

## RESEARCH ARTICLE

## Heart rate dynamics in a marsupial hibernator

Steven J. Swoap<sup>1,2,\*</sup>, Gerhard Körtner<sup>2</sup> and Fritz Geiser<sup>2</sup>

## ABSTRACT

The eastern pygmy possum (*Cercartetus nanus*) is a small marsupial that can express spontaneous short bouts of torpor, as well as multi-day bouts of deep hibernation. To examine heart rate ( $f_H$ ) control at various stages of torpor in a marsupial hibernator, and to see whether  $f_H$  variability differs from that of deep placental hibernators, we used radiotelemetry to measure ECG and body temperature ( $T_b$ ) while measuring the rate of  $O_2$  consumption and ventilation.  $f_H$  and  $O_2$  consumption rate during euthermia were at a minimum ( $321 \pm 34$  beats  $\text{min}^{-1}$ ,  $0.705 \pm 0.048$  ml  $O_2$   $\text{g}^{-1}$   $\text{h}^{-1}$ ) at an ambient temperature ( $T_a$ ) of  $31^\circ\text{C}$ .  $f_H$  had an inverse linear relationship with  $T_a$  to a maximum of  $630 \pm 19$  beats  $\text{min}^{-1}$  at a  $T_a$  of  $20^\circ\text{C}$ . During entry into torpor at a  $T_a$  of  $20^\circ\text{C}$ ,  $f_H$  slowed primarily as a result of episodic periods of cardiac activity where electrical activity of the heart occurred in groups of 3 or 4 heart beats. When  $T_b$  was stable at  $24^\circ\text{C}$  in these torpor bouts, the episodic nature of  $f_H$  had disappeared (i.e. no asystoles) with a rate of  $34 \pm 3$  beats  $\text{min}^{-1}$ . For multi-day bouts of deep torpor,  $T_a$  was lowered to  $6.6 \pm 0.8^\circ\text{C}$ . During these deep bouts of torpor,  $T_b$  reached a minimum of  $8.0 \pm 1.0^\circ\text{C}$ , with a minimum  $f_H$  of 8 beats  $\text{min}^{-1}$  and a minimum  $O_2$  consumption rate of  $0.029 \pm 0.07$  ml  $O_2$   $\text{g}^{-1}$   $\text{h}^{-1}$ . Shivering bouts occurred in deep torpor about every 40 min, during which ventilation occurred, and  $f_H$  was elevated to 40 beats  $\text{min}^{-1}$ . The duration of the QRS complex increased from 12 ms during euthermia to 69 ms at a  $T_b$  of  $8^\circ\text{C}$ . These findings demonstrate the dynamic functioning range of  $f_H$  to be about 600 beats  $\text{min}^{-1}$  ( $\sim 80$ -fold), one of the largest known ranges in mammals. Our study shows that despite a separation of  $\sim 160$  million years, the control and function of the cardiac system seems indistinguishable in marsupial and placental hibernating mammals.

KEY WORDS: Torpor, Hibernation, Cardiac, ECG, Metabolism

## INTRODUCTION

The physiological processes that are engaged during bouts of torpor in mammalian hibernation are numerous and complex. These processes include, but are certainly not limited to, abandonment of euthermic thermoregulation (Heller, 1983), apnea which can last seconds to minutes (Lyman, 1965; Milsom and Jackson, 2011), and active suppression of metabolic rate (Geiser, 2004). Further, shared cardiovascular changes in animals that use torpor include bradycardia (Zosky and Larcombe, 2003; Swoap and Gutilla, 2009; Morhardt, 1970; Harris and Milsom, 1995) mediated by the autonomic nervous system (ANS) and peripheral vasoconstriction (Swoap and Gutilla, 2009; Osborne

et al., 2005) mediated by the sympathetic nervous system (SNS). Other indicators of altered autonomic function during a bout of torpor include SNS activation of white adipose tissue (Swoap and Weinshenker, 2008) with the resultant drop in leptin and elevation in free fatty acid release, and withdrawal of SNS activity from brown fat (Cannon and Nedergard, 2004) in those organisms that have this heat-generating organ.

Heart rate ( $f_H$ ) during torpor has been examined in several placental mammals such as the 13-lined ground squirrel, the marmot, the black bear and long-eared bats (Tøien et al., 2011; Currie et al., 2014; Eagles et al., 1988; Hampton et al., 2010). Placental mammals show three distinguishing features of  $f_H$  during hibernation (Milsom et al., 1999). First, all display low  $f_H$  (between  $\sim 8$  and 12 beats  $\text{min}^{-1}$ ) during bouts of deep torpor. Second,  $f_H$  slows during entrance into torpor in part as a result of asystoles, or skipped beats. Third,  $f_H$  during deep torpor is elevated periodically, and that elevation is associated with ventilation (ventilatory tachycardia). Ventilatory tachycardia is also seen in deep torpor in a marsupial (*Cercartetus concinnus*: Zosky and Larcombe, 2003); however, little is known about the variability in  $f_H$  during entrance into torpor in a marsupial hibernator. Further, in animals such as mice and Djungarian hamsters that utilize exclusively daily torpor during which the minimum body temperature ( $T_b$ ) is around  $20^\circ\text{C}$  and the length of the torpor bout is measured in hours and not days or weeks, the minimum  $f_H$  is much higher, around 70 beats  $\text{min}^{-1}$  (Hudson and Scott, 1979; Swoap and Gutilla, 2009). An increase in  $f_H$  variability during entrance into torpor is also seen in these mammals that use daily torpor (Mertens et al., 2008; Vicent et al., 2017).

Marsupials diverged from placental mammals about 160 million years ago (Luo et al., 2011) and are interesting from an evolutionary point of view because they have lost functional brown fat (Oelkrug et al., 2015), which is widely considered crucial for thermogenesis during rewarming from hibernation. The objectives of the current experiment therefore were to measure the  $f_H$  and  $f_H$  variability characteristics in the marsupial eastern pygmy possum (*Cercartetus nanus*) at different depths of torpor to examine whether cardiac function especially during deep torpor shows any marsupial idiosyncrasies related to their different form of thermogenesis. This arboreal mammal is found in southeastern Australia and, from an experimental point of view, has an advantage because it enters torpor at a wide range of ambient temperature ( $T_a$ ), resulting in shallow and short bouts of torpor at high  $T_a$ , as well as deep and multi-day bouts of torpor at low  $T_a$  (Geiser, 1993; Bartholomew and Hudson, 1962). Because the eastern pygmy possum can undergo both shallow brief torpor and deep hibernation, this organism allows for the examination of  $f_H$  control in both of these situations. The eastern pygmy possum can hibernate without food for up to an entire year, longer than known for any other species, and also expresses some of the longest torpor bouts measured to date in the laboratory of up to 1 month (Geiser, 2007). This attribute of varied torpor bout duration and depth allows for examination of the full dynamic range of  $f_H$  control in a mammal.

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## MATERIALS AND METHODS

### Animals

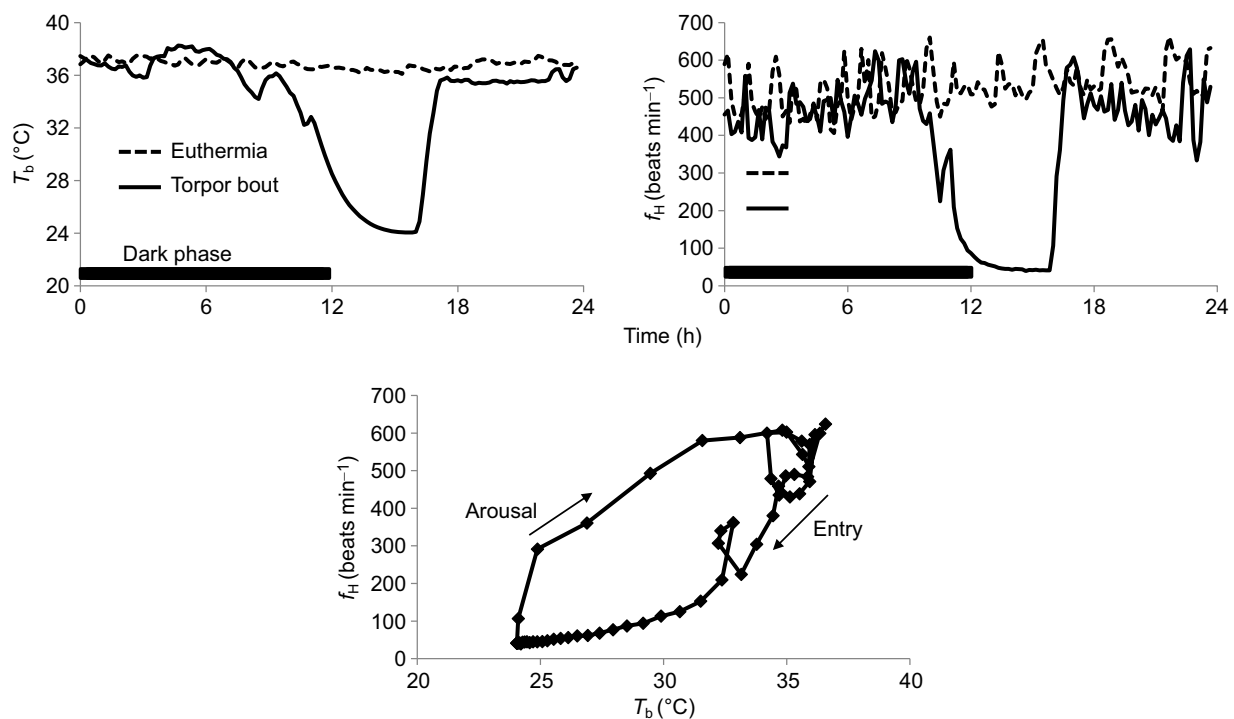
Five eastern pygmy possums, *Cercartetus nanus* (Desmarest 1818), were caught using nest boxes near Dorrigo, NSW, Australia (30°22'S, 152°34'E). The mean minimum monthly  $T_a$  in Dorrigo is 4.4–15.0°C and the mean maximum  $T_a$  is 14.4–24.1°C. The highest recorded maximum  $T_a$  is 36.3°C and the lowest recorded minimum  $T_a$  is –3.5°C. The animals were housed singly in cages with sawdust bedding and nest boxes containing bedding material on a 12 h:12 h light:dark schedule at a  $T_a$  of approximately 22°C. Animals were fed apples, walnuts, sunflower seeds, rolled oats and a mixture of high protein baby cereal, honey, a vitamin supplement, boiled eggs or protein powder and pureed fruit. Food was not provided *ad libitum* because pygmy possums tend to become obese in captivity. Water was freely available. Throughout the experiments, body mass ranged from 25 to 44 g (mean 35 g). All experiments were approved by the University of New England Animal Ethics Committee and were performed in accordance with the guidelines described by the National Health and Medical Research Council.

### Radiotelemetry implantation

Each animal was implanted with an electrocardiogram (ECG) telemeter that (1) detects electrical signals across the heart, (2) measures  $T_b$  and (3) measures locomotor activity (ETA-F10, Data Sciences International, St Paul, MN, USA). For implantation, animals were anesthetized using 5% isoflurane in oxygen gas and maintained with 2.5–3% isoflurane for the duration of the implantation procedure. The telemeter was implanted in the abdominal cavity and ECG leads were placed subcutaneously, approximating a lead II configuration, and held in place by sutures used to close the body wall. Wound clips (7 mm size reflex clips, Fine Science Tools, Foster City, CA, USA) were used to close the

abdominal incision. During the post-operative recovery period, animals were housed individually at 30°C in cages placed half atop a heating pad. The pygmy possums were allowed to recover for 10 days before any experimentation began.

Telemeter data (ECG recordings and  $T_b$ ) were monitored for a period of 10 s, once per minute, using receivers beneath the home cage (RPC-1, Data Sciences International). After approximately 4 weeks of sampling, each possum was placed individually into a 0.5 l metabolic chamber at an initial flow rate of ~300 ml air min<sup>-1</sup> without food and water. These measurements began in the morning and were conducted during the daytime, the period of rest of pygmy possums. Air from the chamber was dried and directed into a Sable FC-1B oxygen analyzer for determination of oxygen content. Flow rate was measured with a mass flowmeter (Omega FMA-5606, Stamford, CT, USA). The  $T_a$  of the chamber, measured to the nearest 0.1°C with a calibrated thermocouple inserted ~1 cm into the respirometry chamber, was initially set to 25°C to measure  $f_H$  and oxygen consumption of euthermic possums. After 3 h, the  $T_a$  of the chamber was raised to approximately 32°C, and lowered again to 25°C at a rate of 1.6°C h<sup>-1</sup> to measure  $f_H$  and O<sub>2</sub> consumption as a function of  $T_a$  and to determine basal values. The possum was moved back to its home cage after completion of the  $T_a$  ramp in the metabolic chamber. To examine deeper torpor bouts, the possums were each placed again in the metabolic chamber in the late afternoon and left there overnight with the  $T_a$  of the chamber set initially to 13–14°C. After the possum had entered torpor in the morning, as assessed in real time by the  $T_b$  of the animal, the air flow rate was lowered to approximately 150 ml min<sup>-1</sup> to increase the O<sub>2</sub> differential, and the  $T_a$  was lowered by 2–3°C approximately every hour to a minimum of 5–8°C. Sampling from the telemeters was continuous in this condition. Once the possum aroused, it was placed back into its home cage at 22°C. To assess the link between ventilation and  $f_H$



**Fig. 1. Typical heart rate ( $f_H$ ) and body temperature ( $T_b$ ) tracings of a pigmy possum housed at 22°C.** This pigmy possum entered a spontaneous bout of torpor (food *ad libitum*) on one day (solid line) and remained euthermic (dashed line) on another (top). The dark phase is the first 12 h of the 24 h period, and is marked by a black bar. These typical tracings show a minimum  $T_b$  during torpor of 24°C and a minimum  $f_H$  of 35 beats min<sup>-1</sup>. The resulting hysteresis curve plotting  $f_H$  as a function of  $T_b$  is also shown (bottom) for the spontaneous bout of torpor (solid line), with arrows indicating directionality of entry into and arousal from torpor.

control during torpor, the possums were placed in the metabolic chamber once again and held at 15°C overnight in the absence of water and food. A pressure transducer (MPX2010, Motorola, Denver, CO, USA) was used to monitor chamber pressure, from which ventilation frequency was determined (Cooper and Withers, 2010). For  $f_H$ ,  $f_H$  variability and ECG noise analysis, raw data files of ECG recordings from the telemeters were imported into Ponemah Physiology Platform software (Data Sciences International). Noise detection was enabled and waveforms were analyzed.

### Data analysis

All results are reported as means±s.e.m. Statistical analyses were performed in SPSS 15.0 (IBM Corp., Armonk, NY, USA). Repeated measures ANOVAs were performed and, when significance was shown, were followed with a *post hoc* Tukey test.  $P < 0.05$  was considered statistically significant.

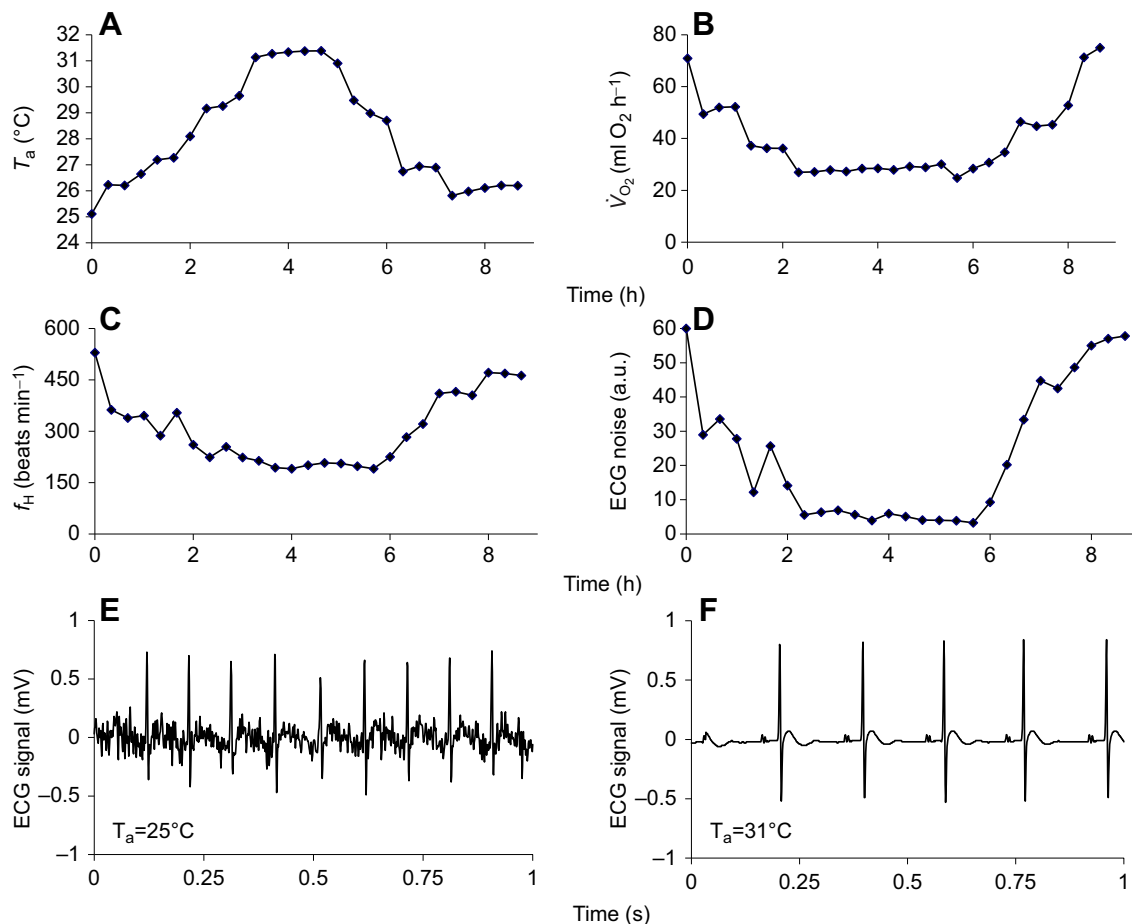
### RESULTS

When pygmy possums were housed in their cages in a room maintained at a  $T_a$  of 22±1°C and  $T_b$  and ECG tracings were monitored, three of the five possums periodically entered a torpor bout despite the presence of food and water (Fig. 1). At this  $T_a$  and on days when the possums did not enter torpor, the average 24 h  $f_H$  for the group was 623±17 beats min<sup>-1</sup> ( $n=5$ ), with an average  $T_b$

over the same time frame of 35.5±0.4°C. On those days that the possums entered torpor spontaneously (Fig. 1),  $f_H$  fell significantly to a minimum of 34±3 beats min<sup>-1</sup> ( $n=3$ ) with a minimum  $T_b$  of 24.1±0.1°C. The relationship between  $f_H$  and  $T_b$  in the animals that entered torpor was complex (Fig. 1).  $f_H$  significantly dropped before the onset of the reduction in  $T_b$ . This was followed by a more gradual decrease in  $f_H$  as  $T_b$  declined.  $f_H$  increased dramatically during the arousal period, which typically took less than 60 min.

Over 10 h, the  $T_a$  of the metabolic chamber housing an implanted pygmy possum was ramped from 25°C to 31–32°C and back down to 25°C (Fig. 2A). Fig. 2E,F shows typical ECG tracings from a pygmy possum at a  $T_a$  of 25 and 31°C. From these ECG tracings, both  $f_H$  and noise from the ECG tracing were quantified together with the rate of O<sub>2</sub> consumption ( $\dot{V}_{O_2}$ ). A typical response to the  $T_a$  ramp in  $\dot{V}_{O_2}$ ,  $f_H$  and noise on the ECG tracing is shown in Fig. 2B–D. The calculated lower critical temperature of the thermoneutral zone from  $\dot{V}_{O_2}$  measurements was 30.0±0.3°C, similar to that found earlier for this same species (Song et al., 1997). The lowest  $\dot{V}_{O_2}$  in the euthermic possum was 0.705±0.048 ml O<sub>2</sub> g<sup>-1</sup> h<sup>-1</sup> ( $n=5$ ) at a  $T_a$  of 31.0±0.2°C and a  $T_b$  of 35.4±0.3°C. Similarly, the minimum  $f_H$  at a  $T_a$  of 31°C was 294±31 beats min<sup>-1</sup>, and the noise in the ECG tracing dropped to 5.1±1.0% of the noise value at a  $T_a$  of 25°C.

When housed individually in a metabolic chamber at 13–14°C without food and water overnight for monitoring  $T_b$ , ECG tracings



**Fig. 2.**  $f_H$ , O<sub>2</sub> consumption rate ( $\dot{V}_{O_2}$ ) and shivering in the pygmy possum are sensitive to  $T_a$  changes from 25 to 31°C. (A)  $T_a$  of the metabolic chamber was raised from 25°C to 31–32°C and back to 25°C over a period of about 8 h. (B)  $\dot{V}_{O_2}$  of a possum in the metabolic chamber. (E,F) Typical electrocardiograms (ECGs) from the same possum at a  $T_a$  of 25°C (the beginning of the ramp, E) and 31°C (the middle of the ramp, F). In addition to the elevated  $f_H$  easily seen at 25°C versus 31°C (C), the noise on the ECG tracing was elevated at the lower  $T_a$  (D).  $\dot{V}_{O_2}$  and noise from the ECG tracing reached a minimum in this possum at a  $T_a$  of 29°C. See Results for group data.

and  $\dot{V}_{O_2}$ , all possums entered a bout of torpor within 24 h of residence in the metabolic chamber. Before the bout of torpor (i.e. euthermic, with a  $T_b$  of  $35.5 \pm 0.3^\circ\text{C}$ ), the average  $f_H$  of the possums was  $624 \pm 11 \text{ beats min}^{-1}$ , not significantly different from the  $f_H$  when housed at  $22^\circ\text{C}$  while not in the metabolic chamber. The average  $\dot{V}_{O_2}$  of the non-torpid pygmy possums at this  $T_a$  of  $13\text{--}14^\circ\text{C}$  was  $3.76 \pm 0.29 \text{ ml O}_2 \text{ g}^{-1} \text{ h}^{-1}$ . Once the possums entered torpor,  $T_b$  dropped to approximately  $15^\circ\text{C}$  ( $14.9 \pm 0.4^\circ\text{C}$ ), which was  $1.3 \pm 0.3^\circ\text{C}$  above  $T_a$ . The minimum  $\dot{V}_{O_2}$  at this  $T_a$  fell to  $0.110 \pm 0.021 \text{ ml O}_2 \text{ g}^{-1} \text{ h}^{-1}$ , and the  $f_H$  dropped to  $25 \pm 2 \text{ beats min}^{-1}$  ( $n=5$ ). When the  $T_a$  of the chamber was then lowered to  $5\text{--}8^\circ\text{C}$  over a period of several hours (see Fig. 3),  $T_b$  fell to an average of  $8.0 \pm 1.0^\circ\text{C}$ , which was  $1.2 \pm 0.4^\circ\text{C}$  above  $T_a$ . The  $\dot{V}_{O_2}$  at this  $T_a$  was  $0.029 \pm 0.007 \text{ ml O}_2 \text{ g}^{-1} \text{ h}^{-1}$  and the minimum  $f_H$  dropped to  $9 \pm 1 \text{ beats min}^{-1}$  ( $n=5$ ). The lowest  $f_H$  sustained in any possum during periods of a stable  $f_H$  was  $8 \text{ beats min}^{-1}$  in a possum with a  $T_b$  of  $6.0^\circ\text{C}$ . Attempts to lower the  $T_a$  below  $5^\circ\text{C}$  evoked arousals in the possums (data not shown).

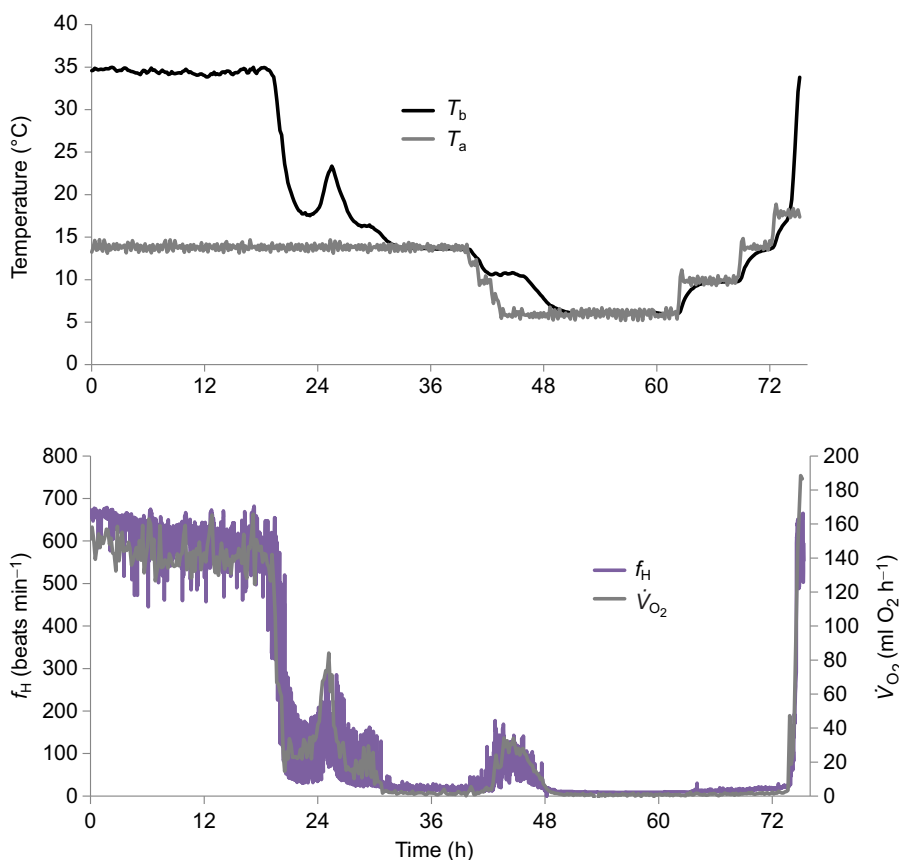
ECGs were examined during three phases of these deep torpor bouts shown in Fig. 3: pre-torpor, entrance into torpor, deep torpor. Before the possums entered torpor,  $f_H$  was fast and had little variability (see ECG tracing in Fig. 4A and the accompanying Poincaré plot). During entrance into torpor (Fig. 4B), the ECG displayed skipped beats, with the heart beats occurring episodically in sets of 3 or 4. The variability of  $f_H$  is easily seen on the accompanying Poincaré plot. During deep torpor, a consistent pattern was observed as shown in Fig. 4C,D. The  $f_H$  was slow and steady for minutes at a time, followed by a slowly increasing  $f_H$ , then a burst of ECG noise and an associated elevation of  $f_H$ , typically to about  $40 \text{ beats min}^{-1}$ . This elevation lasted 20–30 s, at which point the  $f_H$  of the possum returned to a slow and steady level of  $8\text{--}12 \text{ beats min}^{-1}$ . The QRS complex was also examined at three  $T_b$

throughout these deep bouts of torpor. The duration of the QRS complex within the ECG changed as a function of  $T_b$ . As Fig. 5 shows, the duration of the QRS increased 5.8-fold at a  $T_b$  of  $8^\circ\text{C}$  ( $69 \pm 9 \text{ ms}$ ) relative to the QRS duration in euthermia ( $12 \pm 1 \text{ ms}$ ). During arousal,  $f_H$  was quickly obscured by the noise on the ECG tracing, presumably a result of the massive shivering that occurs during emergence from torpor in this species.

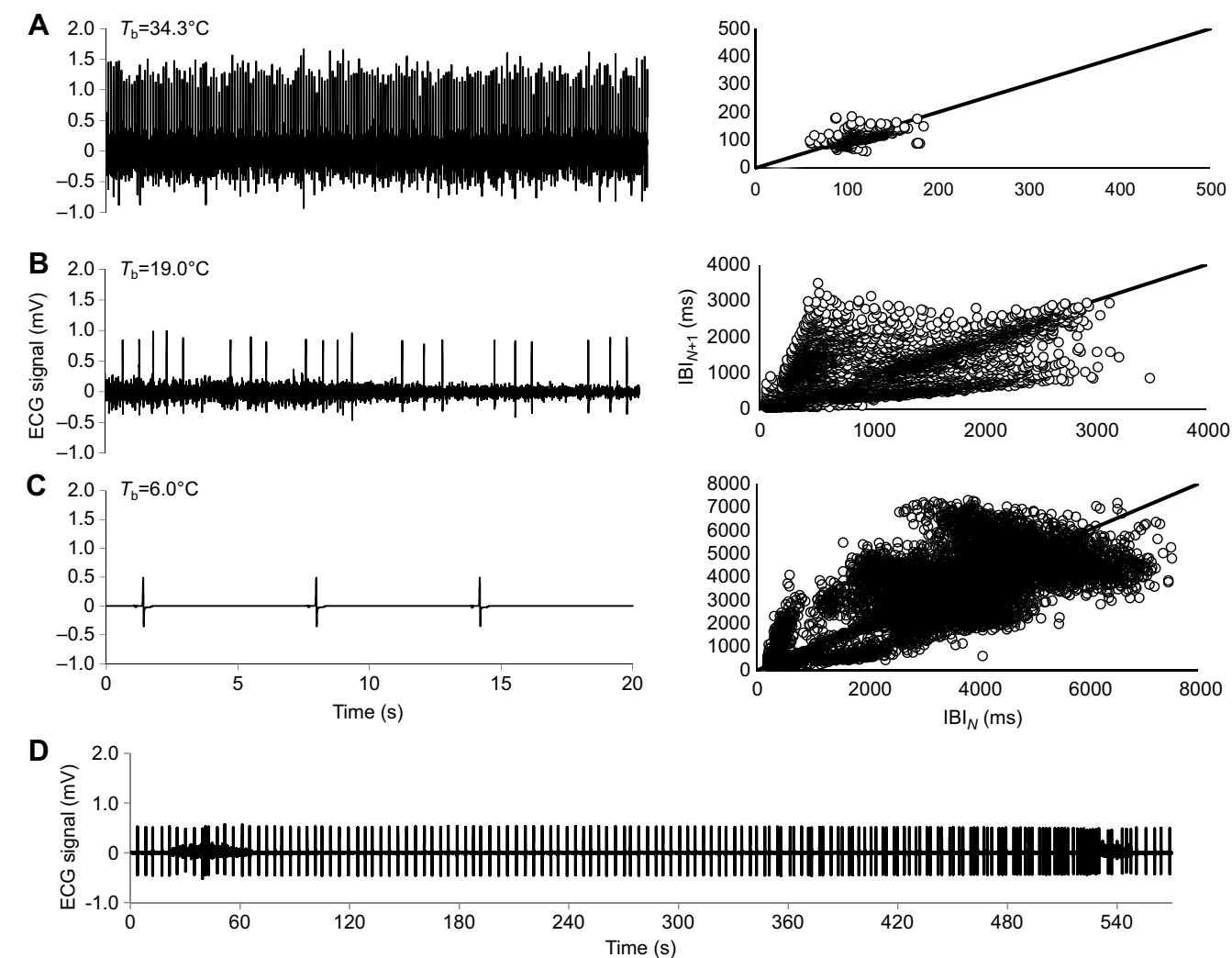
To examine whether the short bouts of tachycardia during deep torpor (Fig. 4D) were related to ventilation patterns, the implanted possums were placed in a metabolic cage with a pressure transducer for determination of breathing activities with simultaneous acquisition of  $T_b$  and  $f_H$  at a  $T_a$  of  $14^\circ\text{C}$ . A typical tracing during euthermia is shown in Fig. 6A. During deep torpor, periods of apnea were clearly discernible between bouts of breathing. A typical bout of breathing during torpor is shown in Fig. 6B, with the region between 120 and 180 s shown on an expanded scale in Fig. 6C. The bouts of increased  $f_H$  during deep bouts of torpor in the pygmy possum occurred as the animal began to breathe.  $f_H$  slowed again during the subsequent apnea bout.

## DISCUSSION

Mammalian  $f_H$  can be tremendously flexible, varying greatly throughout the day, even on a beat-to-beat basis. This flexibility in  $f_H$  is of particular interest in hibernators, where  $f_H$  falls as low as a few beats per minute in a deep bout of torpor. The maximum  $f_H$  for the eastern pygmy possum appears to be about  $625 \text{ beats min}^{-1}$ , similar to an earlier estimate (Bartholomew and Hudson, 1962). The  $f_H$  was very responsive to  $T_a$  above  $25^\circ\text{C}$ , falling to  $325 \text{ beats min}^{-1}$  at thermoneutrality at a  $T_a$  of  $31^\circ\text{C}$ , as has been shown before (Bartholomew and Hudson, 1962). This same relationship between  $f_H$  and  $T_a$  exists in rats and mice. Importantly, the slope of the  $f_H/T_a$

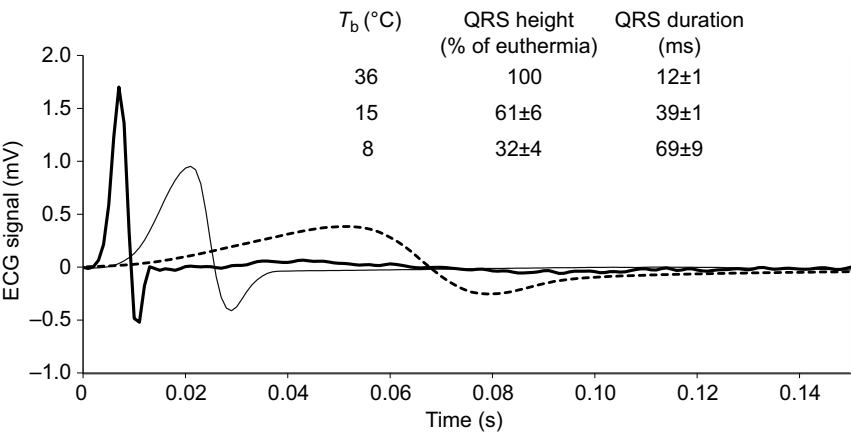


**Fig. 3. Typical tracings of pygmy possum rate of oxygen consumption ( $\dot{V}_{O_2}$ ),  $f_H$  and  $T_b$  during prolonged torpor bouts.** Possums were housed in a metabolic chamber set to a  $T_a$  of  $13\text{--}14^\circ\text{C}$ . Once the possum went into torpor, the  $T_a$  was lowered to  $5^\circ\text{C}$  over a 4 h period. After 22 h at this temperature, the  $T_a$  was then slowly raised. The possum spontaneously aroused from torpor when the  $T_a$  reached  $19^\circ\text{C}$ .  $\dot{V}_{O_2}$ ,  $f_H$  and  $T_b$  were simultaneously measured throughout the entire run. See Results for quantification of these variables.



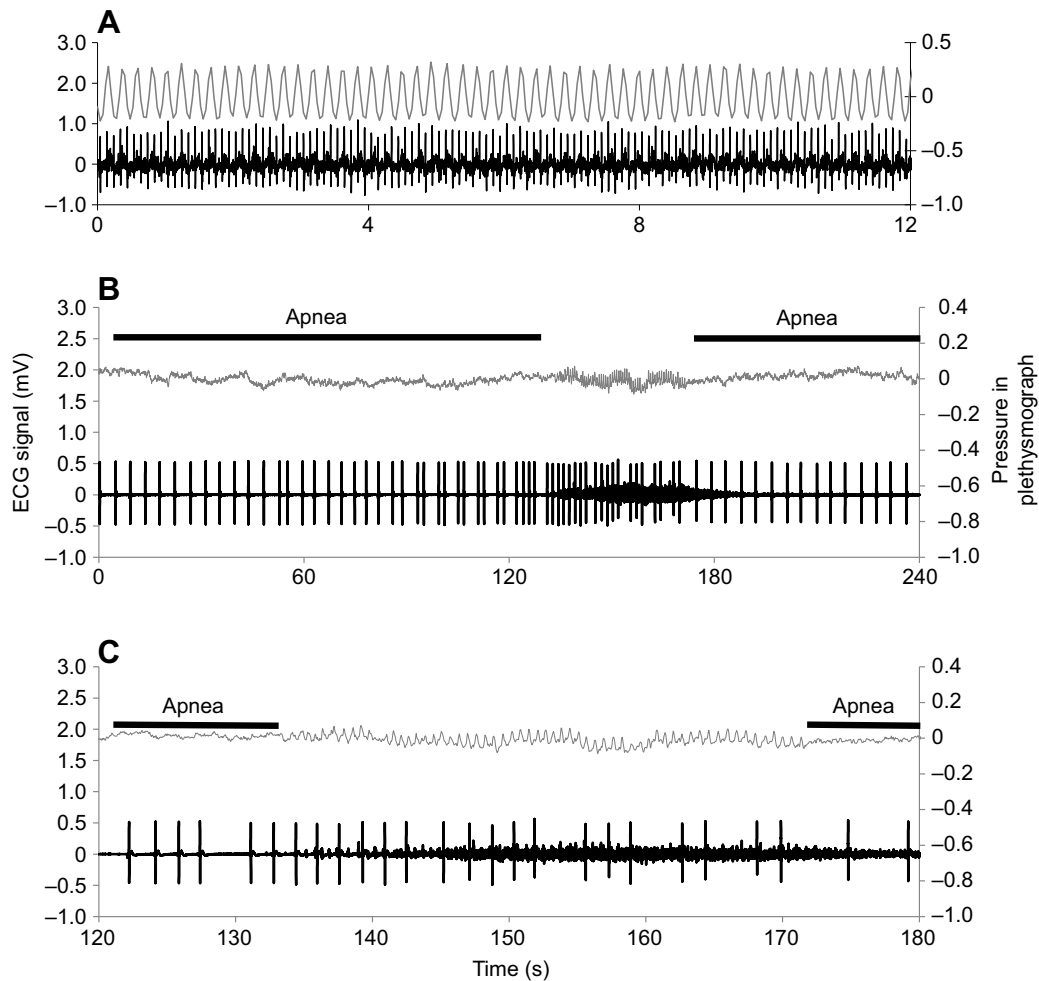
**Fig. 4. Typical ECG tracings and  $f_H$  variability during deep torpor in the pygmy possum for different sections of the torpor bout.** To the right of each ECG tracing is a Poincaré plot, which plots any interbeat interval ( $IBI_N$ ) with the following  $IBI$  ( $IBI_{N+1}$ ). The ECG tracing and accompanying Poincaré plot are shown (A) before the bout of torpor, (B) during descent into torpor and (C) during deep torpor. In addition, a longer time scale during deep torpor is shown (D) to illustrate the bursts of  $f_H$  that occurred in this pygmy possum about every 8 min during deep torpor. The Poincaré plots contain ~65,000, 30,000 and 35,000 data points for A–C, respectively.

relationship is much less steep in rats and mice (8 and 15 beats  $\text{min}^{-1} \text{ } ^\circ\text{C}^{-1}$ , respectively) than it is with the possum (~50 beats  $\text{min}^{-1} \text{ } ^\circ\text{C}^{-1}$ ) between 25 and 31°C (Swoap et al., 2004). However, at  $T_a$  below 22°C when the possums were euthermic, the  $f_H$  stayed unchanged, measured herein at 624 and 623 beats  $\text{min}^{-1}$  at 23 and 15°C, respectively. This lack of change of  $f_H$  at cooler temperatures has been seen previously, including a measured  $f_H$  of euthermic possums of 600–650 beats  $\text{min}^{-1}$  at a  $T_a$  of 5°C



**Fig. 5. QRS complex duration is dependent on  $T_b$ .** The duration of the QRS complex of the pygmy possum ECG is shown at three different  $T_b$  (euthermia 36°C, solid line; 15°C, thin line; 8°C, dashed line) throughout a deep torpor bout.





**Fig. 6. Typical concurrent plethysmograph and ECG tracings in the pygmy possum during eutheria and torpor.** ECG tracings (black line) and plethysmograph tracings (gray line) are shown during (A) eutheria ( $T_b=35.1^\circ\text{C}$ ) and (B) deep torpor ( $T_b=8.1^\circ\text{C}$ ). The region between 120 and 180 s in B is shown on an expanded scale in C for illustration of individual breaths. The tracings in deep torpor show the coincidence of breathing and elevated  $f_H$  during deep bouts of torpor.

(Bartholomew and Hudson, 1962). This suggests that  $f_H$  is at its maximum at  $22^\circ\text{C}$ , and that the SNS has a much greater influence than the parasympathetic nervous system (PNS) over the possums' heart period at  $T_a$  below  $22^\circ\text{C}$ . It is unclear whether cardiac output continues to increase as  $T_a$  drops through elevation in stroke volume in response to the elevated metabolic demand at cooler temperatures.

All animals that use torpor, either hibernation or daily torpor, exhibit a large drop in  $f_H$  during entrance into torpor. This is true for eastern pygmy possum as well (present study and Bartholomew and Hudson, 1962). However, the extent to which  $f_H$  falls differs between animals that use daily torpor and hibernators. With the lower absolute  $T_b$  in hibernators, it is not surprising that the absolute minimum  $f_H$  in hibernators is substantially lower (5–10 beats  $\text{min}^{-1}$ ) compared with the minimum  $f_H$  (70–150 beats  $\text{min}^{-1}$ ) in animals that use daily torpor (Milsom et al., 1999; Morhardt, 1970; Swoap and Gutilla, 2009; Zosky, 2002). However, even for any given  $T_b$  that both groups of animals can achieve, the hibernators have a substantially lower  $f_H$ . For example, during entrance into torpor, the eastern pygmy possum had a  $f_H$  of  $\sim 35$  beats  $\text{min}^{-1}$  at  $24^\circ\text{C}$ , similar to that seen in placental hibernators, woodchucks, hedgehogs and ground squirrels (Lyman, 1958; Harris and Milsom, 1995). This  $f_H$  for hibernators

at a  $T_b$  of  $24^\circ\text{C}$  is only a small fraction (20–45%) of what is observed in animals that use daily torpor at the same  $T_b$ , including placental mammals (the mouse and Djungarian hamster) and in the fat-tailed dunnart, a marsupial (Swoap and Gutilla, 2009; Mertens et al., 2008; Zosky, 2002), supporting the view that hibernators and daily heterotherms differ not only ecologically but also functionally (Ruf and Geiser, 2015).

The relationship between  $T_b$  and  $f_H$  showed marked hysteresis (Fig. 1), suggesting that the control of  $f_H$  during entrance into torpor and emergence from torpor are the result of different phenomena. Hysteresis loops are also observed with  $f_H$  and  $T_b$  in the mouse (Swoap and Gutilla, 2009; Morhardt, 1970), and when examining metabolic rate as a function of  $T_b$  in dunnarts (Geiser et al., 2014), and in the QT interval of the ECG as a function of  $T_b$  in the Djungarian hamster (Mertens et al., 2008). Interestingly, the breadth of the  $f_H/T_b$  loop is much greater in the eastern pygmy possum than in other hibernators as well as in animals that use daily torpor. For example, at a  $T_b$  of  $30^\circ\text{C}$ , the difference in  $f_H$  between entrance and exit from torpor is about 400 beats  $\text{min}^{-1}$  in the eastern pygmy possum. For hibernators, such as the ground squirrel, and in animals that use daily torpor, such as the mouse and Djungarian hamster,  $f_H$  breadth is approximately 200–275 beats  $\text{min}^{-1}$  (Lyman, 1965; Swoap and Gutilla, 2009; Mertens et al., 2008).

The variability of  $f_H$  during entrance into torpor is common to all mammals that enter torpor (Milsom et al., 1999), and the eastern pygmy possum is no different (Fig. 4). There appear to be at least two sources of  $f_H$  variability during torpor and entrance into torpor that are both linked to the ANS. First, the appearance of asystoles (skipped beats) during entrance into torpor (Fig. 4) is seen in many hibernators and is a consequence of elevated PNS activity during entrance into torpor (see Milsom et al., 1999, for a review). While we did not perform any pharmacological experiments in the current study, administration of atropine, a muscarinic receptor antagonist, into the closely related western pygmy possum during torpor significantly elevates  $f_H$  and eliminates asystoles (Zosky and Larcombe, 2003). The second source of variability in  $f_H$  (Fig. 4) occurred during the periods between bouts of apnea in deep torpor in the eastern pygmy possum.  $f_H$  was elevated from 8 to 12 beats  $\text{min}^{-1}$  during apnea to approximately 40 beats  $\text{min}^{-1}$  during ventilation in deep torpor (Fig. 6). This ventilation-associated tachycardia is also seen in several other hibernating species, such as bears and ground squirrels, and is indicative of altered ANS activity (Dawe and Morrison, 1955; Tøien et al., 2011; Milsom et al., 1999; Harris and Milsom, 1995). Indeed, atropine administration during torpor in the western pygmy possum eliminates ventilation-associated tachycardia (Zosky and Larcombe, 2003). The low  $f_H$  of 8 beats  $\text{min}^{-1}$  we measured here is in contrast with a previous study where this same species had a  $f_H$  of 28 beats  $\text{min}^{-1}$  during torpor (Bartholomew and Hudson, 1962). While it is difficult to know the source of the difference between these studies, Bartholomew and Hudson (1962) were surprised at the relatively high torpid  $f_H$  and concluded the animals were likely disturbed when instrumented during the bout of torpor. Notably, we used radiotelemetry here with indwelling lines that minimize disturbance of the possums.

We have shown here that the eastern pygmy possum has an enormous dynamic range in  $f_H$  of 600 beats  $\text{min}^{-1}$ , from 625 to 8 beats  $\text{min}^{-1}$  (an 80-fold difference), to match the metabolic flexibility (greater than 100-fold) in torpor and euthermia. The animal's  $T_b$  during torpor plays a role in this flexibility, which can be seen by the  $Q_{10}$  of the QRS duration of approximately 2 (Fig. 5), similar to that seen in the Djungarian hamster (Mertens et al., 2008). Two other much smaller mammals have been reported to have a greater dynamic range in  $f_H$ , the Etruscan shrew (Fons et al., 1997) and the Gould's long-eared bat (Currie et al., 2014). The 2.4 g shrew has a dynamic range of 900 beats  $\text{min}^{-1}$  (euthermic rate of 1000 beats  $\text{min}^{-1}$  and torpid rate of 100 beats  $\text{min}^{-1}$ ) whereas the 9 g bat has a range of 800 beats  $\text{min}^{-1}$  (euthermic rate of 800 beats  $\text{min}^{-1}$  and torpid of 8 beats  $\text{min}^{-1}$ ). The rapid 600 beats  $\text{min}^{-1}$  increase in  $f_H$  (and likely cardiac output) during recovery from torpor in the pygmy possum is probably important for meeting  $O_2$  demand for metabolic rate elevation and heat production, such as shivering in skeletal muscle, during rewarming as has been suggested for the shrew (Fons et al., 1997). To sum, despite the lack of functional brown fat,  $f_H$  control in this marsupial hibernator throughout a bout of deep torpor appears indistinguishable from that of a placental hibernator in terms of depth, appearance of skipped beats and ventilation-associated tachycardia. However, there appears to be a large difference in minimum  $f_H$  between hibernators and mammals that use daily torpor, even when measured at the same  $T_b$ .

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#### Competing interests

The authors declare no competing or financial interests.

#### Author contributions

Conceptualization: S.S., F.G.; Methodology: S.S., F.G.; Formal analysis: S.S., F.G.; Investigation: G.K., F.G.; Resources: S.S., F.G.; Data curation: S.S.; Writing - original draft: S.S.; Writing - review & editing: S.S., G.K., F.G.; Supervision: G.K., F.G.; Project administration: F.G.; Funding acquisition: F.G.

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#### References

- Bartholomew, G. A. and Hudson, J. W. (1962). Hibernation, estivation, temperature regulation, evaporative water loss, and heart rate in the pygmy possum, *Cercartetus nanus*. *Physiol. Zool.* **35**, 94–107.
- Cannon, B. and Nedergard, J. (2004). Brown adipose tissue: function and physiological significance. *Physiol. Rev.* **84**, 277–359.
- Cooper, C. E. and Withers, P. C. (2010). Comparative physiology of Australian quolls (*Dasyurus*; Marsupialia). *J. Comp. Physiol. B* **180**, 857–868.
- Currie, S. E., Körtnier, G. and Geiser, F. (2014). Heart rate as a predictor of metabolic rate in heterothermic bats. *J. Exp. Biol.* **217**, 1519–1524.
- Dawe, A. R. and Morrison, P. R. (1955). Characteristics of the hibernating heart. *Am. Heart J.* **49**, 367–384.
- Eagles, D. A., Jacques, L. B., Taboada, J., Wagner, C. W. and Diakun, T. A. (1988). Cardiac arrhythmias during arousal from hibernation in three species of rodents. *Am. J. Physiol.* **254**, R102–R108.
- Fons, R., Sender, S., Peters, T. and Jurgens, K. (1997). Rates of rewarming, heart and respiratory rates and their significance for oxygen transport during arousal from torpor in the smallest mammal, the Etruscan shrew *Suncus etruscus*. *J. Exp. Biol.* **200**, 1451–1458.
- Geiser, F. (1993). Hibernation in the Eastern Pygmy Possum, *Cercartetus nanus* (Marsupialia, Burramyidae). *Aust. J. Zool.* **41**, 67–75.
- Geiser, F. (2004). Metabolic rate and body temperature reduction during hibernation and daily torpor. *Ann. Rev. Physiol.* **66**, 239–274.
- Geiser, F. (2007). Yearlong hibernation in a marsupial mammal. *Naturwissenschaften* **94**, 941–944.
- Geiser, F., Currie, S. E., O'Shea, K. A. and Hiebert, S. M. (2014). Torpor and hypothermia: reversed hysteresis of metabolic rate and body temperature. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **307**, R1324–R1329.
- Hampton, M., Nelson, B. T. and Andrews, M. T. (2010). Circulation and metabolic rates in a natural hibernator: an integrative physiological model. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **299**, R1478–R1488.
- Harris, M. and Milsom, W. (1995). Parasympathetic influence on heart rate in euthermic and hibernating ground squirrels. *J. Exp. Biol.* **198**, 931–937.
- Heller, H. C. (1983). The physiology of hibernation. *Science* **220**, 599–600.
- Hudson, J. W. and Scott, I. M. (1979). Daily torpor in the laboratory mouse, *Mus musculus* var albino. *Physiol. Zool.* **52**, 205–218.
- Luo, Z.-X., Yuan, C.-X., Meng, Q.-J. and Ji, Q. (2011). A Jurassic eutherian mammal and divergence of marsupials and placentals. *Nature* **476**, 442–445.
- Lyman, C. P. (1958). Oxygen consumption, body temperature and heart rate of woodchucks entering hibernation. *Am. J. Physiol.* **194**, 83–91.
- Lyman, C. P. (1965). Circulation in mammalian hibernation. In: *Handbook of Physiology* (ed. W. F. Hamilton), pp. 1–45. Washington, DC: American Physiological Society.
- Mertens, A., Stiedl, O., Steinlechner, S. and Meyer, M. (2008). Cardiac dynamics during daily torpor in the Djungarian hamster (*Phodopus sungorus*). *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **294**, R639–R650.
- Milsom, W. K. and Jackson, D. C. (2011). Hibernation and gas exchange. *Comp. Physiol.* **1**.
- Milsom, W. K., Zimmer, M. B. and Harris, M. B. (1999). Regulation of cardiac rhythm in hibernating mammals. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **124**, 383–391.
- Morhardt, J. E. (1970). Heart rates, breathing rates and effects of atropine and acetylcholine on white-footed mice (*Peromyscus*) during daily torpor. *Comp. Biochem. Physiol.* **33**, 441–457.
- Oelkrug, R., Polymeropoulos, E. T. and Jastroch, M. (2015). Brown adipose tissue: physiological function and evolutionary significance. *J. Comp. Physiol. B* **185**, 587–606.
- Osborne, P. G., Sato, J., Shuke, N. and Hashimoto, M. (2005). Sympathetic (alpha)-adrenergic regulation of blood flow and volume in hamsters arousing from hibernation. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **289**, R554–R562.
- Ruf, T. and Geiser, F. (2015). Daily torpor and hibernation in birds and mammals. *Biol. Rev.* **90**, 891–926.
- Song, X., Körtnier, G. and Geiser, F. (1997). Thermal relations of metabolic rate reduction in a hibernating marsupial. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **273**, R2097–R2104.

- Swoap, S. J. and Gutilla, M. J.** (2009). Cardiovascular changes during daily torpor in the laboratory mouse. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **297**, R769–R774.
- Swoap, S. J. and Weinshenker, D.** (2008). Norepinephrine controls both torpor initiation and emergence via distinct mechanisms in the mouse. *PLoS ONE* **3**, e4038.
- Swoap, S. J., Overton, J. M. and Garber, G.** (2004). Effect of ambient temperature on cardiovascular parameters in rats and mice: a comparative approach. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **287**, R391–R396.
- Tøien, Ø., Blake, J., Edgar, D. M., Grahn, D. A., Heller, H. C. and Barnes, B. M.** (2011). Hibernation in black bears: independence of metabolic suppression from body temperature. *Science* **331**, 906–909.
- Vicent, M. A., Borre, E. D. and Swoap, S. J.** (2017). Central activation of the A1 adenosine receptor in fed mice recapitulates only some of the attributes of daily torpor. *J. Comp. Physiol. B* **187**, 835–845.
- Zosky, G.** (2002). The parasympathetic nervous system: its role during torpor in the fat-tailed dunnart (*Sminthopsis crassicaudata*). *J. Comp. Physiol. B Biochem. Syst. Environ. Physiol.* **172**, 677–684.
- Zosky, G. R. and Larcombe, A. N.** (2003). The parasympathetic nervous system and its influence on heart rate in torpid western pygmy possums, *Cercartetus concinnus* (Marsupialia: Burramyidae). *Zoology* **106**, 143–150.