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Thermal physiology of a reproductive female marsupial, *Antechinus flavipes*

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Abstract

Reproduction is an energetically costly, but necessary, process for animals. Small mammals in particular are at a disadvantage as they experience high heat losses to their environment, substantially increasing their energetic costs. However, many small mammals save energy by using torpor, a reduction in metabolic rate and body temperature (T_b). *Antechinus flavipes* is a small dasyurid marsupial endemic to Australia that uses torpor during adverse conditions. Females usually produce only one litter in their life. In our study we aimed to quantify the thermal physiology of reproductive female *A. flavipes* held under natural ambient conditions in an outdoor aviary, and also under stable conditions indoors, during gestation, parturition and lactation. Throughout gestation, *A. flavipes* displayed similar variations in torpor use and T_b to reproductively unsuccessful females (did not produce young). Torpor use increased with decreasing ambient temperature in the outdoor aviary and ceased when held under stable ambient conditions indoors. Interestingly, during parturition/early lactation T_b was tightly controlled and daily T_b variation decreased by ≥2.3°C in comparison to all other reproductive states. Additionally, daily T_b variations during lactation were slightly smaller than those of gestating and reproductively unsuccessful females, however, this data is only preliminary. Our results reveal that the flexibility of the thermal physiology of female *A. flavipes* is important throughout the reproductive period, except during parturition/early lactation when a stable T_b is likely beneficial to the early stages of development.

Keywords: body temperature, gestation, lactation, mammal, parturition, torpor

Introduction

The reproductive period is an energetically expensive time for mammals that often requires additional food intake or the use of an energy saving mechanism (Farmer 2003; McAllan and Geiser 2014). Mating often involves searching for a mate, which may take time away from foraging resulting in a negative energy balance for both males and females. After mating however, the main energetic costs are often harboured by females, with additional nutrients needed for the developing young throughout gestation and the energy required during parturition (Farmer 2003). Once offspring are born, in mammals young are fed via lactation, which requires more energetic investment than the other reproductive periods (Harlow et al. 1985; Thompson and Nicoll 1986; Farmer 2003; Canale et al. 2012; McAllan and Geiser 2014). This is particularly pertinent to marsupials as, unlike eutherian mammals, their young are born extremely altricial and most of the growth and development of the young occurs after parturition in the pouch (Renfree and Shaw 1996).

Balance between energy use and acquisition are vital not only for the survival of the mother, but also to ensure the healthy development of young (Harlow et al. 1985). However, maintaining a high and stable T_b requires large amounts of energy, particularly for small mammals that lose large amounts of heat to the environment due to their large surface area to volume ratios. Some mammals overcome this by using torpor, a reduction in metabolic rate and T_b, which significantly decreases energy requirements (Ruf and Geiser 2015). While a stable T_b is favourable for offspring development during gestation, some species use torpor during this period, likely to save energy during inclement weather and delay parturition until conditions are more favourable (Geiser and Masters 1994; Farmer 2003; Geiser et al. 2005; Willis et al. 2006; Körtner et al. 2008; Stawski 2010; Canale et al. 2012; McAllan and Geiser 2014). On the other hand, some heterothermic mammals are known to decrease the variability of T_b during gestation and lactation if conditions are favourable and therefore abandon torpor use altogether during these reproductive periods, suggesting that homeothermy may be more beneficial during reproduction (Levesque and Lovegrove 2014; Levesque et al. 2014). However, data on the thermal physiology of females during parturition are limited to monotremes and eutherian mammals (Scribner and Wynne-Edwards 1994; Nicol and Andersen 2006) and none are currently available for marsupials.

In our current study we quantified data on the thermal physiology of yellow-footed antechinus (*Antechinus flavipes*), a small dasyurid marsupial, during gestation, parturition and lactation. Antechinus are polyandrous and exhibit semelparity, all males die after the mating period and most females die once their young have been weaned (Körtner and Geiser

1995; Fisher et al. 2006; McAllan and Geiser 2006). However, some females live for a second year to produce another litter (Rojas et al. 2014). During adverse environmental conditions antechinus are known to use torpor to save energy (Geiser 1988; Rojas et al. 2014). *Antechinus flavipes* present a unique example to examine the thermal physiology of female semelparous marsupials during different stages of reproduction, for which data are lacking.

Methods

All *A. flavipes* were captured during the austral autumn (March – April) in 2014 using Elliott traps (Elliott Scientific Equipment, Upwey, Australia) at Aberbaldie Nature Reserve (31°04'24"S, 151°25'34"E) in New South Wales, Australia. Animals were then transferred to animal holding facilities at the University of New England and kept for 2 months before surgeries were performed in late June 2014. To measure core T_b, each individual was implanted with a temperature-sensitive transmitter (1.8 to 2.1 g, Sirtrack, Havelock North, New Zealand) into the intraperitoneal cavity when they were non-reproductive (see Stawski et al. 2015 for details on the surgical procedure). Transmitters were calibrated at temperatures between 15°C to 40°C ($R^2 \geq 0.99$) before implantation and weighed <10% of each individuals body mass (as recommended by Rojas et al. 2010). All transmitters were active from calibration and were therefore recording body temperature as soon as they were implanted into the animals. Following surgery, animals were allowed to recover for one week and they were then transferred to a large outdoor aviary on 26 June 2014, where four females (22.8 ± 1.9g) were held together with three males. The experiment was undertaken from July to October 2014, which is known to encompass the reproductive period for *A. flavipes* (McAllan and Geiser 2006). On the 14 August 2014 all individuals were transferred to individual cages and placed in an animal housing facility indoors. This was to ensure the successful birth of any potential litters as the young were required for another experiment. Ambient temperature (T_a) in the outdoor aviary and the indoor facility was measured with iButtons (± 0.5°C, iButton thermochron DS1921G, Maxim Integrated Products, Inc., Sunnyvale, California, USA) at 10 minute intervals. Body temperature was recorded at 10 minute intervals using a data logger/receiver attached to an antenna that picked up the unique frequency of each transmitter (Körtner and Geiser 2000) and data for each individual were collected for as long as the transmitter batteries lasted. Animals were provided food and water *ad libitum* throughout the whole experiment. All of the procedures were approved by the University of New England Animal Ethics Committee (AEC13-088) and the New South Wales National Parks and Wildlife Service (SL100791).

The torpor threshold of 32.5°C was calculated using equation 4 from Willis (2007) using the mean body mass of all females in this study (22.8 ± 1.9g, measured just prior to being transferred to the outdoor aviary) and the mean T_a the females were exposed to in the outdoor aviary. Torpor bout duration was defined as time periods that T_b dropped below this threshold for >30 minutes (Geiser and Masters 1994). Individual date of conception was calculated as 31.5 days before the first day of the parturition period, which was based on previous studies on *A. flavipes* (Marlow 1961; McAllan and Geiser 2006) and, importantly, several antechinus species are known to show little flexibility in gestation length (Selwood 1980; Cockburn 1992). The daily T_b range was calculated for each individual, as well as the minimum T_b of each torpor bout to represent torpor depth. The mean of all of the individuals means ± 1 standard deviation (SD) (n = number of individuals, N = number of recorded observations) were then calculated for the following groups: reproductively unsuccessful (females that did not produce young, although may have been pregnant and aborted the young before birth), gestating (individuals that produced young), parturient/early lactation (giving birth and early lactation) and lactating (young attached to teats). Linear mixed effects models (packages 'lme4', Bates et al. 2015, and 'MuMIn', Bartoń 2015, in R v. 3.0.1, R Core Team, 2014) were used to determine the effect of daily mean T_a on daily mean T_b; individuals were accounted for as a random effect.

Results and Discussion

When located in the outside aviary under natural weather conditions (T_a range: -1 to 28.9°C; mean T_a: 9.2 ± 1.8°C, N = 22 days) the T_b of all four female *A. flavipes* fluctuated similarly, including the two that produced young during the study (Fig. 1). Importantly, the daily T_b range did not differ between reproductively unsuccessful (8.5 ± 0.4°C, n = 4, N = 67) and gestating (9.1 ± 0.4°C, n = 2, N = 21) females, suggesting that at least during early pregnancy *A. flavipes* are able to cope with large variations in T_b. While other heterothermic marsupials show a similar response, many also show a reduction in T_b variation after conception (Geiser and Masters 1994; Geiser et al. 2005; Körtner et al. 2008). These large fluctuations in T_b revealed that torpor was expressed by all individuals on 63.6 ± 0.1% (n = 4, N = 88) of all days when housed outdoors. Total daily torpor bout duration and torpor depth did not differ between reproductively unsuccessful (torpor bout duration: 144.3 ± 52.9 min, n = 4, N = 41;

torpor depth: 29.4 ± 0.5, $n = 4$, $N = 41$) and gestating (torpor bout duration: 159.5 ± 51.6 min, $n = 2$, $N = 15$; torpor depth: 29.0 ± 0.3, $n = 2$, $N = 21$) females (Fig. 1).

Throughout the period when *A. flavipes* were in the outdoor aviaries, the daily mean T_b of each female exhibited a positive correlation with daily mean T_a ($p < 0.0001$, $t_{1,82} = 7.5$, $R^2 = 0.4$; Fig. 2). Further, the mean T_a on days that torpor was used was 8.6 ± 1.6°C ($N = 60$ animal days), which was colder in comparison to days when individuals remained normothermic (10.2 ± 1.5°C, $N = 28$ animal days). In the wild a drop in T_a often corresponds to a reduction in insect activity (Richards 1989; Pavey and Burwell 2004; Stawski 2012), the primary food source for antechinus. Therefore, even though in the current study individuals were provided with food daily, it is likely that a reduction in T_a still provided a cue for a possible food shortage. Nevertheless, low T_a increases energy expenditure and by using torpor during these periods *A. flavipes* would save large amounts of energy (Ruf and Geiser 2015), which is likely vitally important throughout gestation.

The remainder of the reproductive period in the current study was completed after individuals were transferred indoors to more stable ambient conditions (T_a range: 12.6 to 24.8°C; mean T_a: 17.4 ± 1.3°C, $N = 54$ days). Under these indoor conditions T_a had no effect on T_b for *A. flavipes* ($p = 0.348$, $t_{2,146} = -2.1$, $R^2 = 0.008$). While T_b still fluctuated for reproductively unsuccessful females (5.3 ± 0.2°C, $n = 2$, $N = 42$), in gestating (4.9 ± 0.5°C, $n = 2$, $N = 40$) and lactating (4.2 ± 0.1°C, $n = 2$, $N = 58$) individuals this was slightly reduced and during parturition/early lactation (1.9 ± 0.04°C, $n = 2$, $N = 10$) T_b became even more stable for a period of five days (Fig. 1). Such a large reduction (≥2.3°C) in daily T_b variation during parturition/early lactation has also been documented in the egg-laying echidna (*Tachyglossus aculeatus*) and for two species of hamsters (*Phodopus campbelli* and *P. sungorus*) (Scribner and Wynne-Edwards 1994; Nicol and Andersen 2006), but our data are the first to report this for a marsupial. It is likely that the first few days after birth are important for the mother to produce high quality milk to ensure proper development of young, which requires a high and stable T_b. The daily T_b range experienced by lactating individuals was also reduced in comparison to reproductively unsuccessful and gestating females, but only by 0.7 to 1.1°C, respectively. A similar response was found in gray short-tailed opossums (*Monodelphis domestica*) and tenrecs (*Setifer setosus*) and less variable T_b during lactation is likely beneficial for healthy development and milk production (Harder et al. 1996; Levesque and Lovegrove 2014; Levesque et al. 2014).

Under the more stable ambient conditions, torpor use essentially ceased, with torpor being expressed by reproductively unsuccessful and gestating females on only 5.4 ± 0.1% (n

= 4, *N* = 153) of days. However, previous studies have found that animals are often more reluctant to use torpor in captive conditions (Geiser and Ferguson 2001). Therefore, while lactating *A. flavipes* in the current study did not use torpor, it is uncertain whether free-ranging *A. flavipes* would use torpor during this energetically demanding period. Previous studies have shown that some mammals do indeed cease torpor use during lactation (Geiser et al. 2008; Körtner et al. 2008), whereas others continue to use torpor (Harlow et al. 1985; Geiser et al. 2008; Canale et al. 2012). It has been suggested that by using torpor during gestation, small dasyurids such as *A. flavipes* may be able to store energy for the lactation period and therefore avoid using torpor to guarantee the continued production of high quality milk (Geiser et al. 2008).

Our study reveals that female *A. flavipes* employ varying thermal strategies throughout the reproductive period. Importantly, females continued to use torpor to save energy even when pregnant and housed under variable natural ambient conditions. However, gestating individuals ceased torpor use and maintained a higher T_b when kept in more stable conditions, likely to promote the proper development of young. This response reveals that even though antechinus use torpor often as a last resort (Rojas et al. 2014), it is a vital strategy that may help to ensure the survival of the mother and young during times when food is in short supply or in response to detrimental environmental conditions (Geiser et al. 2005; Willis et al. 2006; Geiser et al. 2008; Canale et al. 2012; McAllan and Geiser 2014). Our data also revealed that T_b was more tightly controlled during parturition and lactation, likely to support the growth and development of the young. Therefore, the flexible thermal physiology of female *A. flavipes* reported in our study would allow them to respond to any sudden changes in the environment and to ensure that all stages of reproduction are successful. However, as captivity is known to influence physiological variables (Geiser and Ferguson 2001), it would be beneficial to conduct this study on a larger sample size of free-ranging *A. flavipes*.

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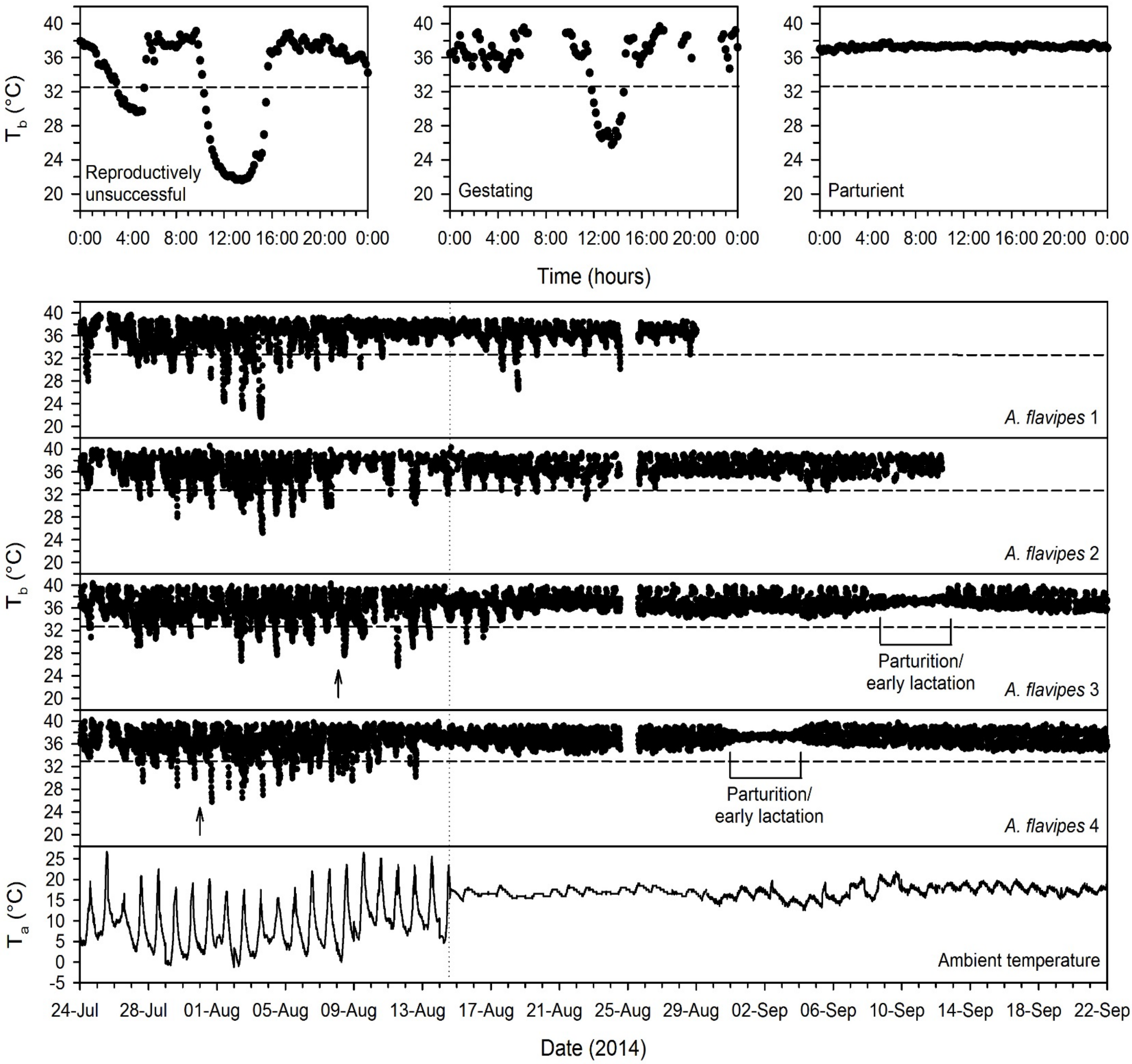

Figure 1. Body temperature (T_b) traces of each individual *Antechinus flavipes* and ambient temperature (T_a) trace over the study period (bottom graph). Only individuals 3 and 4 produced litters. The arrows indicate the approximate conception date for individuals 3 and 4 with parturition beginning 31.5 days later as displayed. The top three graphs represent a single day for a reproductively unsuccessful, gestating and parturient female. The dashed horizontal lines represent the torpor threshold and the dotted vertical line represents the day when individuals were transferred from the outdoor aviary to indoor cages.

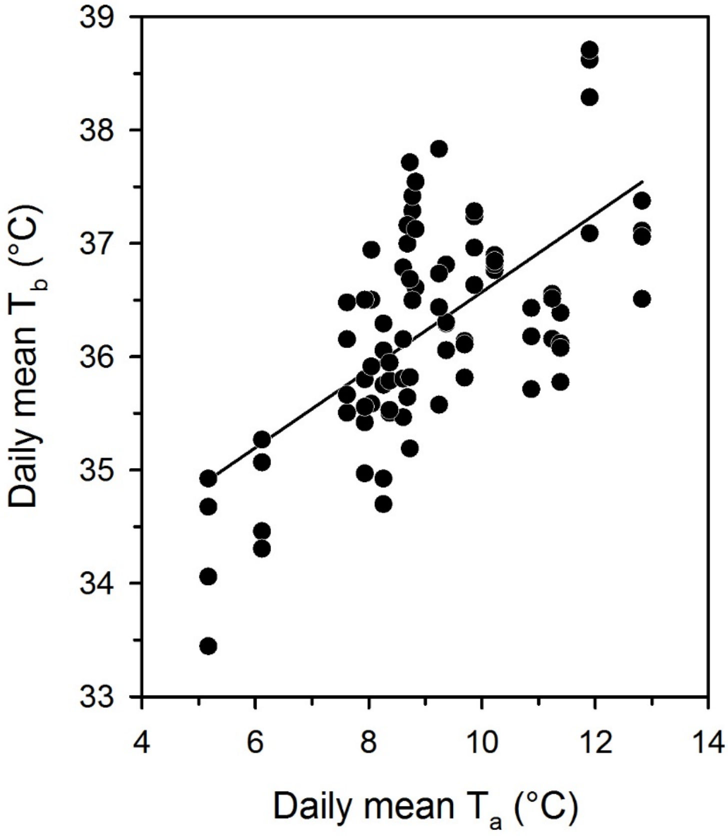

Figure 2. The relationship between daily mean body temperature (T_b) and daily mean ambient temperature (T_a) ($p < 0.0001$, $t_{1,82} = 7.5$, $R^2 = 0.4$) for all individual *Antechinus flavipes* when housed in the outdoor aviaries.