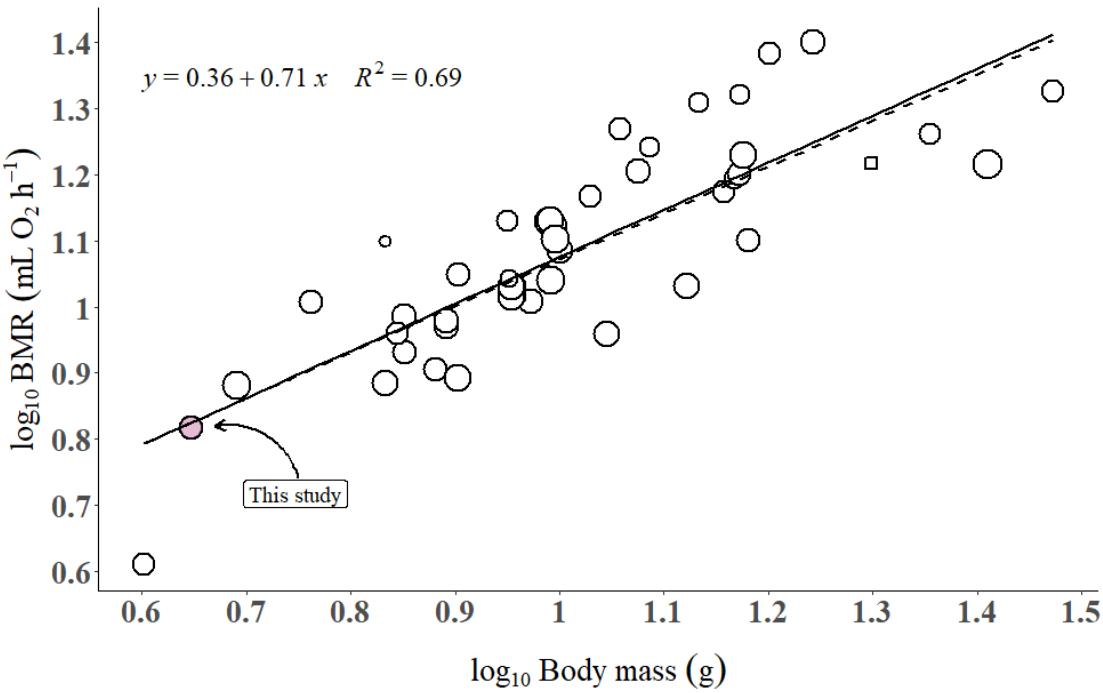

Figure 3.2: The relationship between $\log_{10}$ body mass (g) and $\log_{10}$ BMR (ml O_2 h^{-1}) in vespertilionid bats. The size of the data point represents the precision of the estimate, calculated as the inverse of the standard error corrected for body mass. The larger the circle, the more precise is the estimate. The solid line and the equation shows the linear relationship between body mass and BMR. The dashed line shows the same relationship as the solid line, but calculated using weighted means. The pink circle represents the BMR estimate obtained from the present study. The square shape represents a BMR estimate of one individual, which prevented the calculation of precision.

The pink circle represents the BMR of *M. mystacinus* obtained from the metabolic measurements in the present study. The observed BMR for this species 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. Using the mammalian allometric equation from McNab (2008) in figure 1.2, 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 has a strong effect on BMR, residuals from the relationship between BMR and body mass in figure 3.2 were used to compare the BMR independently of body mass. The bats varied in gender, time of measurement during the year, reproductive state, and in length of captivity, as shown in figure 3.3. Even though no difference is apparent among the categories, enough detailed information was not provided by the original papers to fully consider these effects. A considerable amount of estimates were thus assigned to the category 'NA'. If sufficient information of the estimates were included, the estimates in the 'NA'-category could have been assigned to the other categories, which could potentially change the contribution of estimates to the categories considerably. Hence, more information is needed to take the possible effects from gender, season, reproductivity and captivity into account.

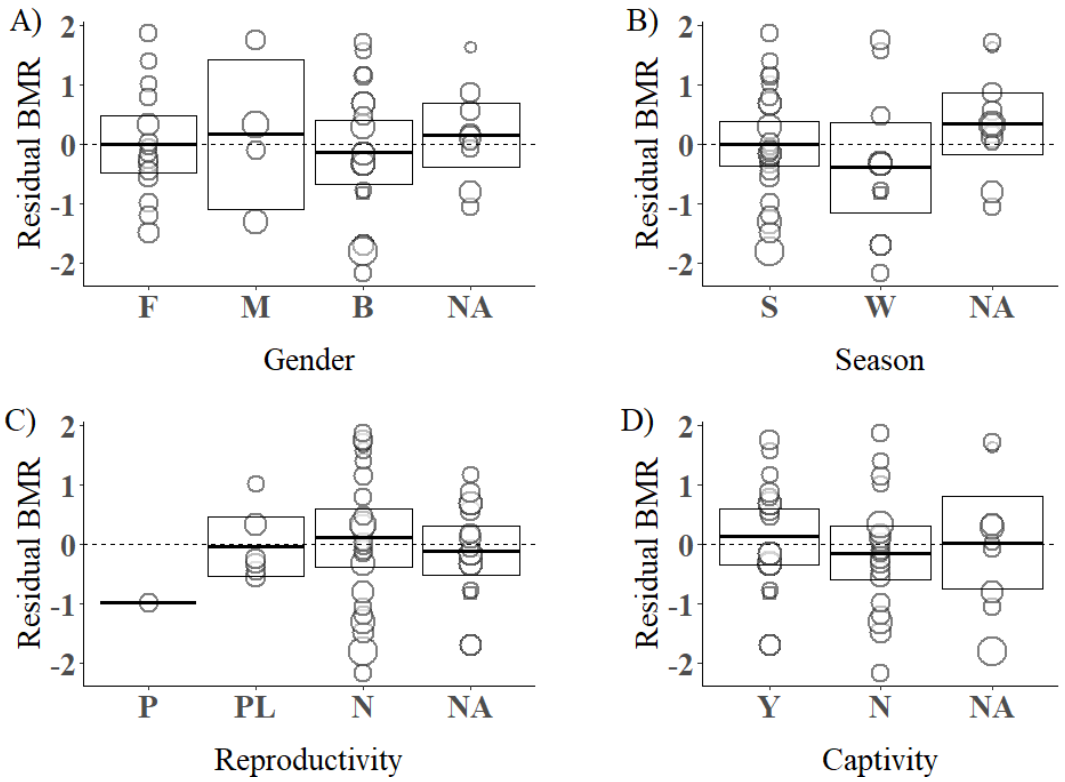

Figure 3.3: The residual BMR of bats classified into the groups A) female (F), male (M), both (B), and information not available (NA); B) summer (S) and winter (W) measurements, and information not available (NA); C) pregnant (P), post-lactating (PL), non-reproductive (N), and information not available (NA); and C) captive (Y) and non-captive (N) bats, and information not available (NA). A residual variation of 0.0 is illustrated by the dashed lines. Bars illustrate means ± 2SE.

Heterogeneity was apparent among the BMR estimates included in the comparative analysis (figure 3.2, figure 3.4). The residual BMR of the vespertilionid species ranged between -2.2 and 1.9. However, no significant phylogenetic signal was found among the species, as shown in figure A3 and table A4 in appendix. This can also be seen in figure 3.4, where a lack of phylogenetic influence on residual BMR is visualised among the species included in the present analysis.

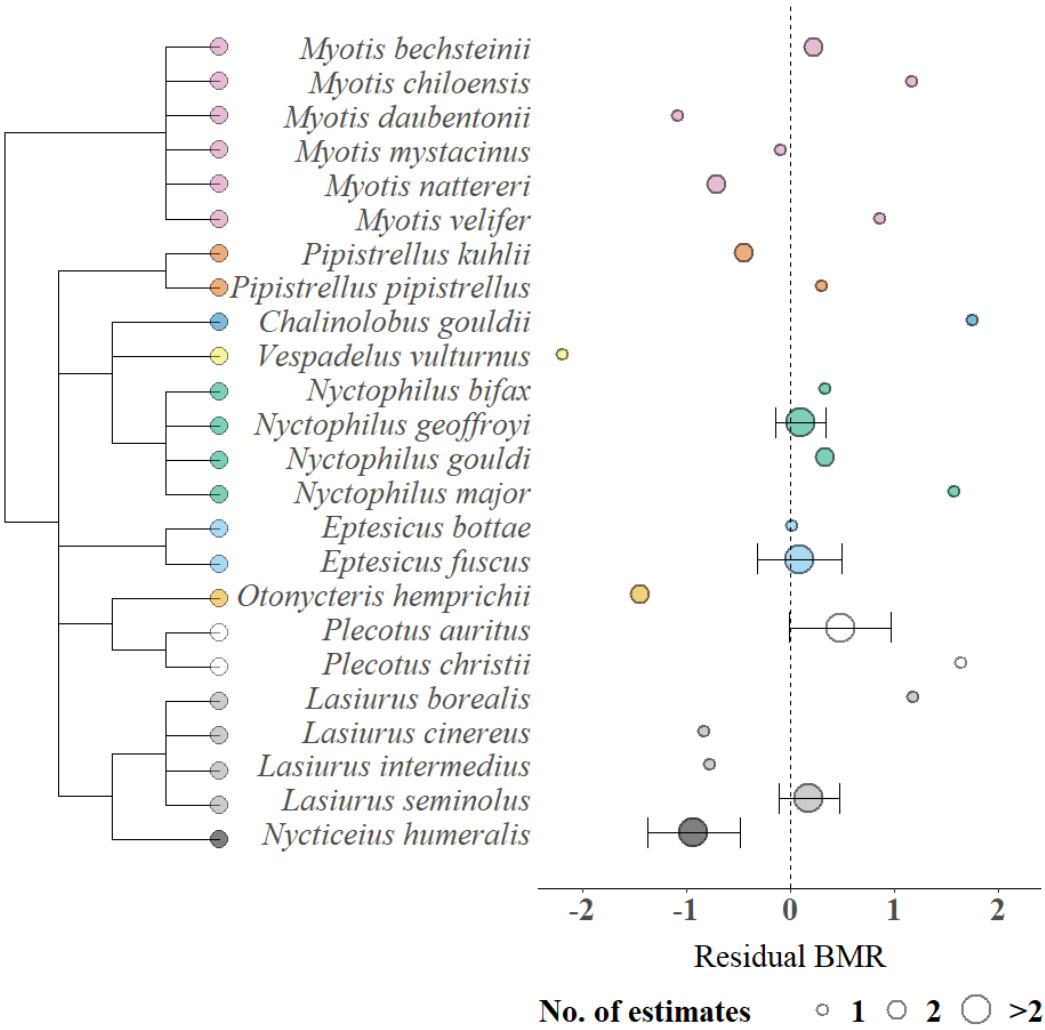

Figure 3.4: The residual BMR of the study species included in the comparative analysis of the present study, calculated from the relationship between body mass and BMR in figure 3.2. The phylogeny is shown to the left to visualise the lack of phylogenetic influence on residual BMR. A residual variation 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.

The distribution of body mass and residual BMR along a latitudinal gradient is shown in figure 3.5 A) and B), respectively. There was no effect of latitude on neither body mass (P = 0.28) nor residual BMR (P = 0.91) among the species included in the present analysis. Very few estimates were obtained from the southern hemisphere, and investigating the hemispheres separately did not affect the relationship.

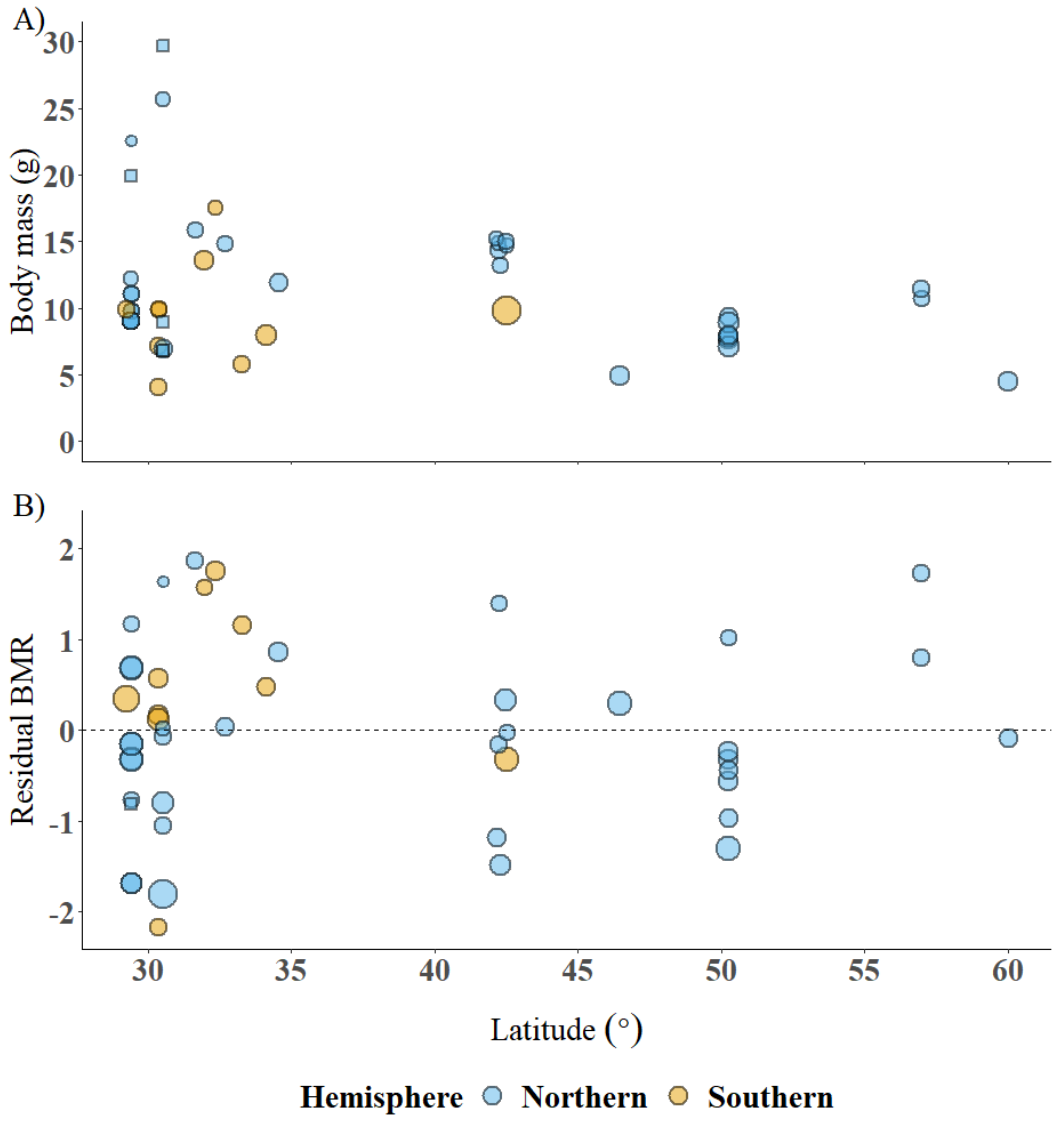

Figure 3.5: A) Body mass as a function of latitude for species from both the southern and northern hemisphere. B) Residual BMR as a function of latitude for species from both the southern and northern hemisphere. Residual BMR was calculated from the relationship between body mass and BMR in figure 3.2. 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.

Even though there was a significant negative relationship between mean environmental temperature and latitude (figure A5 in appendix), there was no significant relationship between residual BMR and mean environmental temperature (P = 0.87, figure 3.6). The relationship did not change if the southern or northern estimates were excluded.

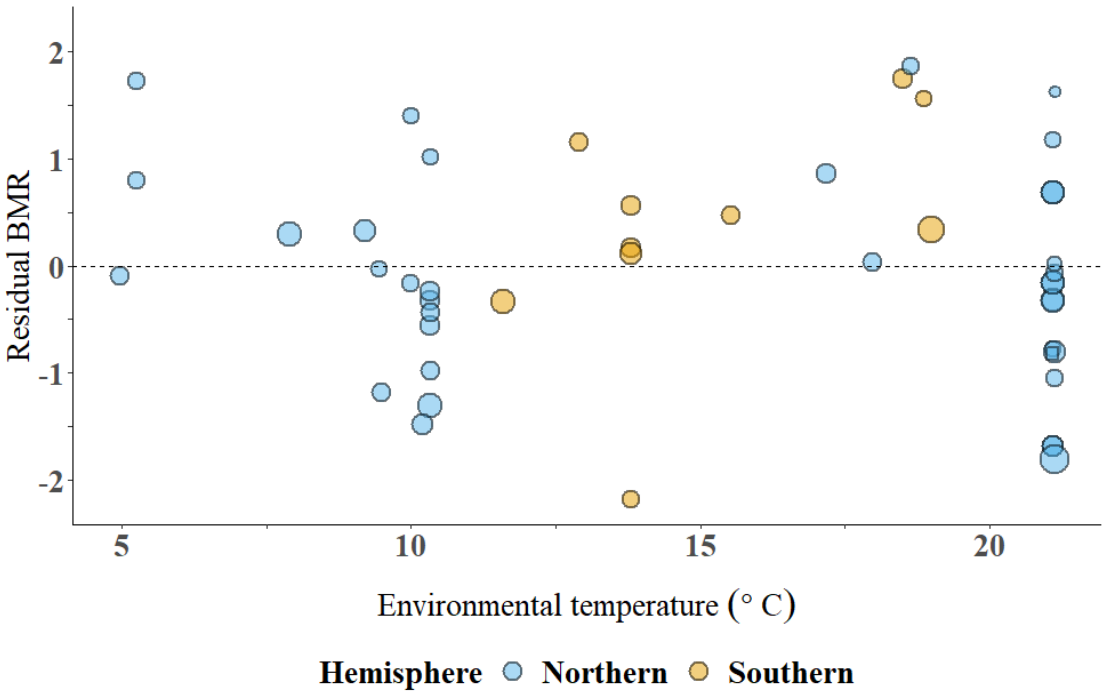

Figure 3.6: Residual BMR as a function of mean environmental temperature for species from both the southern and northern hemispheres. Residual BMR was calculated from the relationship between body mass and BMR from figure 3.2. A residual variation of 0.0 is illustrated by the dashed line. The size of 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. The square shape represents a BMR estimate of one individual, which prevented the calculation of precision.

Discussion

Little information exists on the energetic strategies of *M. mystacinus*. In the present study, a typical thermoregulatory curve was found for this species, including a BMR that fits into the relationship of other vespertilionid bats. The BMR obtained for *M. mystacinus* is the northernmost measure of BMR ever obtained within Vespertilionidae. Consequently, the representation of the latitudinal gradient of BMR estimates is improved. The investigation of BMR within vespertilionid bats along a latitudinal and environmental temperature gradient provides increased understanding of the energetic costs faced in thermally challenging environments.

4.1 Metabolic rates of *M. mystacinus*

In the present study, the thermoregulatory curve for *M. mystacinus* from a population in Norway was presented. The variation in RMR below T_{LC} is well explained by the solid regression line in figure 3.1, and accordingly T_a was found to have a great effect on RMR in *M. mystacinus*. The estimated T_{LC} for these bats is within the range of what is found for other bat species in general, ranging between 25.0 and 34.7°C (Soriano et al., 2002). The T_{LC} of vespertilionid bat species has been found to range between 27.0 and 34.0°C (Genoud, 1993; Genoud and Christe, 2011; Marom et al., 2006; Muñoz-Garcia et al., 2012; Stawski and Geiser, 2011; Willis et al., 2005a,b). As the body mass of insectivorous bat species is generally low, they have a high mass-specific BMR and a high mass-specific thermal conductance and heat loss. This results in a narrow TNZ, which could potentially explain the high T_{LC} in *M. mystacinus* (Withers et al., 2016). However, it has been argued that a clear breakpoint between BMR and RMR is generally difficult to determine for many species, especially heterothermic endotherms such as bats (Boyles et al., 2019). An extrapolation of the regression line for RMR in *M. mystacinus* (figure 3.1) intercepts the x-axis at 36.9°C. This is close to a probable body temperature for vespertilionid bats, which is found to be >32°C in euthermia, although 36.9°C is most likely slightly overestimated (Currie et al., 2015; Geiser and Brigham, 2000; Genoud, 1993; Hosken and Withers, 1997, 1999; Withers et al., 2016).

As mass-specific BMR and mass-specific thermal conductance are in general high for small mammals (Withers et al., 2016), a steep increase in metabolic heat production is expected as T_a declines. However, compared to other mammals, *M. mystacinus* in the present study had a low thermal conductance and thus do not need to increase VO_2 as much as expected to avoid hypothermia. This could indicate that the population has evolved effective insulation mechanisms to cope with the great energetic challenges imposed by the cold environments. As productivity is often lower in these conditions, which limits energy acquisition, it may therefore be an important adaptation for the survival of *M. mystacinus* at its northernmost distribution. However, as only a few data points were obtained at low T_a, these estimates could be over- or underestimated and more data are needed to establish solid conclusions about the thermoregulatory strategies of *M. mystacinus*.

The minimum estimate of the maximal cold-induced metabolic scope observed for *M. mystacinus* in the present study is high compared to other mammals. It exceeds the maximum metabolic scope expected to be achieved through shivering (Piersma, 2011), which for example has been found to be 5.1 for eutherian mammals (Hinds et al., 1993). However, it is difficult to distinguish the effects of shivering from non-shivering thermogenesis in the metabolic setup of the present analysis. A metabolic scope exceeding the expected maximum level is also apparent in other vespertilionid bat species, such as *Nyctophilus bifax* with a maximal cold-induced metabolic scope of minimum 6.25 (Stawski and Geiser, 2011). The high cold-induced metabolic scope of *M. mystacinus* and *N. bifax* may reflect thermoregulatory adaptations to thermally challenging environments encountered by vespertilionid bats in general. It also indicates that temperatures below 12°C, that is the temperature recorded during measurements of the highest VO_2 estimate in the present study, could be very close to the temperature where other heat-producing or energy-saving mechanisms than shivering must be initiated. Such mechanisms could include entering torpor or increasing activity, for example by flying.

4.2 Comparative analysis of BMR in Vespertilionidae

4.2.1 Data selection

As BMR is a trait measured under strict conditions, data selection is an important aspect when conducting a comparative analysis of BMR. In the present study, only observations of BMR with a measure of variation were included, as it enables the evaluation of the quality of the data. Many of the studies that were excluded based on this criteria were also manually evaluated as unclear or poor measurements of BMR by Genoud et al. (2018) in their analysis of BMR in mammals. Even though these BMR observations could indeed be acceptable measurements, enough information is not provided in the original papers to evaluate the quality of the estimates given. Analysing a poor data set can affect the results considerably, in spite of including a large sample size. Consequently, data selection should be done with caution (Boyles et al., 2019; Genoud et al., 2018).

The variations in gender, reproductive state, time of measurement during the year and length of captivity of the bats included in the present study could affect both body mass and BMR (Genoud et al., 2018). However, possible effects of these factors could not be considered in the present analysis, as the sample sizes were low and the categories were not well represented due to insufficient information provided by the original papers. If the required information was provided for these estimates, including them in the correct categories could affect the mean residual BMR of these categories. Therefore, including these poorly represented factors in the present analysis could potentially affect the robustness of the statistical tests, and more data are thus needed to investigate the effects further.

4.2.2 Interspecific variation in BMR

The BMR of *M. mystacinus* was 99% of that predicted for a vespertilionid bat weighing 4.4 g, which indicates that no adaptation in BMR has evolved to inhabit the cold temperatures at a latitude of 60°N in this particular species. However, even though this

population of *M. mystacinus* fits the allometric relationship well, it is possible that southern populations would not. Hence, more studies on this species are required from the entire range of its distribution.

As BMR in *M. mystacinus* was 64% and BMR in vespertilionid bats was on average 65% of that predicted for mammals of the same size, the low BMR in both *M. mystacinus* and Vespertilionidae as a whole could be a general adaptation. A low BMR could for example have evolved to sustain the high energetic requirements encountered by these species, such as that required by flying or inhabiting thermally challenging environments. Flight by bats increases the metabolic rate 17 times above BMR, so the energetic costs are high. However, metabolic rates that are 17 times BMR would increase T_b, and so this scope is most likely an overestimation (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 is still present within the family, and this variation remains to be explained (figure 3.2 and 3.4).

The BMR of vespertilionid bats has been reviewed before (Speakman and Thomas, 2003; Willis et al., 2005b). For example, Willis et al. (2005b) found that the metabolic scaling exponent of vespertilionid bats was 0.89. A measure of variation for this scaling exponent was not reported, however the scaling exponent obtained in the present study of 0.71 had a low SE (0.01). Hence, the two scaling exponents are most likely significantly different from each other. This is not expected, as both studies solely include species of Vespertilionidae. However, it is possible that the scaling exponents differ because the total range of body mass within Vespertilionidae is not represented. The body mass of vespertilionid bats ranged from 2 g to 91 g (Dietz and Kiefer, 2016), whereas the body mass included in the present study ranged from 4.0 g to 19.7 g. Consequently, less than one fifth of the total range in body mass is represented. The range of body mass included in the analysis by Willis et al. (2005b) was not available. However, 11 of their 18 estimates were retrieved from Speakman and Thomas (2003), of which several were excluded from the present study. Therefore, the estimates included in the analyses most likely differ, and it is possible that the full range of the body mass within Vespertilionidae is not represented in either of the analyses. In addition, the study conducted by Willis et al. (2005b) was done 15 years ago, whereas the present study includes more recent data. Nonetheless, BMR data on more species within Vespertilionidae is required to fully represent the range of body mass within the family.

The low representation of vespertilionid species in the present study, and not just of the body mass range, could also affect the analysis of BMR. Vespertilionidae consists of 54 genera, of which 10 are represented in the present study. In addition, 496 vespertilionid species are known (Moratelli et al., 2019), of which BMR was obtained from only 24 species. As the BMR of many taxonomic groups are not reviewed at all, parts of the phylogenetic tree in Vespertilionidae is underrepresented and a phylogenetic correction may not be meaningful (Willis et al., 2005a). Thus, the analysis conducted in the present study could possibly produce a skewed relationship between body mass and BMR. However, as the effect of diet is confounded with phylogeny, and possible dietary effects are avoided in the present study as all bats are insectivorous, it was not expected to find any effect of phylogeny on BMR (figure 3.4, figure A3 in appendix) (Cruz-Neto et al., 2001).

The insectivorous diet of vespertilionid bats could also explain why there is no effect of latitude on body mass in these bats. Due to increased size difference between bat and prey, the ability to detect small prey while flying could be reduced in large bats, and this could impose a constraint on the increase in body mass (Barclay and Brigham, 1991). In addition, occupying the aerial niche could introduce an additional energetic constraint. Investing in a larger body mass would increase the energetic costs of flying, which would require increased rates of energy acquisition. This could be especially challenging when feeding on insects in cold environments. Consequently, a small body mass could be favourable in conditions with low prey availability, such as at high latitudes, which contradicts Bergmann's rule. This could also explain why most large bats are found in the tropics where prey availability is higher (Barclay and Harder, 2003).

As latitude often reflects variations in climatic factors, such as low environmental temperatures encountered at higher latitudes (figure A5 in appendix), it was expected that BMR would increase with latitude to meet the high energetic challenges imposed by cold environmental temperatures. However, no effect of latitude on BMR was found in Vespertilionidae (figure 3.6), which could indicate that other adaptations to high latitude living have evolved. Speakman and Thomas (2003) investigated the effect of latitude on BMR in rodents, and found a significant effect of latitude. However, they found no effect of latitude within 10 families of bats. They suggested that the reason why they found an effect of latitude on BMR in rodents, but not in bats, is that more bats inhabit tropical areas at lower latitudes compared to temperate areas at higher latitudes (Speakman and Thomas, 2003). As figure 3.5 B shows, this is also the case in the present study. This could potentially influence the possibility to reveal an effect of latitude. Due to the differing number of species in tropical and temperate regions, isolating the relationship between BMR and latitude or other climatic factors may be challenging.

Nevertheless, latitude could also be a poor predictor of BMR. Even though environmental temperature decreases with latitude, the climate at two locations of approximately the same latitude could still be quite different. An example from the present study is several BMR estimates obtained from Australia (Currie et al., 2015; Dixon and Rose, 2003; Geiser and Brigham, 2000; Hosken, 1997; Hosken and Withers, 1997, 1999; Stawski and Geiser, 2011; Willis et al., 2005b) and one from Chile (Bozinovic et al., 1985). They are all obtained at approximately the same latitude, but the environmental temperature in Chile at this latitude is lower than the environmental temperature in Australia, as the location in Chile is at a higher altitude. Hence, latitude may fail to represent the different climate types for some locations.

The BMR estimates included in the present study are better represented across a gradient of environmental temperature compared to latitude, as shown in figure 3.5 B and figure 3.6, respectively. It was therefore expected that ambient temperature would be a better predictor for BMR than latitude. However, no effect of ambient temperature on BMR was found among vespertilionid bats in the present study. So how can bats residing in cold areas and at high latitudes survive? Increasing BMR would reduce heat loss in cold environments, but it would also require even more energy in an already energy scarce environment. If BMR is lowered, on the other hand, it could shift the whole curve down, reducing the energy requirements even at low temperatures (Withers et al., 2016). Bats residing in cold conditions could also increase the rate of torpor use to effectively

deal with the cold temperatures. 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, 2003). Hence, it would be interesting to include information on torpor use and migration patterns in a comparative analysis of BMR among species of Vespertilionidae. However, little information is available on the subject (Moratelli et al., 2019).

It is also possible that other environmental factors are better predictors for BMR than both latitude and environmental temperature. Multiple environmental factors are found to correlate with BMR in mammals, such as environmental productivity (Mueller and Diamond, 2001; Lovegrove, 2003; Withers et al., 2016) and rainfall parameters (Lovegrove, 2003; Withers et al., 2016). Bats could be more prone to unfavourable conditions than cold temperatures *per se*, or these factors could reflect unfavourable conditions better than ambient temperature and therefore be a better predictor for BMR. Unfavourable conditions, for example with low and variable prey abundance, could result in low energy gain, which means less energy is available for maintenance processes in the body. This could result in lower BMR. For example, Lovegrove (2000) and MacMillen and Garland Jr (1989) found low BMR within rodent species inhabiting hot deserts with unpredictable rainfall.

Deserts are characterized by variable availability of prey, high ambient temperature during the day and low ambient temperature during the night. Therefore, if the energy gain is low, it could be expected that desert bats have a lower BMR than expected from body mass (Muñoz-Garcia et al., 2016). The desert bat *Otonycteris hemprichii* from the comparative analysis in the present study, measured in the Negev desert in Israel, does indeed have a low BMR compared to the other species in Vespertilionidae (figure 3.4). *Eptesicus bottae*, *Plecotus christii* and the two observations of *Pipistrellus kuhlii* are also measured in the Negev desert. The latter has a BMR lower than what is expected from its body mass, although *E. bottae* has a BMR quite close to that expected from its body mass, and *P. christii* actually has a much higher BMR. This indicates that there are variations in BMR also within desert bats. Muñoz-Garcia et al. (2016) compared these four species to 39 other insectivorous bat species and found no differences between desert and non-desert species.

The Australian bat *Vespadelus vulturnus* is the species with the lowest BMR in the present study. This species was measured during autumn/winter (Willis et al., 2005b), which could potentially explain why BMR is lower than expected from its body mass. Low BMR in this species could reflect preparation to enter multiday torpor during winter season, often characterised by increased body mass and decreased BMR (Genoud, 1990). However, there are also studies that find no effect of season on BMR in vespertilionid bats (Stawski and Geiser, 2011; Willis et al., 2005a), and studies that find conflicting results (Genoud, 1990). As the data included in the present study were obtained both during winter and summer, and some were even difficult to categorise, season should be taken into account when measuring BMR as it may influence BMR in some species. This could help explain any outliers such as *V. vulturnus*.

It has also been suggested that Australian vespertilionids have a lower BMR compared to other vespertilionid bats, which could explain the low BMR of *V. vulturnus* (Willis et al., 2005b). However, all species within *Nyctophilus* and *Chalinolobus* in the present study are also observed in Australia, and both *N. bifax*, *Nyctophilus geoffroyi* and *Nyctophilus gouldi* have a BMR slightly above what is expected from their body mass. Furthermore,

Nyctophilus major and *Chalinolobus gouldii* are among the species with the highest BMR in the present analysis, which contradicts the prediction that Australian bats should have low BMR comopared to other species in Vespertilionidae. Willis et al. (2005b) also tested if Australian vespertilionid bats had lower BMR compared to other vespertilionids, but found no difference in their analysis.

As the effects of both the Australian origin and seasons are mixed, it has been suggested that perhaps low BMR is a general trait of Vespertilionidae. For example, Hosken and Withers (1997) found that *N. major* had a BMR approximately 85% of that predicted for a 13.6 g mammal, and Hosken (1997) found that *C. gouldii* had a BMR 86% of that predicted for a 17.5 g mammal. As both these species are among the bats with the highest BMR compared to other vespertilionid bats in the present study (figure 3.4), this fits nicely with the results from the present study showing that Vespertilionidae had a BMR on average 65% of that predicted for similar sized mammals in general. However, a complete phylogenetically corrected analysis of Vespertilionidae is needed to fully investigate this question.

4.3 Summary and conclusions

The BMR of M. mystacinus was close to that predicted for a vespertilionid bat of the same body mass, however lower than predicted for a mammal. Instead, *M. mystacinus* revealed reduced thermal conductance compared to mammals of the same size, and together with torpor use, this can aid in coping with the cold temperatures encountered at high latitudes. The T_{LC} of 33.1°C may allow these bats to enter torpor even at higher environmental temperatures, which could be favourable as a high cold-induced metabolic scope and thus high energetic costs are revealed for this species. Bat species in Vespertilionidae in general revealed a low BMR compared to other mammals of the same size. Hence, this group have evolved characteristic traits allowing conservative energy use, such as low BMR and torpor. This is consistent with the high energetic costs associated with flying, nocturnal activity and inhabiting energy-scarce environments. It is however unclear how latitude and environmental temperature affects BMR within the family. More information on methodology and the individuals used in each original study could help account for differences between the bat species included in a comparative analysis. Effects of rates of torpor use and roost temperature on BMR are also fruitful avenues of research.

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Appendix

An example of the raw data used to calculate running averages of mass-specific VO_2 from one bat is presented in figure A1. Similar data were obtained from each of the ten bats included in the metabolic measurements of the present study.

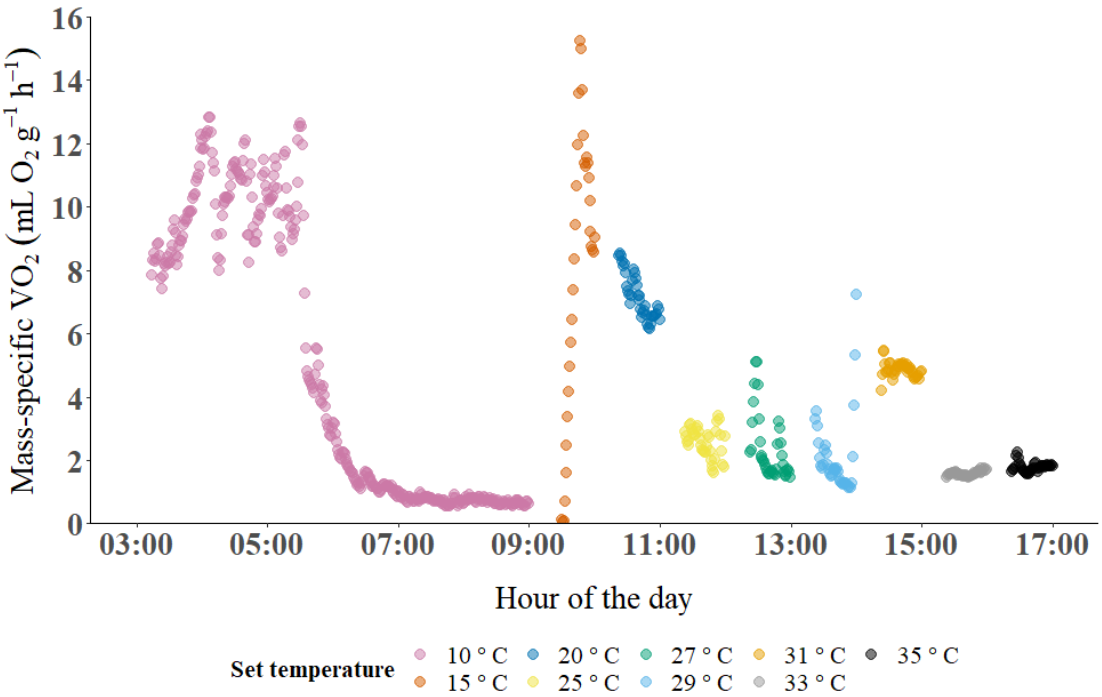

Figure A1: Mass-specific VO_2 (mL O_2 g^{-1} h^{-1}) during metabolic measurements of one bat. Measurements were initiated on the same night of capture and continued throughout the following day. The set temperatures used in the present study are illustrated with different colours. The set temperature, and thus T_a in the respirometry chamber, was increased every hour except for the first set temperature. Baseline measurements of the outside air are removed from this figure.

The variation in mass-specific VO_2 (figure A2 A)) and in the chamber temperature (figure A2 B)) are presented as standard errors (SE) for each average VO_2 estimate included in the thermoregulatory curve of the present study.

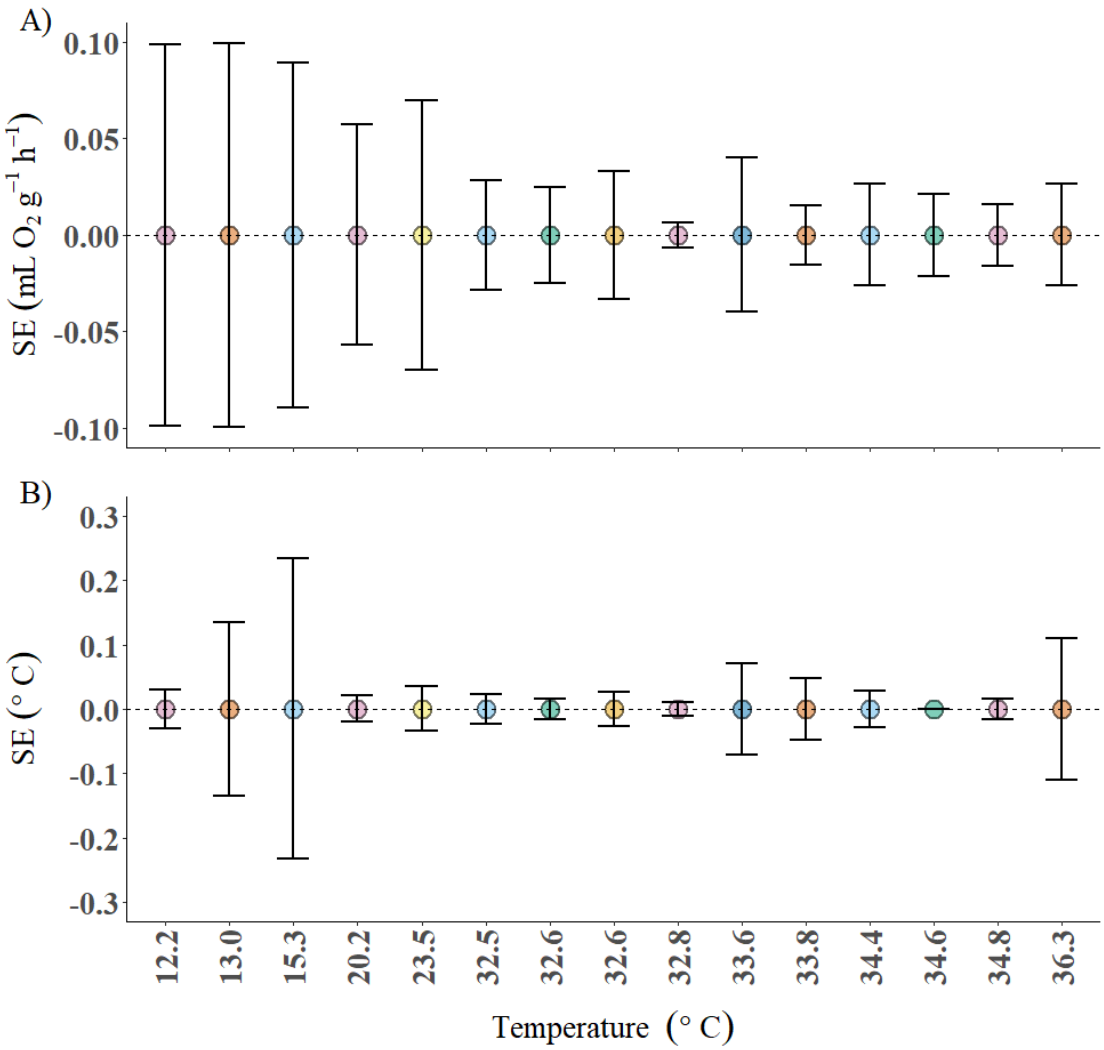

Figure A2: The standard error (SE) of A) mass-specific VO_2 and of B) respirometry chamber temperature for each average VO_2 estimate included in the thermoregulatory curve of the present study.

The papers included in the comparative analysis of the present study are presented in table A1, including the ID of each paper, the references, and how the papers were obtained. Source 1 means that the paper is obtained from the primary search, 2 means it is obtained from the citation search, 3 is from the reference search, and 4 is the data from the present study. Details of how the papers were obtained are presented in figure 2.2. The ID for each paper is used to refer to the paper in table A2 and A3. Table A2 includes the raw data obtained for the comparative analysis in the present study, whereas table A3 includes the additional information obtained for each estimate.

Table A1: References for the included papers in the comparative analysis of the present study. The ID of each paper is used to refer to the given paper in table A2 and table A3. The source refers to how the paper is obtained, where 1 is from the primary search, 2 is from the citation search, 3 is from the reference search and 4 is the present study (see figure 2.2 for more details about the search).

ID	Paper	Source
1	Becker, N.I., Encarnação, J.A., Kalko, E.K., Tschapka, M., 2012. The effects of reproductive state on digestive efficiency in three sympatric bat species of the same guild. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 162, 386–390.	1
2	Becker, N.I., Encarnaçãoao, J. A., Tschapka, M., Kalko, E.K.V., 2013a. Energetics and life-history of bats in comparison to small mammals. Ecological Research 28,249–258.	1
3	Becker, N.I., Tschapka, M., Kalko, E.K.V., Encarnação, J.A., 2013b. Balancingthe energy budget in free-ranging male *Myotis daubentonii* bats. Physiological and Biochemical Zoology 86, 361–369.	1
4	Bozinovic, F., Contreras, L.C., Rosenmann, M., Torres-Mura, J., 1985. Bioenergética de *Myotis chiloensis* (quiroptera: Vespertilionidae). Revista Chilena de Historia Natural 58.	2
5	Currie, S.E., Noy, K., Geiser, F., 2015. Passive rewarming from torpor in hibernating bats: minimizing metabolic costs and cardiac demands. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology 308, R34–R41.	2
6	Dixon, K.J., Rose, R.W., 2003. Thermal energetics of *Nyctophilus geoffroyi* (chiroptera: Vespertilionidae) at the southern limits of its distribution. Australian Journalof Zoology 51, 43–50.	1
7	Geiser, F., Brigham, R.M., 2000. Torpor, thermal biology, and energetics in australian long-eared bats (*Nyctophilus*). Journal of Comparative Physiology B 170, 153–162.	1
8	Genoud, M., 1990. Seasonal variations in the basal rate of metabolism of subtropical insectivorous bats (*Nycticeius humeralis* and *Lasiurus seminolus*): a comparison with other mammals. Revue Suisse de Zoologie 97, 77–90.	3
9	Genoud, M., 1993. Temperature regulation in subtropical tree bats. Comparative Biochemistry and Physiology 104, 321–331.	2
10	Genoud, M., Christe, P., 2011. Thermal energetics and torpor in the common pipistrelle bat, *Pipistrellus pipistrellus* (vespertilionidae: Mammalia). Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 160, 252–259.	1
11	Hosken, D., 1997. Thermal biology and metabolism of the greater long-eared bat, *Nyctophilus major* (chiroptera: Vespertilionidae). Australian Journal of Zoology 45, 145–156.	1

12	Hosken, D., Withers, P., 1997. Temperature regulation and metabolism of an australian bat, *Chalinolobus gouldii* (chiroptera: Vespertilionidae) when euthermic and torpid. Journal of Comparative Physiology B 167, 71–80.	1
13	Hosken, D.J., Withers, P.C., 1999. Metabolic physiology of euthermic and torpid lesser long-eared bats, *Nyctophilus geoffroyi* (chiroptera: Vespertilionidae). Journal of Mammalogy 80, 42–52.	1
14	Marom, S., Korine, C., Wojciechowski, M.S., Tracy, C.R., Pinshow, B., 2006. Energy metabolism and evaporative water loss in the European free-tailed bat and hemprich's long-eared bat (microchiroptera): species sympatric in the Negev desert. Physiological and Biochemical Zoology 79, 944–956.	1
15	McLean, J.A., Speakman, J.R., 2000. Effects of body mass and reproduction on the basal metabolic rate of brown long-eared bats (*Plecotus auritus*). Physiological and Biochemical Zoology 73, 112–121.	1
16	Muñnoz-Garcia, A., Ben-Hamo, M., Pinshow, B., Williams, J.B., Korine, C., 2012. The relationship between cutaneous water loss and thermoregulatory state in kuhl's pipistrelle *Pipistrellus kuhlii*, a vespertillionid bat. Physiological and BiochemicalZoology 85, 516–525.	1
17	Muñnoz-Garcia, A., Larraín, P., Ben-Hamo, M., Cruz-Neto, A., Williams, J.B., Pin-show, B., Korine, C., 2016. Metabolic rate, evaporative water loss and thermoregulatory state in four species of bats in the Negev desert. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 191, 156–165.	3
18	Richardson, C.S., Heeren, T., Widmaier, E.P., Kunz, T.H., 2009. Macro- and microgeographic variation in metabolism and hormone correlates in big brown bats (*Eptesicus fuscus*). Physiological and Biochemical Zoology 82, 798–811.	1
19	Riedesel, M.L., Williams, B.A., 1976. Continuous 24-hour oxygen consumption studies of *Myotis velifer*. Comparative Biochemistry and Physiology Part A: Physi-ology 54, 95–99.	3
20	Skåra, K. H. (unpublihsed). The present study.	4
21	Stawski, C., Geiser, F., 2011. Do season and distribution affect thermal energetics of a hibernating bat endemic to the tropics and subtropics? American Journal of Physiology-Regulatory, Integrative and Comparative Physiology 301, R542–R547.	1
22	Webb, P.I., Hays, G.C., Speakman, J.R., Racey, P.A., 1992. The functional significance of ventilation frequency, and its relationship to oxygen demand in the resting brown long-eared bat, *Plecotus auritus*. Journal of Comparative Physiology B 162,144–147.	2
23	Willis, C.K.R., Lane, J.E., Liknes, E.T., Swanson, D.L., Brigham, R.M., 2005a. Thermal energetics of female big brown bats (*Eptesicus fuscus*). Canadian Journal of Zoology 83, 871–879.	1
24	Willis, C.K.R., Turbill, C., Geiser, F., 2005b. Torpor and thermal energetics in a tiny Australian vespertilionid, the little forest bat (*Vespadelus vulturnus*). Journal of Comparative Physiology B 175, 479–486.	1

Table A2: Raw data obtained from the papers in the comparative analysis. It includes ID of the papers, species names, measures of body mass and BMR, with number of individuals used and a measure of variation for each estimate. The units reported for the variance are given in brackets. ID refers to the papers in table A1.

ID	Species	BM (g)	Var. of BM	N of BM	BMR	Var. of BMR	N of BMR
1a	*Myotis bechsteinii*	9.4	0.9 (SD)	35	52.47 mW	32.36-87.73 (range)	17
1b	*Myotis nattereri*	7.6	0.7 (SD)	27	43.27 mW	17.90-75.09 (range)	17
1c	*Plecotus auritus*	7.8	0.5 (SD)	16	52.12 mW	32.12-72.11 (range)	11
2a	*Myotis bechsteinii*	8.9	0.6 (SD)	23	9.6 mL O_2 h^{-1}	6.0–28.8 (range)	23
2b	*Myotis nattereri*	7.1	0.5 (SD)	18	8.7 mL O_2 h^{-1}	4.0–12.8 (range)	18
2c	*Plecotus auritus*	7.8	0.6 (SD)	13	9.5 mL O_2 h^{-1}	5.9–13.1 (range)	13
3	*Myotis daubentonii*	8.0	0.4 (SD)	5	0.13 mL O_2 min^{-1}	0.01 (SD)	5
4	*Myotis chiloensis*	5.78	0.9 (SD)	11	1.76 mL O_2 h^{-1} g^{-1}	0.28 (SD)	11
5	*Nyctophilus gouldi*	9.9	1.0 (SD)	4	1.34 mL O_2 h^{-1} g^{-1}	0.16 (SD)	4
6	*Nyctophilus geoffroyi*	9.8	0.03 (SE)	5	1.119 mL O_2 h^{-1} g^{-1}	0.186 (SE)	5
7a	*Nyctophilus geoffroyi*	7.1	0.8 (SD)	6	1.36 mL O_2 h^{-1} g^{-1}	0.17 (SD)	6
7b	*Nyctophilus gouldi*	10.0	1.1 (SD)	8	1.22 mL O_2 h^{-1} g^{-1}	0.13 (SD)	8
8a	*Lasiurus seminolus*	9.75	1.32 (SD)	9	1.38 mL O_2 h^{-1} g^{-1}	0.12 (SD)	9
8b	*Lasiurus seminolus*	9.02	1.00 (SD)	10	1.15 mL O_2 h^{-1} g^{-1}	0.13 (SD)	10
8c	*Nycticeius humeralis*	9.02	1.10 (SD)	18	1.19 mL O_2 h^{-1} g^{-1}	0.15 (SD)	18
8d	*Nycticeius humeralis*	11.09	1.04 (SD)	6	0.82 mL O_2 h^{-1} g^{-1}	0.13 (SD)	6
9a	*Lasiurus borealis*	12.2	1.2 (SD)	2	1.43 mL O_2 h^{-1} g^{-1}	0.25 (SD)	2
9b	*Lasiurus cinereus*	19.9	NA	1	0.83 mL O_2 h^{-1} g^{-1}	NA	1
9c	*Lasiurus intermedius*	22.6	3.9 (SD)	2	0.81 mL O_2 h^{-1} g^{-1}	0.21 (SD)	2
9d	*Lasiurus seminolus*	9.8	1.3 (SD)	9	1.38 mL O_2 h^{-1} g^{-1}	0.12 (SD)	9
9e	*Lasiurus seminolus*	9.0	1.0 (SD)	10	1.15 mL O_2 h^{-1} g^{-1}	0.13 (SD)	10
9f	*Nycticeius humeralis*	9.0	1.1 (SD)	18	1.19 mL O_2 h^{-1} g^{-1}	0.15 (SD)	18
9g	*Nycticeius humeralis*	11.1	1.0 (SD)	6	0.82 mL O_2 h^{-1} g^{-1}	0.13 (SD)	6
10	*Pipistrellus pipistrellus*	4.9	0.8 (SD)	28	7.6 mL O_2 h^{-1}	0.8 (SD)	28
11	*Nyctophilus major*	13.6	0.17 (SE)	5	1.5 mL O_2 h^{-1} g^{-1}	0.17 (SE)	5
12	*Chalinolobus gouldii*	17.5	14.9-20.5 (range)	10	1.44 mL O_2 h^{-1} g^{-1}	0.08 (SE)	3
13	*Nyctophilus geoffroyi*	8.0	0.1 (SE)	8	1.4 mL O_2 h^{-1} g^{-1}	0.1 (SE)	8
14	*Otonycteris hemprichii*	25.43	2.09 (SD)	6	3.57 mW g^{-1}	0.21 (SD)	5
15	*Plecotus auritus*	10.7	17% (coef. of var.)	14	82 mW	24 (SD)	14
16	*Pipistrellus kuhlii*	6.99	0.53 (SD)	8	7.26 mW g^{-1}	2.00 (SD)	8
17a	*Eptesicus bottae*	8.95	NA	NA	60.19 mW	28.17 (SD)	6
17b	*Otonycteris hemprichii*	29.71	NA	NA	115.41 mW	51.28 (SD)	6
17c	*Pipistrellus kuhlii*	6.8	NA	NA	41.88 mW	5.42 (SD)	9
17d	*Plecotus christii*	6.8	NA	NA	58.58 mW	31.59 (SD)	7
18	*Eptesicus fuscus*	15.91	0.41 (SE)	17	24.13 mL O_2 h^{-1}	1.72 (SE)	17
19	*Myotis velifer*	11.89	0.20 (SE)	12	1.35 mL O_2 h^{-1} g^{-1}	0.08 (SE)	8
20	*Myotis mystacinus*	4.4	0.11 (SE)	6	1.48 mL O_2 h^{-1} g^{-1}	0.068 (SE)	6
21	*Nyctophilus bifax*	9.9	0.7 (SD)	6	1.28 mL O_2 h^{-1} g^{-1}	0.06 (SD)	6
22	*Plecotus auritus*	11.42	0.96 (SD)	9	0.310 mL O_2 min^{-1}	0.063 (SD)	9
23	*Eptesicus fuscus*	15.0	1.4 (SD)	10	16.98 mL O_2 h^{-1}	2.04 (SD)	10
24	*Vespadelus vulturnus*	4.0	0.69 (SD)	5	1.02 mL O_2 h^{-1} g^{-1}	0.29 (SD)	5

Table A3: Descriptive data of the studies presented in table A2. It includes the ID of the papers, species names, location of where measurements were conducted, year and season of measurements, gender of bats measured, the reproductive state (P = pregnant, PL = post-lactating) and whether the bats had been in captivity prior to the measurements. ID refers to the papers in table A1. * Information obtained by personal communication.

ID	Species	Location	Year	Time of year	Gender	Reproductivity	Captivity
1a	*Myotis bechsteinii*	50°27'10"N, 8°48'58"E	2009	May, Aug.	Female	P	No
1b	*Myotis nattereri*	50°27'10"N, 8°48'58"E	2009	Aug.	Female	PL	No
1c	*Plecotus auritus*	50°27'10"N, 8°48'58"E	2009	Aug.	Female	PL	No
2a	*Myotis bechsteinii*	50°27'10"N, 8°48'58"E	2010	May, Aug.	Female	PL	No
2b	*Myotis nattereri*	50°27'10"N, 8°48'58"E	2010	May, Aug.	Female	PL	No
2c	*Plecotus auritus*	50°27'10"N, 8°48'58"E	2010	May, Aug.	Female	PL	No
3	*Myotis daubentonii*	50°27'10"N, 8°48'58"E *	2010	June	Male	No	No
4	*Myotis chiloensis*	33°28'S, 70°54'W	NA	Nov.-Apr.	Both	No	No
5	*Nyctophilus gouldi*	30°35'S, 151°44'E	2012, 2013 *	May, Jul., Dec. *	NA	NA	Yes
6	*Nyctophilus geoffroyi*	42°53'S, 149°19'E	2000	Autumn	Both	No	Yes
7a	*Nytcophilus geoffroyi*	30°35'S, 151°44'E	1996, 1997	Sep.-May	NA	NA	No
7b	*Nyctophilus gouldi*	30°35'S, 151°44'E	1997	Jan.-Jul.	NA	NA	No
8a	*Lasiurus seminulos*	Florida, USA	1984, 1985	Apr.-Jun.	Both	NA	Yes
8b	*Lasiurus seminolus*	Florida, USA	1984, 1985	Apr.-Jun.	Both	NA	Yes
8c	*Nycticeius humeralis*	Florida, USA	1984, 1985	Apr.-Jun.	Both	NA	Yes
8d	*Nycticeius humeralis*	Florida, USA	1984	Sep.-Dec.	Both	NA	Yes
9a	*Lasiurus borealis*	Florida, USA	1984, 1985	Summer	Both	NA	Yes
9b	*Lasiurus cinereus*	Florida, USA	1984, 1985	Winter	Both	NA	Yes
9c	*Lasiurus intermedius*	Florida, USA	1984, 1985	Winter	Both	NA	Yes
9d	*Lasiurus seminolus*	Florida, USA	1984, 1985	Apr.-Jun.	Both	NA	Yes
9e	*Lasiurus seminolus*	Florida, USA	1984, 1985	Apr.-Jun.	Both	NA	Yes
9f	*Nycticeius humeralis*	Florida, USA	1984, 1985	Apr.-Jun.	Both	NA	Yes
9g	*Nycticeius humeralis*	Florida, USA	1984, 1985	Apr.-Jun.	Both	NA	Yes
10	*Pipistrellus pipistrellus*	46°47'N, 6°31'E	NA	Jun.-Oct.	Both	No	NA
11	*Nyctophilus major*	Perth, Australia	1995, 1996	Autumn, winter	Both	No	Yes
12	*Chalinolobus gouldii*	Perth, Australia	1995	Autumn, winter	Male	No	Yes
13	*Nyctophilus geoffroyi*	34°10'S, 116°50'E	1996	Autumn, winter	Both	No	Yes
14	*Otonycteris hemprichii*	Negev highlands, Israel	2003 *	Jun.-Aug. *	Both	No *	NA
15	*Plecotus auritus*	Scotland, 57°N	1992	Spring, early summer	Female	No	Yes
16	*Pipistrellus kuhlii*	Negev highlands, Israel	2009	Aug.-Sep.	NA	NA	Yes
17a	*Eptesicus bottae*	Negev highlands, Israel	NA	NA	NA	No	NA
17b	*Otonycteris hemprichii*	Negev highlands, Israel	NA	NA	NA	No	NA
17c	*Pipistrellus kuhlii*	Negev highlands, Israel	NA	NA	NA	No	NA
17d	*Plecotus christii*	Negev highlands, Israel	NA	NA	NA	No	NA
18	*Eptesicus fuscus*	31.64°N, 86.74°W	1998	Mar.-Apr.	Female	No	No
19	*Myotis velifer*	Oklahoma, USA	1974	Jan.-Aug.	NA	NA	Yes
20	*Myotis mystacinus*	60°4'23"N, 10°52'20"E	2018, 2019	Jun.-Aug.	Male	No	No
21	*Nyctophilus bifax*	29°24'S, 153°22'E	2008, 2009	Feb.-Mar., Oct.-Nov., Jun.	Male *	No *	No *
22	*Plecotus auritus*	NE Scotland	NA	NA	Both	No	NA
23	*Eptesicus fuscus*	42°47'N, 97°0'W	2001, 2002	Sep., May	Both	PL	NA
24	*Vespadelus vulturnus*	30°35'S, 151°44'E	NA	Autumn, winter	Both	No	No

A phylogenetic signal was not present in the PGLS analysis, with no difference between the linear model of body mass and BMR and the phylogenetic generalised linear model (figure A3). Both models are based on model 2) in table A5, and the phylogenetic information used in the phylogenetic generalised linear model is illustrated in figure 3.4. The lambda estimate retrieved from the PGLS analysis was close to zero and had a p-value of 1 (table A4). Thus, there was essentially no phylogenetic signal in BMR among the 24 vespertilionid species included in the comparative analysis of the present study.

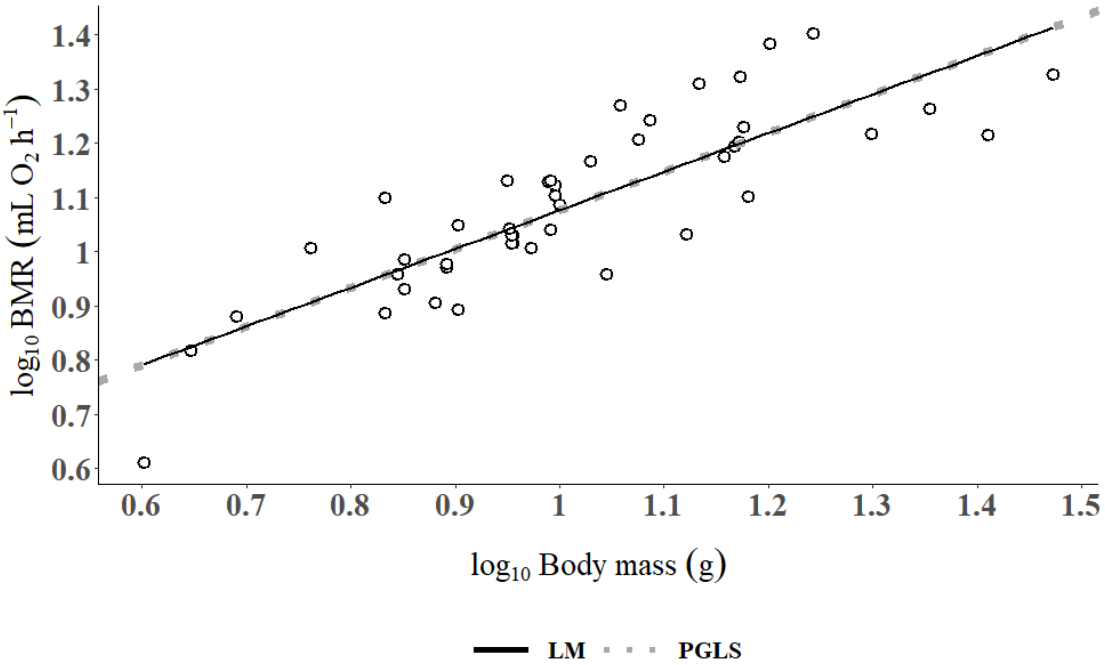

Figure A3: The linear relationship between body mass and BMR on logarithmic scales are illustrated with the solid line, whereas the phylogenetic generalised linear model on logarithmic scales retrieved from the PGLS analysis is illustrated with the dashed, gray line.

Table A4: The hypothesis test for significant phylogenetic signal, with the phylogenetic signal (lambda), the log-likelihood for lambda, the likelihood ratio test when lambda equals 0, and the P-value of the likelihood ratio test. The phylogenetic generalised linear model is based on model 2) in table A5 and the phylogenetic relationship illustrated in figure 3.4.

PGLS	Value
Phylogenetic signal (lambda)	4.19436e-05
log-likelihood (lambda)	-61.3341
Likelihood ratio test (lambda=0)	-0.000549148
P-value (based on likelihood ratio test)	1

The residual variation for each estimate as a function of the mass-specific SE is shown in figure A4. If precise estimates, that is estimates with a low SE, were clustered closer to the mean residual variation of 0.0057 mL O_2 g^{-1} h^{-1}, and the less precise estimates were dispersed where the confidence interval is wider, it could indicate that the residual variation was a result of for example differences in methodology. However, as the most extreme residuals are also precise, it indicates that true heterogeneity exists in BMR among species of Vespertilionidae included in the present study. This heterogeneity can thus be further investigated.

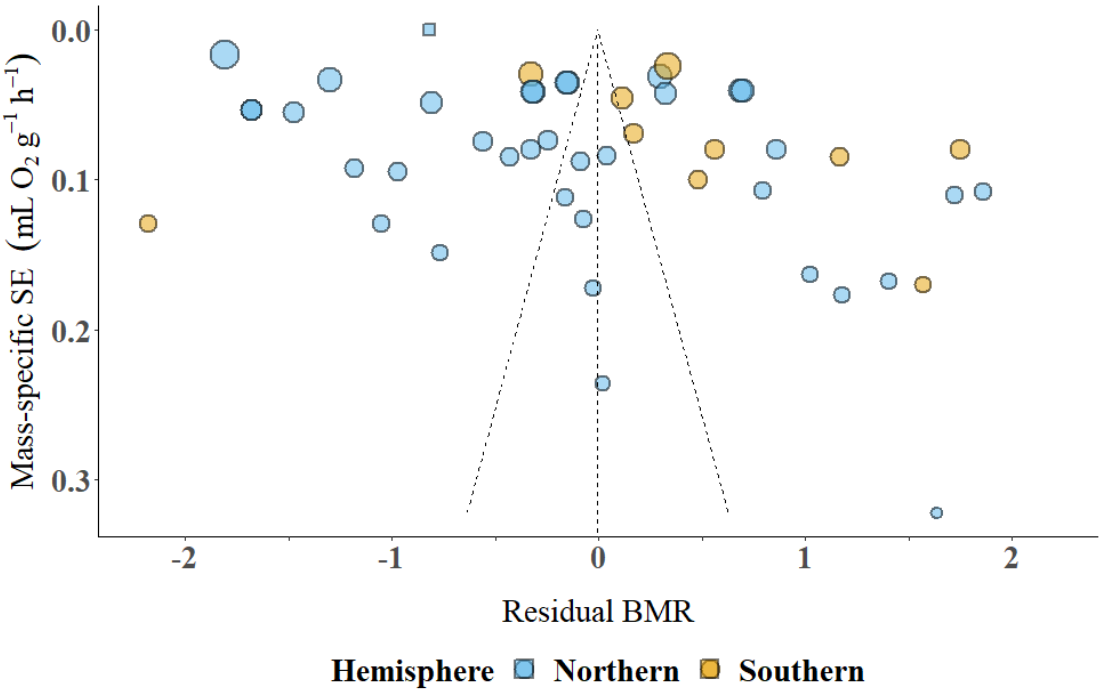

Figure A4: The mass-specific SE (mL O_2 g^{-1} h^{-1}) of residual BMR used to calculate the precision of the BMR estimates. Estimates with low mass-specific SE, and therefore high precision, are represented with increased circle size. Mean residual BMR (0.0057 mL O_2 g^{-1} h^{-1}) for the species included in the analysis and 95% confident intervals are illustrated by the dashed lines.

There was a significant negative effect of latitude on environmental temperature for the areas where the bats included in the comparative analysis of the present study were measured ($P < 0.001$).

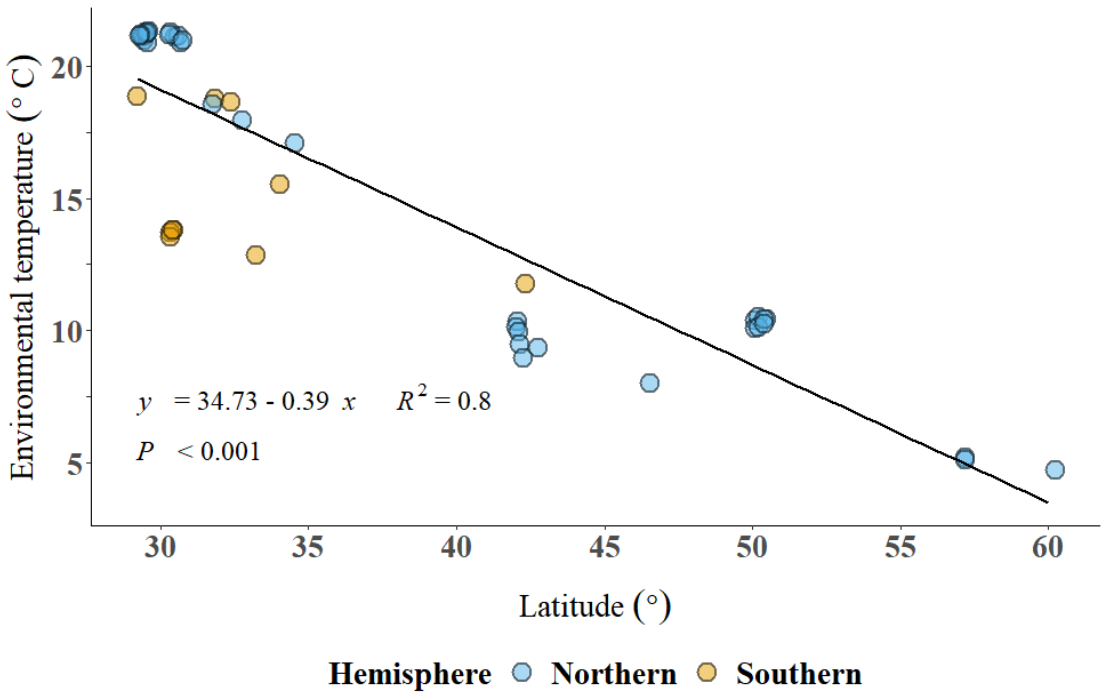

Figure A5: The average environmental temperature (°C) in the areas of which bats included in the present analysis were measured as a function of latitude (°). The temperatures represent yearly average temperature from 2009-2019.

The statistical models that were obtained from the analyses of the present study are presented in table A5. Model 1) is obtained from the my own metabolic measurements, whereas models 2-7) are obtained from the comparative analysis. Linear mixed effect models are presented with random factors, whereas the models without are simple linear models.

Table A5: The models from the statistical analyses presented in the results section of the present study. Each model includes a response variable, the intercept and predictors, with an estimate for each predictor. Standard errors (SE) for each estimate and the confidence intervals are presented, as well as the P-value of the effect. Model 1) is from my own metabolic measurements, whereas models 2-7) are from the comparative analysis. Random factors are presented for linear mixed effect models, with total variation (σ^2) and how much of this The number of observations within each random factor is also presented, in addition to the total observations in the models and the marginal R^2 and conditional R^2. Models without random factors are simple linear models, and instead include the total number of observations in the models, the R^2 and the adjusted R^2.

Coefficient	**Estimates**	**SE**	**CI (95%)**	**P-Value**
Model 1)				
Response: Mass-specific VO_2				
Intercept	14.5046	0.5368	12.7961 – 16.2131	**<0.001**
Temperature	-0.3933	0.0309	-0.4916 – -0.2950	**0.001**
Observations	5			
R^2 / R^2 adjusted	0.982 / 0.976			
Model 2)				
Response: log BMR				
Intercept	0.3770	0.0805	0.2192 – 0.5348	**<0.001**
log Body Mass	0.7071	0.0782	0.5539 – 0.8603	**<0.001**
Random effects				
σ^2	0.01			
Species	0.00			
Author	0.00			
N (Species)	24			
N (Author)	10			
Observations	47			
Marginal R^2 / Conditional R^2	0.664 / 0.751			
Model 3)				
Response: Weighted log BMR				
Intercept	0.3552	0.0837	0.1911 – 0.5194	**<0.001**
log Body mass	0.7292	0.0823	0.5680 – 0.8904	**<0.01**
Random effects				
σ^2	0.01			
Species	0.00			
Author	0.00			
N (Species)	23			
N (Author)	10			
Observations	46			
Marginal R^2 / Conditional R^2	0.669 / 0.757			

Model 4)				
Response: Body mass				
Intercept	13.5973	2.6341	8.4346 – 18.7601	**<0.001**
Latitude	-0.0652	0.0608	-0.1843 – 0.0539	0.283
Random effects				
σ^2	0.90			
Species	31.09			
Author	2.59			
N (Species)	24			
N (Author)	10			
Observations	47			
Marginal R^2 / Conditional R^2	0.011 / 0.974			
Model 5)				
Response: Residual BMR				
Intercept	0.1759	0.7700	-1.3332 – 1.6851	0.819
Latitude	-0.0021	0.0196	-0.0406 – 0.0363	0.914
Random effects				
σ^2	0.81			
Species	0.21			
Author	0.13			
N (Species)	24			
N (Author)	10			
Observations	47			
Marginal R^2 / Conditional R^2	0.000 / 0.296			
Model 6)				
Response: Temperature				
Intercept	34.7319	1.4954	31.7200 – 37.7438	**<0.001**
Latitude	-0.5206	0.0388	-0.5988 – -0.4424	**<0.001**
Observations	47			
R^2 / R^2 adjusted	0.800 / 0.795			
Model 7)				
Response: Residual BMR				
Intercept	0.0133	0.5641	-1.0924 – 1.1189	0.981
Temperature	0.0058	0.0344	-0.0616 – 0.0732	0.866
Random effects				
σ^2	0.80			
Species	0.21			
Author	0.14			
N (Species)	24			
N (Author)	10			
Observations	47			
Marginal R^2 / Conditional R^2	0.001 / 0.307			