

Environmental conditions, rather than season, determine torpor use and temperature selection in large mouse-eared bats (*Myotis myotis*)

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Abstract

We tested whether food availability, thermal environment and time of year affect torpor use and temperature selection in the large mouse-eared bat (*Myotis myotis*) in summer and winter. Food-deprived bats were torpid longer than bats offered food *ad libitum*. Bats placed in a gradient of low (0 °C–25 °C) ambient temperatures (T_a) spent more time in torpor than bats in a gradient of high (7 °C–43 °C) T_a 's. However, we did not observe seasonal variations in the use of torpor. Moreover, even when food deprived in winter, bats never entered prolonged torpor at T_a 's characteristic of their natural hibernation. Instead, bats preferred shallow torpor at relatively high T_a , but they always maintained a difference between body and ambient temperatures of less than 2 °C. Calculations based on respirometric measurements of metabolic rate showed that food deprived bats spent less energy per unit of time in torpor than fed individuals, even when they entered torpor at higher T_a 's. We conclude that T_a likely serves as a signal of food availability and daily torpor is apparently an adaptation to unpredictable changes in food availability, such as its decrease in summer or its increase in winter. Thus, we interpret hibernation to be a second step in the evolution of heterothermy in bats, which allows survival in seasonal environments.

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1. Introduction

Daily torpor and hibernation torpor are commonly used by insectivorous, heterothermic bats in response to adverse weather or food shortage (for reviews see: McNab, 1982; Speakman and Thomas, 2003). In comparison to normothermy, both states of lethargy are characterized by markedly reduced metabolic rate (MR) and lowered body temperature (T_b) and can be distinguished based on physiological characteristics and temporal organization. Average minimum MR is almost 15-times lower in hibernation than in daily torpor; the minimum T_b of hibernating animals is >10 °C lower than T_b of torpid ones; and the duration of average torpor bouts is >30-times longer in hibernation than in daily torpor (Geiser and Ruf, 1995). While arousal from daily torpor occurs daily, daily arousal during hibernation torpor is suppressed, and torpor bouts can last for

80 days (Brack and Twente, 1985; Harris and Milsom, 2000). Moreover, while daily torpor can occur irrespective of season, hibernation, as the name implies, is restricted to winter (Wang, 1989).

Although torpor markedly reduces energy expenditure, there are significant ecological and physiological costs of this state (Humphries et al., 2003b), such as increased susceptibility to predation (e.g., Radzicki et al., 1999; Kokurewicz, 2004), possible accumulation of sleep debt (Daan et al., 1991); and reduced synaptic efficacy (Strijkstra et al., 2003). Furthermore, frequent torpor in reproductive females can result in prolonged pregnancy and slowed growth of young (Racey, 1982). Thus, increased torpor frequency may result in decreased fitness (Grinevitch et al., 1995; Kurta and Kunz, 1988).

Since selection of proper ambient temperatures (T_a) may significantly facilitate thermoregulatory reactions (e.g. Krzanowski, 1961; Gordon, 1993; Speakman and Rowland, 1999), heterothermic bats might use behavioral thermoregulation to maximize energy savings when facing food shortage.

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We hypothesized that thermal preferences of small insectivorous bats, the large mouse-eared bat, *Myotis myotis*, vary according to food availability, the thermal environment, i.e., the range of T_a 's available for selection, and time of year. Since the energy savings of torpor are positively correlated with its depth and length (Geiser and Ruf, 1995), and these are dependent on T_a (French, 1982; Geiser and Kenagy, 1988), we predicted (i) that food deprived bats would enter deeper and longer torpor bouts at lower T_a 's than fed individuals and (ii) that in winter, fasted bats would choose T_a 's similar to temperatures preferred for natural hibernation, i.e. $\sim 7^\circ\text{C}$ (Webb et al., 1996; Nagel and Nagel, 1991) to ensure maximum energy conservation. To test our prediction, we conducted a series of experiments in summer and winter, on fed and food deprived *M. myotis* in warm and cold thermal gradient systems.

We also examined a second hypothesis that T_a 's selected for torpor are a function of a trade-off between benefits of being cool and the energetic costs of torpor. If so, it would mean that bats select temperatures according to the "intended" time to be spent in torpor; namely they would select lower T_a 's for longer torpor bouts. Conversely, bats may choose higher T_a 's for shorter torpor to reduce its total cost. Confirmation of this prediction would indicate that temperature selected for torpor is not a fixed, species-specific value. To test our prediction, we measured energy consumption by indirect calorimetry during torpor at the ambient temperatures that bats selected for torpor in the thermal gradient system during winter experiments.

2. Materials and methods

2.1. Animals and housing

Myotis myotis is widely distributed through western Eurasia, the northeastern limit of its distribution being in Poland (Stutz, 1999). In summer, female bats form nursery colonies in caves and attics, numbering from few to over 800 adult individuals (Zahn, 1999). Average T_a in summer roosts is 20°C to 30°C with maxima reaching 40°C (Zahn, 1999). During summer, *M. myotis* were observed torpid at T_a 's between 20 to 25°C (Heidinger, 1988). In autumn, bats move to their winter quarters to hibernate alone or in clusters of up to several hundred individuals (personal observations). In Poland, bat hibernation season lasts about 4 months and individual torpor bouts of *M. myotis* are 5 to 98 days, with an average of 41.2 days (Harmata, 1987). Under natural conditions *M. myotis* hibernate at T_a 's between -4.0°C and 12.0°C (Webb et al., 1996), while most individuals are found at $\sim 7^\circ\text{C}$ (Nagel and Nagel, 1991; Wojciechowski and Jefimow, personal observations).

In the autumn of 2 consecutive years, we mist-netted six male *M. myotis* (Toruń, central Poland; $53^\circ00'\text{N}$, $18^\circ56'\text{E}$) and transferred them to the laboratory at the Department of Animal Physiology of the Nicolaus Copernicus University in Toruń. During the experimental period mean body mass (m_b) of the bats, measured before each experiment, was 25.2 ± 0.51 g in 1999 (mean \pm SE; $N=6$ in winter and in summer) and 25.4 ± 0.41 g in 2000 ($N=6$ in winter and $N=5$ in summer). While in

captivity, bats were housed under natural photoperiod. At least 1 month prior to winter experiments all bats were transferred to an artificial hibernaculum (modified refrigerator; $T_a=7^\circ\text{C}$, high humidity, constant darkness) to mimic winter conditions and to induce hibernation. During hibernation, bats were fed once a week and water was available *ad libitum*. Bats were placed in their flight cage for 8 h at night to feed and were offered mealworms and crickets *ad libitum*. In spring and summer, bats were maintained continuously in the flight cage at room temperature and under natural photoperiod. Winter experiments were done from December to February which correlates with the middle of hibernation season in Poland, and summer experiments were done in June and July. Upon completion of the summer experiments, bats were released at their site of capture.

All experiments were done under permit no. Opog. 4201/171/98 and Opog. 4201/346/99, Ministry of Environmental Protection, Natural Resources and Forestry and protocols were authorized by the Local Committee for the Ethics in Animal Research.

2.2. Measurements in the thermal gradient system

In both winter and summer, we used the same experimental procedures. To study the effect of food deprivation, individual fed and fasted *M. myotis* were tested in a thermal gradient system that consisted of an aluminum trough ($120 \times 12 \times 10$ cm) divided by half-width partitions into 16 compartments of the same size, and covered with Perspex[®] to permit light entry. The system was heated at one end and cooled at the other, resulting in a linear gradient of T_a . The construction of the gradient did not restrict animal movements during the experiments and wire mesh on the sides of the trough allowed animals to hang in their usual, head-down position while resting. Infrared sensors (photoemitter–photodetector pairs) detected location of a bat while T_a was measured automatically by thermocouples placed in the floor of each compartment. Humidity was not measured, but water condensed in the cold end of the gradient.

At least 1 week before experiments, bats were implanted subcutaneously with a polyethylene cannula (0.8 mm in diameter; PORTEX Ltd., England). The cannula was inserted under the skin of the anaesthetized bat (Forane, Abbott Laboratories Ltd., UK) through a small incision at the back of the neck, and fixed with surgical thread and adhesive to the skin. During experiments, the cannula served as a guide for a type T thermocouple (0.6 mm in diameter, W-TW-36 P2; Physitemp Instruments Inc., USA). At the beginning of each experiment, the thermocouple was inserted into the cannula at the depth of the interscapular brown adipose tissue (BAT) deposits and taped in place. We chose to measure T_b in BAT since it is the major site for nonshivering thermogenesis, and thus gives direct information on changes in T_b , especially during arousals from torpor (Hayward and Lyman, 1967).

A narrow slit in the gradient box's lid allowed movement of the thermocouple suspended above it by an elastic band that let animals to move freely inside the gradient without a load. Locomotor activity of bats was measured using commercial

ultrasound motion sensors that registered movement as all or nothing response. To make sure that ultrasound motion sensors would not affect the behavior of bats, we conducted a series of trial experiments in which we switched sensors on and off. We did not observe any reaction either of active or torpid individuals. All electronic temperature measuring devices were calibrated in a water bath against a mercury thermometer with accuracy ± 0.1 °C.

Experiments were done on fed and food-deprived bats, in winter and summer and in two different thermal environments. Fed bats were offered food (crickets) *ad libitum* every evening before, as well as during experiments. During an experiment, crickets were placed in the gradient after the bats commenced nighttime activity. Food was placed equidistantly along the trough to avoid the effect of food searching on T_a selection. In winter, when all bats were hibernating in the hibernaculum, they were fasted for 7 days before experiments; in summer they were fasted for 2 days at laboratory T_a .

In 1999, fed and fasted bats were tested in the thermal gradient at between 7 °C and 43 °C, the high T_a gradient. In 2000, we examined the effects of a range of available T_a on the bat's responses to food deprivation. Bats were tested in the same thermal gradient system, but with lower T_a 's available (0 °C to 25 °C), the low T_a gradient.

T_a selection, T_b and activity were sampled simultaneously and continuously at 1 Hz and averaged every minute. Activity is presented as a percent of total time of an experiment when the bat was active. Each experiment lasted for 48 h, starting in late afternoon; only data collected during the second 24 h period were analyzed.

2.3. Respirometry

In the autumn 2001, we captured in Toruń four adult male, *M. myotis* (mean $m_b = 24.5 \pm 1.11$ g) for measurement of metabolic rate by indirect calorimetry. For 2 months prior to measurement, the bats hibernated in the artificial hibernaculum and measurements took place in February 2002 at the Department of Animal Ecology of the University in Białystok, north-eastern Poland. Metabolic rate was determined from oxygen consumption measured in an open-flow respirometry

system. Animals were placed in a 0.8 L, T_a controlled (± 0.1 °C) metabolic chamber made of Perspex® and lined with plastic mesh to allow bats to hang in a head-down position. Air from outside the building was dried with anhydrous calcium sulphate (Drierite®, W.A. Hammond Drierite Co. Ltd., USA). Air flow rate was controlled upstream with a mass-flowmeter (β -ERG, Warsaw, Poland) at 100 to 150 mL min⁻¹. Expired air was dried (Drierite), and exhaled CO₂ was removed with Carbosorb® AS (BDH Laboratory Supplies, Poole, England). Oxygen content of the expired air was determined with a 2-channel oxygen analyzer (S-3A/II N 37 M, Ametec, Pittsburgh, USA). The output of the O₂ analyzer (difference between ambient air O₂ and excurrent air O₂) was sampled every 0.006 s, averaged every 3 s and stored for subsequent analysis. Simultaneously, bat T_b was measured with thermocouples as above. T_b was sampled continuously, averaged and saved every 3 s.

In Białystok, prior to and after the measurements bats were kept in an underground bunker that provided excellent conditions for undisturbed hibernation. We did not divide bats into fed and fasted groups. All bats were fed 2 days before measurements and after feeding they were left undisturbed in the hibernaculum. This schedule facilitated entry into torpor during measurements. Respirometry measurements were made at the T_a 's that bats preferred for torpor during experiments in the thermal gradient ($T_a = 16$ °C, 18 °C, 21 °C and 27 °C; see Table 1 for details and description).

Bats were placed in the metabolic chamber at about 08:00 h. Oxygen consumption was recorded continuously until it reached minimum, stable level in torpor, and then for at least 60 min, when the difference between body and ambient temperatures did not exceed 1 to 2 °C. Thereafter, bats were forced to arouse from torpor by shaking the metabolic chamber for 30 s (for comparison see: Thomas et al., 1990). About 15 min after full arousal, as indicated by high body temperature and stable oxygen consumption after a peak during arousal, the recording was completed.

Recordings were corrected using the washout correction of Bartholomew et al. (1981) and the rate of O₂ consumption was calculated using equation (4) of Hill (1972). Total oxygen consumption during entry into and during arousal from torpor was calculated by integrating the area below the $\dot{V}O_2$ curve. O₂

Table 1
Based on the ambient temperatures which *Myotis myotis* selected for torpor in the thermal gradient (column A) we set up the ambient temperatures for the respirometry (column B)

Experiment in thermal gradient	Mean (\pm SE) T_a selected for torpor in the thermal gradient (°C)	T_a for respirometric measurements (°C)	Mean (\pm SE) torpor bout duration in the thermal gradient (min)	Mean (\pm SE) total duration of entry and arousal phases (min) during respirometric measurements	Duration of the maintenance phase of torpor (min)
	A	B	C	D	C–D
Fed; low gradient	15.3 \pm 0.93 (11)	16.0	411.7 \pm 103.34 (13)	258.9 \pm 5.69 (3)	152.7
Fasted; low gradient	17.3 \pm 0.70 (15)	18.0	812.0 \pm 119.91 (10)	174.6 \pm 42.75 (3)	637.4
Fed; high gradient	20.2 \pm 3.08 (5)	21.0	211.6 \pm 51.03 (7)	218.8 \pm 21.90 (4)	–
Fasted; high gradient	26.2 \pm 0.71 (11)	27.00	479.8 \pm 100.84 (14)	170.9 \pm 60.46 (3)	308.9

The mean duration of torpor bouts in the thermal gradient is given in column C. Column D stands for the sum of the durations of entry and arousal during respirometric measurements. Duration of the maintenance phase of torpor in thermal gradient was calculated as the difference between column C and D. Note that since the result of this subtraction was negative for fed bats in the high gradient, we assumed that there was no maintenance phase during torpor under these conditions, and thus all subsequent calculations were made for entry and arousal only. Number of observations is shown in parentheses.

consumption was converted to energy expenditure assuming that consumption of 1 L of O₂ equals 20.083 kJ of energy (RQ=0.85; Schmidt-Nielsen, 1997). Since torpor was artificially terminated, calculations of total oxygen consumption during the phase of maintenance in torpor in relation to T_a were unjustified.

Based on metabolic measurements and data on the torpor bout duration in the thermal gradients we calculated the theoretical cost of torpor in the thermal gradient for fed and food deprived *M. myotis* in winter, in the high and the low T_a gradients. Average torpor bout duration was calculated based on the experiments in the thermal gradients. Duration of the entry into and arousal from torpor as well as the energy consumption during these phases were calculated based on the metabolic measurements at T_a 's, which reflected the average T_a 's selected for torpor in the gradients (above and Table 1).

Duration of maintenance phase (Fig. 6) was calculated as the difference between the mean duration of torpor bout recorded in the thermal gradient and the mean duration of the entry and arousal phases recorded during metabolic measurements at particular T_a 's. Energy consumption during the maintenance phase was calculated by multiplying its duration in the thermal gradient by the metabolic rate recorded during respirometric measurements at appropriate T_a .

2.4. Data analysis

Bats were considered torpid when their T_b was ≤ 30 °C, and the time spent in torpor was calculated as the time with $T_b \leq 30$ °C. The minimum regulated level of T_b during torpor (T_{b-min}) refers to the lowest T_b kept constant (± 1 °C) for at least 15 min. Lengths of day and night were estimated based on the timing of sunrise and sunset at the time of experiments.

The effect of thermal environment, season and food availability on T_b , preferred T_a and activity was analyzed by 3-way analysis of variance (3-way ANOVA). To test for daily variations in analyzed variables we used 4-way ANOVA. When appropriate, LSD post-hoc test was used. In the case of multiple comparisons, when the sample size was small, we used a Kruskal–Wallis ANOVA. For comparisons of two means, Student's *t*-test or Mann–Whitney *U* test was used when appropriate. Pearson's correlation coefficient was applied to test for relationship between two continuous variables.

If bats chewed the thermocouple lead wires, the break was immediately repaired. During lead repair, animals were handled for no more than 10 min and all data collected for 1 h after the repair were discarded from analysis.

In a few cases, when activity sensors failed, we excluded the activity data for the whole experiment, using only T_b and T_a data. All data are presented as mean \pm SE and results were considered statistically significant at $P < 0.05$.

3. Results

3.1. General remarks

M. myotis, when placed in the thermal gradient system, followed a relatively clear pattern of nocturnal activity with

higher activity at night coupled with normothermic T_b . Torpor, if occurring, was recorded mainly during the daytime hours and was terminated at dusk. Preferred T_a depended rather on the range of temperatures available in the thermal gradient than on time of day. Fig. 1 presents examples of the recordings under all experimental conditions in individual bats.

3.2. Activity

Neither total activity nor its daily rhythm were affected by season and on average, bats were active for about 13% of the 24 h experiments. Bats were significantly more active by night than by day [4-way ANOVA: $F(1,68)=39.15$; $P < 0.001$; Fig. 1] and spent in activity almost 20% of the night and only about 8% of the day. However, total daily activity depended both on feeding regimes [3-way ANOVA: $F(1,34)=19.48$; $P < 0.001$] and thermal environment [3-way ANOVA: $F(1,34)=10.25$; $P < 0.01$]. The largest difference between fed and fasted animals was recorded in the low gradient in winter; fed bats were active for $22.60 \pm 4.71\%$ of the experiment, while fasted animals only for $1.06 \pm 0.51\%$. Food restriction also significantly changed the rhythm of daily activity [4-way ANOVA: $F(1,68)=15.63$, $P < 0.001$]. Fed bats were much more active by night than by day (LSD: $P < 0.001$; see Fig. 1 for examples), while food deprived ones reduced nighttime activity to the daytime level. This effect was strongest in the low gradient in summer, when fasted bats aroused from torpor in the afternoon, became active for approximately 1 h, and entered torpor again for the rest of the day and the following night.

3.3. Use of torpor

Bats entered daily torpor in all experiments, but environmental conditions significantly affected torpor use and distribution.

Season and thermal environment affected the time spent in torpor [3-way ANOVA: $F(1,37)=8.70$; $P < 0.01$ and $F(1,37)=107.66$; $P < 0.001$, respectively]. In winter, bats were torpid much longer than in summer; in the low gradient torpor was more than 6-times longer than in the high gradient. The longest torpor bout lasted for over 23 h and was recorded in a food deprived bat in the high gradient, in winter.

Food deprivation resulted in about a 2-fold increase in the time that bats were torpid [3-way ANOVA: $F(1,37)=38.42$; $P < 0.001$, Fig. 2]. However, the effect of food availability was influenced by thermal environment and season [3-way ANOVA: $F(1,37)=11.62$, $P < 0.01$, Fig. 2]. In summer in the low gradient fasted bats were torpid much longer than fed animals ($95.4 \pm 1.34\%$ and $18.3 \pm 7.02\%$, respectively, LSD: $P < 0.001$), while in the high gradient in summer food availability did not affect duration of torpor. In the latter case, torpor was shortest and lasted for less than 2% of the time of experiment (Fig. 2).

Torpor bouts were longer by day than by night [4-way ANOVA: $F(1,74)=9.40$, $P < 0.01$], however, food availability and thermal environment affected daily differences in the use of torpor [4-way ANOVA: $F(1,74)=8.05$; $P < 0.01$]. The most

pronounced day–night differences were recorded in fed bats in the low T_a gradient, both in summer and winter (Fig. 1). These bats always aroused at dusk and were normothermic at night.

3.4. Selection of ambient temperature (T_a)

Irrespective of being normothermic or torpid, bats selected higher T_a 's in the high than in the low T_a gradient [3-way ANOVA: $F(1,37)=168.76, P<0.001$]. Temperature preferences of normothermic bats did not depend on food availability. In the high T_a gradient normothermic bats selected similar T_a 's both in summer

and winter; on average $31.5\pm 0.73\text{ }^\circ\text{C}$. In contrast, in the low T_a gradient, T_a 's selected in summer were higher than in winter [3-way ANOVA: $F(1,37)=9.54, P<0.01; 22.6\pm 0.54\text{ }^\circ\text{C}$ vs. $15.3\pm 0.54\text{ }^\circ\text{C}$, respectively]. We did not observe daily variations in the temperature selection under any experimental conditions.

T_a 's selected for torpor were lower than selected in normothermy only in the high gradient in winter [2-way ANOVA: $F(1,24)=20.16, P<0.001$]. Temperatures selected for torpor depended on the available gradient of T_a 's [1-way ANOVA: $F(1,65)=29.17, P<0.001$, Fig. 3] and were much higher than expected. In the high T_a gradient bats entered torpor at T_a 's higher by $>6\text{ }^\circ\text{C}$ than in the low T_a gradient (LSD: $P<0.001$).

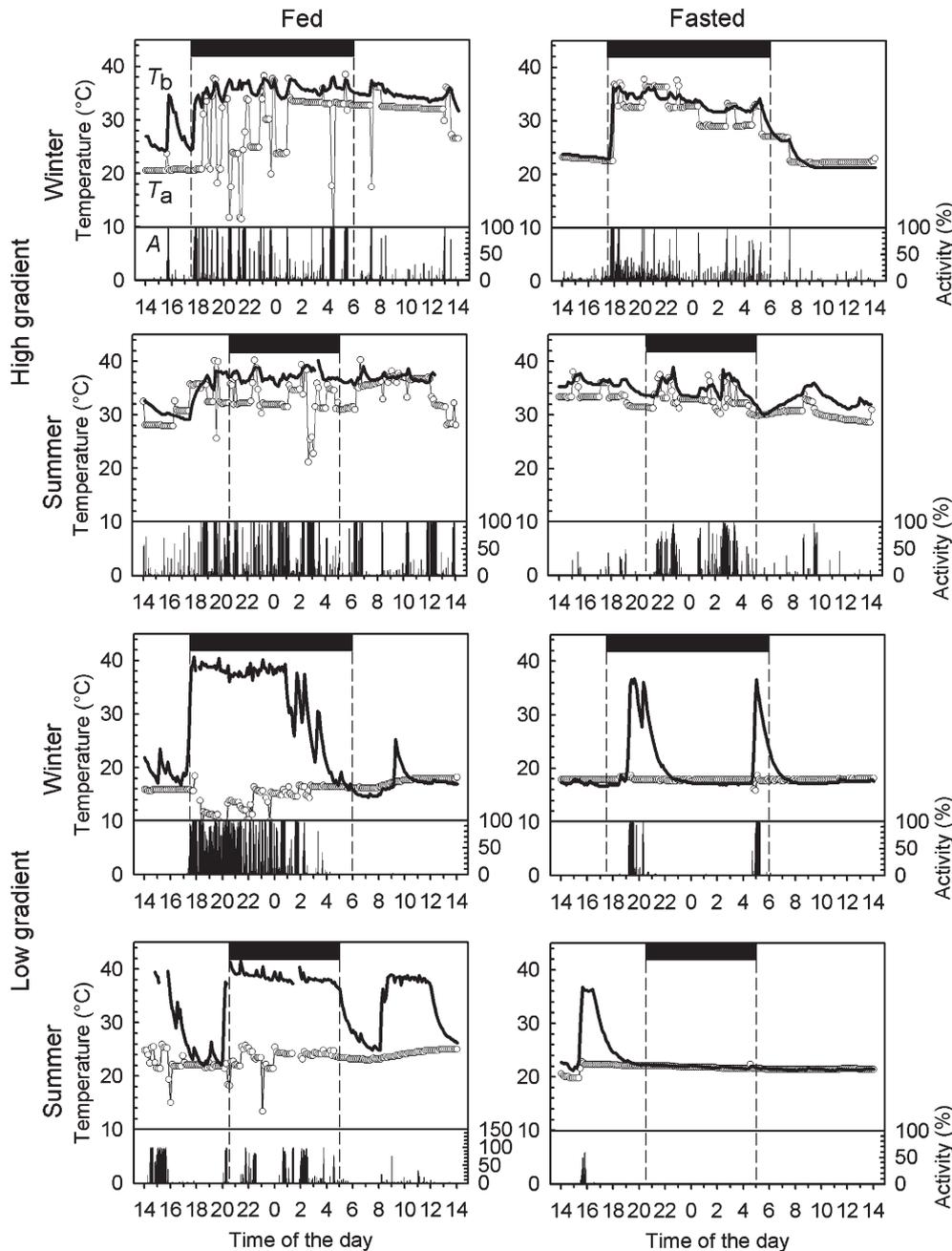


Fig. 1. 24 h recordings of body temperature (T_b , thick black line), selected ambient temperature (T_a , open symbols) and activity (A, black vertical bars) in individual *Myotis myotis* when fed and fasted in summer and winter, during experiments in the high and the low temperature gradient systems. Black horizontal bar on the top of each figure indicates night.

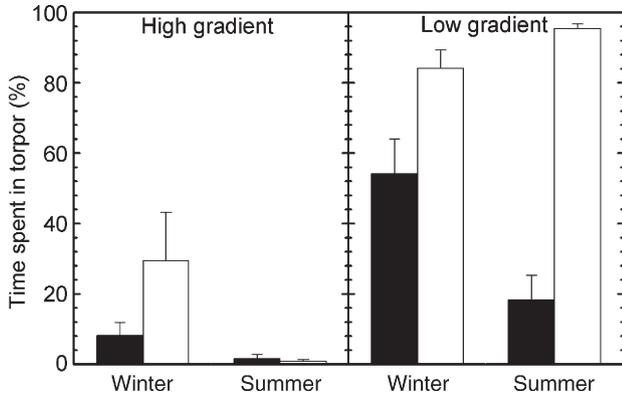


Fig. 2. Time spent by fed (black bars) and fasted (white bars) *Myotis myotis* in torpor in summer and winter during experiments in the high and in the low temperature gradient systems. Time spent in torpor is presented as the % of the total time of 24 h experiment and the data are shown as mean±SE.

Temperatures selected for torpor also differed between seasons, both in the high [Kruskal–Wallis ANOVA: $H(1, N=19)=5.53, P<0.05$] and in the low temperature gradient [1-way ANOVA: $F(1,46)=58.09, P<0.001$]. In summer, bats entered torpor at T_a 's higher than in winter: by $>4.5\text{ }^\circ\text{C}$ in the high T_a gradient and by $>5.5\text{ }^\circ\text{C}$ in the low T_a gradient. The only bats found torpid in the high T_a gradient in summer (3 incidences) selected torpor T_a 's between $29.4\text{ }^\circ\text{C}$ and $30.0\text{ }^\circ\text{C}$.

In summer, both in the high and the low T_a gradient, fed and food deprived bats entered torpor at similar T_a 's. In winter, T_a 's selected by fasted bats were significantly higher than T_a 's selected by fed animals [2-way ANOVA: $F(1,59)=6.21, P<0.05$; LSD: $P<0.01$].

3.5. Changes of body temperature (T_b)

Bats usually maintained high, normothermic T_b at night and became torpid by day (Fig. 1). Food availability significantly affected normothermic T_b [3-way ANOVA: $F(1,37)=25.59; P<0.001$; Fig. 4], while season and thermal environment had no

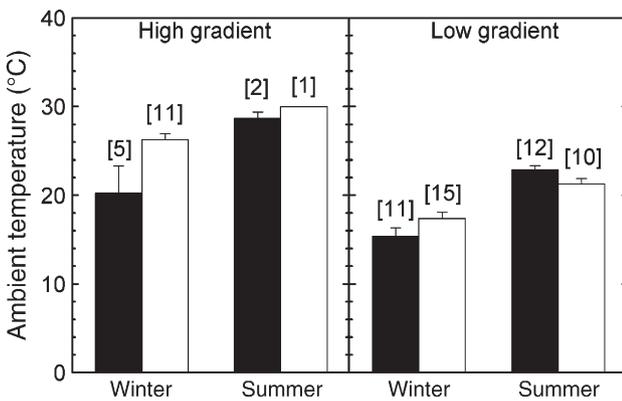


Fig. 3. Mean ambient temperatures (±SE) selected for torpor by fed (black bars) and fasted (white bars) *Myotis myotis* in summer and winter during measurements in the high and in the low temperature gradient systems. Numbers above each bar indicate number of torpor episodes recorded in each experimental setup and thus the number of observations.

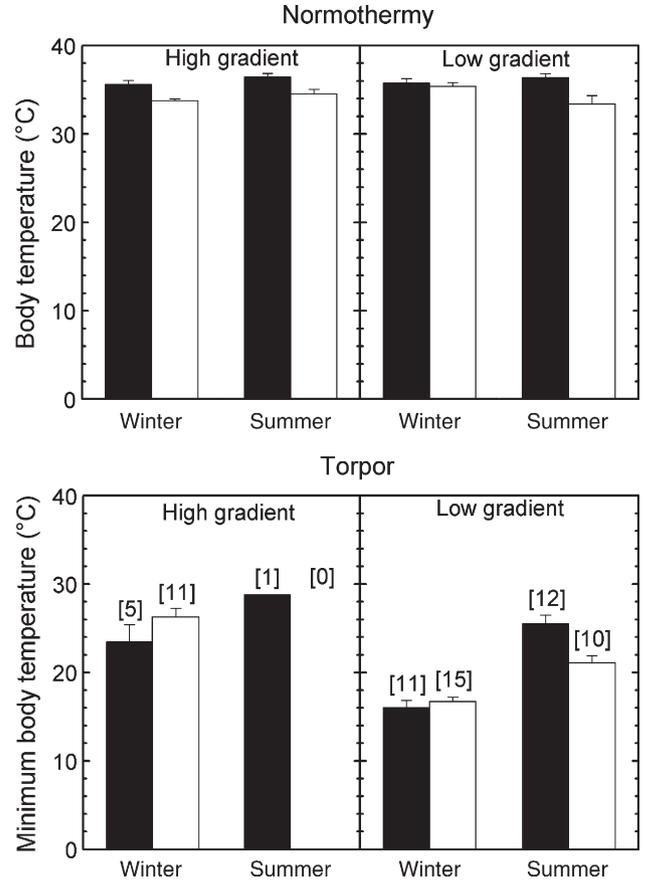


Fig. 4. Body temperatures of fed (black bars) and fasted (white bars) *Myotis myotis* while normothermic (top panel) and torpid (minimum body temperature in torpor; bottom panel). Numbers above bars in the “Torpor” panel indicate number of observations. Note that only when minimum body temperature was stable for at least 15 min it was taken into consideration. This is the reason why these numbers do not match numbers at Fig. 3. All data are shown as mean±SE.

effect. Normothermic T_b of fed bats averaged $36.0\pm0.20\text{ }^\circ\text{C}$, while of fasted bats $34.3\pm0.30\text{ }^\circ\text{C}$.

Average T_b (without differentiating between torpor or normothermy) was higher by night than by day (Fig. 1). This day–night difference was influenced by food availability and the range of T_a 's available in the gradients [3-way ANOVA: F

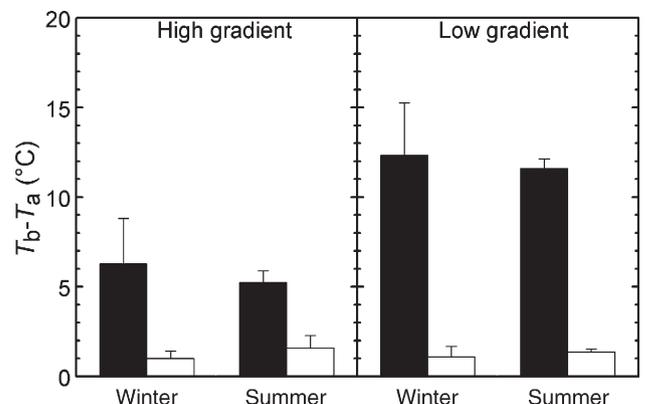


Fig. 5. Mean (±SE) differences between body and ambient temperatures in fed (black bars) and fasted (white bars) *Myotis myotis* during 24 h measurements in the high and in the low temperature gradient systems, in summer and winter.

(1,37)=13.80, $P<0.001$]. The largest day–night differences in T_b were recorded in fed bats in the low T_a gradient (9.2 ± 1.53 °C in winter and 5.0 ± 1.39 °C in summer). In fasted individuals, irrespective of the thermal environment or season, T_b was relatively constant, the day–night difference was smallest (1.2 ± 0.67 °C); and bats, either normothermic or torpid, maintained relatively constant T_b during the experiment. As a consequence, we did not record daily variations in T_b of fasted bats.

3.6. Minimum body temperature in torpor (T_{b-min})

T_b 's of torpid bats were higher than initially expected. Irrespective of the feeding regime, T_{b-min} 's were lower in the low than in the high T_a gradient [2-way ANOVA: $F(1,61)=19.87$, $P<0.001$; Fig. 4]. In the low gradient, T_{b-min} 's in winter were lower than in summer by almost 7 °C [2-way ANOVA: $F(1,44)=85.05$, $P<0.001$]. Moreover, in the low gradient, season influenced the effect of food availability on the T_{b-min} 's [2-way ANOVA: $F(1,44)=11.73$, $P<0.01$]. In summer, fasted bats

maintained lower T_{b-min} 's than fed individuals (21.1 ± 0.79 °C and 25.5 ± 0.95 °C, respectively; LSD: $P<0.001$), while in winter T_{b-min} 's of fed and fasted bats did not differ and averaged 16.4 ± 0.43 °C. In the high T_a gradient in winter, T_{b-min} 's of fed and fasted bats did not differ.

3.7. Body-ambient temperature difference ($T_b - T_a$)

In winter and summer, daily $T_b - T_a$ difference in the high and in the low gradient was affected by food deprivation [3-way ANOVA: $F(1,37)=48.22$, $P<0.001$, Fig. 5]. In fasted bats, $T_b - T_a$ difference was maintained at 1.2 ± 0.24 °C and time of day did not affect it. In fed bats, this difference was larger in the low than in the high gradient (12.0 ± 1.55 °C and 5.7 ± 1.27 °C, respectively; $P<0.001$) and it depended on the time of day [4-way ANOVA: $F(1,74)=7.31$, $P<0.01$]; during daytime, it did not exceed 6.5 °C, while at dusk, with the return to normothermy, it raised to over 11 °C. During torpor $T_b - T_a$ difference in fed bats was almost 2 °C larger than in food deprived individuals ($t_{56}=3.49$, $P(t)<0.001$).

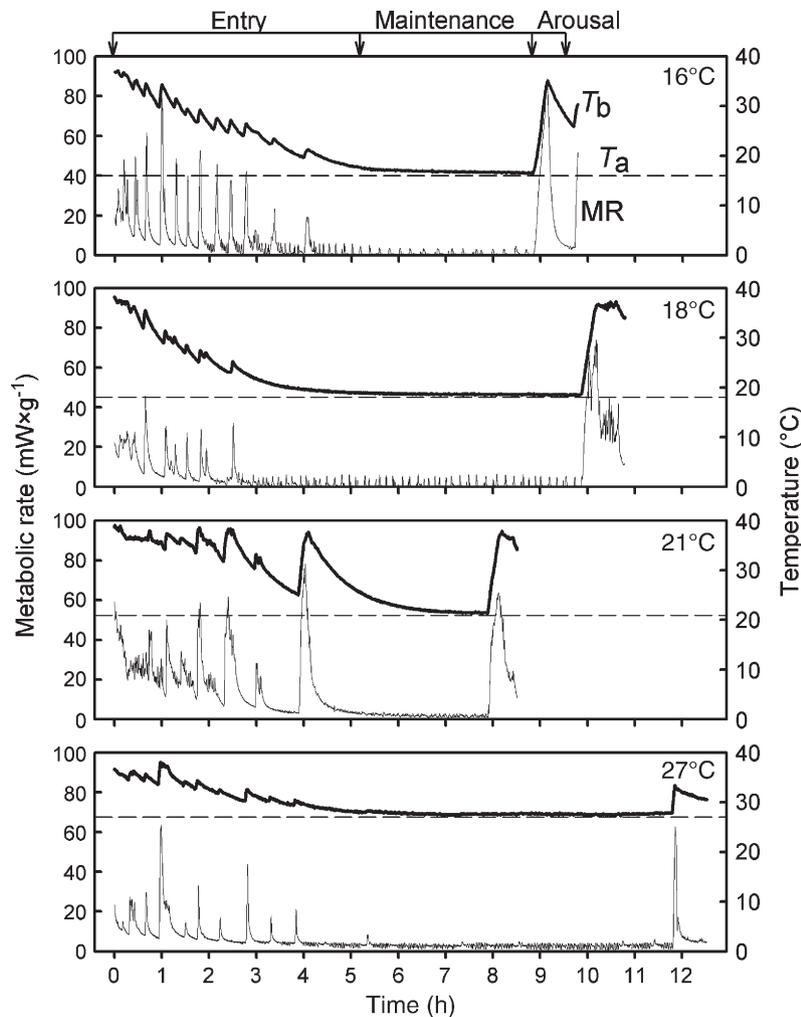


Fig. 6. Changes in metabolic rate (MR, thin line) and body temperature (T_b , thick line) during torpor in individual *Myotis myotis* at 16 °C, 18 °C, 21 °C and 27 °C (as indicated with dashed line and in upper right corner of each graph). Note the characteristic “saw teeth” pattern of changes in T_b and MR during entry into torpor. Arrows above the plot indicate the beginning and the end of each phase of the torpor at T_a of 16 °C.

Table 2

Metabolic rate (MR; mW g^{-1}) at different ambient temperatures (T_a) during entry, maintenance and arousal from torpor in *Myotis myotis*

T_a (°C)	16	18	21	27	r, P
Entry	8.6 ± 0.18 (3)	7.9 ± 0.72 (3)	7.9 ± 1.18 (4)	7.4 ± 0.27 (3)	NS
Maintenance	1.0 ± 0.09 (4)	1.2 ± 0.09 (4)	2.1 ± 0.22 (5)	3.3 ± 0.26 (4)	$0.93, <0.001$
Arousal	41.1 ± 5.20 (4)	45.4 ± 5.98 (4)	45.1 ± 2.35 (5)	41.9 ± 5.84 (4)	NS

All data are shown as mean \pm SE; number of observations is shown in parentheses.

3.8. Energetics of torpor

M. myotis commenced torpor almost immediately after being placed into the metabolic chamber. In most cases, entry into torpor was not smooth, but the decrease of T_b and MR followed “saw teeth”-like pattern (Fig. 6). T_a did not affect the pattern and duration of entry into torpor; both the longest and shortest entries were recorded at $T_a=27$ °C (51 and 260 min, respectively). During the maintenance phase of torpor, MR was low and $T_b - T_a$ difference did not exceed 1 °C. Duration of arousal was inversely correlated with ambient temperature ($r=-0.68, P<0.005$) and the longest arousal (44 min 6 s) was recorded at $T_a=16$ °C, while the shortest (5 min 57 s) occurred at 27 °C.

Neither during entry, nor during arousal from torpor, MR was correlated with T_a . Only during the maintenance phase, MR increased with an increase of T_a ($r=0.93, P<0.001$, Table 2).

Since bats were forced to arouse, we estimated total energy expenditure only for the entry and arousal from torpor. Although energy consumption during entry into torpor did not correlate with T_a , the largest energy consumption was recorded at the lowest T_a ($119.18 \pm 9.07 \text{ J g}^{-1}$; $T_a=16$ °C) and smallest at the highest T_a ($70.04 \pm 24.36 \text{ J g}^{-1}$; $T_a=27$ °C). During arousal, mean energy consumption was inversely correlated with the T_a ($r=-0.75; P<0.001$) (Fig. 7).

3.9. Cost of torpor in the thermal gradients

Detailed data used to calculate the costs of torpor are presented in Table 1, while Fig. 8 presents results of those calculations. Energy consumption during torpor did not differ

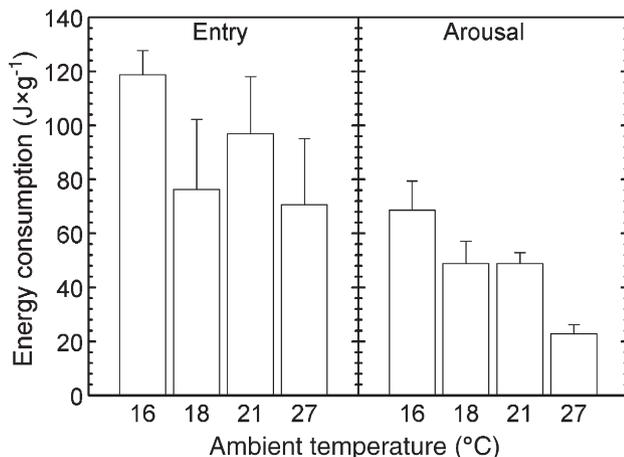


Fig. 7. Mean (\pm SE) energy consumption of *Myotis myotis* during the entry into and arousal from torpor in the metabolic chamber at 16 °C, 18 °C, 21 °C and 27 °C.

between bats at T_a 's=16 and 18 °C (these correspond to T_a 's selected by fed and fasted bats in the low T_a gradient) and between 21 and 27 °C (T_a 's selected by fed and fasted bats in the high T_a gradient) [$P(U)=0.51$ and $P(U)=1.00$, respectively; Fig. 8A].

Hence, we can presume that energetic expenses of torpor per unit of m_b were similar in the low and in the high T_a gradient system, both in fed and fasted bats. However, torpor duration in fasted bats was about two times longer than in fed individuals (Table 1). Thus, when calculated per unit of time (here: 1 h),

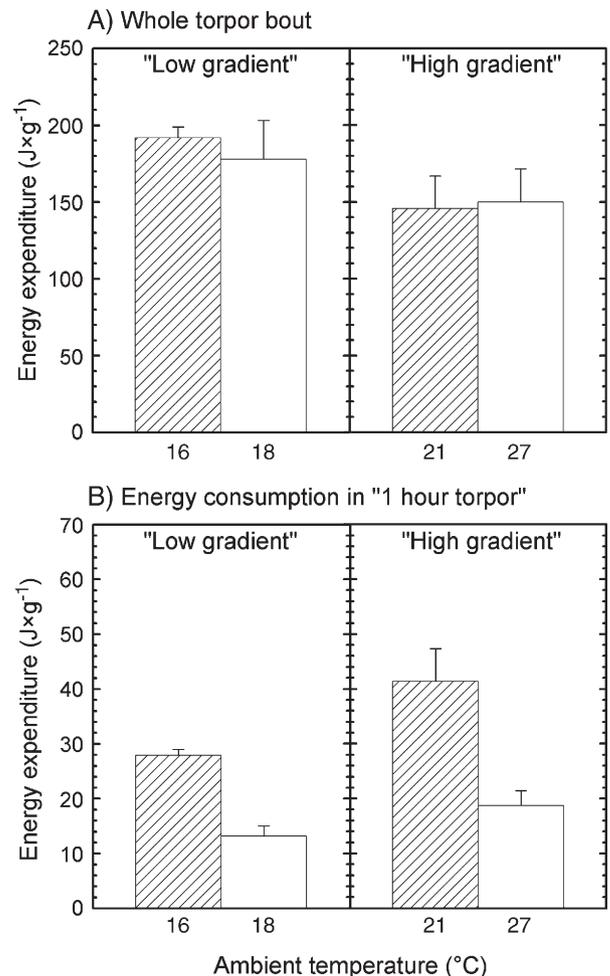


Fig. 8. Theoretical costs of torpor of *Myotis myotis* in the thermal gradients. Values were calculated using data for oxygen consumption and data for the length of torpor bouts in the temperature gradients. Ambient temperatures of 16 °C and 21 °C correspond to T_a 's selected by fed bats in the low and in the high gradient, respectively; T_a 's of 18 °C and 27 °C stand for temperatures selected for torpor by fasted bats in the low and in the high gradient. Panel (A) energy consumption for entire average torpor bout in the thermal gradient. Panel (B) energy consumption during 1 h of torpor.

energy consumption during entry, maintenance and arousal was over two times lower in food deprived than in fed bats [$P(U) < 0.05$ for the “low-gradient bats” and $P(U) < 0.05$ for the “high-gradient bats”, Fig. 8 B]. Moreover, according to our calculations, fed bats in the low gradient spent less energy per unit of time in torpor than fed bats in the high gradient [$P(U) < 0.05$]. We did not observe any difference in energy consumption between animals studied at 18 and 27 °C (Fig. 8B).

4. Discussion

We predicted that food availability would affect thermal preferences and torpor characteristics in *Myotis myotis*. Indeed, fasted bats spent more time in torpor than fed individuals. The range of ambient temperatures available for bats in the thermal gradient was an important factor shaping the response to food deprivation. However, present experiments did not confirm our prediction, that behavioral thermoregulation of fasted bats in winter would reflect the behavior of bats in their natural environment, namely that *M. myotis* would enter prolonged episodes of torpor at T_a close to 7 °C with T_b higher by 1 to 2 °C than T_a . Instead bats entered short, daily torpor at relatively high T_a 's, aroused every night and became active at least for a short time.

One could argue that the discrepancy between our laboratory results and those observed under natural conditions is due to the effect of captivity on bat behavior. Geiser et al. (2000) showed that the laboratory animals of different taxa either enter shorter and shallower torpor or even avoid it, while in nature they enter longer and deeper torpor readily and regularly. However, the results of Geiser and Ferguson (2001) also suggest that if animals are kept in captivity for less than 1 year, their torpor patterns may be representative of animals in the wild. *M. myotis* used in the present experiments were in captivity for less than 1 year and they hibernated successfully in an artificial hibernaculum in which environmental conditions resembled those in nature. This is why we argue that the lack of prolonged torpor episodes does not result from the experimental procedures, but is a response to changes in the environmental conditions and may be indicative of the general rules governing the use of lethargic states also in wild bats.

4.1. T_a and food availability affect torpor use

Although food deprivation resulted in the decrease of overall activity and in a change in its daily rhythm, it had a more pronounced effect in the low gradient where the highest T_a was about half of that available in the high gradient (25 °C and 43 °C, respectively). In nature, when the weather is good and food availability is sufficient, insectivorous bats follow bimodal, dusk-dawn, pattern of activity, reflecting the activity of flying insects (Swift, 1980; Taylor and O'Neil, 1988). Yet, when nights become cool and insect availability decreases, bats may significantly reduce their activity and even change to a unimodal pattern with a peak at dusk (O'Donnell, 2000; Rachwald et al., 2001). In the present experiments, bats in the colder environment (the low T_a gradient) reduced their dusk

activity to the essential minimum and the rest of experiment often spent in torpor.

Surprisingly, we did not find any seasonal differences in daily activity of bats. The opposite, i.e. clear seasonal rhythms of activity and energy budget, even under constant environmental conditions, has been described in hibernating rodents (Körtner and Heldmaier, 1995; Pengelley and Fisher, 1963; Walker, 1981). However, hibernating chipmunks, *Tamias striatus*, provided in winter with supplemental food shortened their torpor episodes, spent more time normothermic, and when in torpor, their minimum T_b was higher than in control animals (French, 2000; Humphries et al., 2003a). Also bats under natural conditions, may be active during winter and even forage when T_a outside the hibernacula is high enough to ensure successful foraging (Avery, 1985; Park et al., 2000). O'Donnell (2000), based on his observations suggested that minimum T_a at dusk rather than season itself affects foraging activity of *Chalinolobus tuberculatus* in New Zealand. It seems that this conclusion may be true also for European bats, including *M. myotis* in the thermal gradient systems.

Since, in the present, laboratory experiments, T_a significantly affected the response of bats to food restriction and in nature insect (food) availability positively correlates with T_a both in summer and winter (Taylor, 1963; Taylor and O'Neill, 1988), we suggest that bats perceive T_a as a signal of food availability. As such, T_a would modulate bat activity and torpor use, both in the thermal gradient and in nature. T_a could serve as a signal for bats, which apparently change their behavior in response to variations in T_a both in summer and in winter (Audet and Fenton, 1988; Grinevitch et al., 1995; O'Donnell, 2000; Rachwald et al., 2001; Krzanowski, 1961; Avery, 1985; Park et al., 2000).

In our experiments, bats entered torpor mainly during the daytime (Fig. 1). This observation agrees with the general pattern of the temporal organization of torpor (Kirsch et al., 1991; Körtner and Geiser, 2000). However, in periods of extended unfavorable conditions (low T_a , food scarcity), torpor bouts may extend even for the activity phase (Grinevitch et al., 1995; Song et al., 1998). Hibernation, with its prolonged torpor bouts in winter, may be considered an extreme example of this extension. Thus, it is not surprising that food availability and thermal environment affected daily use of torpor. On the one hand, bats provided with food *ad libitum* in the low gradient – irrespective of season – consistently entered torpor during the day, aroused at dusk and remained normothermic for the rest of the night. On the other hand, fasted bats either were in torpor for a very short time as in the high gradient or spent almost the entire experiment in torpor, like in the low gradient.

The response to food deprivation, i.e., extension of torpor, was affected by thermal environment and season. In the low gradient fasting always led to an increase in time that bats were torpid (up to 95% of the experiment). In the high gradient, food-deprived bats either spent more time in torpor in winter or avoided it in summer and remained normothermic. Similarly, the South-African dormouse *Graphiurus murinus* more often entered torpor when non-fasted in the cold environment than when food deprived in higher T_a 's (Webb and Skinner, 1996).

Australian marsupial *Sminthopsis macroura* also fits this pattern of torpor use. In the cold, fasted animals entered torpor, but in the warm, they reduced resting metabolic rate within the range of normothermy (Song and Geiser, 1997).

4.2. T_a selection for winter torpor

We predicted that fasted bats in winter would choose T_a 's characteristic of their natural hibernation. Although in the low and high T_a gradients bats could select T_a 's close to 7 °C, they never did. In the high T_a gradient (7 °C to 43 °C), bats chose T_a 's close to their lower critical temperature ($T_{lc}=28.9$ °C; calculated using the equation of Speakman and Thomas, 2003). Also, in the low gradient (0 °C to 25 °C) bats selected T_a 's higher than 7 °C, but these T_a 's were significantly lower than T_a 's selected in the high gradient. One might suggest that differences in thermal preferences were forced by different range of available T_a 's. However, although the range of T_a 's in both gradients overlapped, bats never selected the same T_a 's and never chose the highest or the lowest T_a 's available in the gradient.

Our results differ from the results of previous experiments on several species of Palearctic insectivorous bats. Harmata (1969) reported that *M. myotis* placed in a thermal gradient system in winter entered torpor at temperatures close to 7 °C. Also brown long-eared bats (*Plecotus auritus*) chose temperatures significantly lower in autumn than in summer, which makes energy conservation and fat storage more efficient (Speakman and Rowland, 1999). The only insectivorous bats that chose torpor at relatively high T_a 's (16 °C to 22 °C) were South African *Miniopterus schreibersii* and *Rhinolophus capensis* (Brown and Bernard, 1994). However, these temperatures were within the range of T_a 's preferred for torpor in nature (Brown and Bernard, 1994). From this perspective, our experiments are the first where insectivorous bats placed in a thermal gradient prefer for torpor T_a 's much higher than observed in nature.

The range of T_a 's available in the environment offers an explanation of these, seemingly paradoxical results. In Harmata's experiments (1969), contrary to ours, the range of available T_a 's in the thermal gradient was almost the same as the range of T_a 's available in natural hibernacula of *M. myotis* — from 0 °C to 12 °C. Nevertheless, although thermal preferences of bats differed, both in Harmata's (1969) and in our experiments (in the high and low gradients) *M. myotis* consistently chose T_a 's that were lower by about 30% than the maximum T_a available in the gradient. Thus, if the gradient of available T_a 's would determine the T_a selection, this should be also true for natural conditions. Indeed, in Poland, over 15,000 of the *M. myotis* hibernate every year in the fortifications of Międzyrzecz Fortified Front (Western Poland; 52°24'N, 15°31'E) and the average T_a where these bats are found is lower by about 30% than the maximum T_a available inside this hibernacula (Kokurewicz and Wojciechowski, unpublished; Fig. 9).

Based on the above, we argue that both under laboratory and natural conditions maximum T_a available in the environment affects thermal preferences of *M. myotis*. If so, then bats do not choose for hibernation a fixed, species specific T_a (for *M. myotis*: ~7 °C), which was previously postulated by laboratory and field

studies (Harmata, 1969; Nagel and Nagel, 1991; Kokurewicz, 2004). It seems that bats' thermal preference is rather a dynamic response to the range of T_a 's available in the environment (hibernacula). Thus, *M. myotis* would prefer places where T_a is lower by about 30% than the highest T_a available in the roosting environment. This may suggest that bats choose environments (e.g. in terms of T_a and humidity) that would guarantee the best conditions for hibernation and winter survival.

4.3. Energetic efficiency of torpor

In the present experiments, T_a 's selected for torpor were higher than expected and these, in turn, resulted in relatively high T_b 's in torpor. The lowest recorded T_{b-min} 's of *M. myotis* were always higher than 7 °C to 8 °C, a T_b which would be expected for bats hibernating in nature. High T_b significantly shortens torpor bouts (French, 1982; Geiser and Kenagy, 1988) which may result in smaller energy savings. Although, potentially less advantageous, torpor at relatively high T_a 's has been observed in several mammalian species. Hamsters, *Phodopus sungorus* and dormice, *Glis glis* entered torpor even at T_a 's within thermoneutrality (Ruf and Heldmaier, 1992; Heldmaier and Elvert, 2004). Also heterothermic marsupials [*Cercartetus nanus* (Song et al., 2000) and *Sminthopsis macroura* (Song et al., 1998)], and rodents [*Perognathus longimembris* (French, 1976) and *Mesocricetus auratus* (Gumma and South, 1970)] preferred in the laboratory high T_a 's for torpor. Song et al. (1998, 2000) suggested that high T_a 's and thus high T_b 's ensure energy conservation during short torpor bouts since they reduce costs of arousal. Short torpor, in turn, may be favored because of the disadvantages of long-lasting hypothermia and benefits resulting from being normothermic (French, 1976; Song et al., 1998; Humphries et al., 2003b).

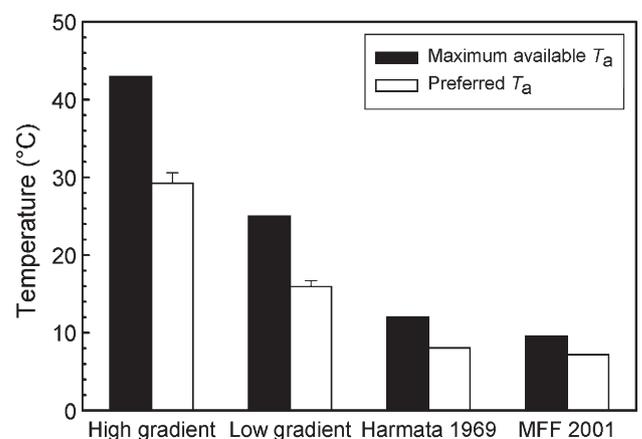


Fig. 9. Maximum ambient temperatures available in the environment (black bars) and ambient temperatures which *Myotis myotis* selected for torpor in winter (white bars). "High gradient" and "low gradient" indicate data collected in the present study (preferred T_a 's are summarized data for fed and fasted bats). "Harmata, 1969" indicates data collected by Harmata (1969) in his study using the thermal gradient where T_a 's ranged from 0 to 12 °C. "MFF 2001" indicates data collected by Kokurewicz and Wojciechowski (unpublished) in Międzyrzecz Fortified Front in February 2001; T_a 's in this hibernaculum ranged between 0 °C and 9.6 °C.

It seems that the reduction of the difference between T_b and T_a , rather than the T_b itself, tells more about the energetic efficiency of torpor. Small $T_b - T_a$ difference coupled with even slight reductions in T_b may result in significant energy savings both during normothermy and torpor (Studier, 1981; Webb et al., 1993). In the present experiments, bats did not enter deep and prolonged torpor; however, they achieved significant reduction of $T_b - T_a$ difference either by entering short torpor or by selecting proper T_a 's. Fasted bats selected T_a 's higher than expected and even remained normothermic for the entire experiment in the high gradient in summer, but they always maintained $T_b - T_a$ difference equal to or lower than 2 °C (Fig. 5).

Respirometry gave support for the idea that even shallow torpor at high T_a may result in significant energy savings when coupled with selection of proper T_a . Although in both gradients fasted bats in winter selected higher T_a 's for torpor than non-fasted ones, our calculations suggest that they spent half as much energy as fed individuals. Thus, in both gradients, fasted animals selected T_a 's at which total costs of torpor were minimized and the energy consuming phases of torpor – entry and arousal – were shortened. Although fasted bats in both T_a gradients remained in torpor for a considerable time, torpor was always shorter than 24 h and bats aroused at least once a day, even for less than 1 h. Thus we suggest that selection of higher T_a 's for torpor was related to intended arousal with the beginning of the activity phase. If the intended torpor bout would exceed 24 h, only selection of low T_a 's could provide energy savings.

4.4. Concluding remarks

The results of present experiments provoked us to ask a question about the evolution of heterothermic states in insectivorous bats. Based on the results of present experiments and data from the literature, we attempt to attribute the ability to enter daily and seasonal torpor to the predictability of seasonal changes in environmental conditions and to the unpredictability of food resources, in summer and in winter.

Evolution of the patterns of heterothermy and differences in the animals' basal metabolic rates (BMR), have been associated with the zoogeographical origin of species (Lovegrove, 2000, 2003). Seasonal heterothermy, i.e. hibernation, evolved in predictable and seasonal environments (e.g. in the Holarctic zone). In unpredictable environments (Australasian and Afrotropical zones), where inter-seasonal changes in weather and in resource availability are low, but inter-annual variability is high, the predominating strategy is daily heterothermy, i.e. daily torpor. In the evolution of heterothermy, low BMR is regarded as promoting daily torpor (Lovegrove, 2000). The BMR of insectivorous bats from the North Temperate Zone (considered a highly predictable one) is lower than predicted by Kleiber's allometric relationship for mammals (McNab, 1982; Speakman and Thomas, 2003), indicating evolution of daily rather than hibernation torpor. However, these bats, like *M. myotis*, also hibernate.

Thus, we propose that daily torpor is a primary adaptation of Palearctic insectivorous bats to stochasticity in food availability

resulting from unexpected cold in summer and warmer weather in winter. In this comparison, we consider hibernation to be a secondary adaptation to predictable, seasonal changes in weather conditions. As laboratory and field data suggest, hibernation can be arrested when outside temperature increases, indicating food availability. Insectivorous bats could benefit from being active and foraging during the periods of increased food availability on the one hand, and from energy savings due to daily torpor on the other. Such plasticity in the thermoregulatory reactions and behavior would ensure survival in a changing environment. Taking into account general change of climate, namely gradual increase of winter temperatures that affect geographical distribution of hibernating bats and also significantly affect their winter energy budgets (Humphries et al., 2002), such an evolutionary origin of hibernation as a facultative response to climatic conditions would be of vital importance for these mammals.

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