

The role of basking in the development of endothermy and torpor in a marsupial

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Abstract Marsupials have a slow rate of development and this allows a detailed examination of thermoregulatory developmental changes and stages. We quantified the cooling rates of marsupial dunnarts (*Sminthopsis crassicaudata*) at 40–56 days (d) old, and torpor and basking behaviour in animals given the option to bask in four age groups from 60 to 150 d. The development of thermoregulation was a continuum, but was characterised by three major thermoregulatory stages: (1) at 40 d, animals were unable to maintain a constant high body temperature during short-term cold exposure; (2) at 60 d, animals could maintain a high T_b for the first part of the night at an ambient temperature of $15.0 \pm 0.7^\circ\text{C}$; later in the night, they entered an apparent torpor bout but could only rewarm passively when basking under a heat lamp; (3) from ~90 d, they expressed prolonged torpor bouts and were able to rewarm endogenously. Young newly weaned 60 d animals were able to avoid hypothermia by basking. In this case, basking was not an optional behavioural method of reducing the cost of rewarming from torpor, but was essential for thermoregulation independent of the nest temperature. Results from our study suggest that basking is a crucial behavioural trait that permits young marsupials and perhaps other juvenile altricial mammals to overcome the developmental stage between poikilothermy early in development and full endothermy later in life.

Keywords Basking · Development · Marsupial · *Sminthopsis* · Thermal energetics · Torpor

Introduction

Most mammals and birds are altricial at birth with little or no insulation, little coordination, and a low metabolic rate (MR), and are, therefore, unable to maintain a high and constant body temperature (T_b) independently (Hill 1976). Endothermic thermoregulation develops gradually with growth, increased insulation, and neural control (Morrison and Petajan 1962; Hill 1976). Marsupials are extreme examples of mammalian altricial development; they are born at <1% of the maternal body mass (Tyndale-Biscoe and Renfree 1987), have an extremely low MR (Hulbert 1988) and essentially non-functional lungs, but they can satisfy the required oxygen uptake via their skin (Mortola et al. 1999). As the rate of growth in marsupials is approximately half that of placental mammals, which show similar, but brief developmental stages, marsupials permit a detailed sequential examination of the morphological and physiological changes during development (Janssens et al. 1997).

For a large part of marsupial development, the low MR is of little thermoregulatory consequence, because the T_b of the developing young, in most species, is buffered by the mother's pouch, which has a temperature similar to that of the mother's core T_b (Bartholomew 1956). However, near the time of pouch exit, the young must be able to regulate their T_b and it has been shown that increased size, insulation, and increased heat production permit them to do so (Janssens et al. 1997). The problem that the young of small marsupials must overcome when they leave the pouch at approximately 10–40% of the mother's size (Russell 1982;

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Tyndale-Biscoe and Renfree 1987) is their relatively large heat loss due to their large surface area to volume ratio and high thermal conductance (Geiser and Brigham 2012). In small adult mammals, such energetic challenges are commonly dealt with by entering torpor and temporarily reducing MR and T_b to conserve energy (Ruf and Geiser 2015). This is also the case in some growing juveniles, which use torpor during development to reduce energetic costs and enhance growth on limited resources (Giroud et al. 2014). However, although torpor saves large quantities of energy, the enormous increase in heat production required during the arousal process at the end of a torpor bout (Lyman et al. 1982) is not only costly, but also physiologically challenging (Currie et al. 2015). While this increase in heat production at a low T_b and the associated energetic costs may be possible for adults, it is not likely the case for all endothermic developmental stages of juveniles with a large relative surface area and a limited capacity to increase MR. One possible solution would be to use passive rewarming or basking permitting arousal with a relatively low MR (Geiser et al. 2004; Currie et al. 2015). Behavioural thermoregulation in the form of basking to reduce thermoregulatory costs in mammals has been well documented, however, basking during rewarming from torpor or for normothermic thermoregulation in mammals has only been observed in the adults of a few species (Schradin et al. 2007; Warnecke et al. 2008; Signer et al. 2011; Rojas et al. 2012; Thompson et al. 2015; Geiser et al. 2016). Although huddling and passive rewarming via raised nest temperatures in juvenile fully endothermic mammals have been observed (Sealander 1952; Arnold 1988; Schradin et al. 2006; Gilbert et al. 2007; Terrien et al. 2011), there are no data on whether and at what developmental stage juveniles use behavioural thermoregulation when offered radiant heat to rewarm from torpor and when normothermic.

The aim of our study was to provide detailed measurements on the developmental thermoregulatory stages in a marsupial. To achieve this, we measured developmental changes in body mass, thermoregulation, thermal energetics, basking behaviour, and insulation in the fat-tailed dunnart (*Sminthopsis crassicaudata*, adult body mass 12–18 g, and hereafter ‘dunnart’). This arid-zone marsupial weighs approximately 10 mg at birth, is weaned at ~60–65 days (d) of age, and the females are sexually mature at ~5 months (Godfrey and Crowcroft 1971). Dunnarts use torpor in both the laboratory, and the field and adults are known to bask during rewarming from torpor in the wild (Warnecke et al. 2008). However, the expression of torpor and the use of basking at different developmental stages have not previously been investigated in this or any other species. To some extent, this has been due to the problem of measuring T_b continuously in very small animals, but with the development of tiny 0.1 g temperature-sensitive transponders,

small body size has become less of a constraint (Wacker et al. 2012). We predicted that the slow developmental rate of marsupial dunnarts would allow us to determine intermediate developmental stages that have not been previously identified.

Materials and methods

Animal care

Four dunnart breeding pairs produced four litters (litter sizes of eight, six, six, and five young). Animals were housed in cages (55×38×22 cm) with wood shaving substrate and nesting boxes filled with shredded paper. Animals were fed a mixture of “Whiskas” cat biscuits (26.0% crude protein and 9.5% crude fat) soaked in water overnight and tins of “Whiskas” tinned cat food (7.0% crude protein and 5.5% crude fat) 7 days a week. Water and food were always available if not otherwise specified. Animals were kept under the natural Summer photoperiod (30.50°S, 151.65°E) from LD 14:10 to 13:11 and at an ambient temperature (T_a) of 18.0 ± 2.0 °C.

Development of endothermy (experiment one)

Four pouch young from one litter (Table 1) at 40 d (age group one) were removed from the nest at the same time and placed individually in 1 L glass jars at T_a 18.0 ± 0.3 °C. A fine, lubricated (Sylk natural personal lubricant, Geneva Marketing PTY LTD, Surry Hills, Australia) and pliable thermocouple was used for rectal temperature (T_{rec}) measurements (calibrated to the nearest 0.1 °C between 10.0 and 40.0 °C in ~5.0 °C increments in a water bath with a precision thermometer traceable to a national standard). The thermocouple was inserted only 5 mm rectally, because the young animals are so small (crown-rump length ~20 mm, body mass 3–4 g at the beginning of measurements), and no damage was caused by this insertion. Rectal temperature measurements were taken within 10 s of the animal being removed from the mother/nest and then every 10 min for 60 min.

To quantify the changes in hair length, and as a proxy for insulation (Stuedel et al. 1994; Wacker et al. 2016), four individual hairs taken from the mid-dorsal area of each animal were straightened using 100% ethanol. Prior to measurements, a small clump of hair (approximately 10–15 hairs) had been soaked in ethanol for 24 h to verify that ethanol did not damage the hair structure. Photographs of each hair were taken using a digital camera (IC80 HD Integrated Stand Alone Digital Camera for Stereo Microscopes, Leica Heerbrugg Switzerland) attached to the microscope (M80 Modular Routine Stereo Microscope with 8:1 Zoom,

Table 1 Details of experiment one on development of endothermy in fat-tailed dunnart pouch young and experiment two on torpor induction in dunnart pouch young

Age group #	Age (days)	Body mass (g ± SD)	<i>n</i>	Treatment	
Experiment one					
1	40	3.3 ± 0.1	4	Cooling to T_a	
2	48	4.8 ± 0.1	4	Cooling to T_a	
3	56	6.7 ± 0.3	4	Cooling to T_a	
Age group #	Age (days)	Body mass (g ± SD)	<i>n</i>	Treatment first week	Treatment second week
Experiment two					
4	60–70	8.8 ± 0.2	8	No radiant heat available	Offered radiant heat
5	90–100	11.9 ± 1.1	11	No radiant heat available	Offered radiant heat
6	120–130	13.6 ± 0.5	11	No radiant heat available	Offered radiant heat
7	150–160	15.4 ± 1.1	11	No radiant heat available	Offered radiant heat

Animals in experiment two were measured on two occasions: once without access to radiant heat and once with radiant heat. Only eight animals in age group four were measured as three were not large enough at this age to safely implant with transponders

Leica Heerbrugg, Switzerland) and the length of the hair was measured. At the end of the measurements, the animals were weighed, four hairs removed for measurement of total hair length and morphometrics taken (pes length, head length, tail length, and crown-rump length). All measurements were repeated at 48 d (age group two) and 56 d (age group three).

For comparison, cooling rates were also determined for three deceased dunnarts (Petajan and Morrison 1962); one juvenile (45 d, 3.8 g), one small adult (273 d, 10.2 g), and one large adult (760 d, 14.1 g) were warmed to 37.0 °C (animals placed in water proof bags in a water bath) and a calibrated thermocouple inserted rectally. Rectal temperature measurements (T_{rec}) were taken at T_a 18.3 ± 0.7 °C every 10 min for 60 min after removal from the water bath and a cooling constant calculated (Petajan and Morrison 1962).

Thermoregulation, basking, and torpor (experiment two)

Eleven juvenile (~60 d, age group four) fat-tailed dunnarts were implanted with calibrated (as for thermocouples) transponders (IPTT-300, 0.13 g, 14 mm × 2 mm, Bio Medic Data Systems, Seaford, Delaware, USA) to measure subcutaneous temperature (T_{sub} , Wacker et al. 2012).

Over approximately 21 h open-flow respirometry was used to measure MR as the rate of oxygen consumption. Outside air was dried (Silica gel) and pumped to the respirometry chamber (300 ml volume Perspex cylinder, 10 cm long, sealed with rubber stoppers containing an air inlet on one side and outlet on the other) and then through a mass flow meter (Omega FMA-5606, Stamford, CT, USA) at a rate of ~350 ml min⁻¹. This resulted in 99% equilibrium times of <4 min. A sub-sample of 150 ml min⁻¹ was

analysed for O₂ content (FX301-01R, Sable System International, Henderson, Nevada, USA). The oxygen analyser was calibrated prior to measurements against high-purity compressed Nitrogen (BOC GASES) and a calibration gas (O₂ = 19.9 ± 0.03% in Nitrogen, BOC GASES). The flow meter was calibrated using a custom-made bubble meter. Oxygen consumption was calculated using Eq. 3a of Withers (1977) assuming an RQ of 0.85.

The respirometry chamber was large enough to allow for free movement by the animals and was placed within a temperature-controlled cabinet maintained at 15.0 ± 0.7 °C; the artificial photoperiod was similar to natural (LD 14:10, 30.50°S, 151.65°E). The T_a within the respirometry chamber was measured with a calibrated thermocouple (HH-71T, Omega Stamford, CT, USA). A 2 × 2 cm piece of paper towel was secured to the base of the chamber to allow for the absorption of urine and faeces, but the animal was unable to burrow underneath. A cardboard roll (3 cm in length) was provided for refuge. Oxygen concentration was measured in sequence; respirometry chamber once/min for 12 min followed by a reference (outside air) measurement once/min for 3 min. The transponder signal was read with a DAS-7006/7R/S Handheld Reader (Bio Medic Data Systems, Seaford, Delaware, USA). The reader was modified and connected to a computer and read automatically via a program written in Microsoft Visual Basic V6. Transponder readings were taken at the same time as oxygen consumption readings.

Animals were measured once a week for 2 weeks (1 week without radiant heat access and the following week with access to radiant heat, and individuals were randomised) at each age group (Table 1); when the animals were 60–70 d (age group four), 90–100 d (age group five), 120–130 d (age group six), and 150–160 d (age group seven). The heat lamp (SunGlo basking spot lamp,

50 W tight beam Colour Rendering Index of 98, 6700 K colour temperature, ExoTerra, Montreal, Canada) was placed at a 45° angle and 20 cm away from the ceiling of the chamber tube. By giving the animals the cardboard tube refuge, they were able to choose whether or not to bask. It is known from the previous work that torpid dunnarts at a T_b of ~20 °C can move at ~0.3 m s⁻¹ (Rojas et al. 2012). Animals were placed in the respirometry chamber between 1530 and 1630 (AEST) overnight, without food and water to induce torpor. Animals were weighed immediately before being placed into and immediately after being removed from the chamber and a linear mass loss over the measurement period was assumed. The chamber was maintained at T_a of 15.0 ± 0.7 °C even with the basking lamp on and a natural photoperiod was used. If the animal was torpid the following morning, and once its T_{sub} was between 19.5 and 20.0 °C, the heat lamp was switched on to encourage basking and aid rewarming. A camera (Swann Secura View, Security Monitoring Kit, Melbourne Australia) in the chamber was used to observe whether the animal was resting, active, basking, or not basking.

Data analysis

Development of thermoregulation (experiment one)

Numeric values are presented as means ± SD for 'n' the number of individuals. Linear mixed effects models were used to determine the effect of age on, body mass, and length of the hair on cooling rate in 40, 48, and 56 d dunnarts. Conventional least-square linear regression analyses were also used for comparison. Cooling rates are the maximum rate of cooling over 10 min and were calculated over the first 10 min, the animals were removed from their mother (since this is when cooling rate was steepest and changed the most with age). The cooling constants over the entire cooling process were calculated from the slope of log₁₀($T_{rec} - T_a$) over time (Petajan and Morrison 1962).

Thermoregulation, basking, and torpor (experiment two)

Numeric values are presented as means ± SD for 'n' the number of individuals. Torpor was defined as a T_{sub} below the threshold of 30.0 °C (Barclay et al. 2001) and torpor bout duration (TBD) was defined as the time, the animal's T_{sub} was below 30.0 °C. The oxygen consumption during resting periods of normothermic individuals was calculated using the average of three 5-min intervals of the lowest, stable, non-torpid values. Cooling rates were calculated from the maximum rate of cooling over 10 min and rewarming rates from the maximum rate of rewarming over 10 min. Normothermic and basking apparent thermal conductance (C) was calculated by dividing total RMR by the $T_{sub} - T_a$

differential and converted to J h⁻¹ °C⁻¹ using a conversion factor of 20.1 (Schmidt-Nielsen 1997). Mass-specific conductance (J g⁻¹ h⁻¹ °C⁻¹) was also calculated for comparison with other studies. Linear mixed effects models were used to determine the effect of body mass on basking and non-basking RMR, TBD, rate of cooling during torpor entry and rate of active rewarming, maximum rewarm rate, total and mass-specific thermal conductance, and head length. The conventional least-square linear regression analyses were also used for comparison. Repeated-measures ANOVAs were used to determine differences between means. Regressions for maximum rewarm rate as a function of body mass and RMR as a function of body mass in basking and non-basking animals were compared using ANCOVAs. All statistical analyses were performed with R Studio version 0.99.489.

Results

Development of endothermy (experiment one)

At 40 d, dunnarts ($n=4$, body mass 3.3 ± 0.1 g) that had been removed from their nest, cooled rapidly, and almost to T_a ($T_a = 18.0 ± 0.3$ °C) over 30 min (Fig. 1a). Within the first 10 min, the animals had reduced T_{rec} by 6.5 ± 1.1 °C; by 40 min, T_{rec} was only 0.6 ± 0.2 °C above T_a ; and at 60 min, T_{rec} was 18.6 ± 0.2 °C. Animals at 48 d ($n=4$, body mass 4.8 ± 0.1 g, $T_a = 18.0 ± 0.1$ °C) cooled soon after being removed from their nest, but only by 2.1 ± 0.4 °C, during the 60-min period. Between 20 and 30 min, all animals rewarmed slightly (by 0.5 ± 0.5 °C) despite T_a remaining constant, and then cooled again. At 56 d ($n=4$, body mass 6.7 ± 0.3 g, $T_a 18.0 ± 0.2$ °C), T_{rec} was almost constant (starting T_{rec} of 33.1 ± 0.6 °C, ending T_{rec} of 33.4 ± 0.2 °C). The maximum rate of cooling over 10 min decreased with age; mean cooling rate at 40 d was 0.65 ± 0.11 °C min⁻¹, 0.14 ± 0.05 °C min⁻¹ at 48 d, and 0.03 ± 0.02 °C min⁻¹ at 56 d. Cooling constants (in log₁₀ °C min⁻¹) also decreased with age (0.0188 ± 0.0012 at 40 d, 0.0012 ± 0.0003 at 48 d, and 0.0001 ± 0.0001 °C at 56 d). Cooling constants were larger in living dunnarts than in deceased animals, as expected (Petajan and Morrison 1962).

The maximum rate of cooling over 10 min was correlated with both body mass (Fig. 1b, $r^2=0.85$, $p<0.001$, $df=7$, $t = 8.67$, $\log_{10}y = -0.86x + 1.60$), and the total length of the individual animal's hair (Fig. 1c, $r^2=0.81$, $p<0.001$, $df=7$, $t = 9.67$, $\log_{10}y = -0.84x + 1.54$). Hair length increased with age: 3.1 ± 0.1 mm at 40 d, 4.8 ± 0.6 mm at 48 d, and 6.2 ± 0.8 mm at 56 d, and was

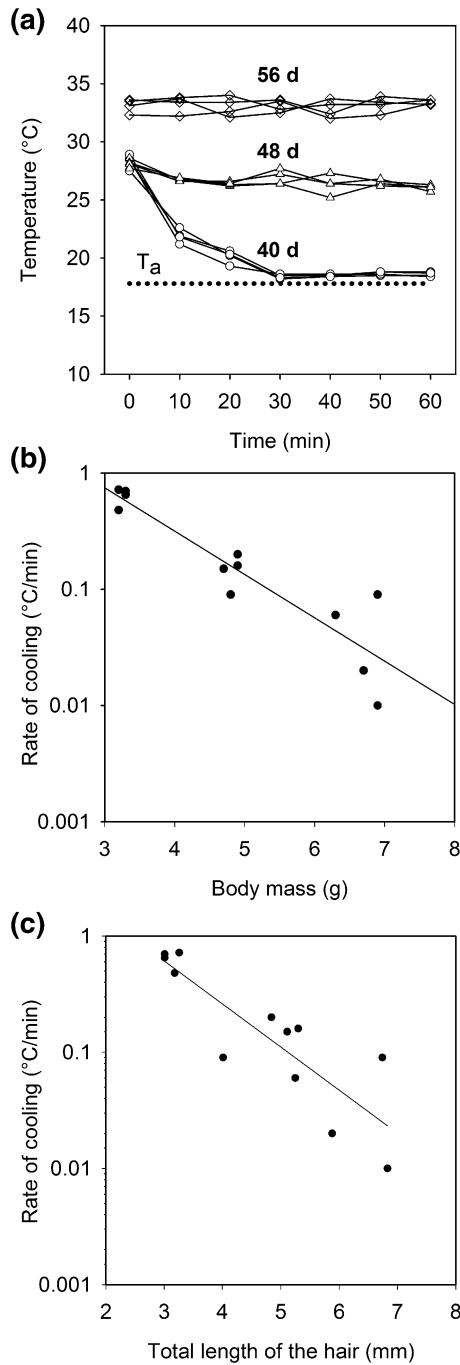


Fig. 1 Development of endothermy in the fat-tailed dunnart. The decrease in T_{rec} (a) over 60 min at a T_a (dotted line) of $18.0 \pm 0.3^\circ\text{C}$ for four individuals at 40 (circles), 48 (triangles), and 56 (diamonds) days of age. Correlation between body mass and the rate of cooling during the first 10 min after being removed from the nest (b, $r^2=0.85$, $p<0.001$, $\log_{10}y = -0.86x + 1.60$) and the length of the animal's hair and the rate of cooling (c, $r^2=0.81$, $p<0.001$, $\log_{10}y = -0.84x + 1.54$). $T_a = 18.0 \pm 0.3^\circ\text{C}$

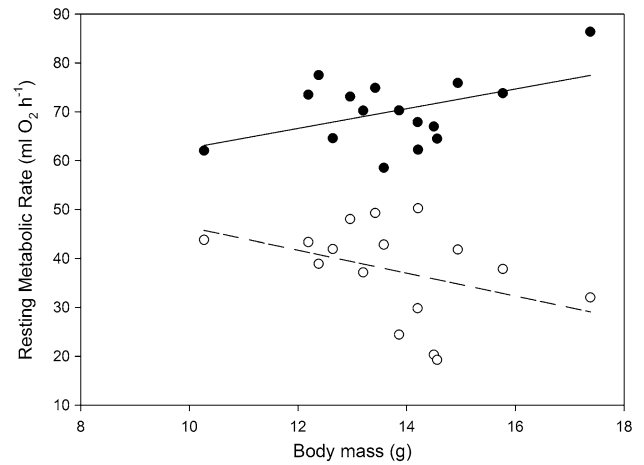


Fig. 2 Non-basking RMR (filled circles, $r^2=0.29$, $p=0.0048$, $y=2.02x+42.41$) and basking RMR (open circles, $r^2=0.21$, $p=0.047$, $y=-2.35x+69.82$) as a function of body mass in fat-tailed dunnarts

strongly correlated with body mass ($r^2=0.89$, $p<0.001$, $df=7$, $t=16.58$, $y=0.85x+0.52$).

Thermoregulation, basking, and torpor (experiment two)

The resting metabolic rate (RMR) at T_a $15.0 \pm 0.7^\circ\text{C}$ increased linearly with body mass in non-basking ($r^2=0.29$, $p=0.048$, $y=2.02x+42.41$) but decreased linearly in basking ($r^2=0.21$, $p=0.047$, $y=-2.35x+69.82$) dunnarts (Fig. 2). The RMR when animals were basking was on average 54% that of non-basking individuals and the two regressions differed significantly (ANCOVA $F=115.10$; $p<0.001$).

Total thermal conductance (C) in normothermic non-basking dunnarts increased with age; $53.91 \pm 2.89\text{Jh}^{-1}\text{C}^{-1}$ (mass-specific $5.72 \pm 0.01\text{Jg}^{-1}\text{h}^{-1}\text{C}^{-1}$) in age group four, $63.16 \pm 6.42\text{Jh}^{-1}\text{C}^{-1}$ (mass-specific $5.23 \pm 0.31\text{Jg}^{-1}\text{h}^{-1}\text{C}^{-1}$) in age group five, $73.47 \pm 3.97\text{Jh}^{-1}\text{C}^{-1}$ (mass-specific $5.36 \pm 0.24\text{Jg}^{-1}\text{h}^{-1}\text{C}^{-1}$) in age group six, and $77.2 \pm 6.40\text{Jh}^{-1}\text{C}^{-1}$ (mass-specific $5.09 \pm 0.32\text{Jg}^{-1}\text{h}^{-1}\text{C}^{-1}$) in age group seven. Both total C ($r^2=0.87$, $p<0.001$, $df=11$, $t=7.74$, $y=4.22x+13.70$) and mass-specific C ($r^2=0.34$, $p<0.001$, $df=11$, $t=13.49$, $y=-0.09x+6.49$) were correlated with body mass.

In basking dunnarts, total thermal C ($r^2=0.72$, $p<0.001$, $df=11$, $t=6.33$, $y=-0.42x+8.50$) and mass-specific thermal C ($r^2=0.37$, $p<0.001$, $df=11$, $t=47.96$, $y=-2.59x+71.65$) were also correlated with body mass, and both were substantially lower than in non-basking

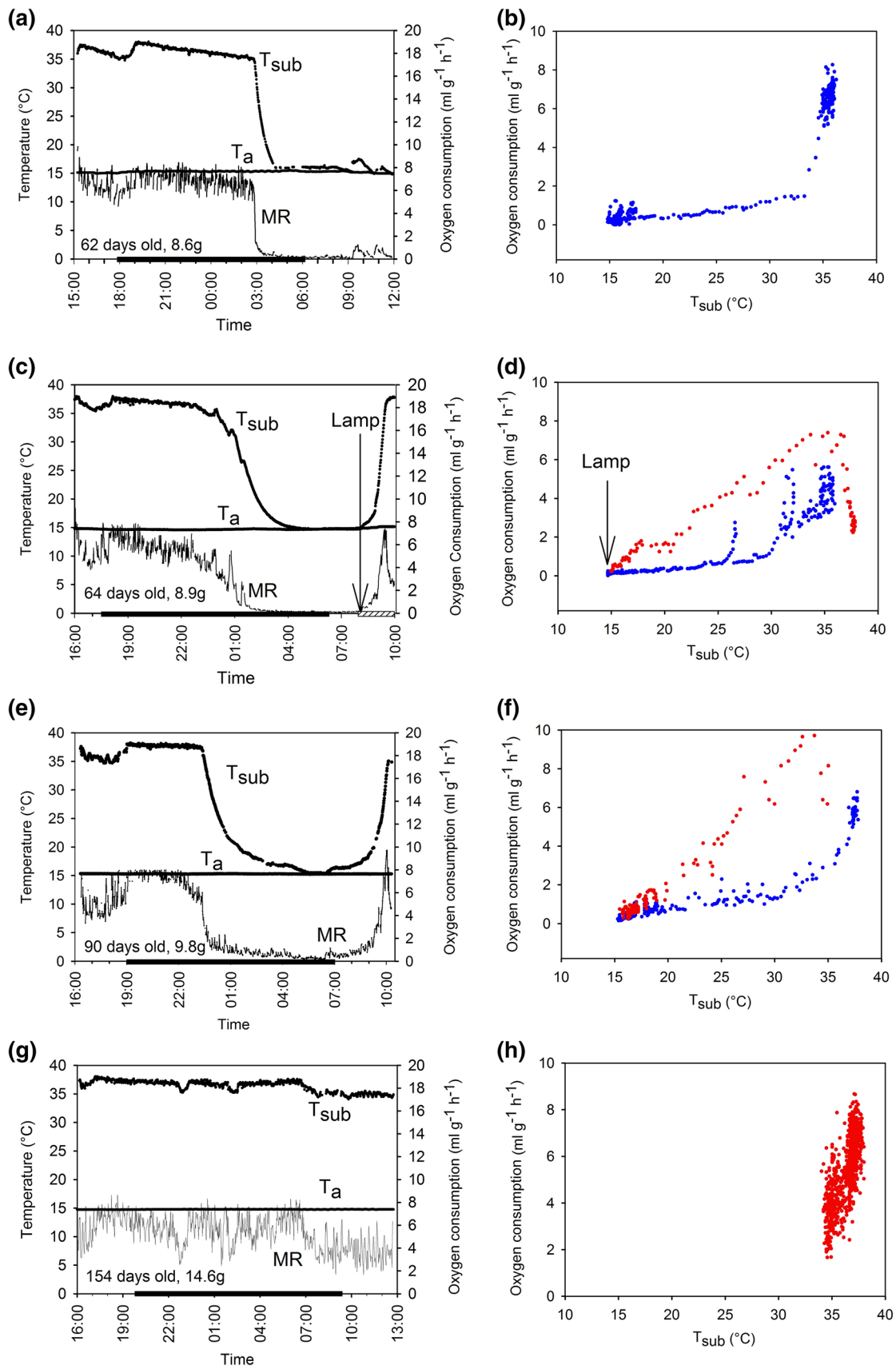


Fig. 3 Development of torpor in juvenile fat-tailed dunnarts with oxygen consumption and T_{sub} traces on the *left-hand side* and their corresponding plots of oxygen consumption as a function of T_{sub} on the *right-hand side* for **a, b** a dunnart in age group four (8.6 g) and unable to rewarm, **c, d** a dunnart in age group four (8.9 g), able to rewarm with the aid of a basking lamp, **e, f** a dunnart in age group five (9.8 g), a long, deep torpor bout with endogenous rewarming, **g, h** a dunnart in age group seven (14.6 g) and no torpor entry. *Dark bars* represent scotophase. *Cross hatched bar* in Fig. 3b represents the time, the basking lamp was on. Cooling data are in *blue*, rewarming in *red*. (Color figure online)

animals (both total C and mass-specific C , $p < 0.001$). Basking dunnarts had a mean total C of $46.29 \pm 6.18 \text{ Jh}^{-1} \text{ } ^\circ\text{C}^{-1}$ (mass-specific $4.11 \pm 0.28 \text{ Jg}^{-1} \text{ h}^{-1} \text{ } ^\circ\text{C}^{-1}$) in age group 4, $43.19 \pm 2.27 \text{ Jh}^{-1} \text{ } ^\circ\text{C}^{-1}$ (mass-specific $3.60 \pm 0.47 \text{ Jg}^{-1} \text{ h}^{-1} \text{ } ^\circ\text{C}^{-1}$) in age group five, $34.79 \pm 10.00 \text{ Jh}^{-1} \text{ } ^\circ\text{C}^{-1}$ (mass-specific $2.56 \pm 0.78 \text{ Jg}^{-1} \text{ h}^{-1} \text{ } ^\circ\text{C}^{-1}$) in age group six, and $31.55 \pm 9.94 \text{ Jh}^{-1} \text{ } ^\circ\text{C}^{-1}$ (mass-specific $2.08 \pm 0.79 \text{ Jg}^{-1} \text{ h}^{-1} \text{ } ^\circ\text{C}^{-1}$) in age group seven.

Torpor use and expression changed substantially with age. At 60–70 d (<9 g), all animals (large enough to implant with transponders, $n=8$) were normothermic for ~7 h and then showed an apparent torpor entry (at ~0300 h in the example in Fig. 3a), with an initial rapid reduction of MR and a little change in T_{sub} , followed by a slow reduction in T_{sub} (Fig. 3b). These individuals were unable to rewarm despite attempts at increasing MR and T_{sub} (two such attempts after 0940 in Fig. 3a) and were, therefore, deemed to be hypothermic (Geiser et al. 2014). All of the eight animals in this age group showed this pattern. However, when they were given access to radiant heat, all animals moved under the heat lamp and were able to rewarm from a low T_{sub} (example in Fig. 3c). When the heat lamp was switched on at 0800, MR gradually increased to values well above the phase of torpor/hypothermia as T_{sub} was raised partially passively from ~15 to 19 °C. Only after T_{sub} exceeded 20 °C at 0900 did MR further increase, peaking at 0930, before returning to resting MR. T_{sub} increased immediately in all animals when the heat lamp was switched on, but MR increased immediately in only six animals (Fig. 3d), whereas in two, it remained rather low until T_{sub} reached ~25 °C.

At 90–100 d (body mass 11.9 g, example in Fig. 3e), 9 of the 11 juvenile dunnarts expressed a long, deep torpor bout. At this age, 5 of the 11 animals were able to rewarm actively and spontaneously, whereas the other six were unable to rewarm at T_{a} 15 °C (but could rewarm at higher T_{a} s when removed from the respirometer). All animals capable of active rewarming at low T_{a} showed a similar pattern to that in Fig. 3f, with T_{sub} decreasing gradually with MR during cooling, with a much higher MR during the rewarming phase. At 120–130 d (body mass >13.0 g, Fig. 3g, h), seven of the animals no longer entered torpor

regularly and four individuals expressed shallow and brief torpor bouts.

During torpor entry, smaller animals cooled more quickly than larger animals and the rate of cooling was inversely correlated with body mass (Fig. 4a, $r^2=0.49$, $p < 0.001$, $df=7$, $y = -0.01x + 0.24$). Torpor bout duration of animals capable of actively rewarming was inversely correlated with body mass (Fig. 4b, $n=11$, Fig. 4 $r^2=0.57$, $p < 0.001$, $df=7$, $y = -41.25x + 853.08$). On average, at 90–100 d dunnarts had very long torpor bouts (6.6 ± 0.9 h), which decreased to 5.0 ± 1 h at 120–130 d and lasted only 4.3 ± 1.0 h at >150 d with 64% of animals in the last age group not using torpor at all. Both the maximum rewarming rate over 10 min during basking and the maximum active (non-basking) rewarming rate increased steeply (Fig. 4c, basking: $r^2=0.87$, $p < 0.001$, $y = 0.06x - 0.41$, non-basking: $r^2=0.81$, $p < 0.001$, $y = 0.07x - 0.58$) with body mass and the two regressions differed significantly (ANCOVA $F=21.14$, $p < 0.001$). The animals' body mass did not change significantly between runs with and without a basking lamp ($p < 0.157$).

When offered a heat lamp during bouts of torpor, 100% of dunnarts basked by moving out from under the cardboard tube refuge and into the direct path of the radiant heat, but juveniles and adults differed in their basking behaviour. All young animals at 60–70 d basked with their heads directed towards the heat lamp. At 90–100 d, nine of 11 animals also basked in this manner. All the older animals (>120 d) basked by turning their backs to the heat lamp. Head length in the youngest animals (60–70, 90–100 d), which all basked by exposing their heads to the radiant heat, was positively correlated with the rate of passive rewarming ($r^2=0.71$, $p < 0.001$, $df=7$, $t=178681.9$, $y=0.71x - 15.74$). No correlation between head length and passive rewarming rate was observed in older animals (>120 d), likely because these always basked with their back to the heat lamp rather than with their head.

Discussion

Our data show that the development of marsupial endothermy involves three major steps. Initially, when individual heat loss was high and heat production was low, animals were poikilothermic when removed from the nest and incapable of physiological thermoregulation, and so were metabolically similar, but not identical, to ectotherms (Hill 1976; Geiser et al. 2006). Second, when heat production increased with growth and when insulation was improved, the animals were able to maintain a constant high T_{b} for several hours during cold exposure, but eventually entered an apparent torpid stage. However, since they were unable to rewarm, most likely because of

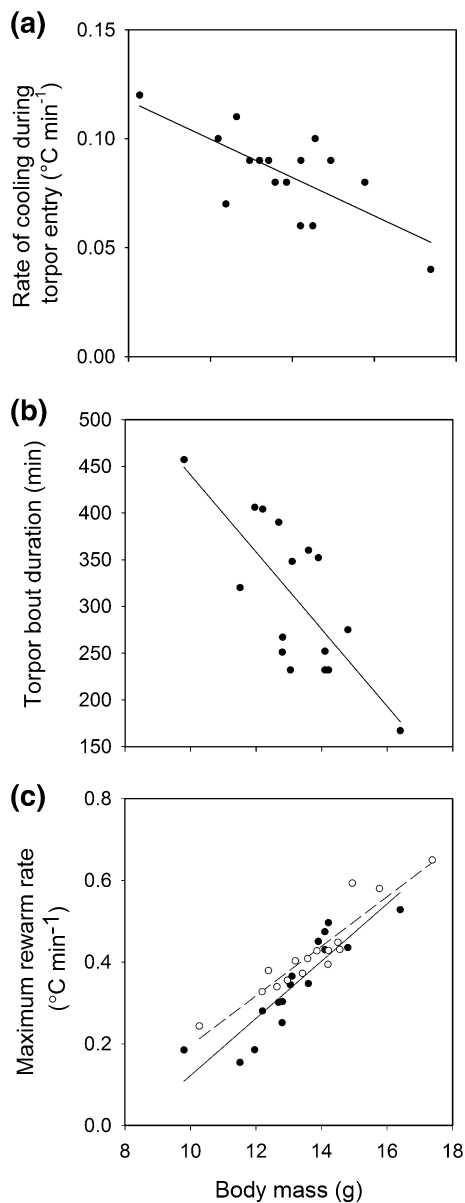


Fig. 4 Maximum rate of cooling during torpor entry over 10 min (**a**, $r^2=0.49$, $p<0.001$, $y = -0.01x+0.24$), torpor bout duration (**b**, $r^2=0.57$, $p<0.001$, $y = -41.25x+853.08$), and the maximum rewarming rate over 10 min during radiant heat-assisted (**c**, open circles, $r^2=0.87$, $p<0.001$, $y=0.06x - 0.41$) and active rewarming (**c**, filled circles, $r^2=0.81$, $p<0.001$, $y=0.07x - 0.58$) as a function of body mass in dunnarts. Data for animals in age group four were excluded; as at this age, the animals were unable to rewarm without the aid of a basking lamp

the low T_b limiting heat production, by definition, these animals were hypothermic (Hill 1976; Geiser et al. 2006). Importantly, during this stage, when animals were offered the opportunity to bask, they were able to rewarm despite their low capacity for increasing MR, similar to behavioural thermoregulation in reptiles. Third, when animals

were subadults and adults, physiological thermoregulation was fully developed, torpor including endogenous arousal was expressed and normothermy could be maintained even under prolonged cold exposure.

The poikilothermic stage early in the thermal development of dunnarts has been observed in many endothermic species (Dawson and Evans 1960; Morrison and Petajan 1962; Westman et al. 2002; Geiser et al. 2006; Gilbert et al. 2010). These individuals are unable to maintain a high T_b during cold exposure as MR cannot fully compensate for heat loss due to a high relative surface area, limited insulation, and large total thermal conductance, and leading to a reduction in T_b . However, very young small marsupials (up to ~40 d) typically rely on pouch or nest temperature to maintain T_b , and without this shared heat, they become hypothermic (Geiser et al. 2006).

In contrast, at 60 d, dunnarts were partially endothermic and could maintain T_{sub} for some time independently of the T_a and could also enter an apparent state of torpor. Dunnarts at this age cooled more quickly than dead individuals due to high thermal conductance (Bradley and Deavers 1980) and were unable to produce enough heat to rewarm from torpor at a T_a of 15°C, and, therefore, by definition were hypothermic. Importantly, the decline of T_b and MR showed the pattern expressed during entry into daily torpor and hibernation (i.e., a physiologically controlled process characterised by an initial rapid reduction in MR followed by a reduction in T_{sub} that further reduced MR), which differs from the pattern during entry into cold-induced hypothermia where animals try to maintain a high T_b via a high MR, but fail to do so, because heat loss exceeds heat production (Geiser et al. 2014). The rapid decline of MR and T_b during the entry phase strongly suggests that it was not caused by energy depletion, which is characterised by a slow reduction in MR and T_b , but instead was an ‘intended’ and regulated torpor entry. While these dunnarts were unable to rewarm endogenously, they could move and position their body under a basking lamp and use the external heat source to rewarm. This suggests that juveniles in the wild, even before being fully endothermic, may also experience a reduction of T_b in the second part of the night or early morning and then bask to rewarm.

Although field studies in the Australian arid zone have been conducted for decades, basking during rewarming, which usually occurs in the late morning, has only recently been observed in adults in the wild (Geiser et al. 2002; Warnecke et al. 2008). Since juveniles are small and hard to see and perhaps more cautious than adults, it is possible that juveniles that are not yet fully endothermic bask as they always do (when normothermic or torpid) when either given the opportunity to bask in captivity. It is this transitional stage in the development of thermoregulation that has not been observed or recognised before: torpor entry

without the capacity of endogenous rewarming but with the ability to use passive rewarming to raise T_b to normothermic values. Our data further support the hypothesis that the ability to enter torpor develops before mammals are fully endothermic (Geiser et al. 2002; Geiser 2008). However, the development of the intermediate heterothermic stage occurs at such a young age that animals are unable to rewarm without the aid of passive rewarming, for example via basking in the sun.

Basking is not restricted to juveniles; no matter what age, sex, or body mass, when a radiant heat source was available all animals basked when rewarming from torpor. Interestingly, basking dunnarts leave the refuge head-first to warm their anterior before the posterior but only expose half of the body. All juvenile fat-tailed dunnarts in our study basked leading with their head, likely because, at this age, the head is proportionally very large and this increase in surface area allows them to rewarm more quickly. In the youngest of these dunnarts, basking is not an optional method for reducing energy thermoregulatory expenditure as in adults (Bartholomew and Rainy 1971; Warnecke et al. 2010; Wacker et al. 2016), but rather is an essential part of being able to rewarm from an otherwise hypothermic condition. Non-torpid animals also chose to bask when a heat lamp was present, but, in these cases, radiant heat mainly reduced the energetic costs of thermoregulation. Our observations of juvenile fat-tailed dunnarts and data from studies on related species show that torpid animals both in the wild and the laboratory with a T_b of $\sim 15^\circ\text{C}$ are able to move from their nest and actively seek a radiant heat source and, at the same time, are also able to respond to and avoid approaching predators (Geiser et al. 2002; Warnecke et al. 2008; Rojas et al. 2012).

Our data indicate that heterothermy is an intermediate stage between poikilothermy and endothermy in the development of thermoregulation in marsupials and perhaps other altricial mammals, and suggest that basking is a crucial step in the development of heterothermy in this species. This heterothermic step requires that animals have access to an external heat source, not as an option to reduce the energetic costs of rewarming, but as an essential requirement for the ability to rewarm from torpor. Basking behaviour in such young marsupials has not been observed before and our data suggest that juvenile dunnarts at the time of weaning, when heat loss is high, may need a radiant heat source to rewarm, so they can reach or maintain a high T_b independently of nest temperature.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethics approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

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