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## Thermal physiology of pregnant and lactating female and male long-eared bats, *Nyctophilus geoffroyi* and *N. gouldi*

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**Abstract** During roosting in summer, reproductive female bats appear to use torpor less frequently and at higher body temperatures ( $T_b$ ) than male bats, ostensibly to maximise offspring growth. To test whether field observations result from differences in thermal physiology or behavioural thermoregulation during roosting, we measured the thermoregulatory response and energetics of captive pregnant and lactating female and male long-eared bats (*Nyctophilus geoffroyi* 8.9 g and *N. gouldi* 11.5 g) during overnight exposure to a constant ambient temperature ( $T_a$ ) of 15°C. Bats were captured 1–1.5 h after sunset and measurements began at 21:22±0:36 h. All *N. geoffroyi* entered torpor commencing at 23:47±01:01 h. For *N. gouldi*, 10/10 males, 9/10 pregnant females and 7/8 lactating females entered torpor commencing at 01:10±01:40 h. The minimum  $T_b$  of torpid bats was 15.6±1.1°C and torpid metabolic rate (TMR) was reduced to 0.05±0.02 ml O<sub>2</sub> g<sup>-1</sup> h<sup>-1</sup>. Sex or reproductive condition of either species did not affect the timing of entry into torpor ( $F=1.5$ ,  $df=2$ , 19,  $P=0.24$ ), minimum TMR ( $F=0.21$ ,  $df=4$ , 40,  $P=0.93$ ) or minimum  $T_b$  ( $F=0.76$ ,  $df=5$ , 41,  $P=0.58$ ). Moreover, sex or reproductive condition did not affect the allometric relationship between minimum resting metabolic rate and body mass ( $F=1.1$ ,  $df=4$ , 37,  $P=0.37$ ). Our study shows that under identical thermal conditions, thermal physiology of pregnant and lactating female and male bats are indistinguishable. This suggests that the observed reluctance by reproductive females to enter torpor in the field is predominantly because of ecological rather than physiological differences, which reflect the

fact that females roost gregariously whereas male bats typically roost solitarily.

**Keywords** Bats · Energy use · Reproduction · Thermoregulation · Torpor

**Abbreviations** MR: Metabolic rate · RMR: Resting metabolic rate ·  $T_a$ : Ambient temperature ·  $T_b$ : Body temperature · TMR: Torpid metabolic rate · TNZ: Thermoneutral zone

### Introduction

Owing to their small size, insectivorous bats require high metabolic rates (MR) for thermoregulation at ambient temperatures ( $T_a$ ) below their thermoneutral zone (TNZ) (Bradley and Deavers 1980). Thermoregulatory energy expenditure can therefore be costly over their diurnal rest phase (Kurta et al. 1987). This energetic cost is reduced in many bats, however, by entering torpor for part of the roost day during summer and for prolonged periods during winter hibernation (e.g. Park et al. 2000; Turbill et al. 2003a). Torpor results in a reduction in body temperature ( $T_b$ ) to within 1–2°C of  $T_a$  over a wide range of  $T_a$  above the  $T_b$  set-point (~2°C in many temperate bats) and is accompanied by a substantial depression in MR (Geiser 2004). However, while torpor results in large energy savings, the associated low  $T_b$  and depressed MR impede the normal function of some physiological processes. Torpor may be particularly detrimental during reproduction, which usually requires high MR and energy expenditure (Kurta et al. 1987; Kurta et al. 1990; Thompson 1992). Nevertheless, torpor has been observed in a variety of pregnant and lactating mammals (Geiser 1996). In bats, torpor at low  $T_b$  slows or interrupts foetal growth during pregnancy (Racey 1973; Hoying and Kunz 1998) and retards milk production during lactation (Wilde et al. 1999), which can lead to reduced postnatal growth rates (Hoying and Kunz 1998). Torpor may be especially costly for

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reproductive bats inhabiting the temperate zone where juvenile bats must develop quickly and acquire enough body fat over the short warm season to survive winter hibernation (Racey and Swift 1981; Thomas et al. 1990; Kunz et al. 1998).

Field studies have found differences in thermoregulatory behaviour among pregnant and lactating female and male *Eptesicus fuscus* (Audet and Fenton 1988; Hamilton and Barclay 1994; Grinevitch et al. 1995; Lausen and Barclay 2003) and, to a lesser extent, *Myotis lucifugus* (Chruszcz and Barclay 2002). While reproductive female bats regularly enter torpor in summer, they use torpor less frequently and maintain a higher minimum  $T_b$  during torpor than male bats at the same time of the year. In particular, lactating females avoid using deep torpor (a reduction in  $T_b$  by  $>10^\circ\text{C}$ ) in comparison to pregnant or male bats. The apparent difference in torpor use suggests that pregnant and/or lactating bats differ physiologically from males in their thermoregulatory response to low  $T_a$ . Alternatively, thermoregulatory behaviours in the field may reflect differences in roosting ecology and the use of behavioural thermoregulation by females. In summer, male bats typically roost solitarily in poorly insulated roost structures (Kunz and Lumsden 2003; Turbill et al. 2003a), whereas reproductive female bats congregate in maternity colonies and select warm, insulated roost sites (Kerth et al. 2001; Sedgeley 2001; Lourenco and Palmeirim 2004). Maternity roosts can also be heated by up to  $10^\circ\text{C}$  above external  $T_a$  caused by the release of body heat by the colony (Dwyer and Harris 1972; Kunz 1974; Hall 1982). Furthermore, females and their young in maternity colonies are able to reduce their thermal conductance by huddling in a cluster, which can provide a  $\sim 50\%$  reduction in the energetic cost of thermoregulation at  $T_a$  below the TNZ (Kurta 1985; Kurta et al. 1987; Hayes et al. 1992). Hence, reproductive females are likely to experience considerably different  $T_a$  and heat loss during roosting than males, which may also explain apparent differences in their use of torpor.

While it is difficult to accurately measure the  $T_a$  within occupied roosts and thermal conductance of wild bats (e.g. see Kunz 1974), the thermal physiology of male and reproductive female bats can be compared under identical conditions in the laboratory (Studier and O'Farrell 1972; Kurta et al. 1987; Cryan and Wolf 2003). Assuming that captive bats exhibit natural thermoregulatory behaviours (Geiser et al. 2000), the presence or lack of physiological differences can be used to predict the importance of ecological and environmental differences in roosting conditions to the behaviour of wild bats.

To assess whether the observed differences are physiological or ecological, we tested the thermoregulatory response of pregnant and lactating female and male *Nyctophilus geoffroyi* and *N. gouldi* to cold exposure overnight in captivity. In the field, male *N. geoffroyi* and *N. gouldi* roost solitarily under peeling bark or in other poorly insulated tree structures and in summer frequently

enter torpor during the early morning (Turbill et al. 2003a). During summer torpor, minimum  $T_{\text{skin}}$  are often around  $15^\circ\text{C}$  and close to the daily minimum of external  $T_a$ . Pregnant and lactating female *N. geoffroyi* and *N. gouldi* roost in small maternity colonies of 10–20 bats within cavities typically in the trunk of trees (Lumsden et al. 2002). Preliminary data suggest that in the wild, females use torpor less frequently than males and have a higher minimum  $T_b$  during torpor (Turbill 1999).

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## Materials and methods

### Experimental procedures

We used open-flow respirometry to measure the oxygen consumption of pregnant and lactating female and male long-eared bats, *N. geoffroyi* ( $n=23$ ) and *N. gouldi* ( $n=28$ ) during exposure to  $T_a$  of  $15.0 \pm 1.1^\circ\text{C}$  overnight in captivity. This  $T_a$  reflected the minimum  $T_a$  experienced by males at the field site in summer and allowed a decrease of  $T_b$  by  $\sim 20^\circ\text{C}$  during torpor. The study was conducted during the Austral spring/summer from 27 October to 12 December 2003. Bats were captured using mist nets from Imbota Nature Reserve ( $30^\circ 35'\text{S}$ ,  $151^\circ 41'\text{E}$ , 1,000 m elevation), located near Armidale on the Northern Tablelands of New South Wales, Australia, and immediately transported ( $\sim 10$  km) to the laboratory at the University of New England. The climate of the Northern Tablelands is cool-temperate, with an average daily minimum  $T_a$  of  $9.8^\circ\text{C}$  and a maximum of  $24.3^\circ\text{C}$  at Armidale during November. Pregnant bats were identified by gentle palpation of their abdomen for the presence of the skull of a foetus and lactating females by the presence of exposed skin surrounding the nipples and swollen mammary glands visible beneath the skin. Pregnant bats were captured between 27 October and 18 November and lactating bats between 13 November and 18 December. Measurements of adult male bats were distributed evenly throughout the study period.

Most bats were captured within 1.5 h of sunset (time of sunset: 18:12–18:49 h) and measurements of bats in respirometry chambers commenced at  $21:22 \pm 0:36$  h and not later than 22:48 h. Measurements continued overnight and bats were removed from respirometry chambers the next morning between 08:00 and 09:30 h (average duration of measurements:  $11:18 \pm 0:41$  h). Bats were exposed to the natural photoperiod during measurements using a shaded, 15 W incandescent light-globe. The  $T_b$  of bats was measured ( $\pm 0.1^\circ\text{C}$ ) using a digital thermometer (Omega, Stamford, USA) immediately after removing them from the respirometry chambers (within 20 s) by inserting a fine calibrated thermocouple  $\sim 1$  cm into the rectum. Bats were weighed prior to and after the period of measurements, and a linear rate of mass loss was assumed for calculation of mass-specific MR. To identify recaptures, bats were temporarily marked by clipping a small patch of mid-dorsal fur. Bats were then placed in cloth bags for the

rest of the day and released after dark at their capture site.

Measurements for several lactating bats that were captured early in the lactating period were of a shorter duration. These bats had given birth recently and their young were presumed to have been at risk of dehydration if left alone in their tree cavity roost over a 24-h period. Thus, measurements of females in early lactation ( $n=7$ , *N. gouldi*;  $n=1$ , *N. geoffroyi*) were completed at  $3:30 \pm 0:30$  h and lasted  $6:20 \pm 0:27$  h. This allowed release at their capture site prior to dawn (time of sunrise: 4:45–05:03 h).

## Respirometry

The MR of bats was measured as the rate of oxygen consumption using open-flow respirometry. Oxygen consumption of bats was measured using a single channel oxygen analyser (FOX, Sable Systems International Inc., Las Vegas, USA). The analyser was insulated within a foam and cardboard box, and located in a temperature-controlled room ( $\pm 2^\circ\text{C}$ ). A sub-sampling design was employed so that the rate of flow of the sample air through the analyser remained constant ( $\sim 125 \text{ ml min}^{-1}$ ) throughout measurements.

Bats were placed in respirometry chambers (diameter 45 mm, length 100 mm, volume 0.140 l) made from cylindrical, clear Perspex tubes lined internally with plastic mesh and sealed at each end using rubber stoppers containing an inlet and outlet for air. These were hung vertically inside a temperature-controlled cabinet. A thermocouple was inserted 5 mm into the chamber and covered by a plastic lid measured  $T_a$  to the nearest  $0.1^\circ\text{C}$ .

Outside air was pumped through Silica gel to remove moisture and rotameters (Aarlborg 7908, New York, USA) controlled the rate of airflow to the chambers. After passing through the chambers, excurrent air was again dried using Silica gel and the rate of airflow was measured using a mass flow meter (Omega FMA-5606, Stamford, USA). A flow rate of  $300 \text{ ml min}^{-1}$  was used throughout, which was sufficient to maintain the oxygen content in the excurrent air above 20%. The excurrent air from the three chambers, along with a reference channel of dried outside air, were sub-sampled in sequence every 3 min using solenoid valves controlled by a computer. Thus, a measurement for each bat was obtained every 12 min. Data from the digital output of the oxygen analyser were used for all measurements and transferred to a computer using a serial connection. The millivolt outputs from the flow meter and the thermocouples and analogue output from the oxygen analyser were transferred to a computer via a 14 bit A/D converter. The analogue data from the analyser were used to calibrate the A/D converter by comparison to the digital data before each day of measurements.

Oxygen consumption of bats was calculated using Eq. 3a of Withers (1977). An RQ of 0.85 was assumed

for all measurements, which may have resulted in a maximum error of  $\pm 3\%$ , if the RQ was actually 0.7 or 1.0 (Withers 1977). Bats were considered to have entered torpor when their oxygen consumption was reduced below the published basal MR  $-1\text{SD}$  for each species (Geiser and Brigham 2000). Torpor bouts were clearly demarcated by a distinct and sustained reduction in MR. Times of arousal were recorded as the first measurement of oxygen consumption above basal rates  $+1\text{SD}$ . Minimum resting metabolic rates (RMR) and torpid metabolic rates (TMR) were calculated for each individual from the average of three relatively stable consecutive measurements (i.e. over 36 min). Minimum RMR values were not obtained from ten bats because they did not display stable resting values before entering torpor. Average rate of energy expenditure ( $\text{kJ h}^{-1}$ ) of bats was calculated by integrating the whole bat MR over the measurement period. Data from lactating female bats released prior to dawn were excluded from these calculations, as measurements did not occur over the same time period and were not comparable to other bats.

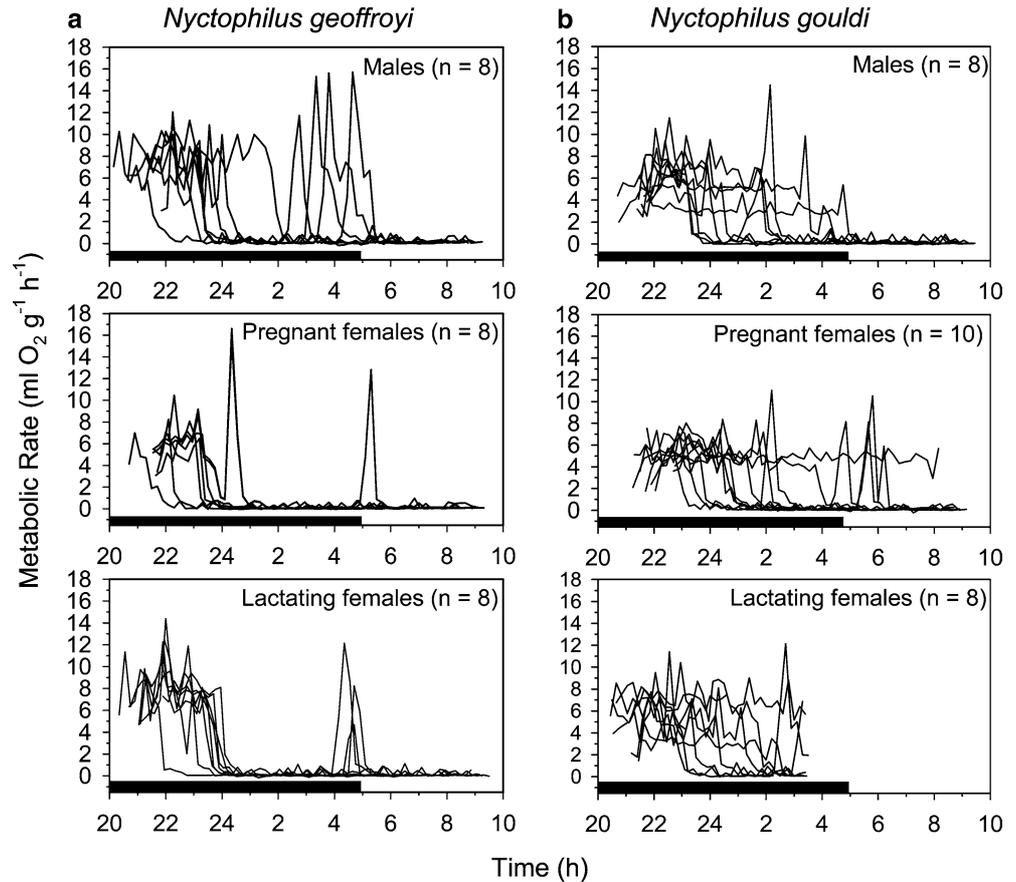
Statistical analyses were carried out using Minitab Statistical Software V13 to compare variables between the species and among sex/reproductive classes within each species. Student's *t* test, ANOVA and general linear modelling (GLM) analyses were used to test for significant differences ( $P < 0.05$ ) among groups.

## Results

During exposure to  $T_a$  of  $15^\circ\text{C}$  overnight, all male and all pregnant and lactating female *N. geoffroyi* entered torpor (Fig. 1a). In *N. gouldi*, all males and 9/10 pregnant and 7/8 lactating females exhibited torpor (Fig. 1b). As lactating female *N. gouldi* were removed from respirometry chambers early at  $\sim 3:30$  h, it is possible that the single normothermic lactating bat may have entered torpor had it remained in the respirometer for longer because another lactating *N. gouldi* entered torpor at 02:36 h, within 30 min of the end of measurements.

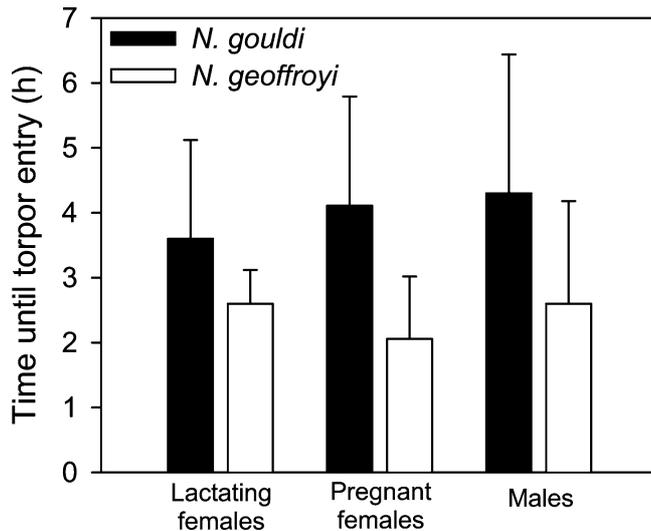
The time from when bats were placed into chambers until torpor entry did not differ significantly among sex/reproductive classes within each species (*N. geoffroyi*:  $F=1.5$ ,  $df=2$ , 19,  $P=0.24$ ; *N. gouldi*:  $F=1.1$ ,  $df=2$ , 21,  $P=0.34$ ; Fig. 2). However, relative to the start of measurements, *N. geoffroyi* entered torpor significantly earlier (after  $2:31 \pm 0:55$  h) than *N. gouldi* ( $03:41 \pm 01:42$  h) ( $T=3.0$ ,  $df=36$ ,  $P<0.01$ ; Fig. 2). Hence, the average time of torpor entry was  $23:47 \pm 01:01$  h for *N. geoffroyi* and  $01:10 \pm 01:40$  h for *N. gouldi*. After entering torpor, 11/49 (22%) bats aroused once during the night at  $03:52 \pm 01:20$  h and were normothermic for  $0:58 \pm 0:07$  h before reentering torpor. Arousals occurred in at least one bat from each sex/reproductive class in both species. The proportion of the overall time of measurements spent in torpor (excluding data for lactating bats that were removed early from chambers) was significantly

**Fig. 1** Metabolic rates of male, pregnant female and lactating female *Nyctophilus geoffroyi* (a) and *N. gouldi* (b) during exposure to  $T_a$  of 15°C overnight in captivity. Dark horizontal bars show time of lights off (natural photoperiod)



greater for *N. geoffroyi* ( $78 \pm 10\%$ ) than for *N. gouldi* ( $67 \pm 15\%$ ;  $T = 2.5$ ,  $df = 29$ ,  $P < 0.05$ ). The proportion of time spent in torpor did not differ among male, pregnant

female and lactating female *N. geoffroyi* ( $F = 1.02$ ,  $df = 2$ ,  $19$ ,  $P = 0.38$ ) or between male and pregnant female *N. gouldi* ( $T = 1.09$ ,  $df = 14$ ,  $P = 0.30$ ).



**Fig. 2** Time from commencement of measurements until torpor entry by *N. geoffroyi* and *N. gouldi*. Two bats, a pregnant and lactating female *N. gouldi*, did not enter torpor. Torpor entry occurred significantly earlier in *N. geoffroyi* than in *N. gouldi* ( $T = 3.0$ ,  $df = 36$ ,  $P < 0.01$ ), but did not differ significantly among males, pregnant females and lactating females within each species (*N. geoffroyi*:  $F = 1.5$ ,  $df = 2$ ,  $19$ ,  $P = 0.24$ ; *N. gouldi*:  $F = 1.1$ ,  $df = 2$ ,  $21$ ,  $P = 0.34$ )

The minimum  $T_b$  of all bats that entered torpor was  $15.6 \pm 1.1^\circ\text{C}$  ( $n = 45$ ), which was  $0.6 \pm 0.6^\circ\text{C}$  above  $T_a$ . The minimum  $T_b$  in torpor did not differ significantly between species ( $T = 0.6$ ,  $df = 44$ ,  $P = 0.6$ ) or among sex/reproductive classes within each species ( $F = 0.76$ ,  $df = 5$ ,  $41$ ,  $P = 0.58$ ). The  $T_b$  of the two *N. gouldi* that remained normothermic were  $36.3^\circ\text{C}$  (pregnant) and  $31.1^\circ\text{C}$  (lactating).

The body mass of bats differed significantly between species (*N. geoffroyi*:  $8.9 \pm 1.5$  g, *N. gouldi*:  $11.5 \pm 2.1$  g;  $T = 5.2$ ,  $df = 47$ ,  $P < 0.001$ ) and among sex/reproductive classes within each species (Table 1; *N. geoffroyi*:  $F = 35.9$ ,  $df = 2$ ,  $20$ ,  $P < 0.001$ ; *N. gouldi*:  $F = 62.9$ ,  $df = 2$ ,  $25$ ,  $P < 0.001$ ), with pregnant females having the greatest mass and males the least mass.

Owing to the expected allometric relationship between body mass and MR, GLM using  $\log_{10}$  body mass as a covariate were fitted to the data to examine differences in  $\log_{10}$  RMR and  $\log_{10}$  TMR between species and among male, pregnant and lactating female bats within species (Table 1). We found no significant effect of sex/reproductive condition on RMR at  $T_a$  15°C ( $F = 1.11$ ,  $df = 4$ ,  $37$ ,  $P = 0.37$ ) and the relationship between RMR and body mass (i.e. slope of the line) was the same for both species ( $F = 0.17$ ,  $df = 1$ ,  $35$ ,  $P = 0.68$ ). However, the elevation ( $y$  intercept) of this relationship differed sig-

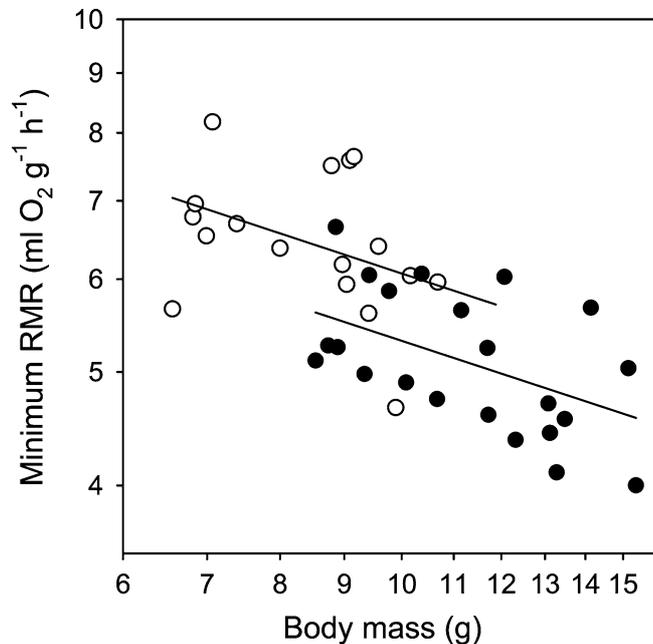
**Table 1** Body mass, minimum torpid metabolic rate (TMR) and minimum resting metabolic rate (RMR) of male, pregnant female and lactating female *Nyctophilus geoffroyi* and *N. gouldi* measured during exposure to  $T_a$  of 15°C overnight in captivity

Species	Sex/condition	Mass (g)	Minimum TMR (ml O <sub>2</sub> g <sup>-1</sup> h <sup>-1</sup> )	Minimum RMR (ml O <sub>2</sub> g <sup>-1</sup> h <sup>-1</sup> )
<i>N. geoffroyi</i>	Lactating	9.0 ± 0.2 (8)	0.05 ± 0.01 (8)	6.74 ± 0.93 (6)
	Pregnant	10.3 ± 1.2 (8)	0.05 ± 0.02 (8)	5.77 ± 0.76 (4)
	Male	7.1 ± 0.5 (7)	0.08 ± 0.05 (7)	6.74 ± 0.76 (7)
	Combined	8.9 ± 1.5 (23)	0.06 ± 0.02 (23)	6.52 ± 0.88 (17)
<i>N. gouldi</i>	Lactating	11.2 ± 0.7 (8)	0.05 ± 0.02 (5)	5.40 ± 1.01 (6)
	Pregnant	13.8 ± 1.1 (10)	0.05 ± 0.01 (7)	4.64 ± 0.53 (8)
	Male	9.3 ± 0.7 (10)	0.05 ± 0.01 (10)	5.51 ± 1.08 (8)
	Combined	11.5 ± 2.1 (28)	0.05 ± 0.01 (23)	5.15 ± 0.70 (22)

Numbers represent means ± SD for number of individuals in brackets. Bats were exposed to  $T_a$  of 15°C overnight

nificantly between the species ( $F=6.3$ ,  $df=1$ , 36,  $P<0.05$ ), which resulted in parallel fitted lines for each species that were significantly related to body mass ( $F=9.2$ ,  $df=1$ , 36,  $P<0.01$ ;  $R^2=0.55$ ; Fig. 3).

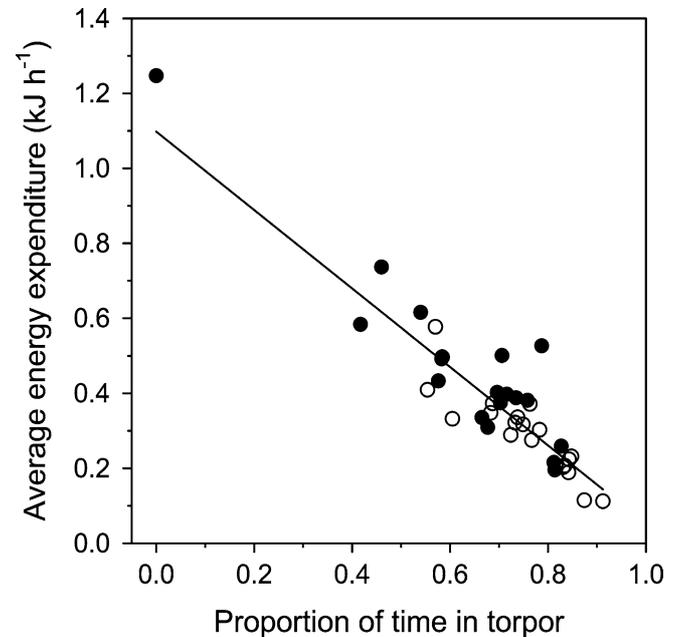
Notwithstanding the negative relationship observed between RMR and body mass, the mean RMR of *N. geoffroyi* ( $6.52 \pm 0.9$  ml O<sub>2</sub> g<sup>-1</sup> h<sup>-1</sup>) was significantly greater than *N. gouldi* ( $5.15 \pm 1.7$  ml O<sub>2</sub> g<sup>-1</sup> h<sup>-1</sup>;  $T=4.9$ ,  $df=24$ ,  $P<0.001$ ; Table 1). For *N. gouldi* (but not for *N. geoffroyi*), mean RMR was significantly less for pregnant females than other sex/reproductive classes ( $F=4.6$ ,  $df=2$ , 19,  $P<0.05$ ).



**Fig. 3** Minimum ( $\log_{10}$ ) resting metabolic rates (RMR) of *N. geoffroyi* (open symbols) and *N. gouldi* (filled symbols) exposed to  $T_a$  of 15°C as a function of ( $\log_{10}$ ) body mass. General linear modeling (GLM) analyses revealed no significant effect of sex/reproductive condition on RMR at 15°C ( $F=1.11$ ,  $df=4$ , 37,  $P=0.37$ ) and resulted in parallel fitted lines for each species that were significantly related to body mass ( $F=9.2$ ,  $df=1$ , 36,  $P<0.01$ ; *N. geoffroyi*:  $\log_{10}$  RMR (ml O<sub>2</sub> g<sup>-1</sup> h<sup>-1</sup>) =  $1.137 - 0.354 \times \log_{10}$  mass (g), *N. gouldi*:  $\log_{10}$  RMR (ml O<sub>2</sub> g<sup>-1</sup> h<sup>-1</sup>) =  $1.080 - 0.354 \times \log_{10}$  mass (g); combined  $R^2=0.55$ )

TMR was not significantly affected by sex/reproductive condition ( $F=0.21$ ,  $df=4$ , 40,  $P=0.93$ ) or species ( $F=0.08$ ,  $df=1$ , 44,  $P=0.78$ ; Table 1). Furthermore, TMR was not significantly related to body mass ( $F=2.85$ ,  $df=1$ , 44,  $P=0.10$ ). The mean minimum TMR at  $T_a$  of 15°C was  $0.059 \pm 0.02$  ml O<sub>2</sub> g<sup>-1</sup> h<sup>-1</sup> for *N. geoffroyi* and  $0.050 \pm 0.01$  ml O<sub>2</sub> g<sup>-1</sup> h<sup>-1</sup> for *N. gouldi* and means did not differ significantly between the species ( $T=1.3$ ,  $df=27$ ,  $P=0.20$ ) or among sex/reproductive classes within *N. geoffroyi* ( $F=1.7$ ,  $df=2$ , 20,  $P=0.22$ ) or *N. gouldi* ( $F=0.95$ ,  $df=2$ , 21,  $P=0.40$ ; Table 1).

As expected, the average rate of energy expenditure (kJ h<sup>-1</sup>) over the measurement period was closely related to the proportion of time spent in torpor ( $F=124$ ,



**Fig. 4** Average rate of energy expenditure (kJ h<sup>-1</sup>) of *N. geoffroyi* (open symbols) and *N. gouldi* (filled symbols) calculated over the measurement period (~11.3 h). As expected, average energy expenditure was closely related to the proportion of time spent in torpor ( $F=127$ ,  $df=1$ , 37,  $P<0.001$ ; average energy expenditure (kJ h<sup>-1</sup>) =  $1.10 - 1.04 \times$ percentage of time in torpor;  $R^2=0.81$ ) and this relationship did not differ significantly between the species ( $F=1.2$ ,  $df=1$ , 37,  $P=0.17$ )

$df=1, 37, P<0.001, R^2=0.81$ ; Fig. 4). This relationship did not differ significantly between the two species ( $F=1.2, df=1, 37, P=0.17$ ). Total energy expenditure varied from 1.3 kJ for a male *N. geoffroyi* that spent 87% of the measurement time ( $\sim 11.3$  h) in torpor to 15.2 kJ for a pregnant *N. gouldi* that did not enter torpor.

## Discussion

Our study shows that sex and reproductive condition have no apparent effect on the thermoregulatory behaviour, energetics and thermal variables of *N. geoffroyi* or *N. gouldi* during exposure to identical cold conditions overnight in captivity. Pregnant and lactating female and male bats within each species responded similarly to cold by entering torpor during the night, as observed in solitary roosting bats in the wild (Turbill et al. 2003a). Moreover, because almost all bats entered torpor, possible nutritional differences at the beginning of measurements did not have an apparent effect on the variables measured. Thus, our results differ from previous studies and suggest that avoidance of torpor by female reproductive bats in the field is unlikely to be a consequence of difference in thermal physiology, but probably results from selection of warm roost microclimates and communal roosting during the maternity season.

Our results, showing no difference in thermoregulatory response between reproductive female and male *N. geoffroyi* and *N. gouldi*, are in clear contrast with the dichotomous response between pregnant female and male captive hoary bats, *Lasiurus cinereus*, found by Cryan and Wolf (2003). These differences may be explained to some extent by differences in experimental procedures between the studies. In our study, measurement of captured bats commenced during the night and usually lasted until mid-morning of the next day. Hence, experimental runs lasted  $>11$  h and included the early morning when free-ranging bats most often enter torpor naturally (Turbill et al. 2003a). In contrast, measurements of *L. cinereus* took place between 08:00 and 16:00 h and experimental runs lasted only 2–4 h (Cryan and Wolf 2003). As 2–4 h are close to the average time prior to torpor entry by *Nyctophilus* (7–15 g) species in our study, it seems possible, therefore, that the much larger *L. cinereus* (25–35 g) did not display their natural thermal response to low  $T_a$  because of the short duration of measurements. Moreover, measurements of captive *L. cinereus* were restricted to the daytime, after the time bats commonly enter their morning torpor bout. The possibility that short-term laboratory data were not representative is further supported by data showing that pregnant female *L. cinereus* use torpor frequently while roosting under natural conditions in the wild (Hickey and Fenton 1996; Willis 2005).

Nevertheless, the experimental approach by Cryan and Wolf (2003) revealed a marked difference in thermoregulatory response between male and pregnant female bats. A strong physiological difference between

the sexes in *L. cinereus* may be required as both male and reproductive female bats roost solitarily in open foliage. Hence, pregnant and lactating female *L. cinereus* cannot gain the thermal benefits from roosting in colonies within insulated structures that are available to most other temperate zone bats. While *L. cinereus* are selective for roosting sites in foliage that provide a relatively warm microclimate (Willis and Brigham 2005), reproductive females probably experience similar roost  $T_a$  to male bats. Consequently, any differences in their natural thermoregulatory behaviours are more likely a reflection of physiological differences than for other temperate zone bat species, which have greater scope for selective behavioural means of reducing thermoregulatory costs while roosting.

In addition, the contrasting results may reflect the greater thermoregulatory costs of *N. geoffroyi* and *N. gouldi*, which are around half to one-third of the body mass of *L. cinereus*. Thermal conductance increases logarithmically with decreasing body mass in mammals (Bradley and Deavers 1980). In addition, *L. cinereus* have a particularly dense pelage that may further reduce their thermal conductance (Shump and Shump 1980; Cryan and Wolf 2003; Willis and Brigham 2005). Hence, mass-specific RMR of *N. geoffroyi* and *N. gouldi* at  $T_a$  of 15°C was approximately 1.4 times greater than in *L. cinereus* (Cryan and Wolf 2003). For reproductive female *N. geoffroyi* and *N. gouldi*, the energetic cost of defending a constant, high  $T_b$  during exposure to  $T_a$  of 15°C overnight evidently outweighed the benefits to sustaining foetal growth or milk production. For example, by employing torpor for an average of 78% of the 11.3 h measurement period, *N. geoffroyi* reduced their energy expenditure by approximately 75% from 13.0 to 3.5 kJ.

The potential detrimental costs of torpor during reproduction are often emphasised, whereas the benefits from energy savings to the fitness of mother and young are possibly underestimated, especially in very small endotherms such as bats. Energy expenditure increases during pregnancy to support foetal growth and further increases to reach highest levels during lactation (Racey and Speakman 1987; Kurta et al. 1989, 1990; Thompson 1992). Thus the use of torpor to reduce thermoregulatory energy expenditure may be an important adaptation of small endotherms to maintain a positive energy balance during reproduction. Indeed, McLean and Speakman (1999) found that the daily energy expenditure of lactating female bats, *Plecotus auritus* ( $\sim 8$  g) held in outdoor aviaries was similar to non-reproductive females, indicating that they used energy-saving mechanisms such as torpor to compensate for the increased energy required for lactation. Torpor may be especially important during sporadic periods of cold or wet weather, when foraging opportunities are reduced and thermoregulatory costs are high (Hickey and Fenton 1996; Körtner and Geiser 2000). Maternity colonies of tree-roosting bats frequently switch between roosts, during which groups of females may sometimes roost as individuals or in smaller sub-groups (Kerth and König

1999; Kunz and Lumsden 2003; C. Turbill, unpublished data). Hence, torpor may be used to compensate for increased thermoregulatory costs at times when reproductive females are roosting solitarily or in smaller groups. Using a model of thermoregulatory costs, Cryan and Wolf (2003) calculated that the energy expenditure of *L. cinereus* at  $T_a$  of 10°C could be reduced by ~60% from a torpor bout lasting only 2 h. Most bats are smaller than *L. cinereus* (Smith et al. 2003) and therefore gain even larger energy savings from torpor. Moreover, short torpor bouts during part of the roost day are likely to have minimal impact on the duration of pregnancy or on daily milk production. Thus, the ability to enter torpor at low  $T_a$  could provide energy savings that result in an overall increase in the fitness of reproductive bats.

Our experimental results support field observations that patterns of torpor use in summer reflect differences in roosting behaviour and roost  $T_a$  between male and reproductive female bats rather than physiological differences. Solitary roosting male bats typically enter torpor on most days in summer (Hamilton and Barclay 1994; Grinevitch et al. 1995; Turbill et al. 2003a, b). The roosts of male bats are often poorly insulated and as a result minimum  $T_b$  of torpid bats is often close to external  $T_a$  in the early morning. However, by exposure to the daily  $T_a$  cycle, male bats can take advantage of passive rewarming from torpor during the day (Turbill et al. 2003a, b; Geiser et al. 2004). In contrast, reproductive female bats, particularly during lactation, use torpor less frequently and minimum  $T_b$  in torpor is usually considerably above external  $T_a$  (Audet and Fenton 1988; Hamilton and Barclay 1994; Grinevitch et al. 1995; Chruszcz and Barclay 2002; Lausen and Barclay 2003). Thermoregulatory costs of females in communal roosts can be reduced substantially owing to decreased heat loss from huddling and an increase in  $T_a$  within the confined roost space (Dwyer and Harris 1972; Kurta 1985; Roverud and Chappell 1991; Hayes et al. 1992; Brown 1999; McKechnie and Lovegrove 2001). In addition, maternity colonies preferentially select a warm and well-insulated roost microclimate, particularly during lactation (Kerth et al. 2001; Sedgely 2001; Chruszcz and Barclay 2002). During exposure to identical thermal conditions in captivity, we found that sex or reproductive condition had no apparent effect on the thermoregulatory behaviour of *N. geoffroyi* and *N. gouldi*. Thus, our study suggests that differences in the use of torpor between male and reproductive female bats in the wild are not physiological, but a consequence of communal roosting by female bats who benefit from behavioural thermoregulation and experience a warmer, more stable roost  $T_a$  than male bats during summer.

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