Journal of Thermal Biology 60 (2016) 162–170

Contents lists available at ScienceDirect

Journal of Thermal Biology

journal homepage: www.elsevier.com/locate/jtherbio

Black or white? Physiological implications of roost colour and choice in a microbat

Anna C. Doty*, Clare Stawski, Shannon E. Currie, Fritz Geiser

Centre for Behavioural and Physiological Ecology, Zoology, University of New England, Armidale, NSW 2351, New England

A R T I C L E I N F O

Article history:
Received 19 April 2016
Accepted 15 July 2016
Available online 17 July 2016

A B S T R A C T

Although roost choice in bats has been studied previously, little is known about how opposing roost colours affect the expression of torpor quantitatively. We quantified roost selection and thermoregulation in a captive Australian insectivorous bat, *Nyctophilus gouldi* (n=12) in winter when roosting in black and white coloured boxes using temperature-telemetry. We quantified how roost choice influences torpor expression when food was provided *ad libitum* or restricted in bats housed together in an outdoor aviary exposed to natural fluctuations of ambient temperature. Black box temperatures averaged 5.1 °C (maximum 7.5 °C) warmer than white boxes at their maximum daytime temperature. Bats fed *ad libitum* chose black boxes on most nights (92.9%) and on 100% of nights when food-restricted. All bats used torpor on all study days. However, bats fed *ad libitum* and roosting in black boxes used shorter torpor and spent more time normothermic/active at night than food-restricted bats and bats roosting in white boxes. Bats roosting in black boxes also rewarmed passively more often and to a higher skin temperature than those in white boxes. Our study suggests that *N. gouldi* fed *ad libitum* select warmer roosts in order to passively rewarm to a higher skin temperature and thus save energy required for active midday rewarming as well as to maintain a normothermic body temperature for longer periods at night. This study shows that colour should be considered when deploying bat boxes; black boxes are preferable for those bats that use passive rewarming, even in winter when food availability is reduced.

1. Introduction

Roost choice is particularly important to small insectivorous bats as they have a large surface area to volume ratio and therefore experience high heat loss at low ambient temperatures (T_a). To minimize energy loss, many insectivorous bats use torpor, an effective energy-saving strategy characterized by a marked reduction in metabolic rate (MR) and body temperature (T_b) (Hock, 1951; Geiser, 2013; Stawski et al., 2014; Ruf and Geiser, 2015). Heterothermic mammals use torpor for a variety of reasons (Geiser and Brigham, 2012), including decreases in available resources (Buffenstein, 1985; Speakman and Racey, 1989; Song and Geiser, 1997; Coburn and Geiser, 1998), to deal with seasonal variations in T_a (Dietz and Kalko, 2006; Stawski and Geiser, 2010), and even to permit reproduction in adverse conditions (Grinevitch et al., 1995; Willis et al., 2006; Stawski, 2010; McAllan and Geiser, 2014).

Although torpor can reduce energy expenditure by up to 99% from that of normothermic values (Geiser and Stawski, 2011), at the end of a torpor bout animals must rewarm to normothermic T_b and the energetic cost of this process, as well as thermoregulation at normothermic T_b, increases with decreasing T_a (Ruf and Geiser, 2015). Thus, the microclimate of a roost can greatly impact the energy expenditure of bats not only at rest and during torpor, but also during the rewarming process.

Bats are known to inhabit a wide range of roost types, such as caves, tree hollows, peeling bark, or leaves to suit varying thermoregulatory, reproductive, and ecological needs (Vonhof and Barclay, 1996; Callahan et al., 1997; Chruszcz and Barclay, 2002; Stawski et al., 2014). For example, the opportunity to roost with other bats appears to be the limiting factor in roost choice for reproductive, cavity-roosting bats (Willis and Brigham, 2007), while hollow entrance size is an important determinant for many Australian tree-roosting species (Goldingay, 2009). Roost choice has direct effects on torpor expression in Rafinesque's big-eared bats (*Corynorhinus rafinesquii*), significantly affecting the number of torpor bouts used per day (Johnson and Lacki, 2013). Therefore the wide range of roost types used by different species of insectivorous bats and other small endotherms can greatly influence an individual's daily energy budget and use of torpor due to their

---

Abbreviations: T_b, Body Temperature; T_{skin}, Skin Temperature; T_a, Ambient Temperature; T_{box}, Box Temperature; DEE, Daily Energy Expenditure; TMR, Torpor Metabolic Rate; RMR, Resting Metabolic Rate; MR, Metabolic Rate; TBD, Torpor Bout Duration

* Corresponding author.
E-mail address: annacdoty@gmail.com (A.C. Doty).

http://dx.doi.org/10.1016/j.jtherbio.2016.07.015
0306-4565/

differing thermal properties (Stawski et al., 2008; Willis et al., 2008; Doucette et al., 2011). Torpor can also vary amongst individuals based on habitat characteristics and resource availability (Encarnação et al., 2012).

Differences in thickness of bark (Nicolai, 1986), tree colour and the position of the sun are all important considerations for tree-roosting bats when deciding where to roost. Some bats prefer more thermally unstable microclimates while others select for cool and/or well-buffered microclimates depending on sex, season, food availability or reproductive status (Law, 1993; Turbill, 2006; Boyles, et al. 2007; Stawski et al., 2008). A cool roost may save bats more energy during periods of deep or prolonged torpor, whereas a warm roost will be beneficial when bats must remain normothermic. Bats are known to choose a cooler and more stable T_a during winter when food availability is low in order to expend comparatively less energy than at high T_a (Speakman and Rowland, 1999). A thermally unstable roost may be cooler at night and in the morning, but as T_a increases animals could take advantage of an increasing roost temperature to passively rewarm to normothermia from torpor (Turbill et al., 2003) and save energy during the rewarming process.

Bat boxes are a commonly used method for providing cost-effective, easily maintained roosting habitat for bats. Boxes are often introduced, for example, into urban areas where roosting trees are compromised or rare, to provide roosts for displaced colonies, to facilitate roosting and thus foraging in crop areas for pest control, or in natural areas that have experienced wildfires, tree thinning, or other situations which have resulted in elimination of bat roosts (Brittingham and Williams, 2000; Smith and Agnew, 2002; Flaquer et al., 2006). However, bat boxes are often painted without consideration to colour, which could dramatically alter the internal temperature of the box/microclimate, and in turn greatly affect the physiology and, importantly, consequent energy use by bats. Some bats from the Northern Hemisphere select dark-coloured roost boxes over lighter ones when given the option (Kerth et al., 2001; Lourenço and Palmeirim, 2004). However these studies were conducted in warm and/or mild climates and thus the physiological implications of roost colour in winter remains unclear. During winter, bats may be more inclined to reduce MR and T_b to minimal levels and thus may not prefer dark boxes when food availability is low. To our knowledge, how roost colour choice may benefit bats in terms of their energy use and savings has not been quantified. It has been suggested that some bats may select dark roosts in autumn/winter, such as burnt trees in wildfire-affected areas (pers. observ; Doty et al., 2016), but the physiological reasons for doing so are uncertain.

The species used in our investigation, Gould's long-eared bat (*Nyctophilus gouldi*), is a small (~10 g) vespertilionid bat that hibernates in south-eastern Australia, and often expresses short bouts of torpor even during spring and summer (Geiser and Brigham, 2000; Turbill, 2006). The torpor metabolic rate (TMR) of these bats can be reduced to an average of 0.06 ± 0.04 ml O_2 g^{-1} h^{-1} at T_a 7.0 ± 0.1 °C, which is only 0.58% of the resting metabolic rate (RMR) at a minimum average T_a of 5.9 °C (Currie et al., 2014). *Nyctophilus gouldi* roost under bark, in hollows or cracks of trees (Lumsden et al., 2002; Churchill, 2009; Webala et al., 2010) and orientate themselves in the roost towards the sun (Turbill et al., 2003), effectively exposing themselves to maximum daytime temperatures.

To gain a better understanding as to why bats actively choose dark roost boxes and if bats from the Southern Hemisphere maintain similar roost choice patterns as those from the Northern Hemisphere, we measured the thermal properties of opposing bat box colours, black and white. We also determined the roost preference and skin temperature (T_{skin}) of non-reproductive *N. gouldi* to quantify any physiological differences when roosting in boxes with potentially different thermal properties. We tested four hypotheses: i) black bat boxes will be warmer than white bat boxes, ii) when fed *ad libitum*, bats will prefer to roost in black boxes in winter because they will save energy whilst rewarming from torpor, and normothermic bats will require less thermoregulatory heat production due to a higher box temperature, iii) when food-restricted, bats will prefer to roost in white boxes in winter in order to reach deeper torpor and save more energy, and iv) box colour choice during the day will be an influencing factor for time spent normothermic at night.

2. Materials and methods

2.1. Experimental protocol

Roosting behaviour and physiological correlates of torpor use in *N. gouldi* were quantified during the Austral winter at the University of New England in Armidale (30°30′S 151°39′E) in NSW Australia, a cool-temperate area surrounded by grazing land and open eucalypt forest. Bats were captured in nearby forest using harp traps (© Faunatech Austbat, Australia) and mist nets (© Ecotone, Poland). They were housed in an outdoor aviary for 12 days and (i) offered mealworms (*Tenebrio molitor* larvae) *ad libitum* and water *ad libitum* for the entire duration of Treatment 1 (June 2014) or (ii) food-restricted (water *ad libitum* for the entire treatment and mealworms *ad libitum* only on days 0, 4 and 8) during Treatment 2 (July 2015).

We report data from seven *N. gouldi* in 2014 (Treatment 1; 4 females body mass 10.5 ± 1.3, 3 males body mass 10.1 ± 0.3; mean body mass for both sexes 10.3 ± 1.0 g) and five *N. gouldi* in 2015 (Treatment 2; 5 males; body mass 10.0 ± 0.7 g). The following methods are identical for both treatments. To measure T_{skin}, temperature-sensitive radio-transmitters (~0.5 g, LB-2NT, Holohil Systems Inc., Carp, Ontario, Canada) were glued using a latex adhesive (B-520; Factor2; Lakeside, Arizona) to the mid-dorsal skin region after removing a patch of fur. Before attachment, transmitters were calibrated in a water bath between 5.0 °C and 40.0 °C using a precision thermometer (0.1 °C resolution); pulse rate was regressed against transmitter temperature ($r^2 > 0.99$) to derive T_{skin} of bats.

After transmitter attachment, bats were released in an aviary ($2 \times 2 \times 5$ m) on campus. Bats were released together to encourage natural roosting behaviours, allowing them to roost communally or solitarily. Six single-chambered plywood boxes (3 Black, 3 White; $25 \times 45 \times 7$ cm) were painted with acrylic paint (White Knight, Villawood, NSW), dried and hung alternating in colour on the wall of the aviary and were exposed to as much direct sunlight as possible throughout the day as dictated by the layout of the aviary, thus facing in a north-northeast orientation. The boxes were therefore exposed to natural fluctuations in T_a and could warm or cool based on T_a and the position of the sun. Weather during both experimental periods was mostly sunny and without rain. To measure box temperature (T_{box}), a temperature data logger (±0.5 °C, DS1921G, iButton Thermochron, Maxim Integrated Products Inc., Sunnyvale, CA, USA) was fitted at the top of each box and covered in mesh to prevent interference from direct contact with bats. External T_a (ambient air temperature outside of the roost) was measured using a temperature data logger (±0.1 °C, DS1922L, iButton Thermochron, Maxim Integrated Products Inc., Sunnyvale, CA, USA) placed in the aviary and in the shade, shielded from solar radiation. The T_{skin} was recorded in 5-min intervals using a multichannel remote receiver/logger and antenna (Körtner and Geiser, 2000) positioned near the aviary. Data from the receiver/logger were downloaded to a laptop computer at the same time each day. Each morning at 08:00 h, the location of the bats in boxes was determined by a receiver (IC-R10; ICOM; OSK, Japan). No disturbance via a rise in T_{skin} was noted at these times. Although the location of the bats was only noted in the morning and it is possible roosting in multiple boxes occurred overnight, the quantified normothermic periods of the bats was likely resultant from the energy savings/expenditures which occurred due to the chosen daytime roost. After 4 days of measurements, at a time when all bats had left their respective roost boxes to feed at night, boxes were removed from the aviary and thoroughly cleaned with soap and water to ensure bats did not choose their roost based on smell. Bats were then captured, weighed and held for <30 min. Dried boxes were replaced an inverse order to assess whether bats chose roosts based on their location alone. Animals were then returned to the aviary, and data from that night were not included in analyses. Because bats (with 2 exceptions) continued to roost only in the black boxes, after 8 days all boxes were again removed and cleaned, but only the white boxes were replaced in their previous locations to determine their effect on the thermal biology of bats for 4 days. After a total of 12 days, bats were removed from their roost boxes prior to evening rewarming. Bats were not captured and weighed every day and boxes were not cleaned every day as this would greatly disrupt the experiment and torpor patterns of the bats via disturbance. At the end of the experiment, transmitters were carefully removed and bats were weighed to ensure no significant weight loss occurred and released at their point of capture that same night.

2.2. Data analysis and statistics

R (V 3.1.3, 2015) and SPSS (V 22, 2014) were used to conduct all statistical analyses. All data are reported as mean ± 1 s.d. for the number of individuals 'n'; the number of observations is 'N'. If the significance level was $P<0.05$, the null hypothesis was rejected.

2.2.1. Box temperatures

ANOVAs were used to compare differences in the average daily maximum, minimum and mean T_{box} black, T_{box} white and T_a for each treatment. The average minimum daily temperature significantly differed between treatments (ANOVA; $F_{2,61}=37.897$, $P<0.001$) as did the average daily temperature (ANOVA; $F_{2,61}=6.767$, $P=0.012$), therefore we report differences in T_{box} black, T_{box} white and T_a between treatments separately. If a difference was detected, a post-hoc Tukey test was used to determine which variables were significantly different between groups.

2.2.2. Roost choice

Roost choice was assessed separately per treatment by quantifying the number of nights each bat chose to roost either in a black box or in a white box when both boxes were available. The number of choices for each box colour was pooled and divided by the total number of choices (the total number of “bat-nights”). On two occasions in Treatment 1, bats roosted in the corner of the aviary (when both black and white roost boxes were offered) and those “choices” were excluded from the analysis.

2.2.3. Skin temperature, torpor patterns and passive and active rewarming

Bats were considered torpid when T_{skin} was <28.0 °C for >30 min. This definition for torpor is generally deemed appropriate considering the T_b-T_{skin} differential of torpid small mammals is generally <2.0 °C (Barclay et al., 1996) and because a number of studies on heterothermic bats in the Southern Hemisphere use $T_b<28$ °C as a torpor threshold (*e.g.* Turbill et al., 2003; Stawski et al., 2009; Bondarenco et al., 2013). The rewarming process involved a combination of one or two steps; i) passive rewarming where T_{skin} tracked T_a either partially or entirely to the normothermia threshold of 28 °C and/or ii) active rewarming where T_{skin} rose well above T_a and resulted in a final T_{skin}-T_a differential of at least 5 °C and T_{skin} was ≥ 28 °C. Bats that partially rewarmed (rewarmed to ≥ 28 °C for <30 min) were still considered torpid.

Total torpor per day was the total amount of time bats spent torpid in one calendar day (24 h; 12:00 h to 12:00 h), expressed as a proportion of the day, arcsine transformed. We also assessed the duration of torpor bouts initiated at any point in the night (night TBD). However, because the duration of torpor bouts initiated at midday and ending at sunset appeared to be affected more by photoperiod than T_a, they were excluded from TBD analysis (but included in the variable total torpor per day and energy expenditure calculations; see Section 2.2.5). Similarly, we assessed the duration of any prolonged normothermia (≥ 28 °C for ≥ 30 min) initiated between dusk and dawn (night normothermia duration). Any bouts of normothermia initiated between midday and dusk were excluded from normothermia duration analysis because, like afternoon torpor bouts, their duration appeared to be limited by photoperiod rather than climate. All variables relating to duration (min) were $\log_{10}$ transformed to reduce heteroscedasticity.

We developed a mixed-effects linear model (LME), using a t-statistic and p-value for indication of significance, and accounting for an uneven number of observations (package nlme; Pinheiro et al., 2014). We fitted a model that included box colour, feeding treatment and minimum daily external T_a and all of their interactions. Using a step-down procedure, the final model was that model which included those factors with significant coefficients. If an individual factor or factor interaction did not significantly contribute to the model but contributed to a significant higher-order interaction, then it remained in the model. The variables assessed were:

- Night torpor bout duration (TBD)
- Night normothermia duration
- Total torpor per day (proportion of the day spent torpid)
- Rate of passive rewarming
- T_{skin} range of passive rewarming
- Mean T_{skin} per night torpor bout
- T_{skin} at the start of active day rewarming
- T_{box} at the start of active day rewarming

The effect of an animal on a given variable was accounted for by using the individual as a random effect. We used all three factors as predictors for each variable. If a torpor bout occurred over multiple calendar days, the minimum daily T_a was averaged for all days the torpor bout occurred. On the two occasions which bats roosted in the corner of the aviary, their subsequent physiological variables were excluded from the analysis. Initially, we used body mass, sex and the presence or absence of other bats in the same box as additional effects in the LME model. Body mass and sex did not have an effect on any of the variables ($P>0.05$), thus they were dropped from the model. However, the presence of other bats in the same box significantly affected the rate of passive rewarming (LME; df=41, value=0.012, s.e.=0.0036, $t=-3.19$, $P=0.003$) and was thus kept in the model as a covariate for that variable, but dropped for all other variables. The high volume of the full statistical results for all variables tested in the LME precluded their inclusion in the manuscript and is thus provided in a separate document (Supplement 1).

2.2.4. Timing of active day rewarming

A Rayleigh test was used to determine whether circular data, *i.e.*; timing of midday rewarming, was not random. A Watson-Williams *F*-test was used to determine if timing of midday rewarming was significantly different between box colours and treatments.

2.2.5. Estimated daily energy expenditure

To assess whether bats used more energy on a daily basis when roosting in either black or white boxes and when fed *ad libitum* or food-restricted, daily energy expenditure (DEE) was estimated. The MR, measured as O_2 consumption (l/h), was estimated for each physiological state and then multiplied by the equivalent proportion of time (h) spent during each state (*i.e.* passive rewarming, active rewarming (day), active rewarming (night), normothermia/activity and torpor). The MR was then converted to kJ by using a conversion factor of 20.083 (Schmidt-Nielsen, 1997).

The estimated TMR was calculated from the equation $\log_{10}$TMR (ml g^{-1} h^{-1})=0.04$^*T_b$ – 1.62 (Currie et al., 2014) derived from *N. gouldi* where T_b was taken from mean subcutaneous temperature (T_{sub}) of individuals during steady-state torpor. Because huddling behaviour is not known to affect TMR in bats (Boratyński et al., 2015), we considered this equation appropriate for our estimation. Only steady-state torpor bouts of > 1 h were included in our analysis. For torpor bouts commencing at night, steady-state torpor was considered to cease at 7:30 h when T_{box} began to increase and resulted in the onset of passive rewarming. T_{skin} of torpid bats overnight was pooled for both black and white boxes because the minimum T_{box} was not different. The RMR was estimated from the equation RMR (ml g^{-1} h^{-1})=11.198–0.34*T_a derived from normothermic *N. gouldi* (S. E. Currie, Cardiorespiratory function and metabolism of heterothermic bats, Ph.D. thesis, University of New England, 2015). The T_a was averaged over the period bats were normothermic either during the day or overnight. As the T_a and, therefore, T_{skin} range of bats passively rewarming in this study were similar to those of *N. gouldi* from Currie et al. (2015), the cost of passive rewarming was considered to be the same. To estimate the cost of active rewarming, MR data of *N. gouldi* (n=11) rewarming at 10 and 15 °C were used (S. E. Currie, Cardiorespiratory function and metabolism of heterothermic bats, Ph.D. thesis, University of New England, 2015). We calculated MR over the average time taken for bats in our study to actively rewarm at similar average T_a of 8.8 ± 0.8 °C and 15.3 ± 0.3 °C. We were, however, unable to account for the cost of flight, as we could not confidently differentiate T_{skin} during flight from bats resting at a normothermic T_{skin} in-roost.

3. Results

3.1. Box temperatures

In treatment 1, the maximum daily T_{box} black was significantly warmer than both T_{box} white and T_a; T_{box} white and T_a did not significantly differ from each other. In treatment 2, maximum daily T_{box} black was also significantly warmer than maximum daily T_{box} white, however both maximum daily T_{box} black and T_{box} white were significantly warmer than maximum daily T_a (Table 1; See Fig. 1 for an example).

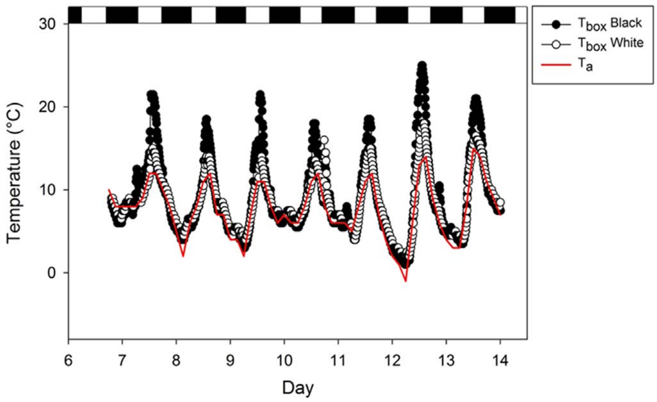

Fig. 1. Daily fluctuations of a T_{box} black, T_{box} white and T_a. The daily maximum T_{box} black (closed black circle) was consistently higher than daily maximum T_{box} white (open circle) and daily maximum T_a (solid red line). The black and white bars at the top of the graph correspond to night and day, respectively. The small increase in the evening in T_{box} white on day 11 may be due to an individual rewarming in close proximity to the temperature data logger. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

In both treatments, the minimum daily T_{box} black was not significantly warmer than T_{box} white, but both minimum daily T_{box} black and T_{box} white were significantly warmer than daily minimum T_a (Table 1).

In treatment 1, the average daily T_{box} black was significantly warmer than T_{box} white, and both average daily T_{box} black and T_{box} white were significantly warmer than T_a. However in treatment 2, the average daily T_{box} black was significantly warmer than T_{box} white and T_a, although average daily T_{box} white was not significantly warmer than T_a (Table 1).

3.2. Roost choice

When given a choice, bats from both treatments chose to roost in the black boxes almost every day. During the first 8 experimental days, when both black and white boxes were available, bats fed *ad libitum* actively chose to roost in black boxes on 92.9% of bat-nights (n=7, N=52). On only two separate occasions did two individuals choose to roost in a white box for one night (3.7% of bat-nights; n=2, N=2) and then immediately returned to a black box the next night. Although bats fed *ad libitum* were given the option to roost alone when offered black and white boxes, they generally chose to roost in the same box as other bats in small groups of < 4. One female bat chose to roost alone in a black box when given the option then, again, alone in a white box after the removal of black boxes. Food-restricted bats displayed a similar pattern of roost choice, but chose to roost in the black boxes on 100% of bat-nights (n=5, N=40) when given a choice between box colours and always roosted in boxes that contained other bats. In both treatments, bats chose to roost in white boxes on 100% of bat-nights when these were the only boxes available (Treatment 1, n=7, N=28; Treatment 2, n=5, N=20).

Table 1
Average daily minimum, maximum and mean T_a, T_{box} white and T_{box} black in winter, separated by treatment. The P-value denotes whether there was a significant difference between groups, as determined by an ANOVA. Different subscript letters show differences between means as determined by a Post-hoc Tukey Test.

	***Ad libitum* (Treatment 1)**						**Food-restricted (Treatment 2)**					
	T_a (°C)	**T_{box} White (°C)**	**T_{box} Black (°C)**	***df***	***F***	***P***	**T_a (°C)**	**T_{box} White (°C)**	**T_{box} Black (°C)**	***df***	***F***	***P***
Daily Maximum	13.9 ± 1.8^a	15.8 ± 2.4^a	20.5 ± 3.3^b	2	17.495	$<$ **0.001**	11.6 ± 3.1^a	17 ± 3.5^b	22.4 ± 3.9^c	2	23.192	**0.036**
Daily Minimum	1.9 ± 2.0^a	5.1 ± 2.3^b	6.0 ± 2.5^b	2	9.563	**0.001**	-2.0 ± 2.3^a	1.0 ± 2.9^b	1.4 ± 4.4^b	2	3.747	$<$ **0.001**
Daily Average	7.3 ± 1.9^a	11.0 ± 1.3^b	13.4 ± 1.2^c	2	40.351	$<$ **0.001**	6.2 ± 3.6^a	7.8 ± 3.7^a	12.6 ± 3.6^b	2	7.550	**0.002**

Table 2
Mean values ± 1 s.d. for listed variables in both black and white boxes when fed *ad libitum* and when food-restricted.

	Food *ad libitum*					**Food-Restricted**				
	Black Box	**N**	**White Box**	**N**	**Difference**	**Black Box**	**N**	**White Box**	**N**	**Difference**
Mean T_{skin} during night torpor bout (°C)	14.8 ± 1.6	51	12.3 ± 1.6	18	2.5	14.3 ± 0.8	37	13.4 ± 1.0	21	0.9
Night TBD (min)	851.3 ± 287.3	51	1207.6 ± 521.4	19	−356.3	914.5 ± 298.0	37	1064.5 ± 72.3	21	−150
Total torpor per day (min)	934.6 ± 225.6	46	1148.2 ± 125.7	21	−213.6	1035.3 ± 114.5	34	1226.5 ± 8.4	15	−191.2
Total torpor per day (%)	64.9 ± 15.7		79.7 ± 8.7		−14.8	71.9 ± 7.9		85.2 ± 0.6		−13.3
Night normothermia duration (min)	397.3 ± 174.8	48	207.7 ± 87.8	11	189.6	318.9 ± 234.4	45	192.5 ± 218.5	20	126.4
Passive rewarming rate (°C/min)	0.044 ± 0.007	32	0.028 ± 0.005	14	0.016	0.041 ± 0.011	7	0.035 ± 0.006	3	0.006
T_{skin} range of passive rewarming (°C)	11.0 ± 2.2	32	7.7 ± 1.7	15	3.3	11.7 ± 3.3	7	9.2 ± 1.5	3	2.5
T_{box} start of active day rewarming (°C)	16.7 ± 1.9	38	12.8 ± 1.8	15	3.9	20.3 ± 4.0	8	13.8 ± 0.4	2	6.5
T_{skin} start of active day rewarming (°C)	19.6 ± 2.5	39	16.1 ± 2.1	15	3.5	19.5 ± 7.5	8	19.0 ± 0.0	2	0.5
Estimated daily energy expenditure (kJ/day)	11.7	7	9.2	7	2.5	8.1	5	5.0	5	3.1

3.3. *Skin temperature and torpor patterns*

The mean T_{skin} during night torpor bouts was only affected by box colour; those bats roosting in black boxes had a significantly higher mean T_{skin} (Table 2, Table 3; Supplement 1).

Bats fed *ad libitum* typically employed two torpor bouts per day, one initiated at night and one initiated in the day following a period of day normothermia (See Fig. 2A for an example). However, food-restricted bats generally spent the entire day torpid and rarely completely rewarmed to normothermia (See Fig. 2B for an example). Bats fed *ad libitum* usually only displayed one bout of normothermia at night (See Fig. 3A for an example), whereas food-restricted bats generally rewarmed to normothermia once after dusk and, on 19 occasions, rewarmed once again for approximately 10 min (partial rewarming) prior to sunrise (See Fig. 3B for an example).

The total time spent torpid per day was significantly affected by daily minimum external T_a and box colour. Significant interactions were also found between box colour and daily minimum external T_a, treatment and daily minimum external T_a, as well as an interaction between all three factors. The total time spent torpid per day was most significantly affected by box colour and an interaction between treatment and daily minimum external T_a, such that bats spent less time torpid per day when roosting in black boxes or when food-restricted and daily minimum external T_a was high (Table 2, Table 3; Supplement 1).

Night TBD was significantly affected by box colour and daily minimum external T_a; bats spent less time torpid at night when roosting in black boxes or when daily minimum external T_a was high. Night normothermia duration was significantly affected by box colour and an interaction between treatment and daily minimum external T_a such that bats spent more time normothermic at night when roosting in black boxes or when food-restricted and daily minimum external T_a was high (Table 2, Table 3; Supplement 1).

3.4. *Passive and active rewarming*

On most days, bats fed *ad libitum* rewarmed once around midday, but did so more often in black boxes (78%) than in white boxes (65%) and then again at night to feed. Food-restricted bats passively rewarmed to normothermia much less often than bats fed *ad libitum*; 17.5% of the time in black boxes and only 15% of the time in white boxes. The T_{skin} range over which bats passively rewarmed was only significantly affected by box colour, and those bats roosting in black boxes were able to passively rewarm over a greater range (See Fig. 3A for an example). The rate at which bats passively rewarmed was also significantly faster when bats roosted in black boxes or when the daily minimum external T_a was high, and with the presence of other bats in the same roost box (Table 2, Table 3; Supplement 1).

Following passive rewarming, the T_{skin} and T_{box} from which active rewarming began was only affected by box colour; bats roosting in black boxes began active rewarming from a significantly higher T_{skin} and a significantly higher T_{box} (Table 2, Table 3; Supplement 1).

3.5. *Timing of active day rewarming*

Bats fed *ad libitum* and roosting in black boxes began afternoon active rewarming on average 30 min earlier (11:43 h ± 21 min) than in white boxes (12:13 h ± 21 min). These rewarming times were non-random in both white (Rayleigh, $z_{15}=34.96$; $r^2=0.92$, $P<0.01$) and black (Rayleigh, $z_{38}=14.11$; $r^2=0.97$, $P<0.01$) boxes and these times differed significantly (Watson-Williams; $F_{1,52}=23.10$, $P<0.01$). In food-restricted bats, active rewarming times were non-random in black boxes (Rayleigh, $z_8=5.51$, $r^2=0.83$, $P<0.01$) and occurred at 12:17 h ± 99 min, but could not be compared to white boxes due to a low sample size. Bats fed *ad libitum* and roosting in black boxes began active rewarming earlier than food-restricted bats roosting in black boxes (Watson-Williams; $F_{1,46}=6.75$, $P=0.012$), however bats roosting in white boxes could not be compared between treatments due to a low sample size ($N=2$).

3.6. *Estimated daily energy expenditure (DEE) and energy expenditure during rewarming*

When food was provided *ad libitum*, bats used, on average, an estimated 3.9 ± 0.5 kJ per day more than food-restricted individuals (pooled data for both box colours). With regard to box colour, bats roosting in black boxes had a greater estimated DEE than individuals that roosted in white boxes (Black; 11.7 and 8.1 kJ per day, White; 9.2 and 5.0 kJ per day, *ad libitum* and food-restricted respectively). The calculated cost of active rewarming, following from passive rewarming, was 0.42 kJ in a black box and 0.9 kJ in a white box. Thus, bats reduced energy expenditure by 53.3% during the midday active rewarming process when roosting in black boxes over white boxes.

4. Discussion

Our data show that black boxes are warmer than white boxes, and that *N. gouldi* fed *ad libitum* almost exclusively select black over white boxes for roosting when restricted to those choices, supporting our hypotheses. However, contrary to our hypotheses, food-restricted bats also chose to roost in black boxes. Lastly, box colour choice had a significant effect on the duration of night normothermia, supporting our hypotheses. When roosting in black boxes, *N. gouldi* i) had a higher mean T_{skin} during night torpor

Table 3
Coefficients (Value) and P-values for all partial effects of individual factors and factor interactions. For those variables which were coded (Box colour and Treatment) the variable shows the inflation from a black box (0) to a white box (1) and fed *ad libitum* (0) to food-restricted (1). We considered that the factor significantly contributed to the model if $P \leq 0.05$. If a cell contains a full stop (.) then that factor did not significantly contribute to the model for the listed variable. A colon (:) in between factors indicates there was a significant interaction between those factors. The effects of treatment were unable to be assessed against the T_{skin} range of passive rewarming and the rate of passive rewarming due to a small sample size in treatment 2. For all relevant statistics for each factor per variable, please refer to Supplement 1. An asterisk (*) denotes that the df is relevant for all remaining factors listed to the right. A double asterisk (**) denotes that huddling also had a significant effect on the variable (df=41, Value=0.012, P=0.003).

	Treatment			**Colour**			**Min Ext T_a**		**Colour: Min Ext T_a**		**Colour: Treatment**		**Treatment: Min Ext T_a**		**Colour: Treatment: Min Ext T_a**	
	df	***Value***	***P***	***df****	***Value***	***P***	***Value***	***P***	***Value***	***P***	***Value***	***P***	***Value***	***P***	***Value***	***P***
Mean T_{skin} during night torpor bout (°C)	.	.	.	114	−1.82	0.004	.	.	.	.	.	.	.	.	.	.
Night TBD (min)	.	.	.	114	0.12	0.009	−0.02	0.014	.	.	.	.	.	.	.	.
Total torpor per day (proportion)	10	0.03	0.666	98	0.18	< 0.001	-0.03	0.014	−0.05	0.014	0.03	0.684	−0.08	< 0.001	0.09	0.018
Night normothermia duration (min)	10	−0.15	0.209	109	−0.20	0.013	0.02	0.507	.	.	.	.	0.07	< 0.024		
Rate of passive rewarming (°C/min)**	.	.	.	41	−0.01	0.029	0.002	0.021	.	.	.	.	.	.	.	.
T_{skin} range of passive rewarming (°C)	.	.	.	44	−2.79	0.005	.	.	.	.	.	.	.	.	.	.
T_{box} start of active day rewarming (°C)	.	.	.	50	−3.76	< 0.001	.	.	.	.	.	.	.	.	.	.
T_{skin} start of active day rewarming (°C)	.	.	.	50	−2.73	0.011	.	.	.	.	.	.	.	.	.	.

bouts, ii) spent less time torpid and more time normothermic, iii) passively rewarmed more often, faster and over a greater range, and iv) started active rewarming earlier in the day and at a higher T_{box} and T_{skin} than in white boxes. When fed *ad libitum*, bats i) spent more time normothermic and less time torpid, and ii) began active rewarming earlier in the day (although this was only compared between black boxes) than when food-restricted. Lastly, when the daily minimum external T_a was high, bats i) spent more time normothermic and less time torpid at night, and ii) passively rewarmed faster than when daily minimum external T_a was low.

The preference of bats fed *ad libitum* to consistently roost in darker and warmer roost boxes was expected and supported by previous studies in the wild that offered different coloured roost boxes on trees and in buildings (Kerth et al., 2001; Lourenço and Palmeirim, 2004). Insectivorous bats may prefer warmer roosting microclimates to remain normothermic and active (Law and Chidel, 2007; Stawski et al., 2008). In particular, the inclination of bats to roost in black boxes, which experienced a higher maximum T_{box}, indicates a preference for roosts that experience a wider daytime T_{box} range, similar to thermally unstable roosts in the wild. Indeed, in our study when bats were required to roost in white boxes, night TBD increased which is similar to the thermal response of TBD in most bats when torpid at lower T_a (Twente and Twente, 1965; French, 1985; Park et al., 2000; Stawski and Geiser, 2010). However, reasons for choosing a warmer roost can be influenced by a suite of factors, of which energetic savings in relation to rewarming is of paramount importance (Lausen and Barclay, 2006).

In contrast, some heterothermic bats may give preference to colder microclimates and/or roost sites in order to reach deeper levels of torpor to save more energy when harsh climatic conditions exist and food is scant (Hall, 1982; Stawski et al., 2008; Ingersoll et al., 2010). Our study therefore posits that when *N. gouldi* were offered food *ad libitum*, they chose to roost in a warmer microclimate to facilitate rewarming for feeding. It is also possible that food-restricted *N. gouldi* checked for food every night, because food was not completely removed and offered on select days, and therefore they continued roosting in the black boxes. Thus, night TBD was not affected by treatment because torpor bouts were often “interrupted” by either partial or full in-roost rewarming prior to dawn. Food-restricted bats may have thus compensated for the cost of arousal required to assess for presence of food by spending more time torpid per day and decreasing normothermia at night.

Bats fed *ad libitum* in our study were not faced with energetic constraints. Therefore, *N. gouldi* may have partitioned more energy for rewarming to feed. Alternatively, free-ranging *N. gouldi* showed a high proclivity for passive rewarming in winter and chose roosts that warmed substantially from direct solar radiation (Turbill and Geiser, 2008), indicating that the species may prefer warmer roosts even when food is limited. Nonetheless, roosting in the black boxes resulted in an estimated 53.3% reduction in energy use, minimizing the cost of passive rewarming. This reduction in energy use by passively rewarming is similar to the calculated savings in a congener, *N. geoffroyi*, of 55% (Turbill et al., 2008). In the same study, the T_{skin} at which *N. geoffroyi* began active arousal (21.4 ± 2.8 °C at $12{:}47 \pm 1{:}03$ h) was similar, although slightly higher, than the T_{skin} at the start of active arousal when roosting in black boxes in our study species (Treatment 1, 19.6 ± 2.5 °C; Treatment 2, 19.5 ± 7.5 °C) (Turbill et al., 2008). Moreover, bats fed *ad libitum* actively rewarmed 30 min earlier when roosting in black than in white boxes because T_{box} black reached a higher temperature at an earlier time than T_{box} white. When roosting in white boxes, bats passively rewarmed for longer, potentially to reach the desired temperature for active rewarming. That bats in white boxes still actively rewarmed prior to maximum T_{box} may

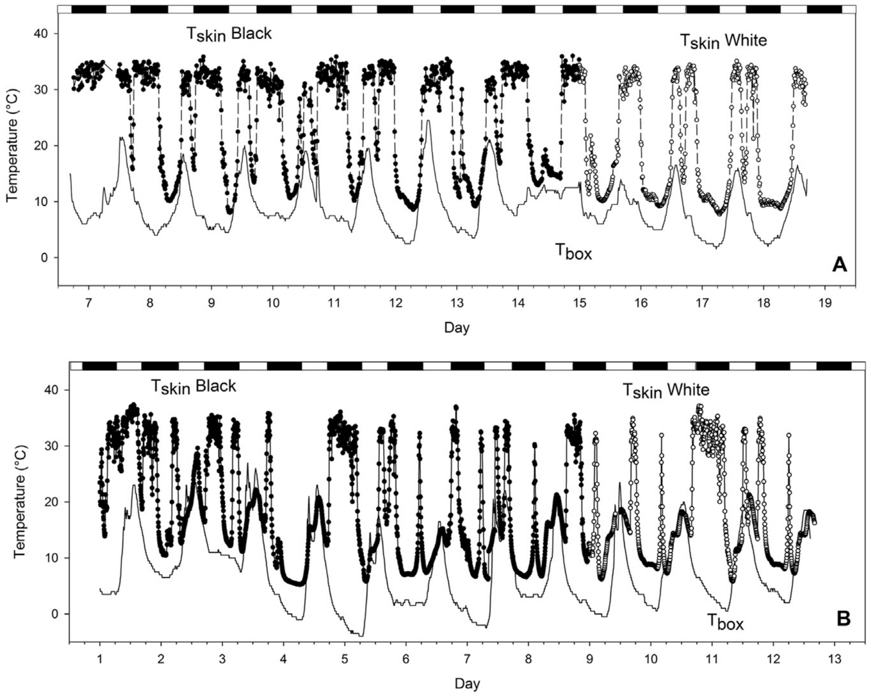

Fig. 2. Representative daily fluctuations of the T_{skin} of bats in treatments 1 and 2. Individual T_{skin} when using black boxes is represented by filled black circles, the T_{skin} when using white boxes is represented by open circles, and the respective T_{box} is represented by a solid line. The black and white bars at the top of the graph correspond to night and day, respectively. The days on the *x*-axis represent the days of the month the experiment took place. (A) T_{skin} fluctuations of a single male bat fed *ad libitum* over the entire study period in June 2014. When using the white boxes, this bat had shorter periods of activity at night. (B) T_{skin} fluctuations of a single male food-restricted bat over the entire study period in July 2015. This bat consistently chose to roost in black boxes, despite food restriction.

have been influenced by other bats warming in the box or may indicate that bats actively rewarmed during the greatest rate of warming in the box. It has been noted under controlled conditions, that torpid *N. gouldi* will rewarm when exposed to rapid changes in T_a (S.E.C. & F.G., unpublished)

Our results show that the overall estimated DEE of bats roosting in black boxes was higher than those required to roost in white boxes regardless of year or food availability. It has been shown in the mouse *Mus musculus* that greater energy intake is associated with higher energy demands, and thus, greater energy expenditure (Selman et al., 2001). Therefore it is likely that bats roosting in black boxes and fed *ad libitum* spent more energy due to exposure to higher T_a and thus used passive rewarming to limit DEE. Active rewarming from low T_b also induces strain on the body through the production of reactive oxygen species as well as pressures on the cardiovascular system (Carey et al., 2003). Passive rewarming reduces these stressors by increasing T_b prior to active rewarming and reducing the time required for rewarming, which likely enables individuals to save antioxidant defenses for times when active rewarming from lower T_b is necessary (Currie et al., 2015). Although overall daily costs were greater when bats chose to roost in black boxes, the benefits gained from passive rewarming may possibly outweigh these costs.

Patterns of roost selection observed here may be further complicated by social thermoregulation. It has been suggested that the costs of rewarming are reduced when individuals that roost together synchronize rewarmings (Arnold, 1993; Nowack and Geiser, 2016). Bats in our study often roosted in small groups < 4, which may explain why bats continued to roost in black boxes when food-restricted even though total estimated energy expenditure of these individuals was higher than in those roosting in white boxes. Social thermoregulation in large groups can significantly reduce energy expenditure during normothermia (Willis and Brigham, 2007), and indeed, the rate of passive rewarming was significantly affected by the presence or absence of other bats in the same box. In addition to the opportunity to huddle, our study shows that bats may select their roost based on the microclimate which provides a sharp increase in T_a to facilitate active daytime rewarming with the least amount of energy expenditure. In addition to rewarming, night normothermia duration was also significantly affected by the day's box colour choice. Our findings are further consistent with studies that show torpor is increased when food is restricted (Kurta, 1991; Wojciechowski et al., 2007) and at low T_a (Geiser and Brigham, 2000; Willis et al., 2005). Thus, the use of warmer boxes may have not only been a strategy to facilitate easier rewarming, but also to forage and/or maintain normothermia longer at night, despite food availability. It would be of additional interest to determine colour preference in roosts with identical thermal properties to understand if bats distinguish preference on colour alone.

5. Conclusion

Our study shows that torpor expression is significantly influenced by roost colour and that bats will choose darker roosts to facilitate midday rewarming or remain normothermic longer at night, despite food availability. As bats are highly influenced by T_a, ensuring appropriate coloured roost boxes are available would be beneficial to enable them to manage their DEE in winter. *Nyctophilus gouldi* prefer to use darker and warmer roosts in winter, therefore it may be in the interest of those seeking to improve artificial bat roosting habitat to provide dark-coloured roost boxes for tree-roosting bat species when T_a is low. However, dark roost boxes can warm to high temperatures in hotter months and warmer climates (Lourenço and Palmeirim, 2004), therefore a variety of box colours should be available to account for seasonal differences in energetic needs as well as to ensure bats will be able to choose cooler roosts when dark boxes exceed the upper limit of their thermal tolerance.

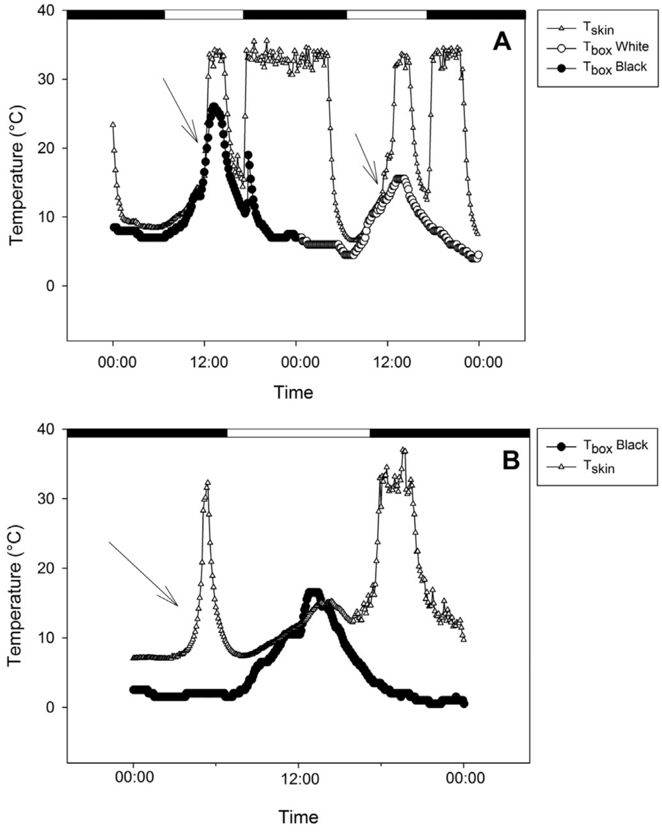

Fig. 3. T_{skin} trace demonstrating rewarming habits of bats in Treatments 1 and 2. Individual T_{skin} is represented by open white triangles, the T_{box} black is represented by filled black circles, and the T_{box} white is represented by open circles. Black bars bordering the top of the graph indicate night, and white indicates day. (A) T_{skin} as a function of time in an individual female bat fed *ad libitum* and the corresponding T_{box} on a day when the bat used a black box followed by a day the bat used a white box. The arrow indicates the point at which active arousal began. Active arousal started at a lower temperature when the individual used a white box. (B) T_{skin} as a function of time in an individual male food-restricted bat and the corresponding T_{box} on a day when the bat used a black box. The arrow indicates the time at which a partial arousal occurred prior to dawn. The bat did not rewarm to normothermia during the day.

Acknowledgements

We thank Dr. Brad Law and Dr. Gerhard Körtner for advice for the study. This study was supported by a Bat Conservation International Grant (GL 1453 24124) awarded to A.D., a Holsworth Wildlife Research Endowment (GL 1444 24155) awarded to A.D., a University of New England Postdoctoral Research Fellowship to C. S. and a grant from the Australian Research Council (DP 130 101 506) awarded to F. G.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.jtherbio.2016.07.015.

References

Arnold, W., 1993. Energetics of social hibernation. In: Carey, C., Florant, G.L., Wunder, B.A., Horwitz, B. (Eds.), Life in the Cold: Ecological, Physiological and Molecular. Westview Press, Boulder, CO, USA, pp. 65–80.

Barclay, R.M.R., Kalcounis, M.C., Crampton, L.H., Stefan, C., Vonhof, M.J., Wilkinson, L., Brigham, R.M., 1996. Can external radiotransmitters be used to assess body temperature and torpor in bats? J. Mammal. 77, 1102–1106.

Bondarenco, A., Körtner, G., Geiser, F., 2013. Some like it cold: summer torpor by freetail bats in the Australian arid zone. J. Comp. Physiol. B 183, 1113–1122.

Boratyński, J.S., Willis, C.K.R., Jefimow, M., Wojciechowski, M.S., 2015. Huddling reduces evaporative water loss in torpid Natterer's bats *Myotis nattereri*. Comp. Biochem. Physiol. A 179, 125–132.

Boyles, J.G., Dunbar, M.B., Storm, J.J., Brack, V., 2007. Energy availability influences microclimate selection of hibernating bats. J. Exp. Biol. 210, 4345–4350.

Brittingham, M.C., Williams, L.M., 2000. Bat boxes as alternative roosts for displaced bat maternity colonies. Wildl. Soc. Bull. 28, 197–207.

Buffenstein, R., 1985. The effect of starvation, food restriction, and water deprivation on thermoregulation and average daily metabolic rates in *Gerbillus pusillus*. Physiol. Zool. 58, 320–328.

Callahan, E.V., Drobney, R.D., Clawson, R.L., 1997. Selection of summer roosting sites by Indiana bats (*Myotis sodalis*) in Missouri. J. Mammal. 78, 818–825.

Churchill, S., 2009. Australian Bats, 2nd ed. Allen & Unwin, Sydney, Australia.

Chruszcz, B., Barclay, R.M.R., 2002. Thermoregulatory ecology of a solitary bat, *Myotis evotis*, roosting in rock crevices. Funct. Ecol. 16, 18–26.

Coburn, D.K., Geiser, F., 1998. Seasonal changes in energetics and torpor patterns in the subtropical blossom-bat *Syconycteris australis* (Megachiroptera). Oecologia 113, 467–473.

Carey, H.V., Andrews, M.T., Martin, S.L., 2003. Mammalian hibernation: Cellular and molecular responses to depressed metabolism and low temperature. Physiol. Rev. 83, 1153–1181.

Currie, S.E., Körtner, G., Geiser, F., 2014. Heart rate as a predictor of metabolic rate in heterothermic bats. J. Exp. Biol. 217, 1519–1524.

Currie, S.E., Noy, K., Geiser, F., 2015. Passive rewarming from torpor in hibernating bats: minimizing metabolic costs and cardiac demands. Am. J. Physiol. Regul. Integr. Comp. Phyiol. 308, R34–R41.

Dietz, M., Kalko, E.K., 2006. Seasonal changes in daily torpor patterns of free-ranging female and male Daubenton's bats (*Myotis daubentonii*). J. Comp. Physiol. B 176, 223–231.

Doty, A.C., Stawski, C., Law, B.S., Geiser, F., 2016. Post-wildfire physiological ecology of an Australian microbat. J. Comp. Physiol. B. http://dx.doi.org/10.1007/s00360-016-1003-3.

Doucette, L.I., Brigham, R.M., Pavey, C.R., Geiser, F., 2011. Roost type influences torpor use by Australian owlet-nightjars. Naturwissenschaften 98, 845–854.

Encarnação, J.A., Otto, M.S., Becker, N.I., 2012. Thermoregulation in male temperate bats depends on habitat characteristics. J. Therm. Biol. 37, 564–569.

Flaquer, C., Torre, I., Ruiz-Jarillo, R., 2006. The value of bat-boxes in the conservation of *Pipistrellus pygmaeus* in wetland rice paddies. Biol. Conserv. 128, 223–230.

French, A.R., 1985. Allometries of the durations of torpid and euthermic intervals during mammalian hibernation: a test of the theory of metabolic control of the timing of changes in body temperature. J. Comp. Physiol. B 156, 13–19.

Geiser, F., 2013. Hibernation. Curr. Biol. 23, R188–R193.

Geiser, F., Brigham, R.M., 2000. Torpor, thermal biology, and energetics in Australian long-eared bats (*Nyctophilus*). J. Comp. Physiol. B 170, 153–162.

Geiser, F., Stawski, C., 2011. Hibernation and torpor in tropical and subtropical bats in relation to energetics, extinctions, and the evolution of *endo*thermy. Integr. Comp. Biol. 51, 337–348.

Geiser, F., Brigham, R.M., 2012. The other functions of torpor. In: Ruf, C., Bieber, C., Arnold, W., Millesi, E. (Eds.), Living in a Seasonal World.. Springer Verlag, Berlin, Heidelberg, New York, pp. 109–121.

Goldingay, R.L., 2009. Characteristics of tree hollows used by Australian birds and bats. Wildl. Res. 36, 394–409.

Grinevitch, L., Holroyd, S., Barclay, R.M.R., 1995. Sex differences in the use of daily torpor and foraging time by big brown bats (*Eptesicus fuscus*) during the reproductive season. J. Zool. 235, 301–309.

Hall, L.S., 1982. The effect of cave microclimate on winter roosting behaviour in the bat *Miniopterus schreibersii blepotis*. Aust. J. Ecol. 7, 129–136.

Hock, R.J., 1951. The metabolic rates and body temperatures of bats. Biol. Bull. 101, 289–299.

Ingersoll, T.E., Navo, K.W., de Valpine, P., 2010. Microclimate preferences during swarming and hibernation in the Townsend's big-eared bat *Corynorhinus townsendii*. J. Mammal. 91, 1242–1250.

Johnson, J.S., Lacki, M.J., 2013. Effects of reproductive condition, roost microclimate, and weather patterns on summer torpor use by a vespertilionid bat. Ecol. Evol. 4, 157–166.

Kerth, G., Weissmann, K., König, B., 2001. Day roost selection in female Bechstein's bats (*Myotis bechsteinii*): a field experiment to determine the influence of roost temperature. Oecologia 126, 1–9.

Körtner, G., Geiser, F., 2000. Torpor and activity patterns in free-ranging sugar gliders *Petaurus breviceps* (Marsupialia). Oecologia 123, 350–357.

Kurta, A., 1991. Torpor patterns in food-deprived *Myotis lucifugus* (Chiroptera: Vespertilionidae) under simulated roost conditions. Can. J. Zool. 69, 255–257.

Lausen, C.L., Barclay, R.M.R., 2006. Benefits of living in a building: big brown bats (*Eptesicus fuscus*) in rocks versus buildings. J. Mammal. 87, 362–370.

Law, B.S., 1993. Roosting and foraging ecology of the Queensland blossom bat (*Syconycteris australis*) in north-eastern New South Wales: flexibility in response to seasonal variation. Wildl. Res. 20, 419–431.

Law, B.S., Chidel, M., 2007. Bats under a hot tin roof: comparing the microclimate of eastern cave bat (*Vespadelus troughtoni*) roosts in a shed and cave overhangs. Aust. J. Zool. 55, 49–55.

Lourenço, S.I., Palmeirim, J.M., 2004. Influence of temperature in roost selection by *Pipistrellus pygmaeus* (Chiroptera): relevance for the design of bat boxes. Biol. Conserv. 119, 237–243.

Lumsden, L.F., Bennett, A.F., Silins, J.E., 2002. Location of roosts of the lesser long-eared bat *Nyctophilus geoffroyi* and Gould's wattled bat *Chalinolobus gouldii* in a fragmented landscape in south-eastern Australia. Biol. Conserv. 106, 237–249.

McAllan, B.M., Geiser, F., 2014. Torpor during reproduction in mammals and birds: dealing with an energetic conundrum. Integr. Comp. Biol. 54, 516–532.

Nicolai, V., 1986. The bark of trees: thermal properties, microclimate and fauna. Oecologia 69, 148–160.

Nowack, J., Geiser, F., 2016. Friends with benefits: the role of huddling in mixed groups of torpid and normothermic animals. J. Exp. Biol. 219, 590–596.

Park, K.J., Jones, G., Ransome, R.D., 2000. Torpor, arousal and activity of hibernating greater horseshoe bats (*Rhinolophus ferrumequinum*). Funct. Ecol. 14, 580–588.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., Team, R.C., 2014. nlme: linear and nonlinear mixed effect models. R. Package version 3, 1–117.

Ruf, T., Geiser, F., 2015. Daily torpor and hibernation in birds and mammals. Biol. Rev. 90, 891–926.

Schmidt-Nielsen, K., 1997. Animal Physiology: Adaptation and Environment. Cambridge University Press, Cambridge, UK.

Selman, C., Korhonen, T.K., Bünger, L., Hill, W.G., Speakman, J.R., 2001. Thermoregulatory responses of two mouse *Mus musculus* strains selectively bred for high and low food intake. J. Comp. Physiol. B 171, 661–668.

Smith, G.C., Agnew, G., 2002. The value of bat boxes for attracting hollow-dependent fauna to farm forestry plantations in southeast Queensland. Ecol. Manag. Restor. 3, 37–46.

Song, X., Geiser, F., 1997. Daily torpor and energy expenditure in *Sminthopsis macroura*: interactions between food and water availability and temperature. Physiol. Zool. 70, 331–337.

Speakman, J.R., Racey, P., 1989. Hibernal ecology of the pipistrelle bat: energy expenditure, water requirements and mass loss, implications for survival and the function of winter emergence flights. J. Anim. Ecol. 58, 797–813.

Speakman, J.R., Rowland, A., 1999. Preparing for inactivity: how insectivorous bats deposit a fat store for hibernation. Proc. Nutr. Soc. 58, 123–131.

Stawski, C., 2010. Torpor during the reproductive season in a free-ranging subtropical bat *Nyctophilus bifax*. J. Therm. Biol. 35, 245–249.

Stawski, C., Turbill, C., Geiser, F., 2008. Prolonged torpor use during winter by a free-ranging bat in subtropical Australia. In: Lovegrove, B.G., McKechnie, A. (Eds.), Hypometabolism in Animals: Hibernation, Torpor and Cryobiology. University of KwaZulu-Natal, Pietermaritzburg, South Africa, pp. 353–360.

Stawski, C., Turbill, C., Geiser, F., 2009. Hibernation by a free-ranging subtropical bat (*Nyctophilus bifax*). J. Comp. Physiol. B 179, 433–441.

Stawski, C., Geiser, F., 2010. Seasonality of torpor patterns and physiological variables of a free-ranging subtropical bat. J. Exp. Biol. 213, 393–399.

Stawski, C., Willis, C.K.R., Geiser, F., 2014. The importance of temporal heterothermy in bats. J. Zool. 292, 86–100.

Turbill, C., 2006. Roosting and thermoregulatory behaviour of male Gould's long-eared bats, *Nyctophilus gouldi*: energetic benefits of thermally unstable tree roosts. Aust. J. Zool. 54, 57–60.

Turbill, C., Körtner, G., Geiser, F., 2003. Natural use of heterothermy by a small, tree-roosting bat during summer. Physiol. Biochem. Zool. 76, 868–876.

Turbill, C., Geiser, F., 2008. Hibernation by tree-roosting bats. J. Comp. Physiol. B 178, 597–605.

Turbill, C., Körtner, G., Geiser, F., 2008. Timing of the daily temperature cycle affects the critical arousal temperature and energy expenditure of lesser long-eared bats. J. Exp. Biol. 211, 3871–3878.

Twente, J.W., Twente, J.A., 1965. Regulation of hibernating periods by temperature. Proc. Natl. Acad. Sci. USA 54, 1044.

Vonhof, M.J., Barclay, R.M.R., 1996. Roost-site selection and roosting ecology of forest-dwelling bats in southern British Columbia. Can. J. Zool. 74, 1797–1805.

Webala, P.W., Craig, M.D., Law, B.S., Wayne, A.F., Bradley, J.S., 2010. Roost site selection by southern forest bat *Vespadelus regulus* and Gould's long-eared bat *Nyctophilus gouldi* in logged jarrah forests; south-western Australia. Ecol. Manag. 260, 1780–1790.

Willis, C.K.R., Turbill, C., Geiser, F., 2005. Torpor and thermal energetics in a tiny Australian vespertilionid, the little forest bat (*Vespadelus vulturnus*). J. Comp. Physiol. B 175, 479–486.

Willis, C.K.R., Brigham, R.M., Geiser, F., 2006. Deep, prolonged torpor by pregnant, free-ranging bats. Naturwissenschaften 93, 80–83.

Willis, C.K.R., Brigham, R.M., 2007. Social thermoregulation exerts more influence than microclimate on forest roost preferences by a cavity-dwelling bat. Behav. Ecol. Sociobiol. 62, 97–108.

Willis, C.K.R., Lovegrove, B.G., McKechnie, A., 2008. Do roost type or sociality predict warming rate? A phylogenetic analysis of torpor arousal. In: Lovegrove, B. G., McKechnie, A. (eds) Hypometabolism in Animals: Torpor, Hibernation and Cryobiology.Interpak Book, Pietermaritzburg, ZA, pp. 373–384.

Wojciechowski, M.S., Jefimow, M., Tęgowska, E., 2007. Environmental conditions, rather than season, determine torpor use and temperature selection in large mouse-eared bats (*Myotis myotis*). Comp. Biochem. Physiol. A 147, 828–840.