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A non-invasive method for quantifying patterns of torpor and activity under semi-natural conditions

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Abstract

Understanding thermal biology in heterothermic endotherms requires that we accurately quantify temporal patterns of torpor use and activity. In many studies this is done using open-flow respirometry or implanted temperature sensitive transmitters. Here we report a method to quantify torpor and activity in cavity living endotherms that does not require surgery or confinement in metabolic chambers. We used temperature dataloggers affixed inside nests to record nest temperatures (T_{nest}) as a proxy for body temperature. We constructed nests so that animals were in direct contact with dataloggers while at rest. Passive infrared motion detectors were used to determine when animals were active in their cages outside nests. We confirmed that the approach accurately quantifies torpor patterns using open-flow respirometry. This method may prove useful in studies addressing temporal patterns of torpor use under semi-natural conditions because it results in little disruption to animals.
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The use of torpor is widespread among mammals and birds and, for many species, it is tremendously important to survival and reproductive fitness (Wang, 1989; Geiser, 2004; Dausmann et al., 2005). Understanding proximate energetic costs and benefits, and ultimate fitness implications of heterothermy requires that we accurately determine when animals are "in" and "out of" torpor. Although conceptually this seems like a simple matter, it has proved surprisingly difficult in practice and many different approaches have been used which vary depending on questions being addressed, species being studied and experimental conditions (Barclay et al., 2001; Willis and Brigham, 2003; Dausmann, 2005).

A wealth of laboratory studies has quantified physiological and energetic consequences of torpor use in birds and mammals (e.g., Cryan and Wolf, 2003; Cooper and Withers, 2004; Lane et al., 2004). Typically these studies employ open flow respirometry to record rate of oxygen consumption of an animal confined in a metabolic chamber over a range of ambient temperatures (T_a). These data are then used to calculate metabolic rate (MR). Surgically implanted temperature-sensitive radiotransmitters are also commonly used to record body temperature (T_b) (e.g., Song et al., 1998; Willis and Brigham, 2003). Both respirometry and implanted transmitters are powerful monitoring tools because they allow us to quantify precisely the timing of torpor onset and arousal. They also allow us to calculate energy use during torpor and normothermia over entire daily T_a and T_b cycles (e.g., Geiser and Brigham, 2000; Willis et al., 2004). However, Geiser et al. (2000) showed that

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patterns of torpor use, especially temporal aspects of torpor such as torpor bout duration, differ dramatically between conspecifics in captive vs. free-living conditions, possibly due to the stress of repeated handling, transmitter implantation and/or confinement in a small, unfamiliar metabolic chamber during oxygen consumption recording (Geiser et al., 2000). Radio-transmitter battery life is limited so long-term studies of individuals with implanted transmitters may require multiple surgeries, which could result in even more pronounced effects on torpor and activity patterns. Thus, while studies under confined captive conditions are essential for quantification of some variables, they may not be sufficient to provide us with a full understanding of the extent of torpor use and its consequences for ecology, behaviour and reproductive fitness.

In animal behaviour research, experiments conducted under semi-natural conditions in enclosures have provided important information about behaviours that are difficult or impossible to observe in completely free-living animals and too sensitive to observer effects to be studied under strictly captive conditions (e.g., Jonsson et al., 2000; Bugnyar and Kotrschal, 2002). This kind of compromise between a field and laboratory approach can also be useful in studies of the thermal biology of heterothermic endotherms (Song et al., 1998; Willis and Brigham, 2003). Here we report a non-invasive technique for quantifying diurnal patterns of torpor use and activity in a small mammal, the stripe-faced dunnart (*Sminthopsis macroura*). Our approach requires no surgical implantation of transmitters and no direct handling or confinement of animals beyond their usual maintenance routine, but still provides an accurate indication of temporal activity cycles and the timing of torpor use.

Stripe-faced dunnarts are small (ca. 20 g) marsupial carnivore/insectivores, native to the arid zone of Australia, which commonly use daily torpor (Song et al., 1998). Like most small Australian mammals, they are nocturnally active and shelter during the day in crevices or cavities (Menkhorst and Knight, 2001). We studied individuals from a captive colony housed at the University of New England in Armidale, NSW, Australia. Animals in the colony live individually in $40 \times 26 \times 16$ cm cages, are fed daily on a mix of wet and dry dog food with ad libitum water, and maintained on natural photoperiod. Each animal is provided with a 1 l cardboard milk carton lined with shredded paper as a nest chamber and they remain inside these chambers during the day and whenever they are inactive.

Previous studies of heterothermic endotherms have employed nest or nestbox measurements of skin temperature (T_{sk}) to estimate torpor use (Godfrey, 1968; French, 1982) and others have used passive infrared (PIR) detectors to monitor activity patterns (Körtner and Geiser, 1995; Song et al., 1998). Our approach combines these monitoring techniques to obtain simultaneous measurements of T_{sk} and behavioural activity. We used PIR detectors (Jaycar Electronics, LA-5017) positioned on the top of dunnart cages as described by Körtner and Geiser (1995) to monitor behavioural activity in the cage when dunnarts were outside of their nestboxes. We logged the number of motion events detected by PIRs in the cages over 10 min intervals for 24 h periods. To simultaneously record T_{sk} we exploited the fact that, like many small mammals, dunnarts use nest chambers while inactive. We affixed small-calibrated temperature dataloggers, accurate to $\pm 0.5\,^{\circ}C$ (iButton DS1921 Thermocron, Dallas Semiconductor, Dallas, USA) on the floor of small nests to record nest temperature (T_{nest}) at 2 min intervals over the same 24 h periods as PIR activity monitoring. Nests were 5 cm in length and 5 cm in diameter and constructed of sturdy (3 mm thick) cardboard tubing. One end of the tubes was left open as an entrance and the other was closed and insulated with several 2-mm-thick layers of cotton fabric. Nests were large enough for single dunnarts to fit completely inside but small enough that they had to sit on the temperature datalogger while in the tube. To provide added insulation and familiar nesting material, shredded paper was placed in the nest entrance. We suspended a second temperature datalogger from the top of each cage to record ambient temperature (T_a) outside the nestbox but inside the cage. Cages were kept in a climate-controlled room and dunnarts used the nest chambers as soon as they were provided, sheltering in them throughout the day and during other periods of inactivity. A representative time course of these data, which demonstrates torpor induced by providing an animal with half rations, is shown in Fig. 1A. Nest temperature approached T_a repeatedly during this recording session but the PIR data allow for clear differentiation between a long-torpor bout and periods when the animal was active in its cage outside the nestbox. By combining the T_{nest} and activity time courses for this individual it becomes possible to quantify temporal aspects of torpor use, such as torpor onset, the start of arousal, the completion of arousal and torpor bout duration. During a recording session for a different individual that remained normothermic with ad libitum food (Fig. 1B) the PIR data indicate that reductions in T_{nest} are unrelated to torpor and reflect periods when the dunnart was active outside of its nestbox.

To confirm that this method allowed us to accurately quantify temporal aspects of torpor use we used airtight "cages" as metabolic chambers for recording metabolic rate (as the rate of oxygen consumption; hereafter MR) concurrent with T_{nest}, T_a, and activity. Chambers were constructed from 21 plastic containers that provided ample space for dunnarts to move freely in and out of their nestboxes. Temperature dataloggers were affixed to

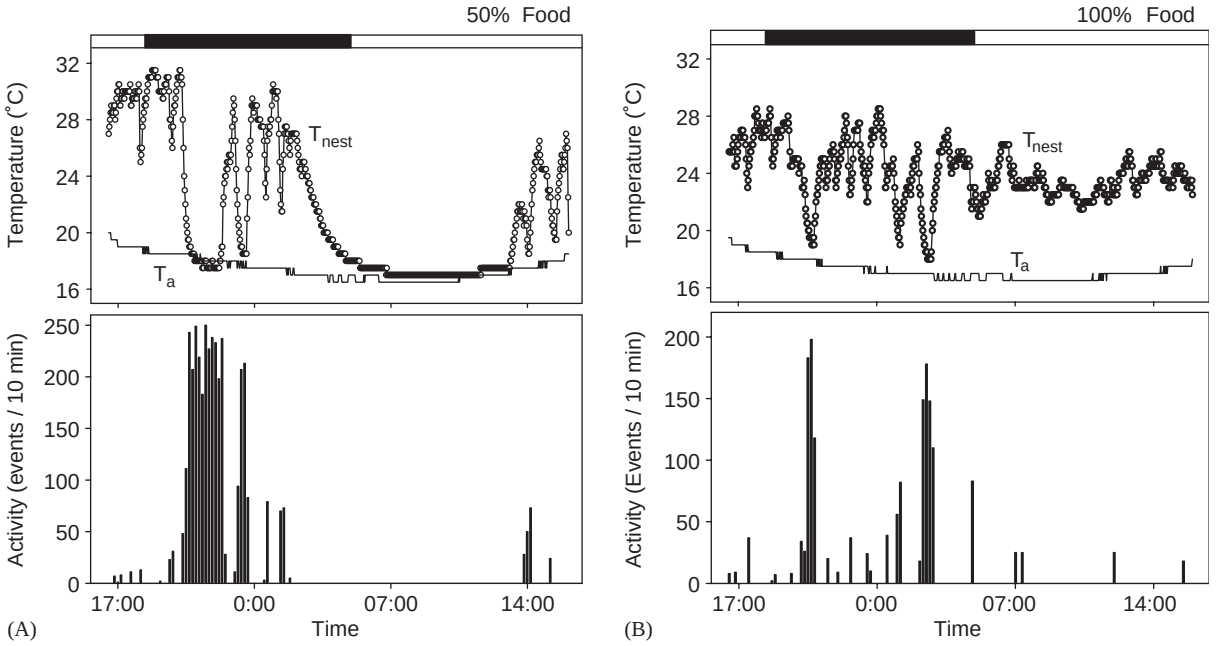

Fig. 1. Representative time courses of nest temperature (T_{nest}), ambient temperature (T_a), and behavioural activity recorded from stripe-faced dunnarts under semi-natural conditions during torpor following half rations (A, 50% food) and normothermia following normal feeding (B, 100% food). The dark bar represents the scotophase.

the floor of nestboxes and to the top of the chambers to monitor T_a as described above and PIR detectors were attached to the top of chambers to record behavioural activity. We placed chambers in a temperature-controlled cabinet at about 18 °C and measured MR using open flow respirometry. We used the respirometry system described by Geiser and Brigham (2000) but with a different single channel oxygen analyser (FOX, Sable Systems International Inc., Las Vegas, USA). Flow rates of air into the chambers were maintained at 450 ml min^{-1}.

A representative time course of the MR recording trials is shown in Fig. 2. Twice during this recording trial T_{nest} fell close to T_a but the activity and MR data show that the first period of reduced T_{nest} was not a torpor bout but a period when the dunnart was active outside of the nest. The second period of reduced T_{nest} was clearly a torpor bout, during which the dunnart was at rest inside the nest, because of the absence of activity and dramatic reduction in MR. The end of the torpor bout was marked by a clear arousal during which T_{nest} increased rapidly. The T_{nest} increase during arousal was concurrent with a typical MR overshoot during the rewarming phase, after which MR stabilised at a normothermic level. T_{nest} during the normothermic period following the torpor bout is less than a typical normothermic T_{sk}, likely because the dunnart is active out of the nestbox during some of this period and perhaps not in direct contact with the temperature datalogger while inside the nest. However, the 4–5 °C differential between T_{nest} and T_a coupled with the activity events during this period make it clear that the dunnart was not torpid. Thus, we have a good record of torpor entry and arousal timing, and activity patterns throughout the entire diurnal cycle. The PIR data confirm the timing of torpor and arousal.

To determine if T_{nest} provided a reliable estimate of torpor use, we tested our ability to quantify the time of torpor entry and arousal, and the duration of torpor bouts, for five dunnarts that entered torpor during respirometry trials. The onset of torpor bouts was obvious in both T_{nest} and MR time courses because both variables fell steeply, initially, and then levelled off at a reduced steady-state level (Fig. 2) and PIR data explained declines in T_{nest} unrelated to torpor use (Figs. 1, 2, see above). Thus we defined the onset of torpor entry as the beginning of the initial steep decline in T_{nest} or MR when PIR data indicated that animals were inactive. We quantified the timing of arousal onset (when T_{nest} and MR showed a rapid increase, Fig. 2) and the completion of arousal (when T_{nest} and MR reached the peak of the rewarming overshoot, Fig. 2) in the same way, and then calculated torpor bout durations based on the torpor onset and arousal completion times identified by T_{nest} and MR. To avoid bias in the analysis we were blind to the timing of fluctuations in MR while quantifying torpor entry and arousal times based on T_{nest}.

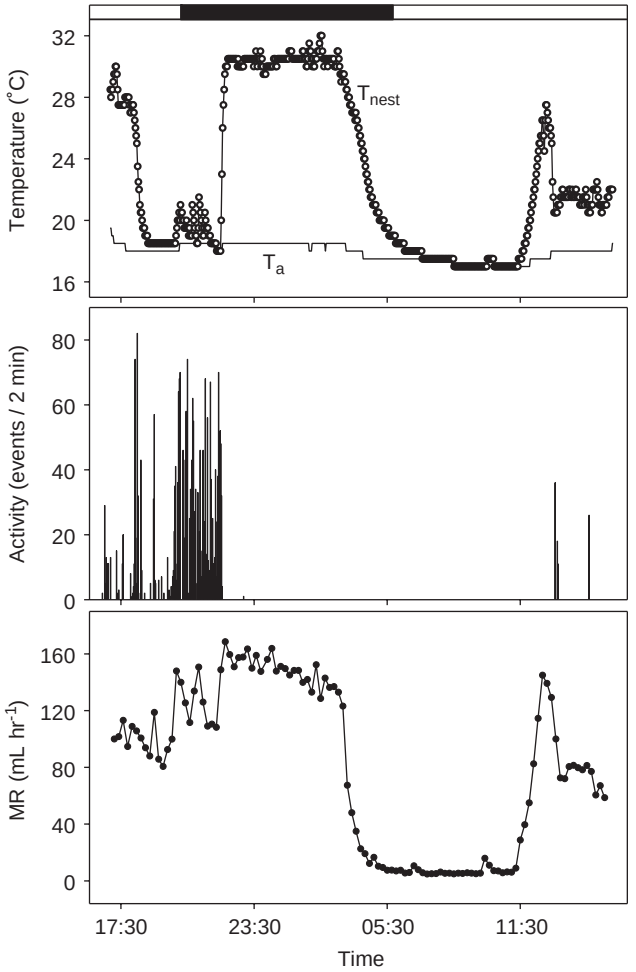

Fig. 2. Representative time courses of T_{nest}, T_a, metabolic rate (MR) and activity recorded from a stripe-faced dunnart under semi-natural conditions. The dark bar indicates the scotophase.

Table 1
Results of linear regression analyses relating the times of torpor entry, arousal onset, arousal completion and torpor bout duration based on measurements of T_{nest} and MR for 5 torpor bouts in 5 stripe-faced dunnarts

Variable	Equation	$F_{1,3}$	r^2	P
Torpor entry	$T_{nest} = 0.165 + 0.748$MR	23.1	0.89	0.02
Arousal onset	$T_{nest} = -0.067 + 1.16$MR	137.1	0.98	0.001
Arousal completion	$T_{nest} = 0.075 + 0.905$MR	10.1	0.77	0.05
Bout duration	$T_{nest} = 0.040 + 0.895$MR	32.8	0.92	0.01

Equation results are in units of time, expressed as decimal fractions of a 24 h period (i.e., 12 h = 0.5). The torpor entry equation is based on the period of time from noon on the previous day and arousal equations are based on the periods of time from the previous midnight.

We found strong correlations between T_{nest}- and MR-defined torpor entry, arousal onset and arousal completion, despite a small sample size (Table 1), and these relationships approximated 1:1. T_{nest} exhibited some thermal inertia relative to MR so there was a slight delay in our T_{nest} estimates of torpor onset (43 ± 35 min), arousal onset (17 ± 9 min) and completion of arousal (34 ± 28 min). However, most importantly, the durations of torpor bouts identified based on T_{nest} and MR were highly correlated (Table 1) and were very close to a 1:1

relationship. Bout durations differed only slightly (8 ± 30 min) despite the fact that some bouts lasted up to 12 h. Thus, T_{nest} provided an accurate indication of temporal patterns of torpor use.

Our approach does not provide measurements of T_{sk} that are precise enough to quantify energy expenditure during torpor and activity. It also becomes less effective as T_a increases because the T_{nest}–T_a differential becomes relatively small and difficult to detect. Despite these limitations, however, the approach may prove useful in studies addressing questions about the timing of torpor and activity for a range of cavity nesting and roosting mammals, as well as cavity nesting birds. It will likely be even more effective with more pronounced reductions in T_b typical of hibernators (as opposed to daily torpor users such as dunnarts, Geiser and Ruf, 1995) because the T_{nest} signal will become even more obvious at low T_a. Transmitter implantation is not required, which will reduce stress and eliminate potential physiological effects of anaesthesia and surgery, and long-term monitoring over an entire hibernation season or even multiple years is possible without the need to subject animals to repeated surgery when their transmitters fail. It is inexpensive, the nesting areas are easy to construct, and the use of iButtons dataloggers means there are no thermocouple wires for animals to chew. Finally, it provides a compromise between field and laboratory conditions because animals need not be confined in unfamiliar metabolic chambers but are still maintained in controlled conditions that allow for easy monitoring. Indeed, for many small mammals and birds it would be possible to use this approach in large habitat enclosures that approach natural home range size, or even field conditions if animals return reliably to the same nests or roosts each day. This non-invasive approach combines two useful monitoring tools to provide a more complete picture of torpor and activity patterns under semi-natural conditions.

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