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 (Tb) and energy expenditure in heterothermic endotherms are affected by changes in ambient temperature (Ta) and selection of suitable microclimates, yet most laboratory studies employ constant Ta 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; 7 g) exposed to a diurnal Tb 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 Ta 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 Ta and passive rewarming. The critical Ta (and torpid Tb) 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 Ta 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 Ta, indicating a trade-off between the benefits and energetic costs of normothermia during resting. Our experiment clearly shows that a daily Ta 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 (Ta) above the critical minimum in torpor, lowered body temperature (Tb) 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 Tb 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 Tb 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 Tb and MR during normothermia and the associated energy costs of thermoregulatory heat production.

Thermal energetics typically are studied in the laboratory at constant Ta 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 (Ta) 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 Ta generally employed in the laboratory. Whereas, many animals experience a daily Ta 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 Ta are minimal, and arouse at around midday or in the early afternoon, seemingly in response to a rising Ta in their shelter and some passive rewarming of torpid Tb (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 Ta and it is suggested that this timing may reflect an inherent propensity for arousal coinciding with rising Ta and passive rewarming in the wild (Körtner and Geiser, 2000). Nevertheless, no previous studies have clearly separated the effects of a daily Ta 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 Ta cycle affects Tb 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 $T_a$ 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 $T_a$ 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 $T_a$, reflecting a trade-off between their high thermoregulatory costs and the requirements for physiological processes only possible at normothermic $T_a$ 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 $T_a$ 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 $T_a$ 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’S 151 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 (~10 km 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 one 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 ($T_{skin}$). For small bats, $T_{skin}$ is closely related to $T_a$, particularly during torpor when $T_{skin}$–$T_a$ 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 Köntner 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: 0.140 l) 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 $T_{\text{skin}}$, 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 $T_{\text{skin}}$ 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 $T_{\text{skin}}$ 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 (ml O$_2$ 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 ± 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 $T_{\text{skin}}$ up to 29°C (Fig. 2A). Time of arousal differed significantly among treatment days ($F_{2,25}=169$, *P*<0.001), and was affected by the shift in time of increasing $T_a$ and passive rewarming of $T_a$. 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 $T_a$ (Fig. 3; RM ANOVA: $F_{2,20}=15.5$, *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±0:58 h/25.1±2.3°C ($T_{\text{skin}}$: 24.6±2.8°C), 12:47±1:03 h/22.9±2.6°C ($T_{\text{skin}}$: 21.4±2.8°C) and 15:36±1:18 h/21.8±2.8°C ($T_{\text{skin}}$: 20.1±2.9°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,
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±0.02 ml O2 g⁻¹ h⁻¹ at Ta of 10–12°C (Tskin 11–13°C) to 0.53±0.12 ml O2 g⁻¹ h⁻¹ at Ta of 26–28°C (Tskin 26–29°C) (Fig. 4) [Torpid MR (ml O₂ g⁻¹ h⁻¹) = 0.015×1.14¹^{Ta} (r²=0.78, P<0.001)]. During periods of normothermy, average mass-specific resting MR was 7.0±0.7 ml O₂ g⁻¹ h⁻¹ at a minimum Ta of 13.7±0.7°C and was reduced to 3.0±0.6 ml O₂ g⁻¹ h⁻¹ at a maximum Ta of 26.5±0.7°C.

The cost of arousal by Nycticeius geoffroyi at a constant Ta 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 Ta, arousal costs declined linearly depending on the increase in Ta (and torpid Ta) prior to arousal (Fig. 5) [average cost of active arousal (kJ)=0.84–0.026×Ta; 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)=0.78–0.02¹^{Ta}; P<0.001].
Energy expenditure of bats over the entire rest phase (12 h) increased linearly depending on the time spent normothermic (Fig 6), ranging from a minimum of 0.59 kJ for a bat remaining torpid throughout the day to a maximum of 3.60 kJ for a bat that was normothermic for 5:06 h (mean: 1.98±0.84 kJ). The slope of the relationship between energy consumption and time spent normothermic did not differ significantly among the different thermal regimes (GLM: slope, \(F_{2,43}=2.4\), \(P=0.1\)). 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 06:00 h and 09:00 h 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±0.12 kJ; RM ANOVA: \(F_{1,15}=35.2\), \(P<0.001\)) because of the greater cooling of \(T_a\) and \(T_s\) 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 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 \(T_a\) 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 $T_a - T_b$ 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 $T_a$ 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 $T_a$ 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 midday 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 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 $T_a$ 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 short-term 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*<sub>R</sub> of 13°C was ~0.5 kJ, the cost of a subsequent 3 h normothermic period is reduced by ~1.7 kJ at *T*<sub>R</sub> of 27°C rather than 13°C. The minor cost of arousal relative to continuous normothermic thermoregulation, even at mild *T*<sub>R</sub>, 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*<sub>R</sub> 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 straightforward 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**

| Abbreviation | Description                           |
|--------------|---------------------------------------|
| BMR          | basal metabolic rate                  |
| MR           | metabolic rate                         |
| TMR          | torpid metabolic rate                  |
| TNZ          | thermal neutral zone                   |
| *T*<sub>R</sub> | ambient temperature                  |
| *T*<sub>B</sub> | body temperature                        |
| *T*<sub>skin</sub> | skin temperature                        |

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