



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

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Abstract

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

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

Abbreviations

T_b Body temperature
 T_a Ambient temperature

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

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Introduction

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

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

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

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

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

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

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

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

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

Materials and methods

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Statistical analyses

Body mass

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

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

Metabolic rate

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

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

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

Active thermoregulation

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

Arousal

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

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

Results

Body mass

Average M_b upon capture was 7.82 ± 0.96 g (range 6.6–10.7 g, $N=22$), while forearm length averaged 39.4 ± 1.3 mm (range 36.7–43.2 mm). There was strong evidence that M_b increased with time after sunset (M_b 0.009 g $\text{min}^{-1} \pm \text{SE } 0.003$, $p=0.004$), but no evidence that it increased with days after June 1st ($p=0.86$) or forearm length ($p=0.40$), and there was no difference between sexes ($p=0.48$). In contrast, the average M_b for all *P. auritus* ($N=90$, $n=156$) captured in the study area between 2017 and 2021 was 8.30 ± 1.31 g ($N=156$), which is higher than for the bats included in the present study ($t_{33.3} = -2.08$, $p=0.045$). In addition to a higher M_b with time after sunset, there was also very strong evidence that M_b was positively related to forearm length, and that there was a difference between sexes (Table 1, Fig. 1). Because females have an earlier reproductive period and had greater M_b than males (females: 8.62 ± 1.32 g, $N=112$; males: 7.49 ± 0.91 g, $N=44$, $t_{113.4} = 6.1$, $p < 0.0001$), and as females had longer forearms (females: 39.6 ± 1.2 mm, $N=112$; males: 38.9 ± 1.4 mm, $N=44$, $t_{64.0} = 3.2$, $p=0.002$) we fitted linear mixed-effects models for each sex separately (Table 1).

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

Metabolic rate

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

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

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

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

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

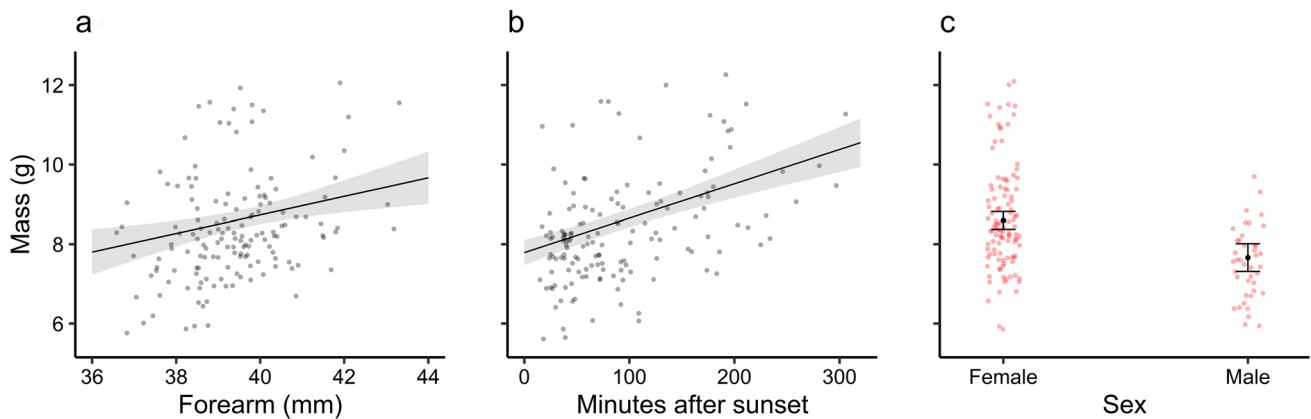


Fig. 1 Fixed effects influencing body mass (g) in captured *P. auritus* ($N=89$, $n=157$) in Nittedal, Norway between 2017 and 2021. Body mass increased with **a** forearm length (mm), **b** time after sunset (min), and **c** was higher in females than in males. In **a** and **b** solid

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

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

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

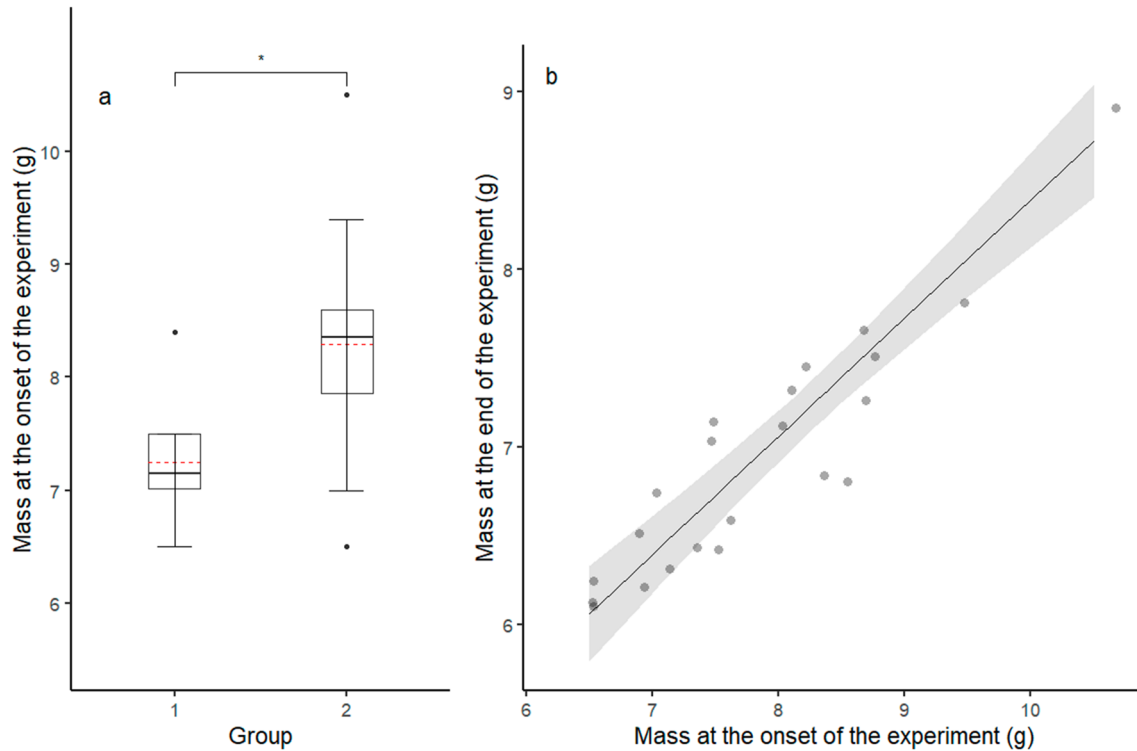


Fig. 2 Body mass (g) at **a** the onset of the experiment was higher in bats that exited torpor before the estimated lower critical temperature (T_{lc}) of the TNZ (Group 2), than those that did not (Group 1).

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

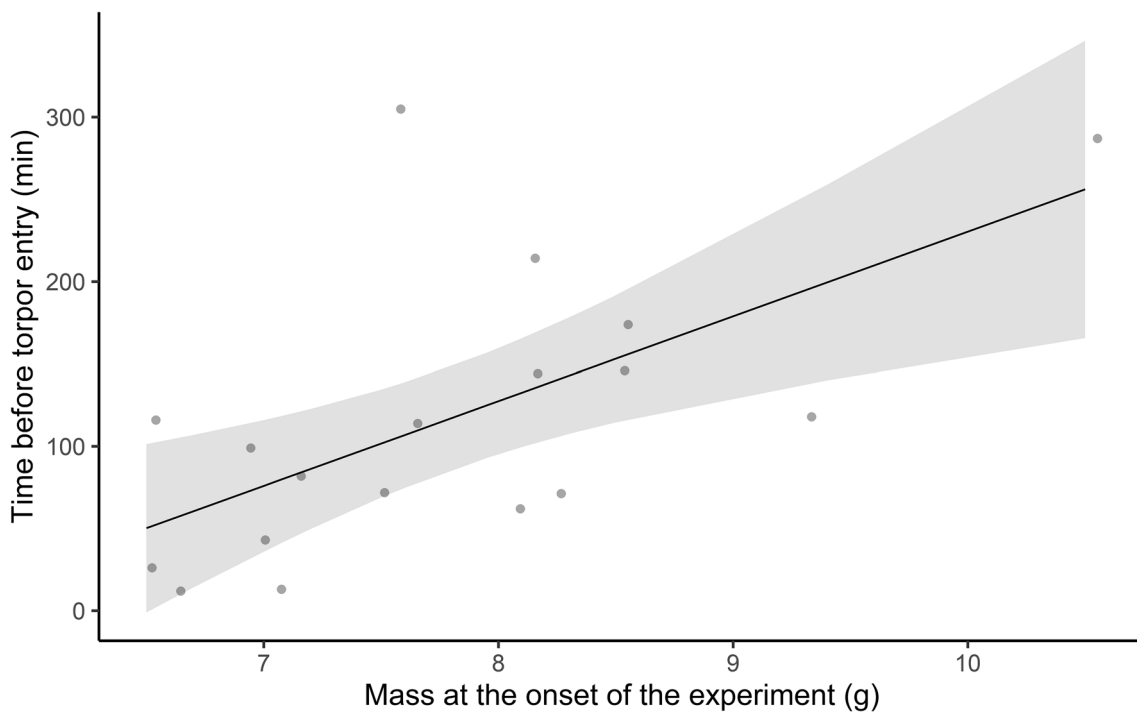


Fig. 3 Bats with a higher body mass (g) spent more time exploring the new environment before entering torpor. Solid lines show estimated relationships, shaded polygons show 95% confidence limits and dots show measured values

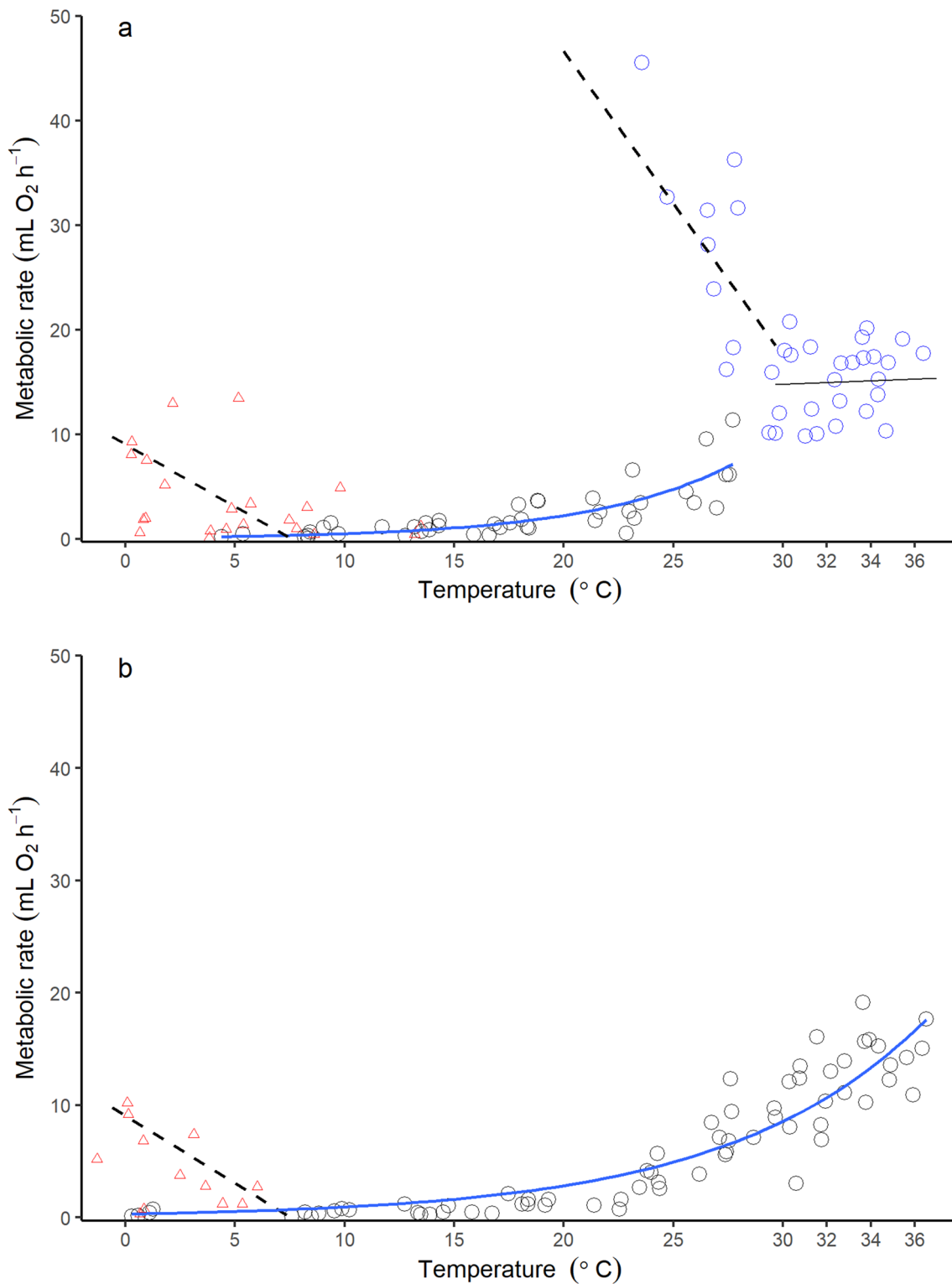


Fig. 4 Metabolic rate ($\text{mL O}_2 \text{ h}^{-1}$) as a function of ambient temperature (T_a °C). Metabolic rate of bats which **a** exited torpor before the TNZ, and bats which **b** remained torpid at temperatures above T_{lc} . The dotted line in **a** shows the increase in metabolism with decreasing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

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

Active thermoregulation during torpor

When exposed to T_a s below 6.7 °C, as indicated by the intercept of the thermoregulatory curve of thermoregulating bats and the exponential curve of TMR, bats which showed active thermoregulation increased TMR by 1.30 mL O₂ h⁻¹ °C⁻¹ ($N=10$, $n=21$; Fig. 4). Average M_b of the bats that increased $\dot{V}O_2$ at low T_a was marginally higher (8.34 ± 1.06 g, $N=10$) at the onset of the experiment then the average M_b of those that did not increase $\dot{V}O_2$ (7.46 ± 0.81 , $N=8$, $t_{16.0}=1.99$, $p=0.064$).

Arousal

Bats that exited torpor below the T_{lc} aroused at 22.9 ± 3.5 °C ($N=11$), while bats that remained torpid into the TNZ aroused at 31.9 ± 3.7 °C ($N=7$). In four cases, arousal was either not apparent, or occurred during baseline measurements. There was strong evidence for a higher $\dot{V}O_2$ during arousal ($t_{11,24}=3.96$, $p=0.002$) in the bats that aroused at a lower temperature (73.78 ± 40.99 mL O₂ h⁻¹), compared to those that aroused later (23.44 ± 7.97 mL O₂ h⁻¹), and increased with decreasing T_a (mL O₂ h⁻¹ = $198.38 - 5.46 \times T_a$, $p < 0.001$). The number of minutes the bats needed to arouse did not differ between the bats which aroused

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

Discussion

By studying how *P. auritus* close to the northern range of its distribution respond physiologically to different temperature conditions, we found that individual state influenced quantitative energetics in this species. As predicted, individual bats with a higher M_b aroused from torpor at lower T_a , while individuals with a lower M_b postponed arousal to higher T_a . Although we observed substantial active thermoregulation at low temperatures in almost half of the measured bats, the relationship between increased $\dot{V}O_2$ at low T_a and M_b was unclear, with only a marginal difference in M_b .

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

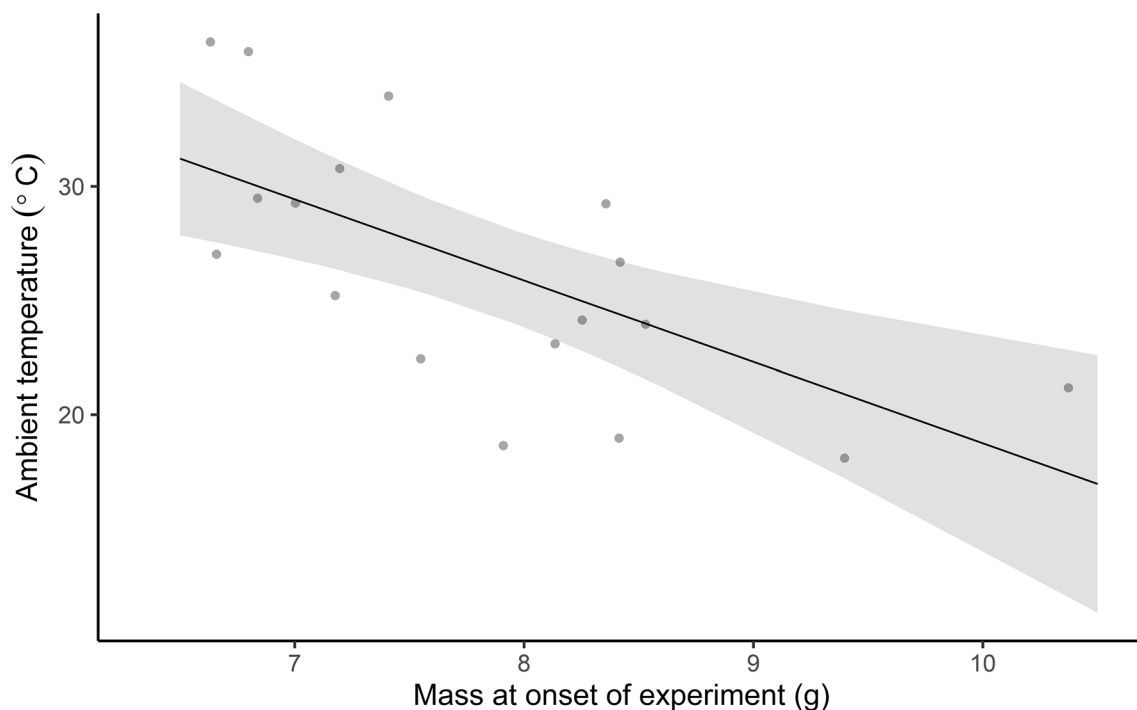


Fig. 5 The ambient temperature (T_a °C) at which bats aroused decreased ($y=58.08 - 4.42x$, $p=0.004$, $N=18$) with increasing body mass (g) at the onset of the experiment

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

TMR was similar between bats that aroused before the T_{1c} , and those that did not. TMR increased exponentially with increasing T_a , and is similar to that of equally sized vespertilionid bats in the southern hemisphere (Geiser and Brigham 2000; Turbill et al. 2008; Stawski and Geiser 2011). Thus, further supporting the notion that torpor in subtropical vespertilionid bats does not differ from temperate vespertilionid bats (Stawski and Geiser 2011; Fjellidal et al. 2022), as torpor is a mechanism to cope with energy limitation at low T_a regardless of the habitat.

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

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

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

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

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

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

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

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

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

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

Declarations

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

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

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