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Measuring subcutaneous temperature and differential rates of rewarming from hibernation and daily torpor in two species of bats

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ABSTRACT

Prolonged and remote measurement of body temperature (T_b) in undisturbed small hibernators was not possible in the past because of technological limitations. Although passive integrated transponders (PITs) have been used previously to measure subcutaneous temperature (T_{sub}) during daily torpor in a small marsupial, no study has attempted to use these devices at T_bs below 10 °C. Therefore, we investigated whether subcutaneous interscapular PITs can be used as a viable tool for measuring T_b in a small hibernating bat (*Nyctophilus gouldi*; *Ng*) and compared it with measurements of T_b during daily torpor in a heterothermic bat (*Syconycteris australis*; *Sa*). The precision of transponders was investigated as a function of ambient temperature (T_a) and remote T_{sub} readings enabled us to quantify T_{sub}–T_b differentials during steady-state torpor and arousal. Transponders functioned well outside the manufacturer's recommended range, down to ~5 °C. At rest, T_{sub} and rectal T_b (T_{rec}) were strongly correlated for both bat species (*Ng* $r^2 = 0.88$; *Sa* $r^2 = 0.95$) and this was also true for *N. gouldi* in steady-state torpor ($r^2 = 0.93$). During induced rewarming T_{sub} increased faster than T_{rec} in both species. Our results demonstrate that transponders can be used to provide accurate remote measurement of T_b in two species of bats during different physiological states, both during steady-state conditions and throughout dynamic phases such as rewarming from torpor. We show that, at least during rewarming, regional heterothermy common to larger hibernators and other hibernating bats is also present in bats capable of daily torpor.

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1. Introduction

Small endothermic animals have large surface area to volume ratios and therefore heat loss over the body surface is substantial. Because of the size–heat loss relationship small endothermic species have to carefully balance energy supply and demands, and many species show pronounced daily and/or seasonal fluctuations of body temperature (T_b), especially at low ambient temperatures (T_a) to reduce the T_b–T_a differential and to minimize this heat loss and energy consumption (Geiser, 2004). Thus many endotherms are not strictly homeothermic but rather heterothermic and therefore are, from a thermal point of view, some of the most interesting.

Unfortunately, remote measurements of body temperature (T_b) in small heterothermic animals were not possible in the past because of the lack of suitable devices for such measurements. However, remote measurements of T_b in heterotherms are crucial for the provision of reliable data because study animals are easily disturbed (Speakman et al., 1991; Luo et al., 2014). Although implantable temperature sensitive devices have been used successfully in free-ranging heterothermic animals (for example; Dausmann, 2005; Bieber and Ruf, 2009; Rojas et al., 2014), they are often limited to animals >20 g and are particularly difficult to use in animals with limited body cavity space such as bats and birds. Therefore past investigations of T_b in undisturbed small animals <20 g have primarily been undertaken using external transmitters that measure skin temperature (T_{sk}). Although there is a correlation between T_{sk} and T_b, external transmitters are also affected by T_a and may not always provide precise measures of T_b; especially when the relationship between T_b and T_a changes, as is the case for heterothermic species during torpor (Barclay et al., 1996; Willis and Brigham, 2003). In addition, lightweight externally adhered transmitters have a limited battery life and are often shed by animals within a short period of time (from a few days to around 1 month) making long-term T_b measurements of small animals very difficult. The development of miniaturized, lightweight temperature-sensitive passive integrated transponders (PITs) enables investigators not only to minimize the stress associated with animal handling but record T_b continuously in unrestrained animals over a range of physiological conditions and prolonged time periods (Roark and Dorcas, 2000; Wacker et al., 2012; Langer and Fietz, 2014).

The majority of bat species are small with many weighing less than 10 g (Simmons and Conway, 2003) which is still considered by some to be prohibitively small for even the lightest available devices. Due to their small size and energetically expensive locomotion, many bats

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use torpor, primarily to minimize energy expenditure during rest, and it is highly likely that the majority of small bat species are capable of entering torpor in one form or another (Stawski et al., 2014b). Nevertheless, our understanding of the thermal biology of many of the smallest bat species remains extremely limited.

To date detailed information on the use of transponders has only been gathered for normothermic individuals or daily heterotherms during shallow torpor ($T_b > 10$ °C). The accuracy and reliability of transponders at T_b below 10 °C, as is often found in hibernators, has not been investigated in detail. Moreover, the use of transponders to measure differentials between core T_b and subcutaneous body temperature (T_{sub}) and their transient changes during torpor entry, steady-state torpor and arousal from torpor has not been undertaken—although such changes have been widely observed in heterothermic mammals (for review, see Lyman, 1982).

The aims of our study therefore were as follows: 1) to assess the accuracy and reliability of transponders at temperatures below 10 °C, 2) to quantify the relationship between core T_b and T_{sub} during normothermia, steady-state torpor and arousal from torpor in a hibernating long-eared bat (*Nyctophilus gouldi*) and 3) to compare these observations with a common blossom bat only capable of expressing shallow daily torpor (*Syconycteris australis*). Both species of bat inhabit the east coast of Australia (Churchill, 2008) and are capable of entering torpor throughout the year (Coburn and Geiser, 1998; Turbill, 2006). *N. gouldi* are insectivorous bats that weigh between 5.2 and 16.5 g, with a minimum rectal T_b during torpor of approximately 2 °C (Geiser and Brigham, 2000). *S. australis* are nectar feeding bats which weigh between 13.7 and 23.0 g with a minimum recorded core T_b around 17 °C (Geiser et al., 1996).

2. Methods

2.1. Study animals and PIT implantation

Eleven *N. gouldi* (*Ng*; 10.5 ± 1.4 g) individuals were captured in mist nets at local bushland surrounding the University of New England (UNE) or at Imbota Nature Reserve and Newholme Stations near Armidale, NSW, Australia (30°35′S, 151°44′E). Bats were transferred to UNE on the night of capture and were housed in large outdoor aviaries (3 m × 1.5 m × 2 m) fitted with hessian cloth for bats to roost and animals were provided mealworms and water *ad libitum*.

Four male *S. australis* (*Sa*; 18.7 ± 1.0 g) were trapped in mist nets at Iluka Nature Reserve on the north coast of NSW, Australia (29°24′S, 153°22′E). Bats were initially hand-fed to ensure they maintained body weight, but were also given a fruit and protein mixture *ad libitum* (for more detail regarding recipe, see Law, 1992). After transfer to UNE, bats were housed in a large indoor flight cage (2 m × 2 m × 2 m) equipped with branches and large stands of foliage for bats to roost in. The room was kept at T_a 20 ± 2 °C with relative humidity greater than 55%. Before implantation of transponders individuals were given a minimum of three days (up to 14 days) to ensure a stable weight was maintained and that animals had acclimatized to captivity.

Bats were anesthetized for PIT implantation with general isoflurane/oxygen anesthesia (0.5–4%). A small (~3 mm) incision was made in the skin between the shoulder blades for transponder insertion. The skin and transponder were sterilized with 70% ethanol prior to insertion. One or two sutures (4/0 chromic gut, Ethicon, Somerville, USA) were used to close the incision site. The entire process took <15 min. Following the minor surgery bats were placed in individual cages in a warm room (~24 °C) and given 48 h to recover before being returned to their respective holding cages.

This study was conducted under a scientific license provided by the NSW Parks and Wildlife Authority (SL100084) and with Animal Ethics approval from the University of New England (AEC11-016).

2.2. PIT calibrations

Temperature-sensitive PITs (IPTT-300, Bio Medic Data Systems, Delaware, USA) are small (14 mm × 2 mm) and lightweight (0.13 g). All transponders continued to function below the manufacturer's recommended range of use (32–43 °C) down to approximately 10.0 °C, and around 16% of 126 transponders continued to function at 5.0 °C. Forty transponders that continued to work below 10 °C were calibrated to the nearest 0.1 °C with a precision reference thermometer traceable to a national standard in a water bath at temperatures between 5.0 °C and 40.0 °C. Calibrations were taken at approximately 5.0 °C increments. To assess precision and thermal inertia of transponders, three readings were taken at 5 min intervals at each temperature. Drift over time has been shown to be minimal in these devices, with <0.5 °C change over several days (Wacker et al., 2012). Transponder signals were read with a DAS-7009S Handheld Reader (Bio Medic Data Systems, Delaware, USA). Transponders were selected for implantation into bats based on the functional temperature range, correlation coefficient, and intercept of the calibration equation.

2.3. Thermocouple calibration

To measure T_a and rectal T_b of bats a fine gauge (42 SWG) copper constantan thermocouple with digital thermometer (HH81A, OMEGA Engineering, Connecticut, USA) was used. The thermocouple and digital thermometer were calibrated in a water bath against a precision thermometer traceable to a national standard, following similar methods as for PIT calibrations above, and over the same temperature range.

2.4. Normothermia

PIT readings of T_{sub} were compared with rectal temperatures (T_{rec}) to assess accuracy of T_{sub} measurements and how this correlated to core T_b. T_{rec} as a measure of core T_b was taken using a calibrated thermocouple inserted rectally to a depth of 2 cm. For comparisons of resting T_{rec} to T_{sub} animals were placed in individual calico bags within a temperature-controlled cabinet at T_a between 5.0 and 20.0 °C (*N. gouldi*, $n = 7$) or between 12.0 and 30.0 °C (*S. australis*, $n = 4$). Bats were transferred from their holding cages to the cabinet during their active phase following sunset in the evening. A maximum of four bats were measured per night and animals were left undisturbed for at least 2 days between measurements. Animals were not disturbed for at least 45 min prior to initial measurement to ensure they were calm and had adjusted to the T_a. Following exposure to each T_a for at least 1 h, T_{rec} and T_{sub} were recorded within 30 s of one another, always in the same sequence (T_{sub} followed by T_{rec}), and animals were returned to holding cages before midnight each night of measurement.

2.5. Torpor

To assess the relationship between T_{rec} and T_{sub} during induced torpor nine *N. gouldi* and two *S. australis* were kept in individual calico bags in a temperature-controlled cabinet overnight at single constant T_a between 5.0 and 20.0 °C (*N. gouldi*) or 12.0 °C (*S. australis*) without access to food or water. Both species have been shown to enter torpor overnight or in the early morning in laboratory settings (Coburn and Geiser, 1998; Geiser and Brigham, 2000) and therefore measurements of T_{sub} and T_b were taken the following morning after lights on (natural photoperiod) to ensure animals were torpid. Again, recordings of T_{rec} and T_{sub} were taken within 30 s of each other in the sequence T_{sub} followed by T_{rec}. *S. australis* individuals did not enter torpor readily in calico bags and therefore, to demonstrate the relationship between T_{sub} and T_a, supplemental T_{sub} values presented here were taken from subsequent experiments where animals were placed in respirometry chambers at constant T_a between 12.0 and 18.0 °C (for detailed information, see Currie, 2015).

2.6. Arousal from torpor

Rewarming rates and the relationship between T_{sub} and T_{rec} during arousal from torpor were quantified in six bats, five *N. gouldi* and a single *S. australis*. Rewarming of *N. gouldi* individuals either took place when animals were kept in a temperature-controlled cabinet or in the flight cages in the early morning. Rewarming was induced by opening the holding chamber or removing bats from their roosts in the aviary. In either case, a thermocouple was inserted 2 cm rectally and, as it did not move following insertion, *N. gouldi* individuals were not handled for the remainder of the rewarming process and either returned to the temperature cabinet or placed on a table in the outdoor aviaries. Measurement of T_{rec} during rewarming in *S. australis* required more handling and restraint, so in order to minimize the influence of heat transference to the PIT the bat was loosely held in a gloved hand. The first measurements of T_{rec} in all bats were taken within one min of disturbance regardless of location. T_{sub} and T_{rec} were recorded simultaneously every min until animals became too active for accurate measurement (T_{rec} ~35 .0°C). In all cases T_a did not vary over the rewarming period and was approximately 10.0 °C for *N. gouldi* and 18.0 °C for *S. australis*.

2.7. Statistical analyses

Statistical analyses were performed using R v3.1.0. Linear mixed effects models were used to calculate regressions of T_{sub} against T_{rec} at rest for both species and during torpor for *N. gouldi* using the *nlme* package with individual as a random effect (Pinheiro et al., 2014). Ordinary least squares regressions were used to calculate calibration equations of each PIT against water bath temperature. Rates of rewarming were calculated from the initial T_{rec} reading until animals were too active or T_{rec}/T_{sub} stabilized. For overall rewarming rates the first and last T_{rec}/T_{sub} were subtracted from one another and divided by time in minutes. Maximum rewarming rates were either calculated as the maximum value between two consecutive readings (1 min maximum) or the maximum value over a 10 min period. Paired t-tests were used to compare the maximum rate of rewarming over 1 min, 10 min maximum and average rewarming rates between T_{sub} and T_{rec} for *N. gouldi*.

3. Results

3.1. PIT calibrations

Fifteen PITs were selected for implantation into 11 *N. gouldi* individuals and 4 *S. australis* individuals. Transponders continued to function below the minimum temperature of factory calibration (32 °C, Bio Medic Data Systems) down to ~5 °C (minimum water temperature = 4.3 °C, minimum temperature indicated by PIT = 0 °C) (Table 1). Accuracy of the transponders decreased at the lower temperatures, however, the coefficients of determination in all calibration equations were still >0.995 (Table 1) and there was minimal change (<0.2 °C) over 10 min. Transponders had low thermal inertia and equilibrated to water bath temperature within 10 s.

Over all T_a measured, the T_{sub} of both normothermic and torpid animals was within 3 °C of T_{rec}. There was a strong correlation (*Ng* $r^2 = 0.88$, *Sa* $r^2 = 0.95$, $p < 0.001$) between T_{sub} and T_{rec} for both species when normothermic at rest. Average resting T_{sub} was 36.6 ± 1.9 °C for *N. gouldi* and T_{rec} was 37.4 ± 1.3 °C ($n = 7$). For *S. australis* ($n = 4$) the average resting T_{sub} was 36.0 ± 1.8 °C with corresponding T_{rec} of 36.5 ± 1.7 °C. There was no significant effect of T_a on either T_{rec} or T_{sub} in normothermic bats of either species (Fig. 2). However, measurements of T_{sub} in torpid *N. gouldi* ($n = 9$) were closely related to T_a as was T_{rec} and, on average, T_{sub} was within 2 °C of T_a. During steady-state torpor T_{sub} and T_{rec} were also strongly correlated in *N. gouldi* ($r^2 = 0.93$, $p < 0.001$) (Fig. 1) but this was not calculated for *S. australis* because of the small sample size. Occasionally T_{sub} of torpid *N. gouldi* appeared to fall below T_a, likely due to short term changes in T_a, whereas torpid *S. australis* often maintained a $T_{sub} > 2$ °C above T_a (Fig. 2).

3.2. Rewarming

For all animals in torpor, the slightest touch or noise initiated the rewarming process. T_{rec} was taken within the first minute of disturbing bats and therefore was representative of the T_b of animals in torpor. During arousal from torpor T_{rec} lagged behind T_{sub} in both species with a range of 0.1–7.8 °C and an average difference of 3.3 °C (Fig. 3). This difference was most pronounced during the middle of the arousal phase and towards the end of arousal the difference between T_{rec} and T_{sub} fell again to an average of 1.8 ± 0.8 °C.

Maximum rewarming rates for *N. gouldi* calculated over 1 min were significantly higher for T_{rec} (2.5 ± 0.3 °C min^{-1}) than T_{sub} (1.9 ± 0.1 °C min^{-1}) (paired *t*-test, $t = -5.36$, $df = 4$, $p < 0.01$). However, there was no difference in the rate of rewarming between T_{sub} and T_{rec} calculated either as overall rate (paired *t*-test, $t = 0.56$, $df = 4$, $p = 0.61$) or as maximum rate over 10 min (paired *t*-test, $t = -1.37$, $df = 4$, $p = 0.24$). The relationship between T_{sub} and T_{rec} during rewarming in *S. australis* was similar to that found for *N. gouldi*. Overall rewarming rate did not differ between the two body regions and was 0.9 °C min^{-1}, and there was only a very small difference (0.1 °C) between the maximum rewarming rates of T_{sub} (0.9 °C min^{-1}) and T_{rec} (1.0 °C min^{-1}) calculated over 10 min. When only the maximum rate of T_{sub} rewarming in *N. gouldi* was examined, the rate calculated over one minute (1.9 ± 0.1 °C min^{-1}) was significantly higher than the rate calculated over 10 min ($T_{sub} = 1.5 \pm 0.2$ °C min^{-1}; paired *t*-test, $t = 5.09$, $df = 4$, $p < 0.01$).

Table 1
Slope, intercept and r^2 of calibration equations and PIT temperature range in transponders calibrated in water baths prior to implantation in *N. gouldi* or *S. australis*.

Bat ID	PIT temperature range (°C)	Slope	Intercept	r^2
NG01	2.4–38.4	0.8801	3.421	0.9983
NG03	0.5–41.2	0.8735	4.2861	0.9983
NG05	0.1–39.4	0.9028	3.4416	0.9972
NG06	1.5–39.8	0.9213	2.4528	0.9973
NG09	1.3–40.4	0.9256	2.369	0.9984
NG10	2.0–40.3	0.9335	1.9409	0.9983
NG11	2.4–40.3	0.9684	0.9027	0.9982
NG13	2.5–40.4	0.9465	1.6982	0.9988
NG14	2.2–40.0	0.9348	1.8843	0.998
NG17	1.3–40.5	0.9197	2.5288	0.9987
NG18	1.5–40.1	0.9148	2.6737	0.9983
SA01	4.9–40.5	0.8858	3.2609	0.9963
SA02	5.9–40.2	0.9385	1.8229	0.9966
SA03	5.8–40.1	0.9071	3.0883	0.9981
SA04	6.8–40.4	0.9037	3.5961	0.9972

4. Discussion

Our study shows that PITs provide reliable measurements of T_{sub} in two species of small heterothermic bats. We demonstrate that although the accuracy of IPTT-300 transponders is reduced at low temperatures, calibrations of individual transponders still enable reliable measures of T_{sub} during torpor in small bat species, which has not previously been possible. Animals used in laboratory settings are subject to an array of stressors that alter their behavior and physiology and possibly impede the quality of the work. This is even more likely to be the case for wild-caught animals kept in captivity for short periods. Therefore it is important, especially for studies of animal physiology, to minimize the exposure of animals to unnecessary stressors. In particular, during torpor animals are very sensitive to external disturbances (Thomas, 1995) and have even been shown to be able to undertake coordinated

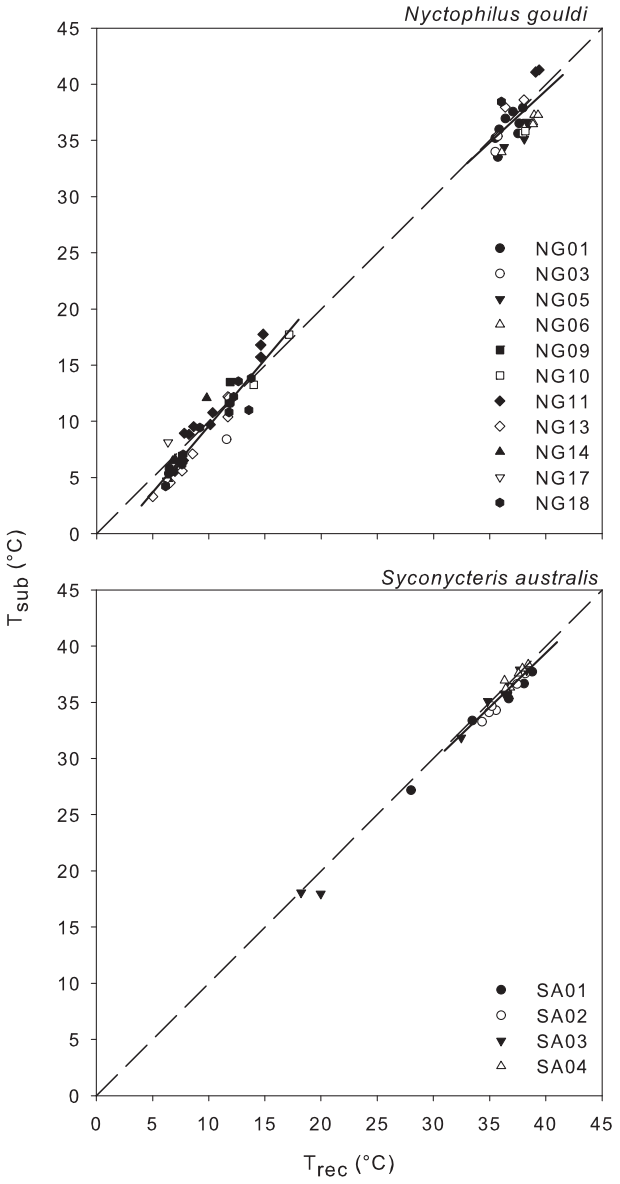

Fig. 1. Subcutaneous PIT temperature (T_{sub}) as a function of rectal temperature (T_{rec}) for individual *N. gouldi* and *S. australis* during rest and torpor. Individuals with a $T_{rec} < 30$ °C were considered torpid. The dashed line represents the line of equality ($T_{sub} = T_{rec}$). In *N. gouldi* individuals T_{sub} was strongly correlated to T_{rec} at rest and during torpor (Rest: $T_{sub} = 0.92(T_{rec}) + 2.52$, $r^2 = 0.88$, $p < 0.001$; Torpor: $T_{sub} = 1.18(T_{rec}) + 2.22$, $r^2 = 0.93$, $p < 0.001$). The correlation between T_{sub} and T_{rec} for *S. australis* at rest was also significant $T_{sub} = 0.97(T_{rec}) + 0.67$, $r^2 = 0.95$, $p < 0.001$.

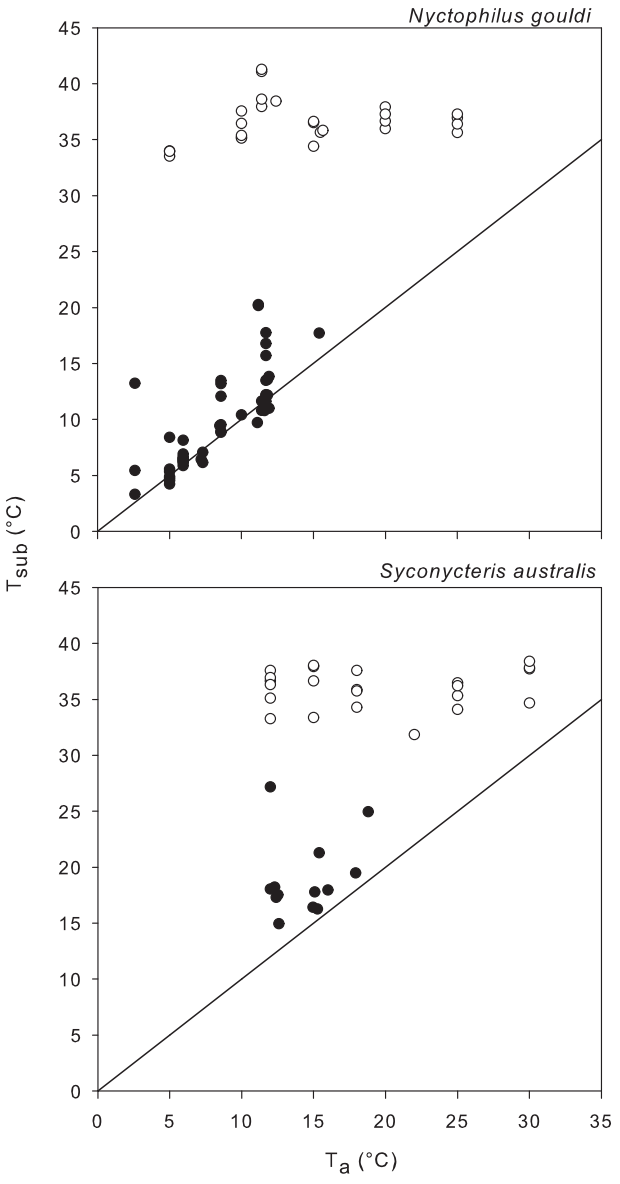

Fig. 2. T_{sub} measurements as a function of T_a in resting (circles) and torpid (filled black circles) *N. gouldi* ($n = 9$) and *S. australis* ($n = 4$). The threshold for torpor was $T_{sub} < 30$ °C. Solid black line indicates $T_{sub} = T_a$.

movement at T_b below normothermy (Rojas et al., 2012). Disturbances to torpid animals, either through touch or non-tactile means such as sound (Luo et al., 2014) or olfactory cues (Stawski et al., 2014a) can increase energy expenditure during torpor (Speakman et al., 1991) or result in premature arousal. As such the ability to remotely measure T_b in these animals is essential to ensure accurate and reliable representations of torpor use. Moreover, in some species the premature induction of arousal from torpor has been shown to have significant effects on the rewarming process resulting in increased rates of rewarming, increased variability of rate, and significant differences in the duration of arousal (Utz and van Breukelen, 2013). In golden mantled ground squirrels (*Spermophilus lateralis*) the effect of induced arousal (by mild shaking) was most pronounced at low T_a and effected the amount of time animals spent normothermic during interbout arousals (Utz and van Breukelen, 2013). Both *N. gouldi* and *S. australis* were very sensitive to disturbance in this study as simply opening the temperature controlled cabinet often resulted in arousals. Although premature induction of arousal likely impacts some features of rewarming, the effect may not be as pronounced in small species, such as bats, that naturally rewarm very quickly. Regardless of the initial disturbance, handling and discomfort associated with rectal measurements of T_b would be most likely to confound results and as such should be minimized.

As the drift of IPTT-300 transponders is minimal, with <0.5 °C difference over 4 days (Wacker et al., 2012) and PITs require no battery, there is new scope for regular monitoring of individuals over long times scales, with retained function of implanted PITs over several years (C.B. Wacker, personal communication). Passive transponders allow for flexible sampling intervals and real-time measurements. In contrast to programmable devices, such as data loggers, that require a fixed sampling frequency designated prior to implantation and data only become available after the device has been removed. Thus PITs provide an exciting opportunity to gain insight into the thermal biology of very small heterothermic species, not only during periods of torpor where timing may not be easily predicted, but during periods of activity which can be extremely energetically demanding such as hovering flight in small

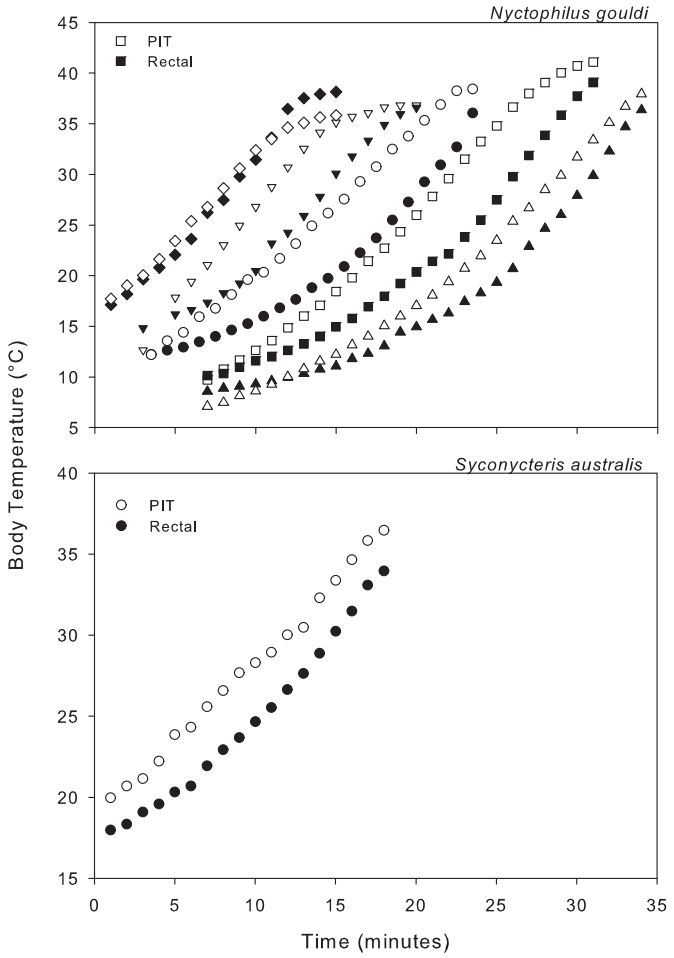

Fig. 3. Simultaneous recordings of subcutaneous PIT temperature (white symbols) and rectal temperature (black symbols) during individual arousals from torpor in *N. gouldi* (n = 5) and *S. australis* (n = 1). Note the different y-axis scaling.

humming birds that only weigh ~3 g. Although the current commercially available transponder system enables us to remotely measure T_b of undisturbed animals, which has previously been very difficult, the lack of automation of the scanner does not allow for regular or continuous recording without the need for wiring a relay to the trigger. In addition, the small range of the scanner (<5 cm with the largest antenna) restricted the scope of this method as a means of T_b measurement in active animals in enclosures and also meant that within a very small respirometry chamber (<300 ml) animals could move out of range. Although larger antennae exist for different transponder systems these are not compatible with the temperature devices used here. An extension of transmission range and automation of the system would therefore greatly improve the scope of this tool and could substantially improve our understanding of animals in different physiological states and possibly different environments.

PITs showed low thermal inertia, unlike larger implantable and external transmitters, which enables more precise measurement of animals during dynamic phases such as entry into torpor and rewarming. The ability to rewarm endogenously from torpor is a defining feature of heterothermic animals and the arousal process is extremely costly (Lyman, 1982). Arousal costs are reduced when rewarming rate is maximized (McKechnie and Wolf, 2004) and the importance of a rapid return to normothermy is evident in both species of bats investigated here as rewarming rates were similar between the two groups, irrespective of torpor pattern expressed. Regardless of the difference in anteroposterior rewarming temperature, the overall rate of induced rewarming and maximum rate of rewarming over 10 min were not found to be different between measurements of T_{sub} and T_{rec} for bats in our study. This suggests that PITs and measurements of T_{sub} are an accurate method for quantifying integrated rates of rewarming. However, the period of time over which rewarming rates were calculated resulted in significantly different maximum values. As the rewarming process in heterotherms is not linear, integration over longer time periods may oversimplify the process (Nicol et al., 2009). Previous investigators have measured maximum rewarming at intervals of 10 min (Geiser and Baudinette, 1990), generally associated with equipment measurement interval and inertia of devices used to record T_b, but also to provide comparative data. In larger species of heterotherms that rewarm more slowly (>1 h) an interval of ~10 min may provide a reasonable measure of maximum rewarming rates, however small bats can rewarm very quickly (minimum 10 min) and as such longer measurement intervals likely underestimate maximum rewarming capacity. When compared to the rewarming rates of other vespertilionid bats taken at T_a of ~20 °C (as reported in Willis, 2008) maximum rates for *N. gouldi* reported here at T_a of 10 °C are much higher (2.5 ± 0.3 °C min^{-1} compared to a range from 0.15 to 1.52 °C min^{-1} of rectal T_b in the literature). This is in part likely reflective of the time over which measurements were taken in the previous studies, which is unfortunately not specified in many reviews. Although the absolute value differed, the maximum rate of rewarming measured from T_{sub} was also higher for *N. gouldi* than previous reported values (1.9 ± 0.1 °C min^{-1}). Therefore, the more frequent sampling interval enabled by low thermal intertia of PITs may provide greater insight into thermal capacities of heterothermic animals.

During rewarming from torpor the anterior portion of the body and hence T_{sub} rewarmed faster than the posterior body/T_{rec} in both species of bat, regardless of minimum T_b at the start of rewarming. These results support previous findings in hibernating placental mammals, including bats, which show that the anterior portion of the body rewarms first (for review, see Lyman, 1982). This antero-posterior differential during rewarming has only previously been reported for a single rodent daily heterotherm, the hispid pocket mouse *Perognathus hispidus* (Wang and Hudson, 1970). Brown adipose tissue is an essential thermogenic organ in small placental mammals and as animals rewarm from torpor there is a dramatic increase in blood flow to this region (Hayward and Ball, 1966; Mejsnar and Janský, 1970). The largest deposition of brown fat is located between the shoulder blades in most small mammals (Smith and Horwitz, 1969) and as PITs in our study were implanted intercapularly, the difference in rewarming of T_{sub} and T_{rec} likely reflects brown fat thermogenesis. In the little pygmy possum (*Cercartetus lepidus*), a marsupial hibernator, initial anteroposterior differences in T_b during torpor were gradually reduced during arousal and this was suggested to be a factor of the animals' small size (Geiser, 1987). However this could also be reflective of their lack of brown adipose tissue as many small bat species, including those in our study, show a marked lag in warming of T_{rec} (Studier, 1974; Hirshfeld and O'Farrell, 1976). The temporal precision of PITs enables fine time scale measurements which demonstrate a differential restriction of blood flow during arousal. This was also reflected in the significant difference between maximum rewarming rates of T_{sub} and T_{rec} calculated over 1 min, which were not substantially different between the daily heterotherm and hibernator. Restriction of blood flow to the peripheries enables animals to effectively rewarm the most critical organs first and slowly return perfusion to the rest of the body (Chatfield and Lyman, 1950; Bullard and Funkhouser, 1962). This generally tends to occur towards the end of the rewarming process. Our results support these findings and show that towards the end of arousal the difference between T_{rec} and T_{sub} declined in both bat species. This difference was minimal in normothermic bats at an average of 1.1 ± 0.8 °C. Consequently small PITs enable quantification of alternate changes in different body regions associated with dynamic physiological transitions such as arousal from torpor and demonstrate that the process of rewarming is similar between the two torpor patterns.

In nature both species of bat in this study roost in thermally labile environments exposing themselves to fluctuating T_a and passive rewarming. *N. gouldi* are known to select roosts to enable frequent passive rewarming in the wild (Turbill, 2006) and this is likely true for *S. australis* as well, as they have been shown to select roosts on the outer, more thermally labile edges of their habitat in winter (Drury and Geiser, 2014). Unlike T_{sk} measured using external transmitters, T_{sub} is less directly impacted by T_a and this method has already been used to show how T_b responds when *N. gouldi* passively rewarm under controlled laboratory conditions (Currie et al., 2015). Passive rewarming saves substantial energy by enabling animals to maximise rewarming rates and minimize arousal times (Geiser et al., 2004; Turbill et al., 2008; Currie et al., 2015). Considering that the time taken to rewarm did not differ between *S. australis* and *N. gouldi*, similar significant energy savings are likely to also occur if these daily heterotherms passively rewarmed. T_{sub} has recently been shown to be a reliable measure of T_b in normothermic free-ranging edible dormice (*Glis glis*, Langer and Fietz, 2014) and the ability to measure T_{sub} in free-ranging bats would also be valuable to improve our understanding of energy use when animals actively expose themselves to fluctuating ambient conditions.

Our study is the first to assess the accuracy of small temperature sensitive transponders as a measure of T_{sub} in bats at low temperatures and during different physiological states. We show that T_{sub} and T_{rec} were strongly correlated in bats during both steady-state conditions of rest and torpor as well as over the transitional phase of rewarming. We also show that, at least for bats, rates of rewarming and differential vasoconstriction do not differ substantially between a daily heterotherm and a hibernator of similar size. T_{sub} measurements of normothermic bats in our study were not a function of T_a and as such provide a more reflective measure of T_b than the commonly used measure of T_{sk}. This suggests that PITs are a viable method for regular recording of T_b across an array of physiological states, and may enable investigation of thermal physiology previously undocumented in the smallest living endotherms.

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