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The use of small subcutaneous transponders for quantifying thermal biology and torpor in small mammals

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ABSTRACT

Remote measurements of body temperature (T_b) in animals require implantation of relatively large temperature-sensitive radio-transmitters or data loggers, whereas rectal temperature (T_{rec}) measurements require handling and therefore may bias the results. We investigated whether ~ 0.1 g temperature-sensitive subcutaneously implanted transponders can be reliably used to quantify thermal biology and torpor use in small mammals. We examined (i) the precision of transponder readings as a function of temperature and (ii) whether subcutaneous transponders can be used to remotely record subcutaneous temperature (T_{sub}). Five adult male dunnarts (*Sminthopsis macroura*, body mass 24 g) were implanted with subcutaneous transponders to determine T_{sub} as a function of time and ambient temperature (T_a), and in comparison to thermocouple readings of T_{rec} . Transponder temperature was highly correlated with water bath temperature ($r^2=0.96-0.99$) over a range of approximately 10.0–40.0 °C. Transponders provided reliable data (± 0.6 °C) over the T_{sub} of 21.4–36.9 °C and could be read from a distance of up to 5 cm. Below 21.4 °C, accuracy was reduced to ± 2.8 °C, but individual transponder accuracy varied. Consequently, small subcutaneous transponders are useful to remotely quantify thermal physiology and torpor patterns without having to disturb the animal and disrupt torpor. Even at $T_{sub} < 21.4$ °C where the accuracy of the temperature readings was reduced, transponders do provide reliable data on whether and when torpor is used.

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1. Introduction

Torpor is recognized as an important energy-conserving mechanism used by many small mammals and birds and is characterized by controlled and pronounced reductions of body temperature (T_b) and metabolism (Florant and Heller, 1977; Geiser and Ruf, 1995; Hiebert, 1990; Levy et al., 2011; Lyman et al., 1982; McKechnie and Lovegrove, 2002). However, quantification of torpor and other aspects of thermal biology can be difficult because rectal readings of T_b require handling and disturbance of the animal, which may cause stress and compromise the results (Bae et al., 2007). The use of radio transmitters and data loggers, on the other hand, is often limited by size, weight and battery life of the device (Rojas et al., 2010). Because of the recommendations of Animal Ethics Committees that transmitters should not exceed 5–10% of the mass of the animal (Gannon and Sikes, 2007; Wilson et al., 1996), transmitter size limits work especially on small mammals that most commonly express torpor.

Abbreviations: T_b , body temperature; T_{sub} , subcutaneous temperature; T_{rec} , rectal temperature; T_a , ambient temperature; T_{water} , water bath temperature; $T_{transponder}$, transponder temperature reading

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Other non-invasive methods such as the use of external temperature-loggers may provide reliable data on temporal variations of T_b , however, these are of limited value when precise thermal data are required (Munn et al., 2010; Willis et al., 2005). The recent development of very small transponders, which can measure temperature in addition to providing animal identification, offers an alternative, but the transponder accuracy over a wide temperature range and their ease of use for animal experimentation has not been determined. Because these transponders are used for veterinary purposes, they have been calibrated and are considered reliable only between 32.0 °C and 43.0 °C (Bio Medic Data Systems Inc., 2008), well above the T_b range that is commonly used for defining torpor in mammals (usually $T_b < 30$ °C, for review see Barclay et al., 2001; Geiser and Mzilikazi, 2011). We aimed to quantify the accuracy of transponders over a wide temperature range and to determine how useful they are for animal experimentation. We compared the transponder measurements of subcutaneous temperature (T_{sub}) with thermocouple measurements of rectal temperature (T_{rec}) and examined temporal and thermal aspects of torpor patterns using the transponders.

The species selected for our experiment was the insectivorous striped-faced dunnart *Sminthopsis macroura* (Marsupialia: Dasyuridae), endemic to arid and semi-arid areas of central and

northern Australia (Morton and Dickman, 2008). The striped-face dunnart has a head and body length of approximately 85 mm and weighs between 20 g and 25 g (Morton and Dickman, 2008). Torpor use has been studied extensively in this species (Körtner and Geiser, 2009), which often enters torpor in the laboratory even when food is available, but especially when food is restricted (Geiser and Drury, 2003; Lovegrove et al., 1999; Song et al., 1995).

2. Material and methods

Twelve transponders (IPTT-300 Bio Medic Data Systems Implantable Programmable Temperature Transponder, Delaware, 0.13 g, 14 mm × 2 mm) were calibrated to the nearest 0.1 °C prior to use in a water bath with a precision reference thermometer (± 0.1 °C) traceable to a National Standard at temperatures between 10.0 °C and 40.0 °C in approximately 5.0 °C increments. All transponders were calibrated on two occasions with a four day period between calibrations. The transponder signal was read with a DAS-7006/7R/S Handheld Reader (Bio Medic Data Systems). Five of the most precise transponders were selected and used in the animal experiments.

Five male captive-bred adult *S. macroura* (body mass 24.3 ± 1.8 g) were implanted with subcutaneous transponders (AEC10/137) between the shoulders under general Isoflurane/oxygen anesthesia. The skin was sterilized with 70% alcohol before the injection. The transponders were injected using individual injectors provided by the manufacturer. A single suture (chromic gut, Ethicon, Somerville USA) was used to seal the hole caused by the injection. The entire process was completed within ten minutes. Animals were allowed to recover for ten days but T_{sub} readings could be taken immediately after the injection. Experimental measurements were commenced after the ten day recovery period. Nest boxes were positioned close to and taped to the side of the animal cage (35 cm × 27 cm × 21 cm) for ease of scanning (Fig. 1). Implanted transponder readings were corrected using the equations obtained from the calibrations (Table 1). Animals were fed daily (dry Whiskas brand biscuits soaked overnight mixed with wet Whiskas tinned food) when not used for experiments, and always had access to water.

To examine daily fluctuations of T_{sub} and spontaneous torpor (with fresh food supplied daily at 15:00 h), animals were exposed in their holding room to an ambient temperature (T_a) of 20.0 ± 2.0 °C for five days, under a photoperiod of L:D 11:13. Animals were scanned every three hours staggered over this five day period to determine temporal fluctuations in T_{sub} . A dim red light was used to allow work at night. Animals were considered to be torpid when T_b/T_{sub} is below 30.0 °C (for review see Barclay et al., 2001; Geiser and Mzilikazi, 2011).

To examine the effect of T_a on T_{sub} animals were exposed in a temperature-controlled cabinet to T_a s of 13.0 ± 1.0 °C, 16.5 ± 0.5 °C, 18.0 ± 0.5 °C, 25.0 ± 0.5 °C and 30.0 ± 0.5 °C

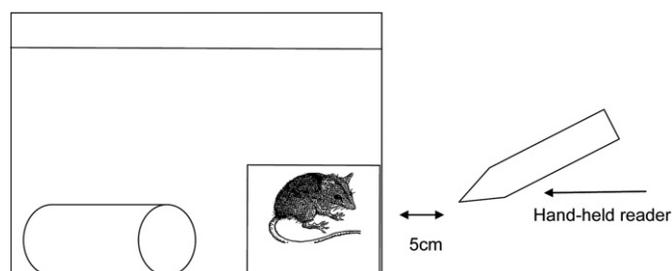


Fig. 1. Animal cage setup. A nest box with nesting material and a cardboard roll for an additional refuge were taped to the sides of the animal cage. Transponders could be scanned without needing to remove the animal from its nest box.

Table 1

Intercept, slope and r^2 values for calibration of transponders in a water bath.

Transponder #	Temperature range (°C)	Intercept	Slope	r^2
1	11.3–38.5	−6.4524	1.1887	0.998
2	11.3–38.5	−4.9877	1.1534	0.999
3	11.1–38.8	−4.0772	1.1069	0.999
4	11.1–38.8	−4.9140	1.1341	0.999
5	12.7–37.0	−7.3353	1.2157	0.998
6	12.7–37.0	−5.4895	1.1674	0.999
7	11.5–38.9	−3.6111	1.0976	0.998
8	11.5–38.9	−9.2343	1.2268	0.998
9	10.4–39.5	−1.2734	1.0253	0.998
10	10.4–39.5	−5.0097	1.1159	0.998
11	11.4–38.0	−5.1583	1.1588	0.996
12	11.4–38.0	−3.2568	1.0789	0.971

overnight without food to induce torpor; transponders were scanned at 08:30–09:00 h on the following morning because this approximates the most likely time for the species to be torpid (Song et al., 1995). Each animal was measured once a week at each of the T_a s. An iButton (DS 1921G, Thermochron, Dallas Semiconductor, Dallas, USA), which was calibrated in a water bath prior to use, was placed close to the animal boxes to record T_a to the nearest 0.5 °C.

To examine the relationship between T_{sub} and T_{rec} , animals were exposed in a temperature-controlled cabinet to T_a 15.0 ± 0.5 °C for one night without food. Between 07:00 h and 08:00 h the following morning animals were again scanned and a calibrated thermocouple, read with an electronic thermometer (Omega HH-71T, Omega Engineering, Stamford CT), was inserted ≥ 1.5 cm rectally to measure T_{rec} to the nearest 0.1 °C within 25 s of the transponder reading (Song et al., 1995). The thermocouple was calibrated to the nearest 0.1 °C prior to use in this experiment using a water bath at temperatures of between 10.0 °C and 40.0 °C in approximately 5.0 °C increments. Torpor was induced by removal of food and each individual measured once a week for three consecutive weeks to determine correlations between transponder and thermocouple measurements.

Statistical tests were performed using Minitab. Linear regressions were fitted with the least squares method. Values expressed as mean \pm standard deviation and means were compared using t -tests.

3. Results

3.1. Transponder calibration

Of the 12 transponders that were calibrated the transponder temperature of ten was highly correlated ($r^2 \geq 0.99$) with the water bath temperature determined by a precision thermometer (Fig. 2a and b, Table 1). The other two also provided good calibrations with $r^2 = 0.97$ and 0.98 (Fig. 2b). However, even the most accurate transponders ($r^2 > 0.99$, numbers one–ten) differed in their thermal properties (ANCOVA: $P < 0.01$, $F_{9,69} = 10.07$). The transponders that were used for implantation into animals drifted by < 0.5 °C between two calibrations conducted four days apart before they were implanted (calculated using linear regressions for each transponder and calibration attempt). Thus, transponders showed little drift and a good repeatability.

3.2. Use of the transponders in living animals

Implanted transponders of animals in nest boxes (i.e. subcutaneous temperature T_{sub} , assumed to be a close proxy of T_b) could

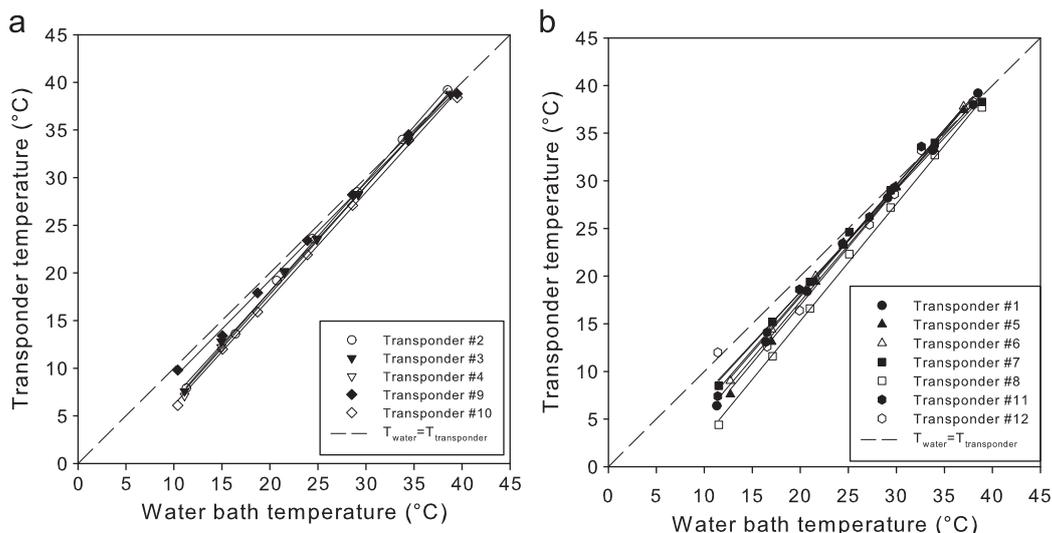


Fig. 2. Calibration of transponders in a water bath. Transponders that were implanted into animals (a) and those that were not chosen for implantation (b). The dashed lines represent $T_{\text{water}} = T_{\text{transponder}}$.

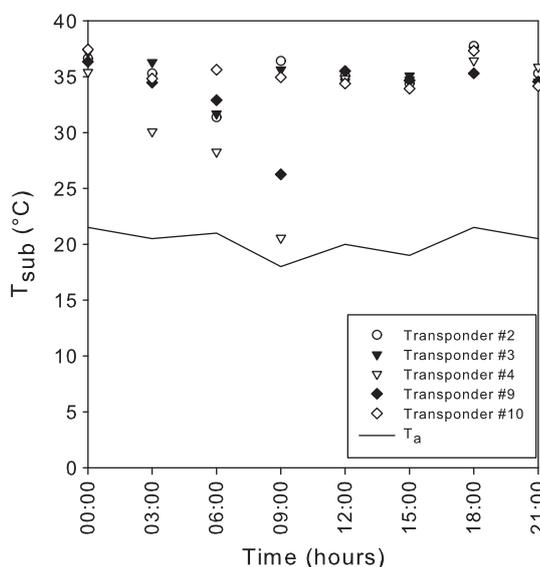


Fig. 3. Transponder readings staggered over a 5-day period at a T_a of 20 ± 2 °C showing spontaneous torpor use in *Sminthopsis macroura*.

be easily read from the outside of the animal cage up to a distance of approximately 5.0 cm with the narrow tip reader model (Bio Medic Data Systems DAS-7006/7S Straight Probe). With a wider tipped reader model that became available after our measurements were completed (Bio Medic Data Systems DAS-7006/7R Round Head Reader), the animals could be scanned from a distance of approximately 5.0 cm and up to 2.5 cm either side of the scanner tip (diameter 7.0 cm).

3.3. Temporal and thermal aspects of torpor

Transponder readings staggered over a five day period with an uninterrupted food supply at a T_a of 20.0 ± 2.0 °C (to determine daily fluctuation of T_{sub}) revealed that spontaneous torpor was used in the mornings (Fig. 3). Of the five animals only two were observed to enter spontaneous torpor, the individual with transponder #9 entered torpor after 06:00 h and the animal with transponder #4 entered torpor at approximately 03:00 h.

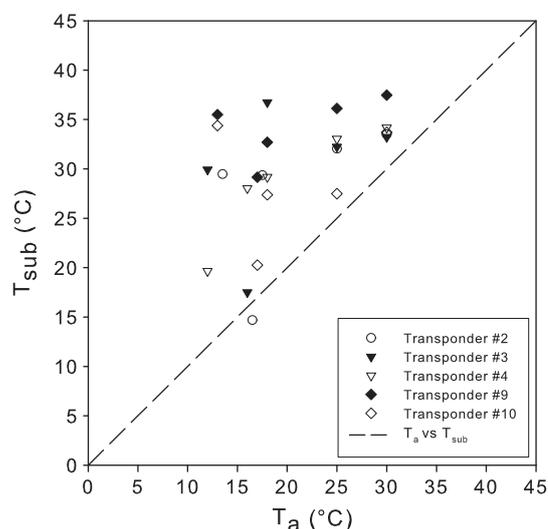


Fig. 4. Subcutaneous temperature, T_{sub} measurements of *Sminthopsis macroura* at 5 T_a s. The diagonal line represents $T_a = T_{\text{sub}}$.

At 15:00 h and a T_a of 19.0 °C all T_{sub} s were between 34.0 °C and 35.5 °C.

Induced torpor (food removed) and torpor depth were strongly affected by T_a (Fig. 4). At a T_a of 30.0 ± 0.5 °C no animals were observed to be torpid, but one animal was observed using torpor when T_a was reduced to 25.0 ± 0.5 °C. The most extensive use of torpor was observed at T_a 16.5 ± 0.5 °C with all five animals displaying torpor. With a further reduction of T_a to 13.0 ± 1.0 °C, two animals displayed shallow torpor with T_{sub} for the individuals with transponders #2 and #3 being 29.5 °C and 29.9 °C, respectively, whereas animal with transponder #4 had a T_{sub} of 19.7 °C.

Mean normothermic (the physiological state during which a heterothermic endotherm displays homeothermic thermoregulation; Geiser, 2011) T_{sub} over the entire T_a range was 34.0 ± 1.0 °C (Fig. 4) and normothermic T_{sub} was not a function of T_a ($y = -0.0392x + 35.16$; $r^2 = 0.02$). At $T_a > 21.4$ °C, the $T_{\text{sub}} - T_a$ differential was positive with a mean of 5.8 °C; the smallest $T_{\text{sub}} - T_a$ differential was 2.5 °C. At $T_a < 21.4$ °C, most $T_{\text{sub}} - T_a$ differentials were also positive with a mean of 11.3 °C. The $T_{\text{sub}} - T_a$ differential

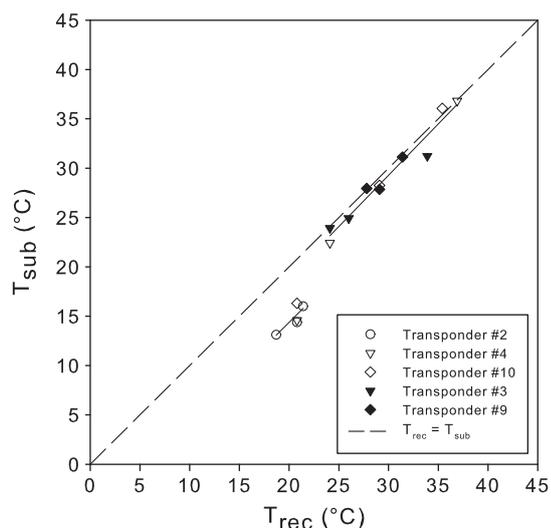


Fig. 5. Individual subcutaneous transponder readings of *Sminthopsis macroura* expressed by different symbols in comparison with thermocouple rectal temperature readings. The r^2 calculated for those values with T_{sub} above $21.4\text{ }^{\circ}\text{C}$ = 0.96. The r^2 calculated for those values with T_{sub} below $21.4\text{ }^{\circ}\text{C}$ = 0.66. The dashed line represents $T_{\text{rec}} = T_{\text{sub}}$.

at T_a $13.0 \pm 1.0\text{ }^{\circ}\text{C}$ increased the mean substantially as more animals at that T_a were not employing daily torpor. However, at T_a $16.5 \pm 0.5\text{ }^{\circ}\text{C}$ one T_{sub} reading (T_{sub} $14.7\text{ }^{\circ}\text{C}$) was below T_a indicating that an error was introduced at this T_a .

3.4. Comparison of rectal temperature and subcutaneous temperature

Rectal T_b and T_{sub} measured at T_a $15.0\text{ }^{\circ}\text{C}$ using transponders were highly correlated (Fig. 5), however, at $T_{\text{rec}} < 21.4\text{ }^{\circ}\text{C}$ transponder readings were less accurate in determining T_{rec} . The average $T_{\text{rec}} - T_{\text{sub}}$ differential for transponders reading below T_a $22.0\text{ }^{\circ}\text{C}$ was $5.6 \pm 1.1\text{ }^{\circ}\text{C}$. The average $T_{\text{rec}} - T_{\text{sub}}$ differential for those transponders reading above T_a $22.0\text{ }^{\circ}\text{C}$ was $0.9 \pm 1.8\text{ }^{\circ}\text{C}$. The animal with transponder #2 was torpid ($T_{\text{sub}} < 25.0\text{ }^{\circ}\text{C}$) on each of the three occasions it was scanned, whereas animals with transponders #3, #4, #9 and #10 were torpid on two occasions they were scanned.

4. Discussion

Our study shows that transponders reliably measure temperature over a wide range of temperatures especially when they have been calibrated. The manufacturer states that the transponders have a physical transmission range of approximately 2.5–5.0 cm (IPTT-300 BMDS data sheet, 2008) and this is in agreement with our measurements. Using the Round Head Probe (scanner/reader) increases the ease and speed of scanning because animals do not need to be directly in front of the scanner. Because of the design of these scanners, we were able to quickly scan the animals on all occasions without needing to disturb them. By staggering measurements over five days we were able to measure the animals and quantify temporal aspects of torpor without disrupting the animal's rest or activity.

The manufacturer also states that the temperature range that has been confirmed to be accurate is between $32.0\text{ }^{\circ}\text{C}$ and $43.0\text{ }^{\circ}\text{C}$ (IPTT-300 BMDS data sheet, 2008). Our comparison of rectal and subcutaneous temperatures shows that transponders were the most accurate at temperatures $> 21.4\text{ }^{\circ}\text{C}$, however, there was some variation in the thermal response and accuracy of individual

transponders varied. Consequently, these transponders provide a reliable alternative method for studies on animal thermal biology as they can be inserted with minor surgery, recovery time is fast and animals show normal movement and activity within 30 min of implantation. Moreover, animals can also be scanned remotely while torpid without disruption to their use of torpor.

Subcutaneous transponder temperature readings over five different T_a s at different times and at a constant T_a of approximately $20\text{ }^{\circ}\text{C}$ show what is expected for the species. Dunnarts enter torpor during the second half of the night or in the morning. The majority of experimental animals used torpor at T_a s of approximately $16.0\text{ }^{\circ}\text{C}$ and $18.0\text{ }^{\circ}\text{C}$ and no animals used torpor at a T_a of about $30.0\text{ }^{\circ}\text{C}$ (Geiser and Drury, 2003; Lovegrove et al., 1999; Song et al., 1995). At the lowest T_a of $13.0\text{ }^{\circ}\text{C}$ only three animals were torpid at the time of scanning and two of these were using shallow torpor. This is most likely because at this T_a , which is below the minimum T_b of captive individuals (Song et al., 1995), the high energetic costs associated with rewarming from a torpor bout are prohibitive (Geiser and Drury, 2003; Lovegrove et al., 1999; Warnecke et al., 2008). The mean normothermic T_{sub} of $34.0\text{ }^{\circ}\text{C}$ at T_a 13.0 – $30.0\text{ }^{\circ}\text{C}$ was similar to the average normothermic T_b of $35.7 \pm 0.9\text{ }^{\circ}\text{C}$ recorded using intraperitoneal transmitters in *S. macroura* (Geiser and Drury, 2003) or $34.3 \pm 0.6\text{ }^{\circ}\text{C}$ in thermo-neutrality (Song et al., 1995), and $34.3 \pm 0.4\text{ }^{\circ}\text{C}$ in another small marsupial the eastern pygmy possum, *Cercartetus nanus* (Song et al., 1997). The smallest $T_{\text{sub}} - T_a$ differential of $2.5\text{ }^{\circ}\text{C}$ is similar to that commonly seen during daily torpor in this species (Lovegrove et al., 1999).

Whereas most readings from transponders in animals were as expected, at a T_a of $16.5\text{ }^{\circ}\text{C}$, the reading of transponder #2 was $14.7\text{ }^{\circ}\text{C}$. One possible explanation is that the temperature of the cabinet before the reading was taken had fallen below that measured when the T_{sub} was taken or that a thermal gradient developed in the cabinet. Another possible explanation is a true measurement error perhaps due to the low temperature, as it is not possible for the T_b to fall below the T_a because a negative thermal differential would require active cooling. Whatever was the reason for this error, such an error is not often relevant for characterizing torpor use.

Important additional considerations beyond accuracy and ease of use are cost, battery replacement and data acquisition. The subcutaneous transponders are much cheaper than radio-transmitters. As they do not require battery replacement they do not require repeated surgeries and therefore can be used for conducting long-term studies lasting over months or years, which is not possible with transmitters and small data loggers. A major advantage over implanted data loggers is that transponder data can be obtained instantly in real time and not only after the device has been removed. Obviously, a useful addition to the transponders would be an automatic system that can scan several individuals over time (an automated system, but using much larger transponders and with a smaller temperature range than used here is commercially available).

Whereas small transponders appear most useful for measuring thermal variables in small animals, they also have potential uses for quantifying temperature gradients or regional heterothermy (a homeothermic organism maintaining a thermal gradient between different parts of the body; Geiser, 2011; Munn et al., 2009), in larger mammals, in addition to temporal heterothermy (a core body temperature that varies from normothermia over time; Geiser, 2011). Recent data on ibex and kangaroos have revealed strong seasonal changes in thermal energetics (Maloney et al., 2011; Signer et al., 2011) likely due to regional heterothermy. Transponders could be used to quantify long-term fluctuations of skin temperatures or temperatures of extremities or other body parts in comparison to core temperatures to gain a

better understanding of how large animals deal with thermal challenges.

Overall, while the transponders are able to read below the factory calibrated range of 32.0–43.0 °C, they do so reliably above 21.4 °C. They can then be used as an accurate tool for qualifying torpor use, but readings at low temperatures should be verified by a reliable established method if exact temperatures are required. For temporal variations of T_b that do not require exact temperature measurements, the transponders are a good alternative to established methods. The possible applications for this technology include not only thermal energetics but also medical research, because the laboratory mouse, *Mus musculus*, is known to enter torpor (Swoap and Gutilla, 2009) and is widely used for medical work.

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