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Torpor, thermal biology, and energetics in Australian long-eared bats (*Nyctophilus*)

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Abstract Previous studies have suggested that Australian long-eared bats (*Nyctophilus*) differ from northern-hemisphere bats with respect to their thermal physiology and patterns of torpor. To determine whether this is a general trait of Australian bats, we characterised the temporal organisation of torpor and quantified metabolic rates and body temperatures of normothermic and torpid Australian bats (*Nyctophilus geoffroyi*, 7 g and *N. gouldi*, 10 g) over a range of air temperatures and in different seasons. The basal metabolic rate of normothermic bats was 1.36 ± 0.17 ml g^{-1} h^{-1} (*N. geoffroyi*) and 1.22 ± 0.13 ml g^{-1} h^{-1} (*N. gouldi*), about 65% of that predicted by allometric equations, and the corresponding body temperature was about 36 °C. Below an air temperature of about 25 °C bats usually remained normothermic for only brief periods and typically entered torpor. Arousal from torpor usually occurred shortly after the beginning of the dark phase and torpor re-entry occurred almost always during the dark phase after normothermic periods of only 111 ± 48 min (*N. geoffroyi*) and 115 ± 66 min (*N. gouldi*). At air temperatures below 10 °C, bats remained torpid for more than 1 day. Bats that were measured overnight had steady-state torpor metabolic rates representing only 2.7% (*N. geoffroyi*) and 4.2% (*N. gouldi*) of the basal metabolic rate, and their body temperatures fell to minima of 1.4 and 2.3 °C, respectively. In contrast, bats measured entirely during the day, as in previous studies, had torpor metabolic rates that were up to ten times higher than those measured overnight. The steady-state torpor metabolic rate of thermoconforming torpid bats showed an exponential relationship with body temperature ($r^2 = 0.94$), suggesting that temperature effects are important for reduction of metabolic rate below basal levels. However, the 75% reduction of metabolic rate between basal metabolic rate and torpor metabolic rate at a body temperature of 29.3 °C suggests that metabolic inhibition also plays an important role. Torpor metabolic rate showed little or no seasonal change. Our study suggests that Australian *Nyctophilus* bats have a low basal metabolic rate and that their patterns of torpor are similar to those measured in bats from the northern hemisphere. The low basal metabolic rate and the high proclivity of these bats for using torpor suggest that they are constrained by limited energy availability and that heterothermy plays a key role in their natural biology.

Key words Bats · Body temperature · Metabolic rate · *Nyctophilus* · Torpor bouts

Abbreviations BMR basal metabolic rate · C thermal conductance · MR metabolic rate · RMR resting metabolic rate · T_a air temperature · T_b body temperature · TMR torpor metabolic rate · TNZ thermoneutral zone · $\dot{V}O_2$ rate of oxygen consumption

Introduction

With the exception of rodents, bats are the only non-marine placental mammals endemic to Australia. It is believed that the first bats migrated to Australia from south-eastern Asia in the mid Tertiary and evolved into several distinct Australian genera (Hall 1984). Despite this evolutionary background and the long separation from bats in other parts of the world, little detailed work has been conducted to determine whether physiological adaptations of Australian bats differ from those found on other continents. It is possible that the aridity,

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unpredictable rainfall, and infertile soils characteristic of the Australian continent are reflected in energetic and thermoregulatory features of bats as have been observed in marsupials (Dawson 1989; Lovegrove 1996). It is known that Australian microbats (Microchiroptera) as well as small megabats (Megachiroptera) use torpor extensively to reduce energy expenditure (Morrison 1959; Kulzer et al. 1970; Morris et al. 1994; Geiser et al. 1996; Hosken 1997; Bartels et al. 1998; Coburn and Geiser 1998; Hosken and Withers 1999). This is most likely because of their large relative surface area, high costs for thermoregulation at low air temperature (T_a), and unpredictable or temperature-dependent food supply as in bats from Europe and North America (Hock 1951; Pohl 1961; Henshaw 1970; Lyman 1970; Kunz 1982; Fenton 1983; Thomas et al. 1990; Speakman et al. 1991; Barclay et al. 1996).

Some recent studies on endemic Australian long-eared bats of the genus *Nyctophilus* (Family Vespertilionidae) suggest that they may differ considerably from northern-hemisphere species with respect to the physiology of torpor. Body temperature (T_b), metabolic rate (MR), and the differential between T_b and T_a reported in several species of these tree-cavity roosting bats (*Nyctophilus geoffroyi*, *N. gouldi* and *N. major*) during torpor are relatively high (Morris et al. 1994; Hosken 1997; Hosken and Withers 1999). Whereas the T_b of many torpid vespertilionids from the northern hemisphere fall to near 0 °C, and the differential between T_b and T_a is often around 1 °C (Hock 1951; Henshaw 1970; Lyman 1970; Geiser and Ruf 1995), torpid Australian long-eared bats appear to maintain a T_b of >10 °C, and a T_b–T_a differential that is more than twofold, and a MR that is up to tenfold that of northern-hemisphere vespertilionids. Some of these differences may be due to climatic factors; however, effects of captivity or experimental conditions may also play an important role (Hosken 1997; Hosken and Withers 1999). With respect to experimental conditions it may be important that all recently published experiments used to quantify physiological variables of torpid Australian long-eared bats (*Nyctophilus* spp.) were conducted with individuals in which torpor was induced during the day and measured 2–3 h after entry into torpor around the time when free-living *Nyctophilus* bats typically arouse (Turbill et al. 1999). In contrast, many measurements on northern-hemisphere bats were conducted overnight to allow them to undergo their natural thermal cycle with torpor entry at night or around dawn, or bats were transferred to the measuring equipment while hibernating (Hock 1951; Pohl 1961; Henshaw 1968; Riedesel and Williams 1976; Thomas et al. 1990; Speakman et al. 1991). Riedesel and Williams (1976) showed that the time to reach minimum MR during torpor in *Myotis velifer* (12 g) was up to 19 h from the beginning of measurements.

The scarcity of data on the thermal biology and energetics of Australian bats also extends to seasonal effects. Present data suggest that Australian microbats undergo short torpor bouts usually lasting for less than a day in summer, and multi-day torpor bouts (i.e. hibernation) in winter (Hall 1982; Ellis et al. 1991; Brigham and Geiser 1998; Turbill et al. 1999) similar to northern-hemisphere species (Audet and Fenton 1988). As torpor bout duration in many heterothermic mammals appears to be related to T_b and MR (Twente and Twente 1965; French 1985; Geiser and Kenagy 1988; Barnes and Ritter 1993), it is possible that bats either change thermoenergetics with season, or that bout duration is to a large extent controlled by external factors such as T_a and food availability. To our knowledge there is no published literature on whether the seasonal change in torpor bout duration of bats in general reflects seasonal changes in MR during torpor.

We quantified patterns of thermoregulation, energetics, and the duration of normothermic periods and torpor bouts in two species of long-eared bats, *N. geoffroyi* and *N. gouldi*, to determine whether they differ from northern-hemisphere bats. We measured the MR of bats overnight to allow them to enter torpor during the night or near sunrise as in the field, and assessed whether these values differed from those obtained from bats measured entirely during daytime. Further, we investigated interrelations between physiological variables associated with torpor and determined whether MR during torpor (TMR) differs among seasons.

Materials and methods

Bats were captured in mist nests in Imbota Nature Reserve (formerly Eastwood State Forest) near Armidale, New South Wales (30°35′S, 151°44′E), an open woodland area at approximately 1000 m altitude. Captured animals were transferred to the laboratory at the University of New England and measured over a period of 1–3 days before being released at the site of capture. To ensure that bats held for more than 1 day maintained condition while in captivity, they were hand fed with mealworms and water once per day while not being measured. Measurements were conducted on a total of 24 *N. geoffroyi* (September 1996 to May 1997) and 9 *N. gouldi* (January to July 1997).

The MR, measured as rate of oxygen consumption ($\dot{V}O_2$), was monitored over periods ranging from several hours to several days over a range of T_as and natural photoperiod. TMR was averaged from minimum measurements that remained constant over at least 30 min. Two types of measurements were performed: (1) overnight and the following day(s), and (2) entirely during daytime.

Most bats were measured beginning in the afternoon, overnight and throughout most of the following day to allow them to undergo their natural daily thermal cycle with torpor entry during the night or near sunrise (Turbill et al. 1999; Figs. 1, 2). Readings were taken in the morning after torpor bouts of at least 4 h (average 10.5 h) at the time when bats are naturally torpid (Turbill et al. 1999). Initially, we followed the protocol of Hosken (1997) and other recent studies of *Nyctophilus* spp., beginning measurements in mid-morning, and taking readings after bats were in torpor for 2–3 h. However, we noted that most bats did not appear to reach steady-state minima, but instead often maintained a MR throughout the day that was about ten times that reported in northern-hemisphere bats. Therefore, we discontinued this protocol and concentrated instead on overnight measurements as outlined above.

Measurements of basal metabolic rate (BMR) were carried out separately. Postabsorptive bats were exposed to T_a 26 °C in the morning and T_a was slowly increased to a maximum of 35 °C in increments of about 2 °C, which lasted for at least 1 h each. The minimum MR of normothermic individuals over at least 30 min at one of these temperature steps was used to calculate the average BMR of each bat. The thermoneutral zone (TNZ) was estimated from the range of T_a in which the BMR of individuals was measured. The MR of normothermic resting individuals (RMR) below the TNZ was also averaged over at least 30 min. Usually RMR at only one T_a could be measured because bats entered torpor before a second measurement at another T_a could be completed. Time of arousal from torpor and entry into torpor and normothermic periods were estimated from measurements of MR. Bats were assumed to have fully aroused when the MR overshoot during arousal episodes reached approximately twice RMR at the same T_a (Geiser 1986). Food and water were not available during measurements of MR which was performed in 500-ml respirometry chambers fitted with a wide plastic mesh to allow for roosting and placed in a temperature-controlled (±0.5 °C) cabinet. The flow rate (100–200 ml min^{-1}) was controlled with rotameters and measured with mass flowmeters (Omega FMA-5606).

The percentage oxygen was measured with Ametek Applied Eletrochemistry S-3 A oxygen analysers. Most measurements were conducted with a dual-channel S-3AII. Occasionally, a single-channel S-3AI, fitted with a high-resolution output board (80335SE), was used. In the dual-channel set-up, the MR of two bats was measured simultaneously every 3 min; solenoid valves switched to a reference channel (outside air) for 3 min once every 15 min. The outputs from the oxygen analyser, the flowmeters, and thermocouples measuring T_a inside the respirometry chamber to the nearest 0.1 °C, were transferred via a logger (Datataker DT100) to a personal computer. In the single-channel set-up, four channels, which included three animal channels and one reference channel (outside air) were scanned in sequence with solenoid valves. Each of the four channels was read for 3 min (i.e. the MR of each bat and the reference channel were measured once every 12 min). Both the output from the oxygen analysers and the flowmeter were transferred with an A/D 14-bit converter card to a personal computer. The T_a inside the respirometry chamber was read to the nearest 0.1 °C with an Omega digital thermocouple thermometer and recorded on the personal computer via the A/D converter card. The MR was calculated using standardised gas volumes and Eq. 3a of Withers (1977). Animals were weighed before and after the experiments and a linear decrease of body mass throughout the experiment was assumed for calculation of mass-specific MR. Mass loss ranged between 0.1 g day^{-1} and 0.4 g day^{-1}.

Thermal conductance (C) was calculated using the equation: $C = \mathrm{MR}(T_b - T_a)$. The Q_{10} was calculated using the equation: $Q_{10} = (\mathrm{MR}_1/\mathrm{MR}_2)^{10/(T_{b1} - T_{b2})}$. T_b was measured to the nearest 0.1 °C with an Omega digital thermometer by inserting a fine calibrated thermocouple probe 1 cm into the rectum. Since the rate of rewarming is slow at the beginning of the rewarming process (Geiser and Baudinette 1990), rectal T_b, which was measured within 15 s of removal from the respirometers, was within 0.2 °C of the T_b before the disturbance. Animals were considered torpid when T_b fell below 30 °C. When no T_b measurements were available, animals were considered to be torpid when their MR fell to that or below that of individuals in which both variables were measured and which had a T_b of less than 30 °C at same T_a. All equipment was calibrated before measurements. Data-acquisition software was written by G. Körtner, B. Lovegrove and T. Ruf.

Results are expressed as means ± standard deviation (SD) for the number of individuals (n) that were measured if not specified otherwise. Differences among or between means were tested using a one-way ANOVA or a Student's t-test as appropriate (Zar 1984). Regression equations were fitted using the method of least squares (Zar 1984). Interrelations between physiological variables, and potential seasonal physiological changes were only tested in *N. geoffroyi*, because it was the only species for which our sample size allowed for meaningful analysis.

Results

Both species of *Nyctophilus* bats showed a high proclivity to enter torpor in captivity. The most common daily pattern of torpor and activity observed was: entry into torpor shortly after bats were placed into the respirometer during the day, torpor throughout the day, arousal near lights off, a short normothermic period, and re-entry into torpor followed by a torpor bout lasting for the rest of the night and the following day (Fig. 1a). Arousals, characterised by an enormous increase in MR near the beginning of the dark phase, began 18 ± 31 min after lights off (*N. geoffroyi*) and 37 ± 55 min after lights off (*N. gouldi*). The total normothermic periods lasted for 111 ± 48 min (*N. geoffroyi*) and 115 ± 66 min (*N. gouldi*). Normothermic periods usually consisted of a period of intense activity and only brief resting phases. When bats were measured overnight, torpor entry almost always occurred during the dark phase. Only on one occasion did a bat (*N. gouldi*) measured overnight enter torpor in the light phase 40 min after lights on. After re-entry into torpor, bouts lasting from early in the dark phase, throughout the day to the beginning of the next dark phase, were frequently observed in both species.

A second arousal after midnight or late during the dark phase followed by a short normothermic period was observed less frequently (Fig. 1b). Torpor bouts between two normothermic periods at night lasted for 368 ± 149 min (*N. geoffroyi*) and 220 ± 194 min (*N. gouldi*).

Occasionally, bats entered torpor for more than 1 day (Fig. 2). The longest torpor bouts, observed at $T_a < 10$ °C, were 41:30 h (*N. geoffroyi*) and 33:36 h (*N. gouldi*), but all of these were interrupted by measurements of T_b and it is likely that bats would have remained torpid for longer if left undisturbed. Partial arousals, during which maximum MR was less than 20% of that during full arousals, were observed in some cases (Fig. 2).

Both *Nyctophilus* species rarely maintained a high T_b at rest during daytime cold exposure. At high T_a, normothermic thermoregulation was more common. BMR of *N. geoffroyi* was 1.36 ± 0.17 ml g^{-1} h^{-1} (T_b = 35.7 ± 0.7 °C; body mass = 7.1 ± 0.8 g; n = 6) and was measured at T_a 29.1–33.2 °C (Fig. 3a, b). The BMR of *N. gouldi* was 1.22 ± 0.13 ml g^{-1} h^{-1} (T_b = 36.0 ± 1.4 °C; body mass = 10.0 ± 1.1 g; n = 8) and was measured at T_a 30.7–32.5 °C (Fig. 4a, b). During cold exposure the RMR of both species increased linearly with decreasing T_a (Figs. 3b, 4b), but the T_b of *N. geoffroyi* (33.6 ± 1.4 °C) was somewhat lower than in the TNZ (Fig 3a).

When torpid, bats substantially reduced MR and T_b (Figs. 1–4). Over a wide range of T_a, bats were thermoconforming and MR and T_b fell with T_a. The TMR of both species showed an exponential decline with T_a above 1 °C (*N. geoffroyi*: TMR (ml g^{-1} h^{-1}) = 0.026×

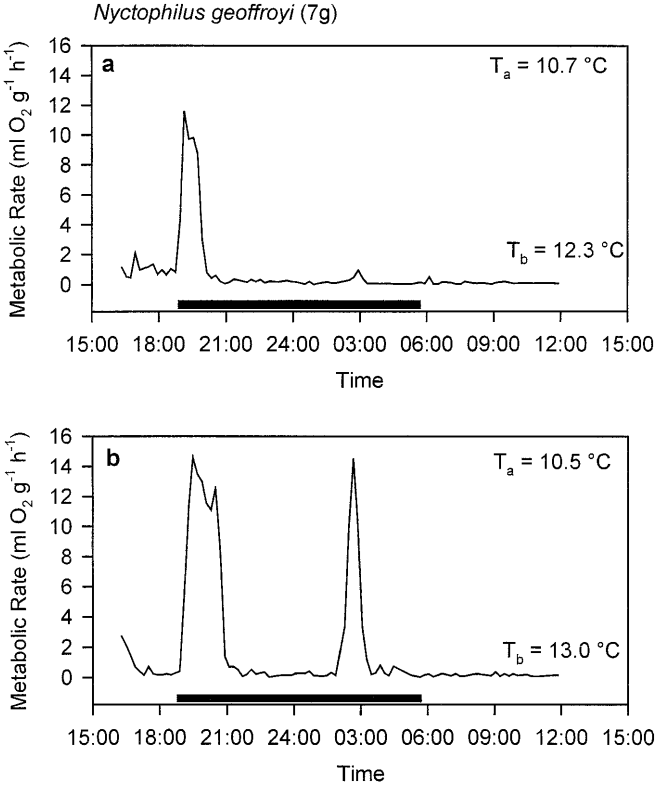

Fig. 1a, b Daily fluctuations of metabolic rate in *Nyctophilus geoffroyi*. One bat (**a**) measured at an air temperature (T_a) of 10.7 °C aroused after lights off, indicated by the substantial increase in metabolic rate (MR), and remained in torpor from early in the dark phase well into the next day when body temperature (T_b) was measured. The other bat (**b**) measured at T_a 10.5 °C aroused twice, early in the dark phase and at about 0300 hours. The *black bars* indicate the dark phase

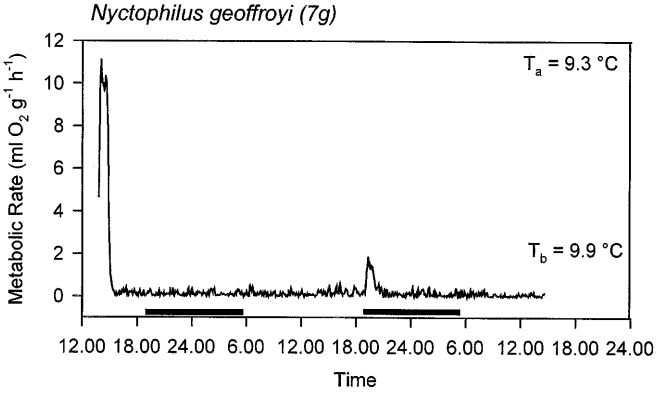

Fig. 2 MR over 2 days in *N. geoffroyi*. The bat was measured at T_a 9.3 °C and remained in torpor from late afternoon on the 1st day, throughout the night and the following day. A partial arousal occurred on the 2nd day shortly after lights off. The *black bars* indicate the dark phase

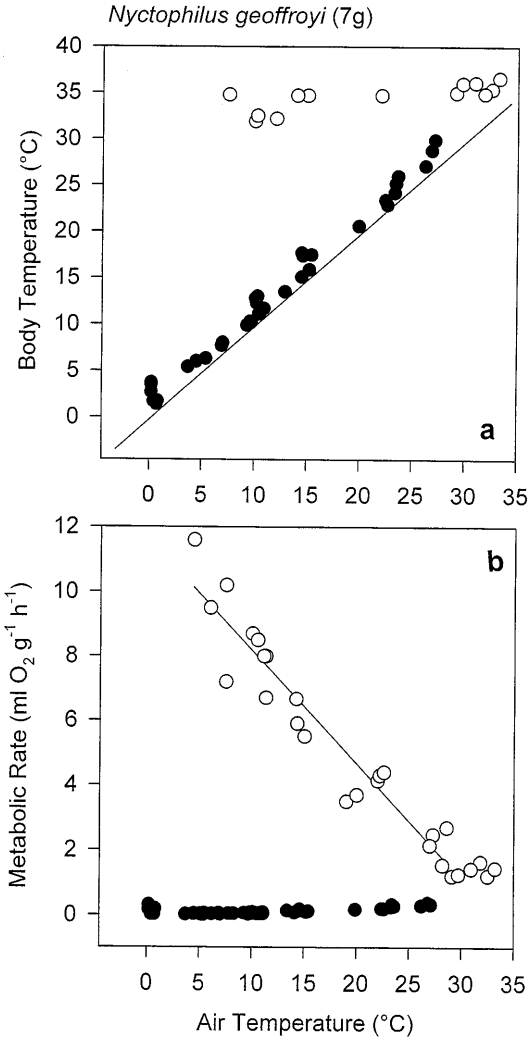

Fig. 3 T_bs (**a**) and MRs (**b**) in normothermic (*open symbols*) and torpid (*filled symbols*) *N. geoffroyi* as a function of T_a. Each point represents one individual measurement. MR in resting normothermic bats (RMR) increased with decreasing T_a: RMR (ml g^{-1} h^{-1}) = 11.69–0.35T_a (°C); $r^2 = 0.91$

$1.102^{Ta(°C)}$, $r^2 = 0.92$; *N. gouldi*: TMR (ml g^{-1} h^{-1}) = $0.022 \times 1.124^{Ta(°C)}$, $r^2 = 0.59$ (Figs. 3b, 4b). The minimum TMR of *N. geoffroyi* was 0.037 ± 0.014 ml g^{-1} h^{-1} ($T_b = 6.3 \pm 3.2$ °C; $T_a = 5.9 \pm 2.7$ °C; $n = 17$). The minimum TMR of *N. gouldi* was 0.052 ± 0.01 ml g^{-1} h^{-1} ($T_b = 10.1 \pm 2.1$ °C; $T_a = 8.9 \pm 1.4$ °C; $n = 6$).

The steady-state TMR of thermoconforming *N. geoffroyi* was related to T_b (Fig. 5). This relationship was best described by an exponential equation ($r^2 = 0.94$; $P < 0.0001$). TMR was around 0.05 ml g^{-1} h^{-1} at T_b below 10 °C and increased about sixfold to values around 0.3 ml g^{-1} h^{-1} at T_b between 25 °C and 30 °C. The Q_{10} was 3.4 between BMR and the minimum TMR and 3.0 for TMR of thermoconforming bats.

Whereas the TMR and T_b of torpid *Nyctophilus* bats declined with T_a over a wide range of T_a, the T_b–T_a differential in thermoconforming torpid bats was not a function of T_a (Figs. 3a, 4a). In *N. geoffroyi*, the T_b–T_a differential of all torpid thermoconforming bats was described by the equation: T_b–T_a (°C) = 1.08 + 0.02T_a (°C) ($r^2 = 0.035$; $P > 0.3$) and the mean T_b–T_a was

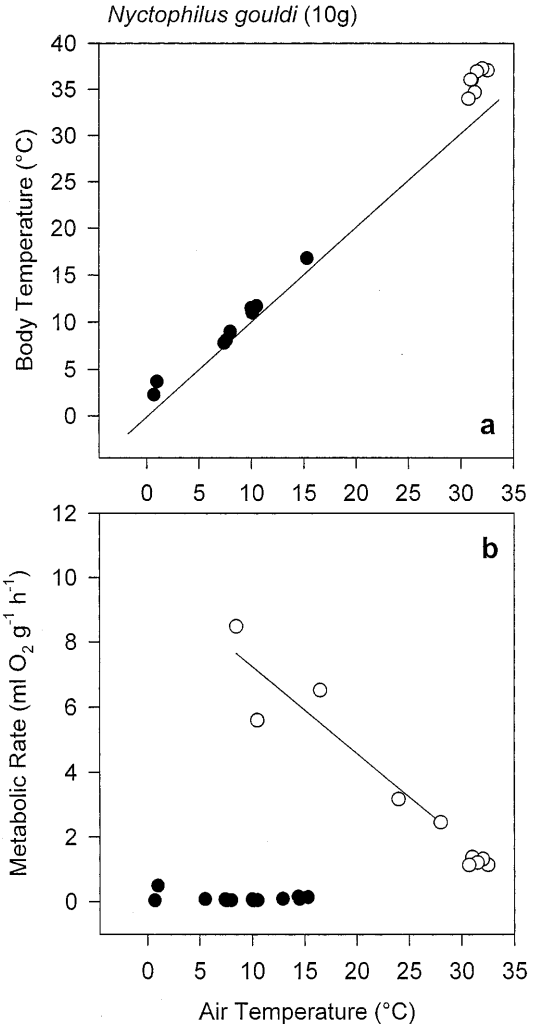

Fig. 4 T_bs (**a**) and MRs (**b**) in normothermic (*open symbols*) and torpid (filled symbols) *N. gouldi* as a function of T_a. Each point represents one individual measurement. MR in resting normothermic bats (RMR) increased with decreasing T_a: RMR (ml g^{-1} h^{-1}) = 9.91–0.26T_a (°C); $r^2 = 0.83$

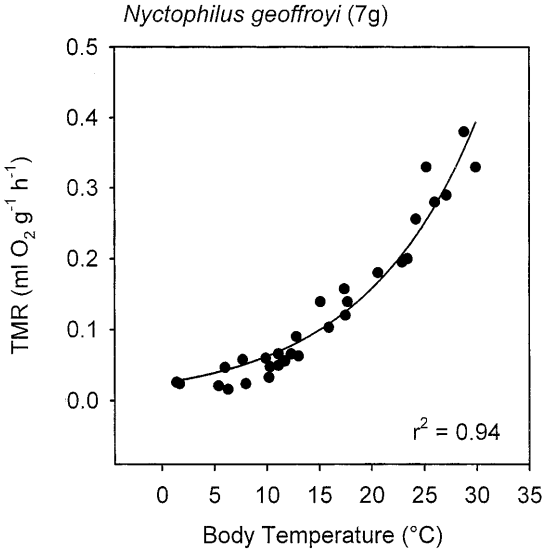

Fig. 5 MRs as a function of T_b in torpid thermoconforming *N. geoffroyi*. Each point represents one individual measurement. MR in torpid bats (TMR) decreased exponentially with T_b: TMR (ml g^{-1} h^{-1}) = 0.0249 × $1.09^{T_b(°C)}$; $r^2 = 0.94$

1.36 ± 0.85 °C. In *N. gouldi*, the T_b–T_a differential of all torpid thermoconforming bats was described by the equation: T_b–T_a (°C) = 1.02 + 0.006T_a (°C) (r^2 = 0.003; $P > 0.9$) and the mean T_b–T_a was 1.08 ± 0.46 °C. It is likely that the constant T_b–T_a differential despite the rise of TMR at high T_a was possible because C increased from values around 0.05 ml g^{-1} h^{-1} °C^{-1} in thermoconforming torpid bats at T_a below 10 °C to values of about 0.1–0.3 ml g^{-1} h^{-1} °C^{-1} at T_a above 15 °C (Song et al. 1997).

At very low T_a, torpid bats increased their TMR to maintain T_b above a threshold (Figs. 3, 4). Most individual *N. geoffroyi* increased TMR at T_a 0.4 ± 0.3 °C by about fivefold to 0.209 ± 0.57 ml g^{-1} h^{-1} and T_b was 2.7 ± 0.9 °C ($n = 5$). These bats had a raised C similar to that in torpid thermoconforming individuals at $T_a > 15$ °C. One *N. geoffroyi* showed no evidence of thermoregulation at T_a 0.4 °C, and the lowest T_b measured for the species was 1.4 °C (Fig. 3a, b). One *N. gouldi* at T_a 1.0 °C regulated T_b at 3.7 °C and had a TMR of 0.42 ml g^{-1} h^{-1}; the lowest T_b measured in this species was 2.3 °C at T_a 0.7 °C (Fig. 4a, b).

TMR differed between bats that were measured overnight versus bats in which torpor was induced in the morning and TMR was measured 2–3 h later (Fig. 6). At T_a 4–10 °C, the TMR of *N. geoffroyi* was 0.04 ± 0.02 ($n = 18$) when measured overnight, which was only about 10% of the value obtained from bats measured entirely during the daytime (TMR = 0.41 ±

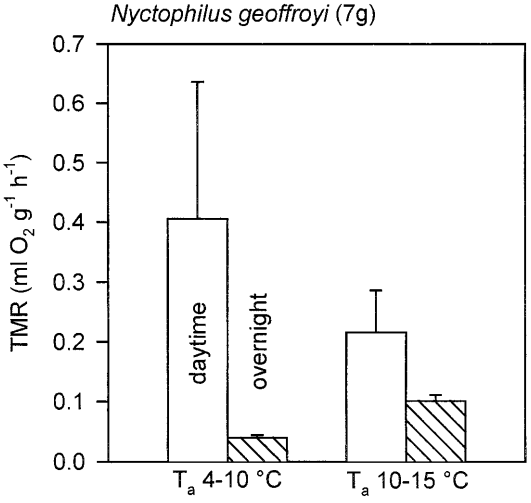

Fig. 6 MRs in torpid *N. geoffroyi* measured overnight (*hatched columns*) and entirely during the daytime (*open columns*) at T_as of 4–10 °C and 10–15 °C. Means ± SE are presented here because of the large variation; means ± SD are provided in the text. TMR at both T_as was significantly greater during the daytime measurements than during the overnight measurements in both T_a ranges

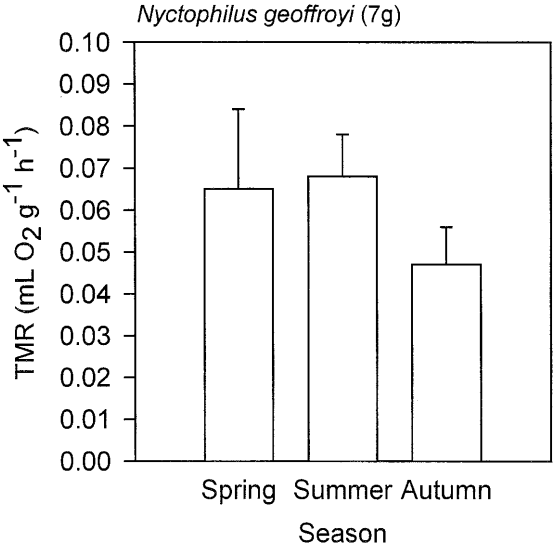

Fig. 7 Metabolic rates (means ± SD) in torpid thermoconforming *N. geoffroyi* measured at similar T_as in spring (T_a 10.3 ± 1.6 °C), summer (T_a 9.8 ± 0.8 °C), and autumn (T_a 10.2 ± 0.6 °C). TMR did not differ among seasons (ANOVA; $P > 0.08$) although TMR in autumn appeared somewhat lower than those in the other seasons. Body mass was indistinguishable among seasons

0.46; $n = 4$; *t*-test; $P < 0.001$). However, the variance in the daytime only sample was very large and the minimum TMR in one bat was close to that measured in overnight experiments. At T_a 10–15 °C, TMR measured entirely during the day was about double the value of those measured overnight (Fig. 6; *t*-test; $P < 0.05$).

The TMR of *N. geoffroyi* did not appear to differ strongly with season (Fig. 7). When measured in a similar T_a range (ANOVA; $P > 0.7$), TMR in spring (0.065 ± 0.019 ml g^{-1} h^{-1}, T_a 10.3 ± 1.6 °C; body mass 7.4 ± 0.3 g; $n = 4$) and summer (0.068 ± 0.01 ml g^{-1} h^{-1}; T_a 9.8 ± 0.8 °C; body mass 7.1 ± 0.7 g; $n = 7$) appeared somewhat greater than TMR measured in autumn (0.047 ± 0.009 ml g^{-1} h^{-1}; T_a 10.2 ± 0.6 °C; body mass 7.6 ± 0.4 g; $n = 4$); however, the means did not differ significantly (ANOVA; $P = 0.083$). Body mass and total TMR (ml h^{-1}) also did not differ among seasons (ANOVA; $P > 0.1$).

Discussion

Our study provides the first evidence that Australian long-eared bats exhibit similar thermal characteristics and patterns of torpor to their relatives from the northern hemisphere. The T_b of both *Nyctophilus* species fell to about 1–3 °C and torpor bouts could last for more than 1 day. TMR of the bats we measured was as low as 0.5% of that in normothermic bats at the same T_a and about 3% of the BMR. As torpor was used frequently and reduced energy expenditure substantially it appears that it plays a central role in the biology of Australian microbats.

Physiological variables of normothermic bats measured here were very similar to those reported in previous studies. Regressions of RMR in *N. geoffroyi* as a function of T_a, and the T_b below thermoneutrality were close to reported results, as were BMR and T_b in the TNZ (Hosken and Withers 1999). However, for both *N. geoffroyi* and *N. gouldi*, BMR was only about 65% of that predicted from allometric equations for bats of equivalent size (Hayssen and Lacy 1985). This suggests that maintenance metabolism in Australian bats is relatively low, as in many other Australian monotreme, marsupial, and placental mammals (Hulbert and Dawson 1974; Dawson 1989; Geiser and Coburn 1999; Hume 1999). This frugal use of energy for maintenance metabolism during normothermia in Australian mammals is likely a reflection of unpredictable climate and food supply and infertile soils of the continent (Hulbert and Dawson 1974; Lovegrove 1996).

The minimum T_b of bats reported here are similar to those of hibernating endotherms of equivalent body mass (Geiser and Ruf 1995). Similarly, the T_b–T_a differential of about 1 °C in torpid thermoconforming *Nyctophilus* is close to that reported for hibernating bats from the northern hemisphere (Hock 1951; Henshaw 1968, 1970). Moreover, the minimum TMRs reported here for Australian long-eared bats fall near the mean for hibernating endotherms (Geiser and Ruf 1995), and are 88% (*N. geoffroyi*) and 126% (*N. gouldi*) of the TMR predicted from the allometric equation for hibernators of the same body mass at a T_b of 5 °C (Geiser 1988). They are also similar to those of northern-hemisphere microbats and the Australian broad-nosed bat (*Scotorepens balstoni*, 7 g) at similar T_b (Table 1). However, physiological variables of torpid *Nyctophilus* bats reported in our study are well below values previously reported for the same species (Morris et al. 1994; Hosken and Withers 1999), which has profound implications for energy expenditure and whether they are able to undergo multi-day torpor bouts. Some of the differences between the studies may be explained by regional differences, as long-eared bats studied previously came from areas that are warmer than the Armidale region. However, the minimum TMRs reported (Hosken 1997; Hosken and Withers 1999) were similar to values reported here, and it appears therefore that the minimum TMR rather than the mean values reported in previous studies represent steady-state torpor in *Nyctophilus*. This interpretation is supported by our data on bats measured entirely during the daytime, following the protocol of previous studies, when TMRs were about ten times the mean value of bats measured overnight (Fig. 6). The large differences between bats measured entirely during the day and those measured overnight are likely to be related to the natural thermal cycle of *Nyctophilus* bats in the wild. Free-ranging *Nyctophilus geoffroyi* invariably enter torpor in the night or early morning and often arouse during the middle of the day when T_a rises (Turbill et al. 1999). If torpor is induced around mid-morning it coincides with the time when free-ranging

Table 1 Physiological variables of torpid northern-hemisphere and Australian microbats. Data for species measured overnight or hibernating (T_b body temperature, *TMR* torpid metabolic rate)

Species	Body mass (g)	Min. T_b (°C)	Min. TMR (ml O_2 g^{-1} h^{-1})	Torpor bout length (days)	Source
Northern hemisphere					
Myotis myotis	25	4	0.04	41	Pohl 1961; Kulzer 1965; Harmata 1987
Myotis lucifugus	5.2	1.3	0.02	31	Hock 1951; French 1985; Thomas et al. 1990
Myotis velifer	12		0.07		Riedesel and Williams 1976
Myotis natteri	8	9	0.031		Kulzer 1965; Speakman et al. 1991
Myotis daubentoni	9		0.07		Speakman et al. 1991
Barbastella barbastellus	7		0.04		Pohl 1961
Pipistrellus pipistrellus	7.4	3	0.07		Kulzer 1965; Speakman et al. 1991
Eptesicus fuscus	22	5	0.03	25	Kulzer 1965; French 1985; Szewczak and Jackson 1992
Rhinolophus hipposideros	6			18	Harmata 1987
Australian					
Nyctophilus geoffroyi	7	2.7	0.037	>1	Ellis et al. 1991; present study
Nyctophilus gouldi	10	3	0.052	>1	Present study
Scotorepens balstoni	7	3.2	0.044		Geiser and Brigham, unpublished data
Miniopterus schreibersii	15			12	Hall 1982

bats often show passive rewarming caused by the increased in T_a and arousal. Torpid bats at that time appear to maintain T_b and MR above minimum steady-state levels perhaps because their normal daily thermal cycle has been interrupted. It is also likely that disturbance by handling during the day results in a substantial increase in MR, as has been shown for several bat species (Speakman et al. 1991; Thomas 1995). Thus, the temporal organisation of an animal's daily cycle must be considered if reliable data on steady-state torpor, suitable for comparison with other species and for investigation of mechanisms of MR reduction, are to be obtained.

Our results support the argument that the reduction of MR during torpor in species capable of undergoing prolonged torpor bouts is due to three major factors (Geiser 1988; Guppy and Withers 1999). First, early in entry into torpor, normothermic thermoregulation ceases (Heller et al. 1977), and the MR can fall to approximately BMR (i.e. by about 85–90% at low T_a in our bats) from RMR without lowering T_b (Geiser 1988; Withers 1992; Song et al. 1996).

Second, as the Q_{10} of 8.6 (i.e. a 75% reduction of MR) between BMR and TMR at T_b of 29.3 °C, is far above that typical for biochemical reactions of 2–3 (Schmidt-Nielsen 1997) and for many, especially larger, hibernating species (Geiser 1988; Nicol et al. 1992), it appears that physiological inhibition is involved in lowering the MR (Geiser 1988; Storey and Storey 1990; Malan 1993; Song et al. 1997; Guppy and Withers 1999; Hosken and Withers 1999; Martin et al. 1999). This interpretation is further supported by the observation that the regression of the logarithm of TMR versus T_b intercepts with T_b at BMR 40.5% below the BMR (Fig. 8) suggesting that physiological inhibition contributes to lowering TMR in small species capable of hibernation. However, in vitro evidence suggests that enzyme activity during hibernation is usually reduced by less than 50%

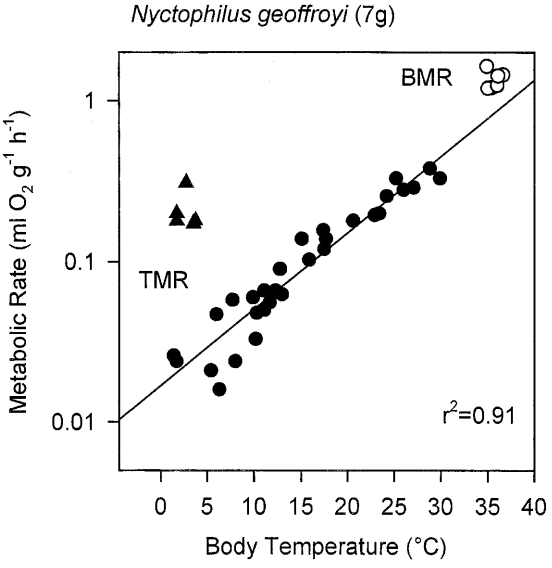

Fig. 8 MRs as a function of T_b torpid (TMR) and normothermic (BMR; *open circles*) *N. geoffroyi*. Each point represents one individual measurement. The linear regression $\log_{10}$ TMR (ml g^{-1} h^{-1}) = $-1.77 + 0.047T_b$ (°C); $r^2 = 0.91$, fitted to the logarithm of MR in thermoconforming torpid bats (*filled circles*) intersected T_b of normothermic bats with BMR at 59.5% BMR. TMR of thermoregulating torpid bats are shown by *filled triangles*

in comparison to active animals (Storey and Storey 1990; Martin et al. 1999), whereas the TMR is as low as 3% of the BMR (i.e. a difference of more than one order of magnitude!).

Thus, the third influence clearly must be the effect of lowering T_b by about 35 °C. As not enough heat is produced for normothermic thermoregulation, because the threshold for T_b is lowered at torpor entry and thermogenesis is inactivated, T_b begins to decline and this fall of T_b in turn should affect TMR. That TMR and T_b are related is emphasised by the 94% prediction

of *T*MR by T_b in thermoconforming torpid bats (Fig. 5), and by the exponential relationship that is characteristic of effects of temperature on rates (Withers 1992; Schmidt-Nielsen 1997; Guppy and Withers 1999) (Fig 5). Overall, the reduction of MR from RMR to the minimum TMR in *N. geoffroyi* is 99.6%. Most of this reduction (85.9%) can be explained by the fall from RMR to BMR, 5.7% is explained by metabolic inhibition (from BMR to the predicted TMR at T_b 35.7 °C; Fig. 8), and 8.0% is explained by the fall in T_b assuming that the Q_{10} of about 3 for TMR (Fig. 8) is explained entirely by temperature effects. If only values below the BMR are considered as in many studies on this subject (summarised in Geiser 1988), the 97.3% reduction in MR can be explained by metabolic inhibition (40.5%) and temperature effects (56.8%) applying the assumptions outlined in the previous sentence.

Although *Nyctophilus* in steady-state torpor show no evidence for physiological thermoregulation over a wide range of T_a, regulation of T_b commenced in most individuals when T_a fell below about 1 °C. Evidence for thermoregulation comes from the increase in the T_b–T_a differential and a substantial increase in TMR (Figs. 3, 4, 8), as in other heterothermic endotherms (Heldmaier and Ruf 1992). Thermoregulatory thresholds during torpor appear to be common to most (all?) heterothermic endotherms (Heller and Hammel 1972; Heller et al. 1977). It appears that extra energy is spent to prevent T_b of torpid animals from reaching temperatures that would cause physiological dysfunction. In hibernating species capable of prolonged torpor, most minimum T_b lie within the range 0–10 °C (Geiser and Ruf 1995). A reduction much below 0 °C is obviously not possible because of the implications of the freezing of body fluids (Barnes 1989). Higher minimum T_b appear to be a reflection of the thermal environment experienced during hibernation (Kulzer 1965). Increased thermoregulatory costs and in turn more frequent arousals from torpor and the associated increase in energy expenditure at T_a below the T_b threshold (Geiser and Kenagy 1988; Ransome 1990) should represent a strong selective pressure to maintain minimum T_b below or near the T_a experienced for most of the hibernation season, especially in species relying to a large extent on stored energy during winter. While this argument may appear plausible from an energetic point of view, why should Australian bats living on a warm continent let T_b fall to such low temperatures just a few degrees above the freezing point of water? Although most of Australia is warm in summer, more than 80% of the continent experiences regular frost in winter, and the average minimum T_a in winter for most of the southern half of Australia is below 6 °C (Colls and Whitaker 1993). As tree roosts provide limited thermal buffering, and tree-dwelling bats usually are torpid when T_a is low, it is likely that Australian bats in many regions regularly experience T_b between 1 °C and 5 °C, as do pygmy possums (*Cercartetus* spp.), honey possums (*Tarsipes rostratus*) and feathertail gliders (*Acrobates pygmaeus*) living in the same habitats (Geiser 1987; Withers et al. 1990; Geiser and Ruf 1995).

Seasonal changes in TMR of *N. geoffroyi* were not pronounced. Spring and summer TMR were almost identical, but there was some indication of a slight decrease in autumn. It is therefore possible that TMR in winter are even lower and that TMR shows some seasonal acclimatisation. Unfortunately, we were unable to find *N. geoffroyi* in the winter of our study, most likely because they were hibernating. However, as our summer data were similar to those obtained in northern hemisphere bats during hibernation in winter, it is likely that TMR show only small seasonal changes and that the known seasonal changes in torpor patterns and bout duration in bats are to a large extent determined by environmental conditions. Clearly, more detailed field data on torpor patterns and environmental variables are needed to put our results on thermal biology and energetics in perspective.

Most *Nyctophilus* investigated here displayed short bouts of torpor and aroused daily after sunset. The abrupt signal of lights off in the laboratory at the beginning of the dark phase appears to be a strong trigger for arousals even at low T_a. However, it is clear from the present study and other laboratory and field investigations that *Nyctophilus* spp. are capable of prolonged torpor and most likely hibernate for part of the winter (Ellis et al. 1991; Brigham and Geiser 1998; Turbill et al. 1999). Prolonged bouts of torpor also conform with the TMR of the bats measured here. TMR in *Nyctophilus* was similar to that of hibernating species and only about 10% of that in species which enter daily torpor exclusively and are unable to undergo prolonged torpor (Geiser and Ruf 1995). It thus appears that although *Nyctophilus* bats may frequently arouse on a daily basis, their short bouts of torpor do not resemble daily torpor, but are, physiologically speaking, short bouts of hibernation. It would be interesting to investigate whether this is also the case for other bats from temperate climates and whether short torpor bouts in microbats from the tropics constitute daily torpor or short hibernation.

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