Timing of the daily temperature cycle affects the critical arousal temperature and energy expenditure of lesser long-eared bats

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SUMMARY

Daily patterns of body temperature (T_b) and energy expenditure in heterothermic endotherms are affected by changes in ambient temperature (T_a) and selection of suitable microclimates, yet most laboratory studies employ constant T_a to measure metabolic rates. In particular, exposure to a daily temperature cycle, even within rest shelters, may be important in timing of torpor and arousal and determining resting energy costs in wild animals. We tested how captive bats (*Nyctophilus geoffroyi*; 7g) exposed to a diurnal T_a fluctuation (between 13°C and 27°C), similar to natural conditions in their summer tree roosts, adjusted the timing of daily arousals. To distinguish the effects of T_a and passive rewarming from time of the day, we shifted the heating phase to commence at 06:00 h, 09:00 h or 12:00 h on each day. Bats entered torpor overnight and aroused the next day at a time corresponding to rising T_a and passive rewarming. The critical T_a (and torpid T_b) for arousal was not fixed, however, but was lower when heating occurred later in the rest phase, providing the first evidence that the critical arousal T_a is affected by time of the day. Bats re-entered torpor in response to cooling late in the afternoon, yet always aroused at lights off. A period of normothermic thermoregulation was therefore closely synchronised with maximum daily T_a , indicating a trade-off between the benefits and energetic costs of normothermia during resting. Our experiment clearly shows that a daily T_a cycle affects the thermoregulatory behaviour and energetics of these small bats. More generally, these results demonstrate the critical influence of behavioural decisions on the daily energy expenditure of small heterothermic mammals.

Key words: arousal, bat, daily energy expenditure, passive rewarming, temperature, torpor.

INTRODUCTION

Many small mammals regularly employ short bouts of torpor to partially offset their high rate of thermoregulatory energy expenditure (Geiser and Ruf, 1995). During torpor, metabolic rate (MR) is greatly reduced because of the suspension of thermoregulatory heat production at an ambient temperature (T_a) above the critical minimum in torpor, lowered body temperature $(T_{\rm b})$ and, at least in some heterothermic mammals, biochemical depression of metabolic activity (Hock, 1951; Song et al., 1997; Storey and Storey, 1990; Heldmaier and Ruf, 1992; Geiser, 2004). Although rewarming of T_b during arousal requires an enormous increase in MR, even very brief torpor bouts can provide energy savings to small animals (Tucker, 1962; Hiebert, 1990; Cryan and Wolf, 2003). The major drawbacks of using torpor appear to be the possible negative effect of low $T_{\rm b}$ and depressed MR on biochemical and physiological processes, cognition and behaviours that are likely to be beneficial during normothermia (van Breukelen and Martin, 2002a; Carey et al., 2003). The use and daily timing of torpor should therefore reflect a trade-off between the benefits of elevated $T_{\rm b}$ and MR during normothermia and the associated energy costs of thermoregulatory heat production.

Thermal energetics typically are studied in the laboratory at constant T_a to derive species-specific values of basal, resting and torpid MR. In wild animals, however, behavioural decisions will greatly affect thermoregulatory energy costs during resting. For example, small animals can select among a wide range of thermal microclimates available in terrestrial environments (Wolf and Walsberg, 1996; Kerth et al., 2001; Willis and Brigham, 2005). Moreover, even small differences in use and timing of torpor *versus*

normothermia in response to thermal conditions within shelters will have a large impact on resting energy expenditure (Willis et al., 2004). Season, body fat reserves, food availability and ambient temperature (T_a) are all known to influence an animal's propensity to use torpor (Geiser, 2004). The circadian timing of torpor entry and arousal is also well known (Willis, 1982; Körtner and Geiser, 2000), but the application of these studies to wild populations is often limited by the use of a constant T_a generally employed in the laboratory. Whereas, many animals experience a daily T_a cycle in their resting shelter and this appears to be an important cue for the timing of torpor and arousal (Körtner and Geiser, 2000; Mzilikazi et al., 2002; Turbill et al., 2003a). In general, small nocturnal mammals show a high propensity for torpor in the early morning, when daily $T_{\rm a}$ are minimal, and arouse at around midday or in the early afternoon, seemingly in response to a rising $T_{\rm a}$ in their shelter and some passive rewarming of torpid $T_{\rm b}$ (Davis and Reite, 1967; Schmid, 1996; Körtner and Geiser, 2000; Geiser et al., 2004; Körtner et al., 2008). Captive animals typically arouse several hours before their nocturnal active phase even under constant T_a and it is suggested that this timing may reflect an inherent propensity for arousal coinciding with rising T_a and passive rewarming in the wild (Körtner and Geiser, 2000). Nevertheless, no previous studies have clearly separated the effects of a daily T_a cycle from the influence of endogenous circadian cues and the photoperiod on timing of torpor and arousal patterns.

The present study aims to quantify how a temporal shift in an identical daily T_a cycle affects T_b cycles and thermal energetics of male lesser long-eared bats (*Nyctophilus geoffroyi* Leach 1982). In the wild, male *N. geoffroyi* typically roost solitarily under exfoliated

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bark of trees and torpor patterns closely reflect the external daily $T_{\rm a}$ cycle (Turbill et al., 2003a). However, when these bats occasionally use well-insulated tree roosts, in which daily T_a cycles are reduced in amplitude and delayed, arousals occur later in the day and after less passive rewarming. These observations suggest that the timing and extent of diurnal heating interact in triggering arousals. To distinguish the effects of T_a and time of day on the temporal organisation of heterothermy, we exposed captive bats to a diurnal $T_{\rm a}$ profile that matched natural roost conditions, but shifted the timing of the daily heating and cooling phases. We tested the hypothesis that small heterothermic bats arouse from torpor at a critical threshold of rising T_a and passive rewarming of torpid T_b , reflecting a trade-off between their high thermoregulatory costs and the requirements for physiological processes only possible at normothermic T_b and MR. If so, the timing of torpor and normothermic periods will be independent of time of the day and instead closely reflect the timing of the daily T_a cycle. Alternatively, there may be an interaction between the effects of rising $T_{\rm a}$ and time of the day on the critical T_a for arousal, as suggested by field data. Furthermore, we aimed to quantify the energetic savings gained by timing arousals and normothermic periods to coincide with passive rewarming and maximum daily T_a while day-roosting.

MATERIALS AND METHODS Experimental procedure

We used temperature telemetry and open-flow respirometry to measure the thermoregulatory response and energy expenditure of 13 adult male lesser long-eared bats, Nyctophilus geoffroyi (mass at capture: 7.13±0.7 g) during exposure to a diurnal fluctuation of $T_{\rm a}$ approximately matching that experienced in their natural tree roosts during the warm season. Bats were captured using mist nets and harp traps at Imbota Nature Reserve (30 deg.35' S151 deg.44' E, 1000 m a.s.l.) on the Northern Tablelands of New South Wales, Australia, between 1st March and 13th April (austral autumn) in 2003 and 2004. Captured bats were immediately transferred to the University of New England (~10km away) where metabolic measurements commenced on the night of capture or occasionally on the following afternoon. Measurements for each bat continued over the next 4 days, after which bats were released at the capture site. Each day, bats were removed from respirometry chambers for approximately 1 h after lights-off and following arousal from torpor. During this time, they were weighed, provided with water and hand fed 1.0±0.3 g of mealworms, and weighed again before being returned to their respirometry chamber. Bats remained within 0.5 g of capture body mass while in captivity.

Each bat was exposed to each of three daily T_a patterns, which were identical in profile but temporally shifted so that the heating phase commenced at 06:00 h, 09:00 h or 12:00 h (Fig. 1). Over the first 3 days, the sequence of time of heating was chosen randomly. To test for possible effects of time in captivity, the timing of the T_a profile experienced by each bat on day 1 was repeated on day 4. We found no significant effect of time in captivity on the thermoregulatory response of bats (paired *t*-tests: P>0.05). Bats were exposed to a dim incandescent light from 06:00 h to 18:15 h to mimic the natural photoperiod at that time of the year.

Ambient temperature profile

Respirometry chambers were located in a temperature-controlled cabinet in which a computer program regulated T_a to within 0.5°C of the T_a profile using a 100 W ceramic heating element (Elstein 10T; Northeim, Germany) and the cabinet's built-in cooling element. A fan circulated the air within the cabinet. The bats were not exposed

to radiant heat. The amplitude, rate of heating and cooling, and timing of the T_a profile approximately matched natural variation in diurnal roost T_a experienced by wild bats during the warm season (Turbill et al., 2003a). A minimum T_a of 12.8±0.9°C (±s.d.) was maintained throughout the night and heating commenced the following day at 06:00 h, 09:00 h or 12:00 h (Fig. 1) and lasted for 4 h 40 min at a rate of 0.05°C min⁻¹ until T_a reached a maximum 26.6±0.8°C. A plateau of maximum T_a was maintained for 2 h, before cooling commenced at an identical rate until the minimum T_a was reached or, when heating had commenced at 12:00 h, the end of measurements for that day (i.e. following lights-off and arousal).

Temperature telemetry

Prior to metabolic measurements, a temperature-sensitive radiotransmitter (Titley Electronics, Ballina, Australia; model LT1, 0.45 g) was attached to each bat to measure its skin temperature $(T_{\rm skin})$. For small bats, $T_{\rm skin}$ is closely related to $T_{\rm b}$, particularly during torpor when T_b-T_a differentials are usually 1–2°C (Audet and Thomas, 1996; Barclay et al., 1996; Willis and Brigham, 2003). Each transmitter was calibrated against a precision mercury thermometer (±0.1°C) in a water bath prior to use and attached, after removing a small patch of hair, to the mid-dorsal skin of the bat using a rubber-based adhesive (Skinbond; Smith and Nephew, Mt Waverley, Victoria, Australia). Transmitters were removed from bats at the end of the 4-day measurement period using an alcohol based removal agent (Universal Adhesive Remover; Smith and Nephew). The T_{skin} of bats in respirometry chambers was measured (via inter-pulse interval) every 3 or 4 min using an FM receiver (Yaesu, F-9600; Cypress, CA, USA) connected via an A/D converter to a computer or a datalogger (for details, see Körtner et al., 1998).

Metabolic measurements

Bats were weighed (±0.1 g) immediately prior to measurements and, on subsequent days, immediately after they were removed from respirometry chambers in the early evening (before feeding) and again prior to being re-introduced into the chambers. A linear rate of mass loss was assumed over each day to calculate mass-specific MR values. Respirometry chambers were made from cylindrical, clear Perspex tubes (volume: 0.1401) lined internally with plastic mesh and hung vertically inside the temperature-controlled cabinet. Air flow (75-300 ml min⁻¹) was controlled with rotameters and measured using mass flowmeters (Omega FMA-5606; Stamford, CT, USA). A lower flow rate (75–100 ml min⁻¹) allowed greater accuracy of measurements while bats were in torpor. T_a inside the chambers was measured (±0.1°C) by a thermocouple inserted 5 mm into the chamber. Flow rate and T_a were digitized using a 14-bit A/D converter card and captured using a datalogger (Datataker DT 100F, Data Electronics) before being recorded by computer software, which was written by G.K., B. Lovegrove and T. Ruf.

The percentage oxygen differentials of a sub-sample (flow rate: 50 ml min^{-1}) of air from the respirometry chambers and a reference channel (outside air) were measured using either an Ametek Applied Electrochemistry S-3A/II analyser (Naperville, IL, USA; in 2003) or a Sable Systems FC-1B analyser (Sable Systems International, Las Vegas, NV, USA; in 2004;). Measurements did not differ between analyser systems under similar thermal conditions (*t*-tests, P>0.05). The Ametek S-3A/II was a dual system that enabled measurement of two bats in parallel every 3 min, interrupted by switching to a reference channel every 12 min. The set-up using the Sable Systems analyser switched in series every 3 min between two bats and a reference channel, providing a measurement per bat every 9 min. Switching between channels was controlled using solenoid

values. The outputs from the Ametek analyser, after conversion *via* a 14-bit A/D card, and the digital output from the Sable Systems analyser, were recorded using data-acquisition software onto a personal computer. Rates of oxygen consumption were calculated using STPD volumes from equation 3a of Withers (Withers, 1977) and a respiratory quotient (RQ) of 0.85 was assumed throughout. All equipment was calibrated prior to use.

Data analysis

Torpor entry was defined as the pronounced decline in MR below the mean basal MR minus 1 s.d. published for N. geoffroyi (Geiser and Brigham, 2000). Periods of passive rewarming were characterised by a slow increase in T_{skin} , in parallel to T_a , that were accompanied by a gradual increase in average MR. Arousals were clearly defined by a rapid increase in MR to a maximum peak (overshoot) usually followed by a decrease to resting values, and a concurrent rise in $T_{\rm skin}$ to normothermic levels. Arousal was assumed to last until the last measurement prior to MR having decreased to less than 75% of maximum peak MR or, for occasional cases where peak MR was followed by sustained high values owing to activity, to the last measurement of MR that occurred after T_{skin} reached 30°C. MR was averaged over this period and multiplied by the duration of arousal to calculate total energy expenditure for each arousal. Energy expenditure (kJ) was calculated from oxygen consumption (mlO₂ g^{-1}) using a conversion factor of 20.083 (Schmidt-Nielsen, 1997).

Average mass-specific MR of resting normothermic bats was calculated over >30 min at minimum and maximum T_a . During passive rewarming of torpid bats, average MR was calculated over the duration of 2°C intervals in heating of T_a (also >30 min). Rest phase energy expenditure was calculated by integrating measurements between the times of lights on and off, or until bats had aroused from torpor, which sometimes occurred shortly after lights off. This was considered to be a realistic definition because it is necessary for bats to regain normothermy prior to their normal active phase and emergence from the roost.

Statistical tests were conducted using Minitab Statistical Software V13.1. Null hypotheses were rejected at P<0.05. Values are presented as means \pm 1 s.d. Repeated measures ANOVA (RM ANOVA) was used to compare response variables among treatment days (commencement of heating at 06:00 h, 09:00 h or 12:00 h) within individual bats. To avoid pseudo-replication, mean values were calculated from number of individuals (*n*) rather than observations (*N*). General linear modelling (GLM) was used to analyse the relationship between dependent and independent variables. Regression coefficients and r^2 values were derived from the fitted model for groups that differed significantly.

RESULTS

Bats exposed to the experimental T_a fluctuation exhibited a similar temporal pattern of torpor as observed in the wild (Fig. 1). After being placed into respirometry chambers, bats entered torpor at 23:38±2:18 h; however, on 70% of measurement days, bats aroused briefly during the night, before re-entering torpor before or close to the time of lights on (and natural sunrise). The following morning, commencing at 06:00 h, 09:00 h or 12:00 h, T_a increased from an overnight minimum of 12.8±0.9°C to a maximum of 26.6±0.8°C. Bats aroused from torpor following an increase in T_a on 100% of measurement days when heating commenced at 09:00 h or 12:00 h (Fig. 1, Fig. 2B,C). When heating commenced at 06:00 h, however, bats remained in torpor on 4 out of 15 (27%) measurement days, despite passive rewarming of torpid T_{skin} up to 29°C (Fig. 2A). Time of arousal differed significantly among treatment days



Fig. 1. Representative examples of skin temperature (T_{skin} ; filled circles and dotted line) and metabolic rate (MR; solid line) of captive bats exposed to a diurnal fluctuation in ambient temperature (T_a ; dashed line) with heating commencing (shown by arrow) at (A) 06:00 h, (B) 09:00 h or (C) 12:00 h. The photoperiod spanned from 06:00 h to 18:15 h.

($F_{2,26}=169$, P<0.001), and was affected by the shift in time of increasing T_a and passive rewarming of T_b . However, time of arousal did not precisely reflect the temporal shift in T_a profile, but on days when heating commenced earlier, arousals occurred later than expected, and therefore at higher T_a and after greater passive rewarming of torpid T_b (Fig. 3; RM ANOVA: $F_{2,27}=15.5$, P<0.001; Tukey's test: all pairs, P<0.05). Thus, on days when heating commenced at 06:00 h, 09:00 h and 12:00 h, bats actively aroused at an average time/ T_a of $10:35\pm0:58$ h/ $25.1\pm2.3^{\circ}$ C (T_{skin} : $24.6\pm2.8^{\circ}$ C), $12:47\pm1:03$ h/ $22.9\pm2.6^{\circ}$ C (T_{skin} : $21.4\pm2.8^{\circ}$ C) and $15:36\pm1:18$ h/ $21.8\pm2.8^{\circ}$ C (T_{skin} : $20.1\pm2.9^{\circ}$ C), respectively.

A period of normothermia coincided with the plateau of maximum T_a . On days when heating commenced at 12:00 h, T_a remained at the daily maximum and bats remained normothermic until lights off (Fig. 1, Fig. 2C). On days when heating commenced at 06:00 or 09:00 h, bats re-entered torpor in response to subsequent cooling of T_a , even late in the afternoon, before always arousing again close to the time of lights off (Fig. 1, Fig. 3A,B). Bats re-entered torpor during cooling at a T_a of 24.9±2.8°C and 22.8±2.7°C on days when heating commenced at 06:00 h and 09:00 h, respectively, which did not differ significantly (RM ANOVA: $F_{1,13}$ =2.2, P=0.16).

Normothermic periods lasted longer on days when heating commenced at 09:00 h (3:42±1:26 h), owing to the lower T_a at arousal and torpor re-entry, than on days when heating had commenced at 06:00 (2:01±1:33 h) or 12:00 h (2:50±1:08 h; repeated ANOVA: $F_{2,32}$ =6.1, P<0.01; Tukey's test: 09:00 vs 06:00 h or 12:00 h,

P < 0.05). The duration of normothermic periods was cut short on days when heating had commenced at 12:00 h by the time of lights-off and beginning of the active phase.

During passive rewarming of bats in torpor, mass-specific MR increased exponentially from an average of $0.06\pm0.02 \text{ ml} O_2 \text{ g}^{-1} \text{ h}^{-1}$ at T_a of $10-12^{\circ}\text{C}$ (T_{skin} $11-13^{\circ}\text{C}$) to $0.53\pm0.12 \text{ ml} O_2 \text{ g}^{-1} \text{ h}^{-1}$ at T_a of $26-28^{\circ}\text{C}$ (T_{skin} $26-29^{\circ}\text{C}$) (Fig. 4) [Torpid MR ($\text{ml} O_2 \text{ g}^{-1} \text{ h}^{-1}$)= 0.015×1.141^{T_a} (r^2 =0.78, P<0.001)]. During periods of normothermy, average mass-specific resting MR was $7.0\pm0.7 \text{ ml} O_2 \text{ g}^{-1} \text{ h}^{-1}$ at a minimum T_a of $13.7\pm0.7^{\circ}\text{C}$ and was reduced to $3.0\pm0.6 \text{ ml} O_2 \text{ g}^{-1} \text{ h}^{-1}$ at a maximum T_a of $26.5\pm0.7^{\circ}\text{C}$.

The cost of arousal by *N. geoffroyi* at a constant T_a of 12.8±0.9°C (i.e. at time of lights off on days of heating from 06:00 h or during

the night) was 0.50 ± 0.07 kJ (*N*=45, *n*=13). Following heating and passive rewarming of T_b , arousal costs declined linearly depending on the increase in T_a (and torpid T_b) prior to arousal (Fig. 5) [average cost of active arousal (kJ)= $0.84-0.026 \times T_a$; P<0.001]. Partial passive rewarming under the conditions of heating experienced reduced the cost of active arousal, on average, by 55% to 0.23 ± 0.09 kJ. Including the additional cost of an increase in torpid MR during the period of passive rewarming, the total arousal cost was reduced, on average, by 46% to 0.27 ± 0.03 kJ (Fig. 5) [average total cost of arousal (kJ)= $0.78-0.02 \times T_a$; P<0.001].



Fig. 2. Histograms showing times of arousal (filled vertical bars) and times of re-entry into torpor (open vertical bars) by bats over their rest phase during exposure to a diurnal fluctuation in ambient temperature (T_a ; shown at the top) that commenced heating at (A) 06:00 h, (B) 09:00 h or (C) 12:00 h. Bats were placed into respirometry chambers in the evening (T_a : 13°C) and all had entered torpor during the night or around the time of lights on. All bats aroused in response to passive rewarming when heating commenced at 09:00 h or 12:00 h. When heating commenced at 06:00 h, bats (n=3) remained in torpor throughout on 4 of 15 (27%) days. The horizontal black and white bars above indicate the photophase.



Fig. 3. Ambient temperature (T_a) at the time of active arousal by bats receiving passive rewarming from exposure to a daily fluctuation in T_a when heating commenced at 06:00 h, 09:00 h or 12:00 h (individuals: filled circles and connecting lines; averages: open circles). Bats aroused at lower T_a and therefore after less passive rewarming when heating had commenced later in the day (repeated ANOVA: $F_{2,22}$ =14.4, P<0.001; Tukey's test: all P<0.05).



Fig. 4. Average torpid metabolic rate (TMR; ±1 s.d.) of bats during passive rewarming from heating of T_a (±1°C). Passive rewarming resulted in an exponential increase in average TMR [(in ml O₂ g⁻¹ h⁻¹)=0.015×1.141^{T_a} (°C); r^2 =0.78). Note that at T_a of 29.1°C (lower set-point of thermal neutral zone) TMR during passive rewarming extrapolated to ~50% of BMR measured at same T_a (Geiser and Brigham, 2000).



Fig. 5. Energetic costs of active arousal from torpor as a function of ambient temperature (T_a) for arousals from minimum T_a without passive heating (triangles) and for arousals after diurnal passive heating (filled circles). Passive heating provided a linear reduction in the average cost of active arousal [solid line: energy expenditure for active arousal (kJ)=0.84–0.026× T_a (°C); r^2 =0.87, P<0.001]. During torpor, passive heating resulted in a net increase in energy expenditure for arousal [open circles; dashed line: total energy expenditure for arousal [kJ)=0.78–0.02 T_a (°C); r^2 =0.82, P<0.001].

Energy expenditure of bats over the entire rest phase (12h) increased linearly depending on the time spent normothermic (Fig. 6), ranging from a minimum of 0.59kJ for a bat remaining torpid throughout the day to a maximum of 3.60 kJ for a bat that was normothermic for 5:06h (mean: 1.98±0.84kJ). The slope of the relationship between rest phase energy expenditure and time spent normothermic did not differ significantly among the different thermal regimes (GLM: slope, F_{2,41}=2.4, P=0.1). However, the amount of energy expended for a given time period spent normothermic was significantly greater for bats on days when heating commenced at 06:00 h and 09:00 h in comparison to 12:00 h (GLM: y-intercept, F_{1,43}=287.1, P<0.001; Tukey's test: 12:00 h vs 06:00 h or 09:00 h, P<0.001). The additional energy expenditure of bats on days when heating commenced at 06:00h and 09:00h resulted from the need for a second, completely active arousal near lights off prior to the active phase. The average cost of the second arousal near lights off was significantly greater for bats on days when heating commenced at 06:00 h (0.46±0.09 kJ) than at 09:00 h (0.31±0.12kJ; RM ANOVA: F_{1,15}=35.2, P<0.001) because of the greater cooling of T_a and T_b of torpid bats prior to active arousal on these days.

DISCUSSION

Our study shows that the thermoregulatory behaviour of male lesser long-eared bats is closely related to short-term changes in thermal conditions. As in the field, temporal patterns of heterothermy depended on the extent and timing of the daily T_a cycle (Turbill et al., 2003a). Captive bats were torpid in the morning coinciding with daily T_a minima and aroused after reaching a threshold level of passive rewarming from rising T_a during the day. Importantly, this critical arousal T_a is not a fixed parameter, but depends on the time



Fig. 6. Energy consumption over the rest phase (12 h) of bats exposed to a diurnal fluctuation in T_a when heating commenced at 06:00 h (open circles), 09:00 h (triangles) or 12:00 h (filled circles) as a function of time spent normothermic (0 h represents bats that remained in torpor). The time of commencement of heating had no significant effect on the slope of the relationship between energy consumption and time normothermic [general linear modelling (GLM) slope: $F_{2,41}=2.4$, P=0.10], however, energy expenditure was greater overall on days when heating commenced at 06:00 h or 09:00 h, in comparison to 12:00 h [GLM, y-intercept: $F_{1,43}=287$, P<0.001; fitted line for combined 06:00 h and 09:00 h: rest phase energy expenditure (kJ)=0.734.0+0.513×time normothermic (h); for 12:00 h: energy expenditure (kJ)=0.261+0.513×time normothermic (h); $r^2=0.90$].

of day. Bats synchronised a period of normothermy with the time of the daily plateau of T_a maxima and re-entered torpor in response to cooling of T_a in the afternoon. Selection of poorly insulated rest sites is not uncommon among bats and appears particularly common in solitary roosting males (Kurta and Kunz, 1988; Kurta et al., 1989; Bronner et al., 1999; Chruszcz and Barclay, 2002; Kunz and Lumsden, 2003; Lausen and Barclay, 2003; Turbill et al., 2003b; Turbill, 2006a; Turbill, 2006b). A number of heterothermic mammals also prefer rest sites that receive direct solar radiation, which increases the amplitude of heating of shelter $T_{\rm a}$ during the day (Vaughan and O'Shea, 1976; Humphrey et al., 1977; Hosken, 1996; Kerth et al., 2001; Geiser et al., 2002; Mzilikazi et al., 2002; Mzilikazi and Lovegrove, 2004). By co-ordinating torpor and normothermy with the daily T_a cycle, small species like N. geoffroyi can exploit the high T_a provided in thermally unstable roosts to minimise the energetic cost of arousal and a period of normothermy during the rest phase.

The energy expenditure of torpid bats remained a small fraction of normothermic costs even during exposure to a wide daily fluctuation in T_a . Metabolic rate of torpid bats at the maximum T_a of 27°C, when T_{skin} was passively rewarmed to around 28°C, remained only 15% of normothermic values at the same T_a and 39% of BMR (Geiser and Brigham, 2000). Moreover, if torpid MR is extrapolated to T_a of 29.1°C, the lower limit of the thermal neutral zone (TNZ) in *N. geoffroyi*, the predicted torpid MR remains only ~50% of BMR (Fig. 4). Our experimental design, where torpid animals were slowly warmed to near their TNZ, provides unique support for temperature-independent mechanisms of metabolic depression in small hibernators (Geiser, 1988; Song et al., 1997; Buck and Barnes, 2000; Geiser and Brigham, 2000). Superficially, it could appear that the reduced T_b-T_a differential during torpor (~2°C) compared with during normothermia (~5°C) actually caused the much lower torpid metabolic rate (TMR) relative to BMR at a T_a in the TNZ. However, the lower T_b-T_a differential is clearly a result, not the cause, of the lower TMR (Geiser, 2004). Because the thermal conductance of torpid *N. geoffroyi* at a T_a near the TNZ is similar to that during normothermia (Geiser and Brigham, 2000), the ~5°C lower T_b of torpid bats reflects their reduced heat production at a lower TMR, not the other way around. This is an important point because it shows, firstly, that the energy savings to costs of arousal from passive rewarming are even greater than they would be if TMR was solely a function of temperature, and, secondly, that the energy savings if the bats remain in torpor are relatively little affected by the wide daily T_a variations in their poorly insulated tree roosts.

Although torpid MR remained much below BMR during heating, the exponential relationship with T_a resulted in a greater rate of increase in MR as T_a approached the TNZ. Expressed as a proportion of BMR, TMR increased by 6.6% over 5°C with warming of T_a from 15°C to 20°C, but increased by 12.8% with warming of T_a from 20°C to 25°C. Active arousals were more common after heating of T_a above ~20°C, suggesting that increased MR may play a role in stimulating arousals in response to passive rewarming (Schmid, 1996). This T_b threshold may also reflect a transition to greater levels of translation and protein synthesis (van Breukelen and Martin, 2001) and activity of the brain and central nervous system during torpor (Carey et al., 2003), which could lead to a greater propensity for active arousal.

Laboratory studies under constant T_a have found that whereas times of entry into torpor are variable and occur earlier in the active phase under conditions of energetic stress, times of arousal appear largely fixed according to an endogenous circadian rhythm (Tucker, 1962; Brown and Bartholomew, 1969; Geiser, 1986). By contrast, in wild animals, times of arousal frequently coincide with an initial period of passive rewarming during the day (Geiser et al., 2004), suggesting that these arousals are triggered by a threshold level of exogenous rewarming (Schmid, 1996; Lovegrove et al., 1999; Körtner and Geiser, 2000). Our experiment has shown, by manipulating the timing of diurnal heating relative to the photophase, that arousals in a small bat are not fixed, but triggered to occur during the day according to the timing of passive rewarming from rising T_a , in addition to the strong arousal cue from the photoperiod.

The interaction between T_a and time of the day as a cue for arousal can be represented in a simple model (Fig. 7). This model suggests that arousals are triggered if and when T_a reaches a curvilinear threshold. Furthermore, the model suggests that at T_a below the lower asymptote of the arousal threshold, such as when roosting in cool caves, bats should remain in torpor until dusk. This is the daily pattern observed in captive N. geoffroyi under constant cool temperatures (Geiser and Brigham, 2000). Remaining in torpor early in the morning provides maximum energy savings when daily $T_{\rm a}$ is typically minimal. The progressive sensitivity of N. geoffroyi to a thermal arousal cue over the course of their rest phase somewhat resembles that found in hibernating ground squirrels over multiday bouts of torpor (Bechman and Stanton, 1978). Similarly, it may indicate an increase in the sensitivity of the bat's central nervous system over the course of the torpor bout, perhaps reflecting an expectation of the strong circadian cue for arousal at dusk. Alternatively, from a behavioural perspective, as the time available for a normothermic period during the rest phase diminishes, N. *geoffroyi* may arouse at lower T_a despite the higher cost for arousal and normothermy. Surprisingly, the increasing predisposition for



Fig. 7. An approximate model of the change in the critical threshold T_a that triggers arousal (thermal cue for arousal; thick dashed line) over the rest phase (daytime) in male *N. geoffroyi*. Torpor is entered before or near dawn. Arousal and active rewarming of body temperature (solid line) from torpor is predicted to occur if and when T_a (thin dotted line) reaches the thermal cue for arousal. Bats re-enter torpor in response to cooling of T_a . Torpid bats arouse near dusk.

arousal did not reflect an inclination to remain normothermic later in the rest phase, as *N. geoffroyi* always re-entered torpor in response to a decrease in T_a , despite the need for a second arousal shortly after at dusk. Hence, the timing of normothermic periods was finely tuned to short-term fluctuations in T_a that affected thermoregulatory costs during the rest phase.

The fact that bats aroused well before the beginning of their active phase, whereas, to maximise their energy savings, they could have remained torpid, indicates that some period of normothermia is advantageous prior to the active phase. Their behaviour suggests that, above a threshold T_a , the benefits of normothermia outweigh the thermoregulatory energy cost. Bats were usually motionless in the respirometry chambers during midday bouts of normothermia, possibly indicating these periods were important for physiological rather than behavioural reasons. For wild bats, the risk of predation while roosting under tree bark is likely greatest in the early morning when large diurnal birds that search under bark for arthropods are most active, but bats show a strong tendency for torpor at this time. The short duration of many normothermic bouts and their close synchrony with maximal T_a in captive and wild bats also suggests that alertness to predators is not a primary reason for arousing. The most parsimonious explanation for the apparent preference for normothermia during resting in long-eared bats is to facilitate the numerous biochemical and physiological processes that are retarded by a low $T_{\rm b}$ and MR during torpor bouts. For example, bats may arouse to allow for restorative sleep processes, protein synthesis or even the digestion of food captured the previous night (Storey and Storey, 1990; Daan et al., 1991; van Breukelen and Martin, 2002b).

By co-ordinating torpor, arousal and normothermy with shortterm changes in T_{a} , small bats appear to gain an energetic advantage from selecting roosts containing a wide daily T_{a} cycle. Energy costs at low T_{a} in the early morning are avoided by using torpor, whereas passive rewarming from diurnal heating provides largely reduced energetic cost of rewarming from torpor. However, the energy savings gained from passive rewarming are not as significant for small heterotherms, such as *N. geoffroyi*, as they are for larger species (Lovegrove et al., 1999). Much greater energy savings are gained from the reduction in subsequent thermoregulatory costs. For example, whereas the cost of arousal of *N. geoffroyi* at T_a of 13°C was ~0.5 kJ, the cost of a subsequent 3 h normothermic period is reduced by ~1.7 kJ at a T_a of 27°C rather than 13°C. The minor cost of arousal relative to continuous normothermic thermoregulation, even at mild T_a , promotes a highly dynamic pattern of torpor in these bats, which is remarkably similar to the opportunistic endothermy of bees and moths (Heinrich, 1974). The low rewarming costs are an important advantage of a small body size in a heterothermic endotherm. Moreover, frequent shifting between physiological states allows these bats to exploit thermally unstable day-roosts, which, although cold in the morning, provide a short period of high daily T_a maxima during the day.

Behavioural decisions greatly influence the resting energy expenditure of small heterothermic mammals such as bats. Shelter microclimate, in particular, can determine the timing and energy costs of thermoregulatory behaviour. Moreover, even within an identical thermal regime, variation in duration of torpor versus normothermia by N. geoffroyi resulted in a fivefold difference in rest-phase energy expenditure. Although species-specific values of resting and torpid MR are easily measured and available for many species (Speakman and Thomas, 2003), the extent by which they affect daily energy expenditure is easily outweighed by variation among and within species in their choice of shelter microclimate and heterothermic behaviour. This fact cautions against a straight forward energetic interpretation of these values, especially for small heterothermic endotherms. An analysis that incorporates the potentially large effect of behavioural decisions would provide a more accurate picture of their physiological adaptations to manage a limited energy budget.

LIST OF ABBREVIATIONS

BMR	basal metabolic rate
MR	metabolic rate
TMR	torpid metabolic rate
TNZ	thermal neutral zone
Ta	ambient temperature
T _b	body temperature
$T_{\rm skin}$	skin temperature

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REFERENCES

- Audet, D. and Thomas, D. W. (1996). Evaluation of the accuracy of body temperature measurement using external radio transmitters. *Can. J. Zool.* 74, 1778-1781.
- Barclay, R. M. R., Kalcounis, M. C., Crampton, L. H., Stefan, C., Vonhof, M. J., Wilkinson, L. and Brigham, R. M. (1996). Can external radio-transmitters be used to assess body temperature and torpor in bats. J. Mammal. 77, 1102-1106.
- Beckman, A. L. and Stanton, T. L. (1978). Changes in CNS responsiveness during hibernation. Am. J. Physiol. 231, 810-816.
- Bronner, G. N., Maloney, S. K. and Buffenstein, R. (1999). Survival tactics within thermally-challenging roosts: heat tolerance and cold sensitivity in the Angolan freetailed bat, *Mops condylurus. S. Afr. J. Zool.* 34, 1-10.
- Brown, J. H. and Bartholomew, G. A. (1969). Periodicity and energetics of torpor in the kangaroo mouse, *Microdipodops pallidus*. *Ecology* **50**, 705-709.
- Buck, L. C. and Barnes, B. (2000). Effects of ambient temperature on metabolic rate, respiratory quotient, and torpor in an artic hibernator. Am. J. Physiol. Regul. Integr. Comp. Physiol. 279, R255-R262.
- Carey, H. V., Andrews, M. T. and Martin, S. L. (2003). Mammalian hibernation: cellular and molecular responses to depressed metabolism and low temperature. *Physiol. Rev.* 83, 1153-1181.
- Chruszcz, B. J. and Barclay, R. M. R. (2002). Thermoregulatory ecology of a solitary bat, *Myotis evotis*, roosting in rock crevices. *Funct. Ecol.* 16, 18-26.
- Cryan, P. M. and Wolf, B. O. (2003). Sex differences in the thermoregulation and evaporative water loss of a heterothermic bat, *Lasiurus cinereus*, during its spring migration. J. Exp. Biol. 206, 3381-3390.
- Daan, S., Barnes, B. M. and Strijkstra, A. M. (1991). Warming up for sleep? Ground squirrels sleep during arousals from hibernation. *Neurosci. Lett.* **128**, 265-268.

- Davis, W. H. and Reite, O. B. (1967). Responses of bats from temperate regions to changes in ambient temperature. *Biol. Bull.* 132, 320-328.
- Geiser, F. (1986). Thermoregulation and torpor in the Kultarr, Antechinomys laniger (Marsupialia: Dasyuridae). J. Comp. Physiol. B 156, 751-757.
- Geiser, F. (1988). Reduction of metabolism during hibernation and daily torpor in mammals and birds: temperature effects or physiological inhibition? J. Comp. Physiol. B 158, 25-37.
- Geiser, F. (2004). Metabolic rate and body temperature reduction during hibernation and daily torpor. Annu. Rev. Physiol. 66, 239-274.
- Geiser, F. and Brigham, R. M. (2000). Torpor, thermal biology, and energetics in Australian long-eared bats (*Nyctophilus*). J. Comp. Physiol. B **170**, 153-162.
- Geiser, F. and Ruf, T. (1995). Hibernation versus daily torpor in mammals and birds: physiological variables and classification of torpor patterns. *Physiol. Zool.* **68**, 935-966.
- Geiser, F., Goodship, N. and Pavey, C. R. (2002). Was basking important in the evolution of mammalian endothermy? *Naturwissenschaften* 89, 412-414.
 Geiser, F., Drury, R. L., Körtner, G., Turbill, C., Pavey, C. R. and Brigham, R. M.
- Geiser, F., Drury, R. L., Körtner, G., Turbill, C., Pavey, C. R. and Brigham, R. M. (2004). Passive rewarming from torpor in mammals and birds: energetic, ecological and evolutionary implications. In *Life in the Cold: Evolution, Adaptation and Application* (ed. B. M. Barnes and C. H. Carey), pp. 51-62. Fairbanks, AK: University of Alaska.
- Heinrich, B. (1974). Thermoregulation in endothermic insects. Science 185, 747-756.
- Heldmaier, G. and Ruf, T. (1992). Body temperature and metabolic rate during natural hypothermia in endotherms. J. Comp. Physiol. B 162, 696-706.
- Hiebert, S. (1990). Energy costs and organisation of torpor in the rufous hummingbird (*Selasphorus rufus*). *Physiol. Zool.* **63**, 1082-1097.
- Hock, R. J. (1951). The metabolic rates and body temperatures of bats. *Biol. Bull.* 101, 289-299.
- Hosken, D. J. (1996). Roost selection by the lesser long-eared bat, *Nyctophilus geoffroyi*, and the greater long-eared bat, *N. major* (Chiroptera: Vespertilionidae) in *Banksia* woodlands. *J. R. Soc. West. Aust.* **79**, 211-216.
- Humphrey, S. R., Richter, A. R. and Cope, J. B. (1977). Summer habitat and ecology of the endangered Indiana bat, *Myotis sodalis. J. Mammal.* 58, 334-346.
- Kerth, G., Weissmann, K. and König, B. (2001). Day roost selection in female Bechstein's bats (*Myotis bechsteinii*): a field experiment to determine the influence of roost temperature. *Oecologia* **126**, 1-9.
- Körtner, G. and Geiser, F. (2000). The temporal organization of daily torpor and hibernation: circadian and circannual rhythms. *Chronobiol. Int.* **17**, 103-128.
- Körtner, G., Pavey, C. R. and Geiser, F. (2008). Thermal biology, torpor and activity in free-living mulgaras in arid zone Australia during the winter reproductive season. *Physiol. Biochem. Zool.* **81**, 442-451.
- Kunz, T. H. and Lumsden, L. F. (2003). Ecology of cavity and foliage roosting bats. In Bat Ecology (ed. T. H. Kunz and M. B. Fenton), pp. 3-89. Chicago: University of Chicago Press.
- Kurta, A. and Kunz, T. H. (1988). Roosting metabolic rate and body temperature of male little brown bats (*Myotis lucifugus*) in summer. J. Mammal. 69, 645-651.
- Kurta, A., Bell, G. P., Nagy, K. A. and Kunz, T. H. (1989). Energetics of pregnancy and lactation in free-ranging little brown bats (*Myotis lucifugus*). *Physiol. Zool.* 62, 804-818.
- Lausen, C. L. and Barclay, R. M. R. (2003). Thermoregulation and roost selection by reproductive female big brown bats (*Eptesicus fuscus*) roosting in rock crevices. J. Zool. (London) 260, 235-244.
- Lovegrove, B. G., Körtner, G. and Geiser, F. (1999). The energetic cost of arousal from torpor in the marsupial *Sminthopsis macroura*: benefits of summer ambient temperature cycles. *J. Comp. Physiol. B* **169**, 11-18.
- Mzilikazi, N. and Lovegrove, B. G. (2004). Daily torpor in free-ranging rock elephant shrews, *Elephantulus myurus*: a year-long study. *Physiol. Biochem. Zool.* 77, 285-269.
- Mzilikazi, N., Lovegrove, B. G. and Ribble, D. O. (2002). Exogenous passive heating during torpor arousal in free-ranging rock elephant shrews, *Elephantulus myurus*. *Oecologia* 133, 307-314.
- Schmid, J. (1996). Oxygen consumption and torpor in mouse lemurs (*Microcebus murinus* and *M. myoxinus*): preliminary results of a study in western Madagascar. In Adaptations to the Cold (ed. F. Geiser, A. J. Hulbert and S. C. Nicol), pp. 47-54. Armidale: University of New England Press.
- Schmidt-Nielsen, K. (1997). *Animal Physiology: Adaptation and Environment.* Cambridge: Cambridge University Press.
- Song, X., Körtner, G. and Geiser, F. (1997). Thermal relations of metabolic rate reduction in a hibernating marsupial. Am. J. Physiol. Regul. Integr. Comp. Physiol. 273, R2397-R2104.
- Speakman, J. R. and Thomas, D. W. (2003). Physiological ecology and energetics of bats. In *Bat Ecology* (ed. T. H. Kunz and M. B. Fenton), pp. 430-492. Chicago: University of Chicago Press.
- Storey, K. B. and Storey, J. M. (1990). Metabolic rate depression and biochemical adaptation in anaerobiosis, hibernation and estivation. Q. Rev. Biol. 65, 145-174.
- Tucker, V. A. (1962). Diurnal torpidity in the California pocket mouse. *Science* 139, 380-381.
- Turbill, C. (2006a). Roosting and thermoregulatory behaviour of male Gould's longeared bats, *Nyctophilus gouldi*: energetic benefits of thermally unstable tree roosts. *Aust. J. Zool.* 54, 57-60.
- Turbill, C. (2006b). Thermoregulatory behaviour of tree-roosting chocolate wattled bats (*Chalinolobus morio*) during summer and winter. J. Mammal. 87, 318-323.
- Turbill, C., Körtner, G. and Geiser, F. (2003a). Natural use of heterothermy by a small, tree-roosting bat during summer. *Physiol. Biochem. Zool.* 76, 868-876.
- Turbill, C., Law, B. S. and Geiser, F. (2003b). Summer torpor in a free-ranging bat from sub-tropical Australia. J. Therm. Biol. 28, 223-226.
- van Breukelen, F. and Martin, S. L. (2001). Translational initiation is uncoupled from elongation at 18 degrees C during mammalian hibernation. *Am. J. Physiol. Integr. Comp. Physiol.* 281, R1374-R1379.

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- van Breukelen, F. and Martin, S. L. (2002a). Invited review: molecular adaptations in mammalian hibernators: unique adaptations or generalized responses? J. Appl. Physiol. 92, 2640-2647.
- van Breukelen, F. and Martin, S. L. (2002b). Reversible depression of transcription during hibernation. J. Comp. Physiol. B 172, 355-361.
 Vaughan, T. A. and O'Shea, T. J. (1976). Roosting ecology of the pallid bat,
- Antrozous pallidus. J. Mammal. 57, 19-41.
- Willis, C. K. R. and Brigham, R. M. (2003). Defining torpor in free-ranging bats: experimental evaluation of external temperature-sensitive radiotransmitters and the
- concept of active temperature. J. Comp. Physiol. B 173, 379-389.
 Willis, C. K. R. and Brigham, R. M. (2005). Physiological and ecological aspects of roost selection by reproductive female hoary bats (Lasiurus cinereus). J. Mammal. **86**, 85-94.
- Willis, C. K. R., Lane, J. E., Liknes, E. T., Swanson, D. L. and Brigham, R. M. (2004). A technique for modelling thermoregulatory energy expenditure in freeranging endotherms. In Life in the Cold: Evolution, Mechanisms, Adaptation and Application. Twelth International Hibernation Symposium (ed. B. M. Barnes and H. V. Carey), pp. 209-219. Biological Papers of the University of Alaska, number 27. Fairbanks, AK: Institute of Arctic Biology, University of Alaska.
- Willis, J. S. (1982). The mystery of periodic arousal. In Hibernation and Torpor in Mammals and Birds (ed. C. P. Lyman, J. S. Willis, A. Malan and L. C. H. Wang), pp. 92-103. New York: Academic Press.
- Withers, P. C. (1977). Measurement of VO₂, VCO₂, and evaporative water loss with a flow-through mask. *J. Appl. Physiol.* 42, 120-123.
 Wolf, B. O. and Walsberg, G. E. (1996). Thermal effects of radiation and wind on a small bird and its implications for microsite selection. *Ecology* 77, 2228-2236.