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The Rate of Cooling during Torpor Entry Drives Torpor Patterns in a Small Marsupial*

Chris B. Wacker†
Fritz Geiser
Centre for Behavioural and Physiological Ecology, Zoology, University of New England, Armidale, New South Wales 2351, Australia

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ABSTRACT

To maximize energy savings, entry into torpor should involve a fast reduction of metabolic rate and body temperature (T_b); that is, animals should thermoconform. However, animals often defend against the decrease in T_b via a temporary increase in thermoregulatory heat production, slowing the cooling process. We investigated how thermoregulating or thermoconforming during torpor entry affects temporal and thermoenergetic aspects in relation to body mass and age in juvenile and adult fat-tailed dunnarts (*Sminthopsis crassicaudata*; Marsupialia: Dasyuridae). During torpor entry, juvenile thermoconformers cooled twice as fast as and used less energy during cooling than juvenile thermoregulators. While both juvenile and adult thermoconformers had a lower minimum T_b, a lower torpor metabolic rate, and longer torpor bouts than thermoregulators, these differences were more pronounced in the juveniles. Rewarming from torpor took approximately twice as long for juvenile thermoconformers, and the costs of rewarming were greater. To determine the difference in average daily metabolic rate between thermoconformers and thermoregulators independent of body mass, we compared juveniles of a similar size (~13 g) and similarly sized adults (~17 g). The average daily metabolic rate was 7% (juveniles) and 17% (adults) less in thermoconformers than in thermoregulators, even though thermoconformers were active for longer. Our data suggest that thermoconforming during torpor entry provides an energetic advantage for both juvenile and adult dunnarts and may aid growth for juveniles. While thermoregulation during torpor entry is more costly, it still saves energy, and the higher T_b permits greater alertness and mobility and reduces the energetic cost of endogenous rewarming.

Keywords: torpor patterns, thermoconforming, thermoregulation, energetics, marsupial, torpor depth.

Introduction

Thermoregulation and maintaining a high body temperature (T_b) is energetically costly for all endotherms, especially at a low ambient temperature (T_a), where heat is lost to the environment. To overcome these energetic challenges, many small endothermic species enter torpor (Nowack et al. 2020). During torpor, animals decrease their metabolic rate (MR), resulting in a reduction in their T_b, which further reduces MR and allows them to save up to 90% (or more) of the energy usually expended during normothermia (Lyman et al. 1982; Geiser and Baudinette 1987; Cooper and Withers 2004; Warnecke et al. 2008; Dausmann 2014; Ruf and Geiser 2015; Geiser 2021). By using torpor, animals can better cope with limited food availability, and when food is available, they can incorporate the saved energy into development, growth, and reproduction (Song and Geiser 1997; Geiser and Körtner 2010; Giroud et al. 2014).

The amount of energy saved by using torpor depends on the species, age, and sex of the animal and the pattern of torpor expressed. Torpor patterns are usually characterized by the cooling rate during torpor entry, the depth and duration of the torpor bout, and the rate of rewarming from torpor (Lyman et al. 1982). The rate of cooling during torpor entry is largely determined by body mass (BM; with small animals cooling faster than large animals), by the T_a the animal is exposed to, and to some extent by whether an animal thermoconforms or thermoregulates during torpor entry (Geiser 2021). Thermoconforming animals allow their T_b to decrease rapidly to (or just above) the minimum T_b, whereas thermoregulating animals defend against the decrease in T_b by somewhat increasing their MR, slowing the cooling rate (Nicol and Andersen 2007; Geiser 2021). While both forms of torpor entry are regularly observed, little is known about how they differ physiologically or affect other aspects of the torpor pattern (such as duration or depth) and therefore the quantity of energy the animal saves.

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†Corresponding author; email: cwacker4@gmail.com.

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Torpor patterns can vary greatly between even closely related species, and intraspecific variation is common (Geiser 1988; Levy et al. 2011). For example, after endothermic thermoregulation is established, juvenile heterotherms typically have deeper torpor bouts than adults (Geiser 1988, 2021; Wacker et al. 2017). While a deeper and longer torpor bout increases the energy saved during torpor (Lyman et al. 1982; Geiser and Ruf 2023), rewarming endogenously from a low T_b can decrease total energy savings by up to 80% (Geiser and Drury 2003; Warnecke et al. 2008; Stannard et al. 2015; Wacker et al. 2017). Animals with deep torpor bouts therefore must use proportionally more energy to rewarm from torpor, which may be problematic, as they also need to save as much energy as possible to allocate to growth.

The species used for our experiments was the fat-tailed dunnart (*Sminthopsis crassicaudata*; hereafter, dunnarts). Dunnarts are primarily nocturnal, foraging early in the evening and entering torpor late at night or early in the morning (Morton 1978; Warnecke et al. 2008). Dunnarts undergo daily torpor readily in the laboratory, torpor patterns vary greatly among them, and they have been observed to thermoconform and thermoregulate during torpor entry (Munn et al. 2010; Wacker et al. 2016, 2017). Moreover, during torpor, young dunnarts may become hypothermic and unable to rewarm using endogenous heat production alone (Wacker et al. 2017).

Our study consisted of three parts.

1. We measured the percentage of juvenile and adult dunnarts that became hypothermic, that did not use torpor, or that thermoconformed or thermoregulated during torpor entry. We predicted that most, if not all, of the younger and smaller juveniles would thermoconform during torpor entry to save as much energy as possible and that the older and larger juveniles and adults would thermoregulate during torpor entry because they could afford the energetic cost.

2. We compared the torpor bouts of juvenile and adult dunnarts to identify the parts of the torpor pattern that were affected by BM and developmental stage. We predicted that thermoconforming during torpor entry in juveniles and adults would be energetically cheaper than thermoregulating during torpor entry and lead to deeper and longer torpor bouts and that this effect would be more pronounced in juveniles than in adults.

3. We measured the difference in average daily MR (ADMR) between thermoconformers and thermoregulators independent of BM by comparing the temporal and energetic aspects of the torpor bouts of juvenile dunnarts of a similar BM and adult dunnarts of a similar BM. We therefore compared juveniles, for which maximizing energy saving for growth should be a priority, with fully grown adults, which need energy only for activity, maintenance, and thermoregulation. We predicted that animals that thermoconformed during torpor entry might have similar ADMRs to those that thermoregulated during torpor entry because of the substantial costs of endogenous rewarming from the deeper torpor bouts.

Methods

Animals

All dunnarts were obtained from the University of New England captive breeding colony. Animal cages (55 cm × 38 cm × 22 cm) were equipped with a wood shaving substrate and nest boxes filled with shredded paper. Animals were fed a mixture of Whiskas cat biscuits (Mars Petcare Australia, Wodonga; 26.0% crude protein, 9.5% crude fat) soaked in water overnight and tins of Whiskas cat food (Mars Petcare Australia; 7.0% crude protein, 5.5% crude fat) 7 d a week. Water and food were always available unless otherwise specified. Dunnarts were housed under a natural photoperiod (14L:10D to 13L:11D; 30.50°S, 151.65°E; $T_a = 18.0°C \pm 1.9°C$), and experiments were conducted during late summer.

Twelve dunnart young, randomly chosen from three litters born within 7 d of each other (total of six males and six females, $n = 12$), were used for the juvenile age group measurements (age groups 1–4). Litters sizes were six, six, and five young. As male dunnarts become sexually mature only between 5 and 6 mo of age (Ewer 1968; Godfrey and Crowcroft 1971), all young were kept with their mother throughout the experiment and male young were separated once all measurements had concluded (at 150–160 d of age). One juvenile female died early in the experiment, so all data from this animal were excluded. All animals were identified using transponder microchips (IPTT-300, Bio Medic Data Systems, Seaford, DE; 0.13 g; 14 mm × 2 mm; resolution: 0.1°C).

Because BM and age are strongly correlated ($r^2 = 0.82$, $P < 0.001$, $y = 0.05x + 7.089$) up to and including 160 d, we used this age to mark the end of the juvenile stage. All analyses included BM as a variable. Juveniles were grouped into four groups to standardize measurements and ensure that we obtained torpor bouts that reflected changes in thermal biology that were due to BM and therefore age. Juvenile dunnarts were measured once between 60 and 70 d (age group 1), once between 90 and 100 d (age group 2), once between 120 and 130 d (age group 3), and once between 150 and 160 d (age group 4).

Eight nonbreeding male and eight nonbreeding female adult dunnarts (270–310 d of age, $n = 16$) chosen randomly from the University of New England captive colony were used for the adult age group. These animals had never been mated and were housed individually. MR and subcutaneous temperature (T_{sub}) were measured once under the same conditions as the juveniles, as outlined below.

Measurements of Subcutaneous Temperature and Metabolic Rate

Transponders (IPTT-300, Bio Medic Data Systems; 0.13 g; 14 mm × 2 mm; resolution: 0.1°C) calibrated in a water bath from 10°C to 40°C to the nearest 0.1°C were implanted under the skin between the scapulae in 12 juvenile dunnarts and 16 adult dunnarts to measure T_{sub} (for surgical details, see Wacker et al. 2012). The transponder signal was read with a DAS-7006/7R/S handheld reader (Bio Medic Data Systems), and T_{sub} readings were synchronized with oxygen consumption readings.

MR, determined as the rate of oxygen consumed over approximately 21 h (1600–1300 hours the following day), was measured with open-flow respirometry. Outside air, dried with silica gel, was pumped through the respirometry chamber (300-mL Perspex cylinder; 10 cm long, with rubber stopper seals, an air

inlet at one end, and an outlet at the other end) and then through a mass flow meter (FMA-5606, Omega, Stamford, CT) at a rate of ~350 mL min^{-1}, resulting in 99% equilibrium times of less than 4 min (Lasiewski et al. 1966). A subsample of 150 mL min^{-1} was analyzed for O_2 (FX301-01R, Sable Systems, Henderson, NV). The oxygen analyzer was calibrated before measurements against high-purity compressed nitrogen (BOC Gases) and a calibration gas (19.9% ± 0.03% O_2 in nitrogen; BOC Gases). The flow meter was calibrated using a custom-made bubble meter. Oxygen consumption was calculated using equation (3a) by Withers (1977), assuming a respiratory quotient of 0.85.

The respirometry chamber was placed in a temperature-controlled cabinet to maintain its T_a at 15.0°C ± 0.7°C under a natural photoperiod (14L:10D to 13L:11D; 30.50°S, 151.65°E). A calibrated thermocouple (HH-71 T, Omega) was used to measure the T_a to the nearest 0.1°C in the respirometry chamber. A piece of paper towel (2 cm × 2 cm) was secured to the chamber's floor to absorb urine and feces, and a cardboard tube (3 cm long, open on both ends) was provided as a refuge. Oxygen concentration was measured in sequence: a respirometry chamber measurement once per minute for 12 min followed by a reference (outside air) measurement once per minute for 3 min for comparison. BM was measured immediately before and after measurements, and a linear mass loss over time was assumed. A camera (security monitoring kit, Swann SecuraView, Melbourne) in the respirometry cabinet was used to observe whether the animal was resting or active.

Analyses

Calculations. An animal was considered to have entered torpor when its T_{sub} was below a defined torpor threshold of 30.0°C (Barclay et al. 2001). The rate of cooling during torpor entry was calculated as the maximum cooling rate ($cool_{max}$) over 10 min. Oxygen consumption during torpor entry (cooling MR) was calculated as the total oxygen consumption (mL h^{-1}, converted to mL g^{-1} h^{-1}, assuming a linear mass loss while in the respirometry chamber) from initial entry into torpor to the lowest T_{sub} reached. The camera in the respirometry cabinet was used to observe animals as either resting or active, and these observations were confirmed with metabolic measurements: adult dunnarts have a resting MR (RMR) of approximately 5.0 mL O_2 g^{-1} h^{-1} at T_a = 15.0°C and an active MR of more than 6.0 mL O_2 g^{-1} h^{-1} (Warnecke and Geiser 2010; Wacker et al. 2017). Normothermic RMR was calculated from the total oxygen consumption while the animals were in a resting phase.

The time and energy juveniles and adults spent in five physiological states (resting, cooling, steady-state torpor, endogenous rewarming, and activity) were measured and used to calculate ADMR. Torpor MR (TMR) was calculated from the total oxygen consumption during torpor from when the animal had reduced its T_{sub} to the lowest value until it began to rewarm. Rewarming MR was calculated from the total oxygen consumption from the initial point of rewarming when MR increased to the maximum T_{sub} reached after rewarming from torpor. Activity MR was calculated from the total oxygen consumption while the animals were active (as opposed to resting or torpid). These oxygen consumption totals were converted to joules using a conversion factor of 20.1 J mL O_2^{-1} (Schmidt-Nielsen 1997). The total time the animals spent in each of these states was also calculated. Because dunnarts rest in the afternoon, ADMR was calculated from the total energy expenditure over the approximate 21 h in the respirometry chamber and converted to kilojoules per day by extrapolating to 24 h via an extension of the resting phase. The percentage of BM lost during the approximate 21 h in the respirometer was calculated.

Definitions. Dunnarts were divided into thermoconformers and thermoregulators to identify torpor patterns by assessing individual torpor bouts. There was a clear distinction between animals that thermoconformed during torpor entry, with a fast reduction of T_{sub} (>0.9°C min^{-1}; fig. 1) from torpor entry to steady-state torpor, and those that thermoregulated during torpor entry, with periodic increases in MR that slowed the decrease in T_{sub} (<0.9°C min^{-1}). Torpor bout duration (TBD) was defined as the time an animal's T_{sub} remained below the torpor threshold of 30.0°C.

Statistics. Numeric values are presented as mean ± SD for *n*, the number of individuals.

Frequency of torpor and type of torpor entry in dunnarts. Linear mixed effects models corrected for repeated measures by including the individual's ID as a random effect were used to determine whether BM varied significantly across the five age groups.

Comparing juvenile and adult thermoconformers and thermoregulators: cooling rate and energy used during torpor entry and the effect on torpor patterns. Linear mixed effects models corrected for repeated measures by including the individual's ID as a random effect were used to test the relationships between BM and $cool_{max}$, between $cool_{max}$ and O_2 consumption during torpor entry, between $cool_{max}$ and lowest T_{sub}, and between $cool_{max}$ and TBD.

Energy costs for juvenile and adult thermoconformers and thermoregulators at a similar BM. Linear mixed effects models corrected for repeated measures by including the individual's ID as a random effect were used to test the relationship between $cool_{max}$ and the time and energy juvenile and adult thermoconformers and thermoregulators spent in each physiological state (rest, torpor entry, steady-state torpor, endogenous rewarming, and activity) and whether the type of torpor entry affected the ADMR of juvenile and adult thermoconformers and thermoregulators.

Linear mixed effects models (nlme statistical package; Pinheiro et al. 2022) and repeated-measures ANOVAs (lme4 statistical package; Bates et al. 2015) were performed with R version 4.0.2 and R Studio version 0.99.489 (R Core Team 2021). Conventional least-squares linear regression analyses were also used for comparison (performed in SigmaPlot ver. 11.0.0.77). The sex of the animals was not a significant factor in any statistical analysis.

Results

Frequency of Torpor and Type of Torpor Entry in Dunnarts

BMs were significantly different between all age groups ($F_{16,3}$ = 17.78, $P < 0.001$). Initially, all juvenile dunnarts in age group 1

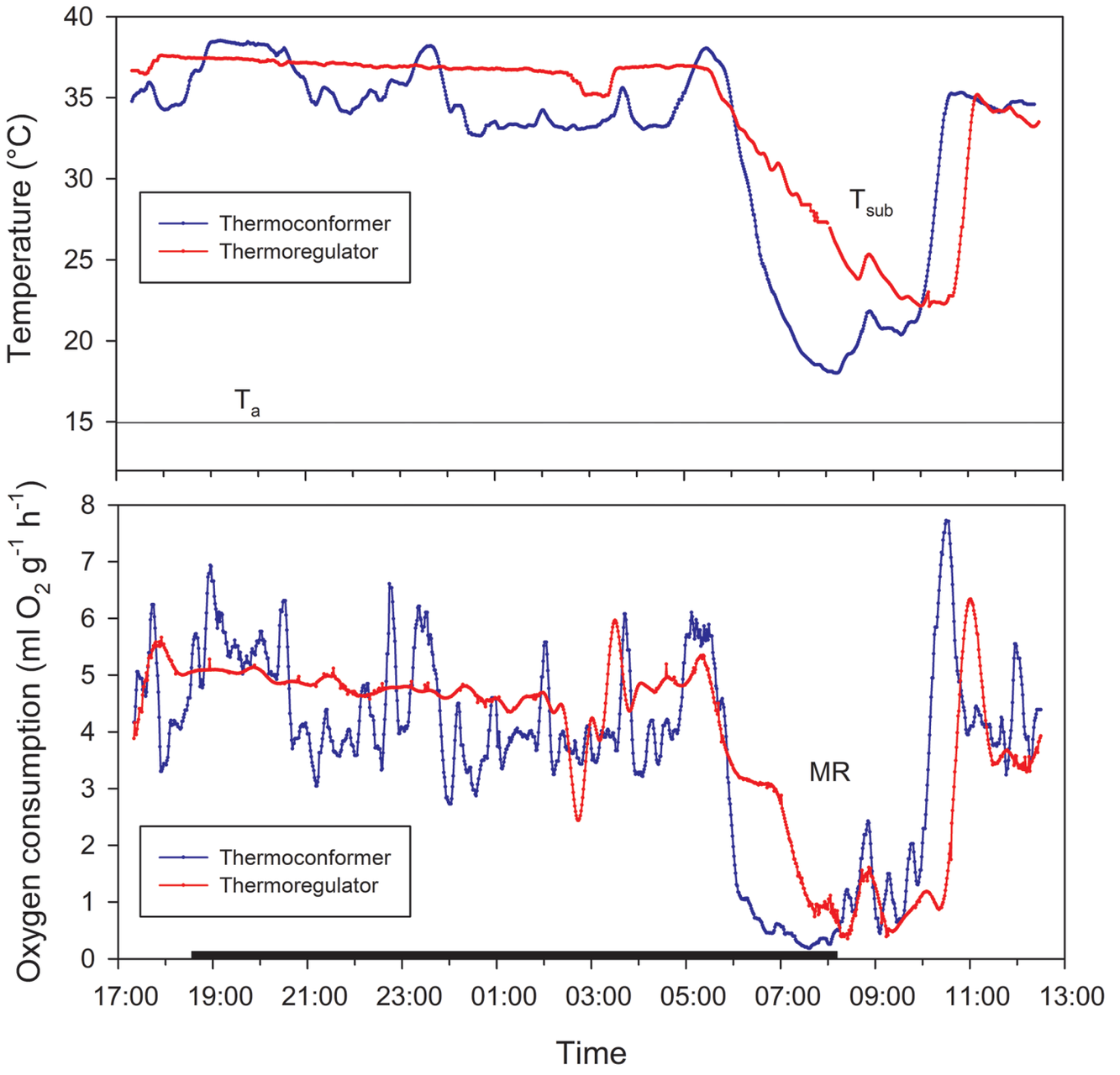

Figure 1. Examples of juvenile dunnarts that thermoconformed (blue line) and thermoregulated (red line) during torpor entry. Note the steeper entry and lower subcutaneous temperature (T_{sub}) of the thermoconformer compared to the thermoregulator. The thick black line on the *x*-axis represents scotophase. MR = metabolic rate; T_a = ambient temperature.

(60–70 d of age; table 1) reduced MR and T_{sub} but could not endogenously rewarm, and instead, they became hypothermic. Hypothermic animals were removed from the chamber, rewarmed in front of a heater, and returned to the nest box. While all animals in juvenile age group 2 (90–100 d of age) entered torpor, only 45% could rewarm and the remaining 55% became hypothermic. Only torpor bouts from the animals that were able to rewarm from torpor were included in our analyses.

Of the five animals in age group 2 (90–100 d of age) that could rewarm, 36% thermoconformed and 9% thermoregulated during torpor entry. Of the animals in age group 3 (120–130 d of age), 82% used torpor and could rewarm. Of the nine animals in age group 3 that used torpor, 46% thermoconformed and 36% thermoregulated during torpor entry. Of the animals in age group 3, 18% did not use torpor. At 150–160 d of age (age group 4), 18% of animals used torpor and thermoregulated during torpor

Table 1: Patterns of torpor entry (thermoconforming or thermoregulating) in juvenile and adult dunnarts

	Animals that became hypothermic			Animals that entered torpor and could rewarm						Animals that did not use torpor		
				Thermoconformed			Thermoregulated					
	%	*n*	BM (g)	%	*n*	BM (g)	%	*n*	BM (g)	%	*n*	BM (g)
Age group 1 (juvenile, 60–70 d; $n = 8$; 10.9 ± .4 g)	100	8	10.9 ± .4	0	0	0	0	0	0	0	0	0
Age group 2 (juvenile, 90–100 d; $n = 11$; 11.7 ± .7 g)	55	6	11.8 ± .4	36	4	11.5 ± 1.1	9	1	12.2	0	0	0
Age group 3 (juvenile, 120–130 d; $n = 11$; 13.5 ± .5 g)	0	0	0	46	5	12.9 ± .2	36	4	13.7 ± .4	18	2	13.6 ± .1
Age group 4 (juvenile, 150–160 d; $n = 11$; 15.1 ± 1.2 g)	0	0	0	0	0	0	18	2	15.6 ± 1.1	82	9	15.0 ± 1.2
Adult (270–310 d; $n = 16$; 18.9 ± 1.1 g)	0	0	0	31	5	17.0 ± 2.53	31	5	18.0 ± 1.75	38	6	18.5 ± .9

Note. Hypothermic animals in age groups 1 and 2 have been included for comparison.

entry, while 82% did not use torpor. Of the 16 adult dunnarts, 62% used torpor, with 50% thermoconforming and 50% thermoregulating during torpor entry. All adult dunnarts that entered torpor rewarmed endogenously.

Comparing Juvenile and Adult Thermoconformers and Thermoregulators: Cooling Rate and Energy Used during Torpor Entry and the Effect on Torpor Patterns

Juvenile dunnarts that thermoconformed during torpor entry had a lower BM (12.6 ± 1.3 g, $n = 9$) than juveniles that thermoregulated during torpor entry (14.0 ± 1.3 g, $n = 7$; $F_{14,2} = 37.12$, $P = 0.001$), while adult thermoconformers (16.8 ± 2.5 g, $n = 5$) and thermoregulators (18.0 ± 1.8 g, $n = 5$) were of a similar (but not statistically different) BM ($F_{8,2} = 1.18$, $P = 0.212$).

Thermoconformers had a higher mean cool_{max} than thermoregulators both in juveniles (thermoconformers, 0.14°C ± 0.05°C min^{-1}, $n = 9$; thermoregulators, 0.06°C ± 0.01°C min^{-1}, $n = 5$; $F_{14,2} = 28.46$, $P < 0.001$; fig. 2*a*) and in adults (thermoconformers, 0.23°C ± 0.02°C min^{-1}, $n = 5$; thermoregulators, 0.16°C ± 0.02°C min^{-1}, $n = 5$; $F_{10,2} = 31.08$, $P = 0.001$). The cool_{max} was 1.7 times faster in adult thermoconformers than in juvenile thermoconformers ($F_{12,2} = 19.60$, $P = 0.002$) and 2.5 times faster in adult thermoregulators than in juvenile thermoregulators ($F_{10,2} = 8.22$, $P = 0.021$).

The mean oxygen consumption during torpor entry (fig. 2*b*) was substantially lower in thermoconformers than in thermoregulators both in juveniles (46%; thermoconformers, 2.50 ± 0.92 mL O_2 g^{-1} h^{-1}; thermoregulators, 4.61 ± 0.82 mL O_2 g^{-1} h^{-1}; $F_{14,2} = 12.63$, $P = 0.010$) and in adults (35%; thermoconformers, 1.32 ± 0.17 mL O_2 g^{-1} h^{-1}; thermoregulators, 2.03 ± 0.68 mL O_2 g^{-1} h^{-1}; $F_{8,2} = 6.15$, $P = 0.032$). Mass-specific energy expenditure during torpor entry in juvenile thermoconformers was nearly twice that in adult thermoconformers ($F_{12,2} = 14.51$, $P = 0.022$), and the difference between juvenile and adult thermoregulators was even greater ($F_{10,2} = 18.32$, $P < 0.001$).

Torpor bouts were deeper (lowest $T_{\text{sub}} - T_{\text{a}}$; fig. 3*a*) in thermoconformers than in thermoregulators both in juveniles (thermoconformers, lowest $T_{\text{sub}} - T_{\text{a}} = 0.9$°C ± 0.7°C; thermoregulators, lowest $T_{\text{sub}} - T_{\text{a}} = 5.6$°C ± 3.4°C; $F_{14,2} = 17.52$, $P < 0.001$) and in adults (thermoconformers, lowest $T_{\text{sub}} - T_{\text{a}} = 1.9$°C ± 1.1°C; thermoregulators, lowest $T_{\text{sub}} - T_{\text{a}} = 5.1$°C ± 0.5°C; $F_{8,2} = 14.11$, $P < 0.001$). Juvenile thermoconformers had a lower minimum T_{sub} than adult thermoconformers (juveniles, 15.9°C ± 0.9°C; adults, 16.8°C ± 1.3°C; $F_{12,2} = 3.18$, $P = 0.036$), whereas in thermoregulators, the minimum T_{sub} in juveniles and adults was similar (juveniles, 20.4°C ± 3.2°C; adults, 20.7°C ± 1.6°C; $F_{10,2} = 1.01$, $P = 0.401$).

Torpor bouts (fig. 3*b*) were longer in thermoconformers than in thermoregulators both in juveniles (thermoconformers, 436.0 ± 75 min; thermoregulators, 264.0 ± 76 min; $F_{14,2} = 9.65$, $P < 0.001$) and in adults (thermoconformers, 476.6 ± 224.7 min; thermoregulators, 192.8 ± 63.5 min; $F_{8,2} = 6.18$, $P = 0.006$). Torpor bouts were of a similar duration in juvenile (435.7 ± 74.9 min) and adult (476.6 ± 224.7 min) thermoconformers ($F_{12,2} = 0.98$, $P = 0.268$) and in juvenile (264.3 ± 76.4 min) and adult (192.8 ± 63.5 min) thermoregulators ($F_{10,2} = 3.18$, $P = 0.051$).

Energy Costs for Juvenile and Adult Thermoconformers and Thermoregulators at a Similar Body Mass

To separate the effects of development and BM, we compared functional variables (fig. 4) of thermoconformers and thermoregulators at a similar (but not significantly different) BM in juveniles (thermoconformers, 13.1 ± 0.8 g, $n = 7$; thermoregulators, 13.4 ± 0.8 g, $n = 5$; $F_{8,2} = 1.48$, $P = 0.391$) and adults (thermoconformers, 16.8 ± 2.5 g, $n = 5$; thermoregulators, 18.0 ± 1.8 g, $n = 5$; $F_{10,2} = 2.19$, $P < 0.183$). Both juvenile and adult thermoconformers and thermoregulators differed

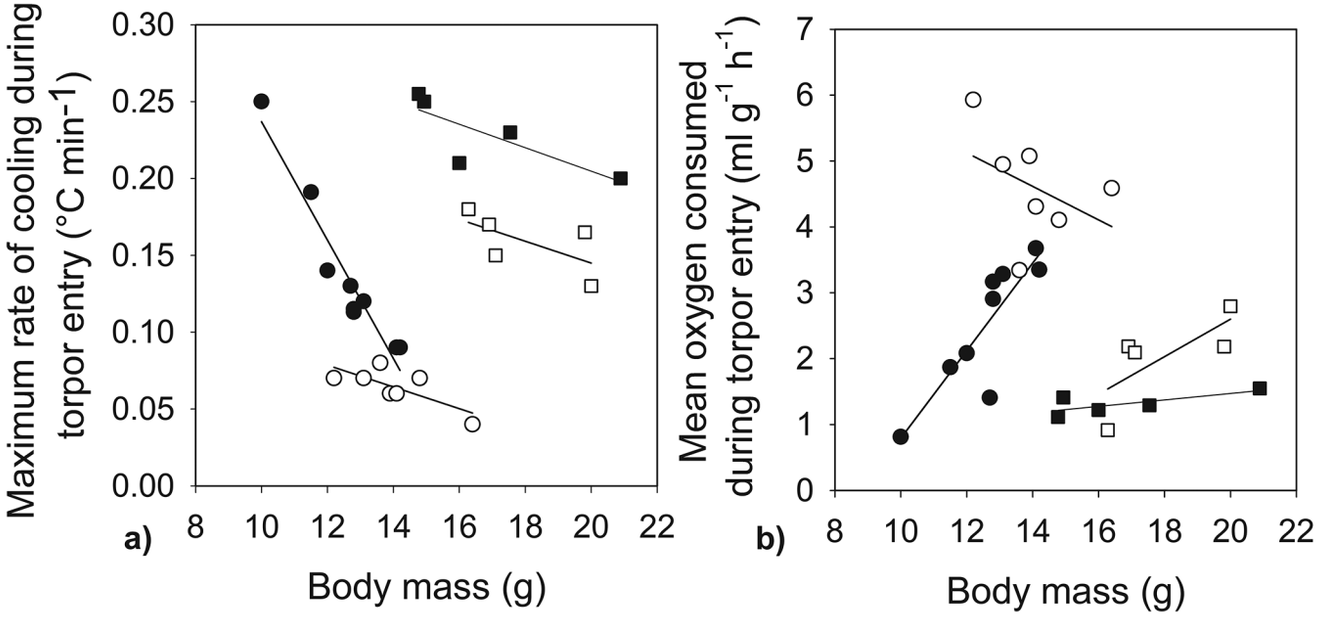

Figure 2. *a*, Maximum rate of cooling over 10 min as a function of body mass in juvenile dunnarts that thermoconformed (filled circles; $r^2 = 0.93$, $P < 0.001$, df = 8, $y = -0.039x + 0.622$) and thermoregulated (open circles; $r^2 = 0.55$, $P = 0.048$, df = 6, $y = -0.011x + 0.164$) during torpor entry and in adult dunnarts that thermoconformed (filled squares; $r^2 = 0.64$, $P = 0.104$) and thermoregulated (open squares; $r^2 = 0.41$, $P = 0.245$) during torpor entry. *b*, Mean oxygen consumed during entry into torpor as a function of body mass in juvenile thermoconformers (filled circles; $r^2 = 0.75$, $P = 0.003$, df = 8, $y = 0.664x - 5.852$) and thermoregulators (open circles; $r^2 = 0.17$, $P = 0.356$) and adult thermoconformers (filled squares; $r^2 = 0.17$, $P = 0.281$) and thermoregulators (open squares; $r^2 = 0.53$, $P = 0.163$).

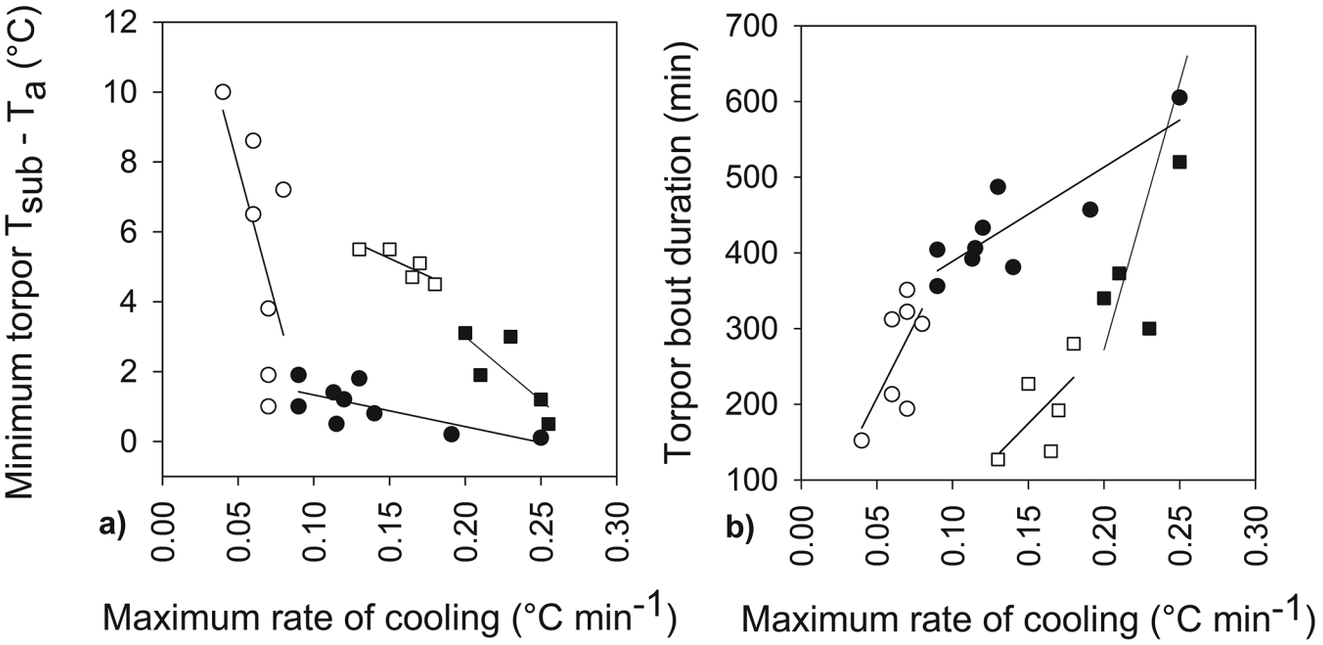

Figure 3. *a*, Torpor depth (lowest subcutaneous temperature [T_{sub}] − ambient temperature [T_a]) as a function of maximum rate of cooling during torpor entry in juvenile thermoconformers (filled circles; $r^2 = 0.52$, $P = 0.028$, df = 8, $y = -908x + 2.24$) and thermoregulators (open circles; $r^2 = 0.36$, $P = 0.198$) and adult thermoconformers (filled squares; $r^2 = 0.61$, $P = 0.135$) and thermoregulators (open squares; $r^2 = 0.72$, $P = 0.163$, df = 4, $y = -19.87x + 8.22$). *b*, Torpor bout duration as a function of maximum rate of cooling during torpor entry in juvenile thermoconformers (filled circles; $r^2 = 0.74$, $P = 0.003$, df = 8, $y = 1{,}243.47x + 264.48$) and thermoregulators (open circles; $r^2 = 0.43$, $P = 0.110$) and adult thermoconformers (filled squares; $r^2 = 0.57$, $P = 0.139$) and thermoregulators (open squares; $r^2 = 0.39$, $P = 0.259$).

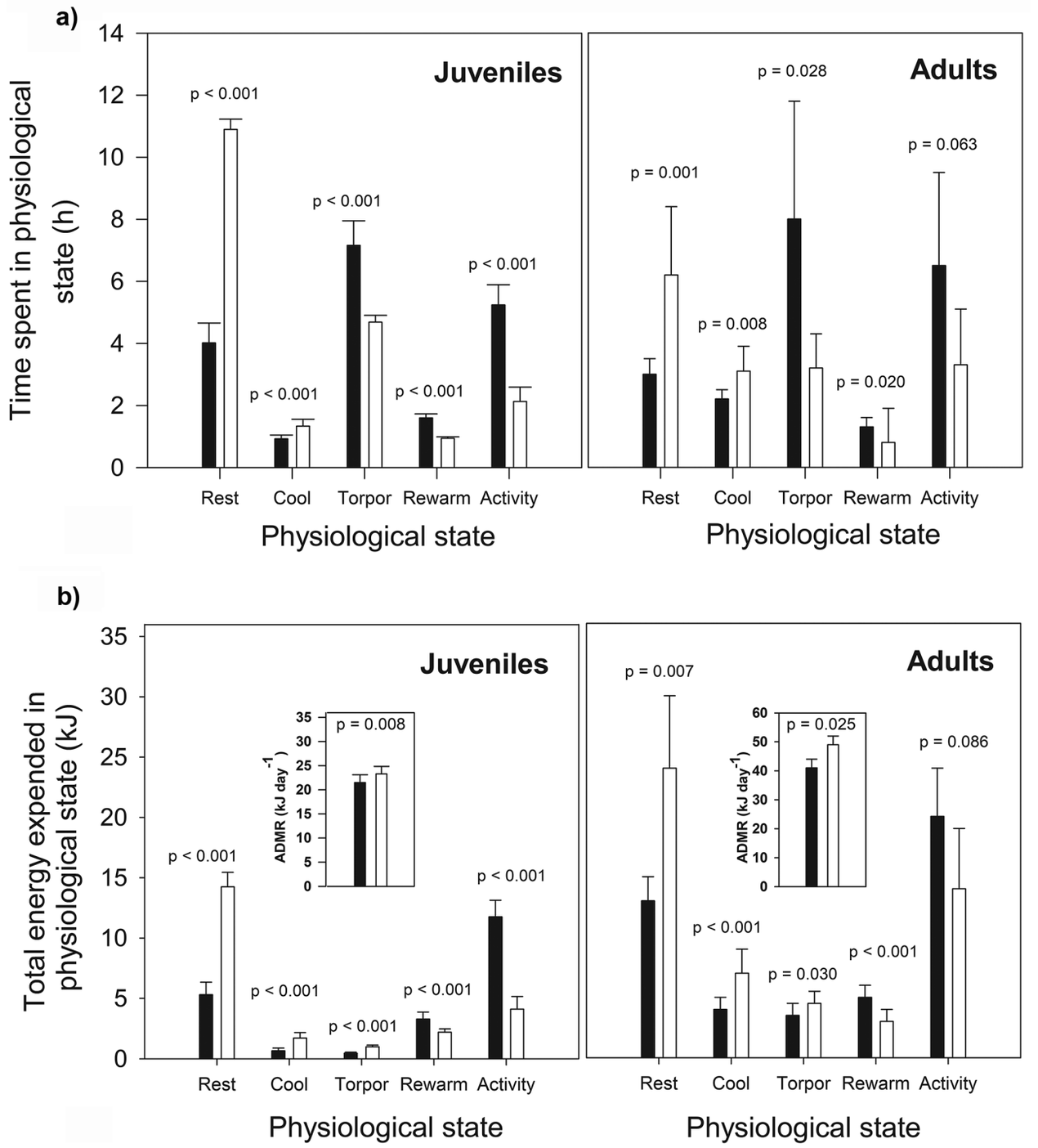

Figure 4. *a*, Comparison of the time juvenile and adult thermoconformers (black bars) and thermoregulators (white bars) spent in each of the five physiological states. *b*, Quantity of the energy juvenile and adult thermoconformers (black bars) and thermoregulators (white bars) used in each of these states during approximately 21 h in the respirometer, including the average daily metabolic rate (ADMR). *P* values (repeated-measures ANOVA) are included.

significantly in the time they spent in four of the five physiological states and in the energy they used while in those states. The *P* values comparing juvenile thermoconformers with juvenile thermoregulators and adult thermoconformers with adult thermoregulators are included in figure 4.

Resting. Thermoregulators spent more time and energy resting than thermoconformers both in juveniles (thermoregulators, 11.8 ± 0.3 h, 14.2 ± 1.2 kJ; thermoconformers, 4.0 ± 0.6 h, 5.3 ± 1.02 kJ) and in adults (thermoregulators, 6.2 ± 2.2 h, 24.1 ± 5.9 kJ; thermoconformers, 3.0 ± 0.5 h, 13.2 ± 2.0 kJ);

however, while juvenile thermoregulators spent 60% more time resting than adult thermoregulators ($F_{8,2} = 28.55$, $P < 0.001$), because of their smaller size they used only 60% of the energy ($F_{8,2} = 3.62$, $P = 0.039$). Juvenile thermoconformers spent 25% more time resting than adult thermoconformers ($F_{10,2} = 16.58$, $P = 0.007$) but used only 30% of the energy ($F_{10,2} = 40.08$, $P < 0.001$) during the resting phase.

Torpor Entry (Cooling). By cooling more quickly during torpor entry (mean time of the whole cooling phase: juvenile thermoconformers, 0.9 ± 0.1 h; juvenile thermoregulators, 1.3 ± 0.2 h; adult thermoconformers, 2.2 ± 0.3 h; adult thermoregulators, 3.1 ± 0.8 h) and thermoconforming during torpor entry, thermoconformers used substantially less energy during entry into torpor than thermoregulators (juvenile thermoconformers, 0.67 ± 0.22 kJ; juvenile thermoregulators, 1.72 ± 0.45 kJ; adult thermoconformers, 3.9 ± 1.1 kJ; adult thermoregulators, 7.0 ± 0.9 kJ). Juvenile thermoconformers spent less than half the time ($F_{10,2} = 34.34$, $P < 0.001$) and used less than one-fourth the energy ($F_{10,2} = 58.16$, $P < 0.001$) entering torpor as adult thermoconformers. Juvenile thermoregulators spent less than half the time cooling during torpor entry as adult thermoregulators ($F_{8,2} = 29.62$, $P < 0.001$) and spent 75% less energy entering torpor than adult thermoregulators ($F_{8,2} = 12.85$, $P = 0.001$).

Torpor. Juvenile thermoconformers had longer torpor bouts (thermoconformers, TBD = 7.2 ± 1.3 h; thermoregulators, TBD = 4.4 ± 1.2 h) and spent less energy during torpor (thermoconformers, 0.46 ± 0.08 kJ; thermoregulators, 1.00 ± 0.12 kJ) than juvenile thermoregulators. Adult thermoconformers also had longer torpor bouts (thermoconformers, TBD = 8.1 ± 3.8 h; thermoregulators, TBD = 3.2 ± 1.1 h) and spent less energy during torpor (thermoconformers, 3.5 ± 0.9 kJ; thermoregulators, 4.4 ± 1.2 kJ) than adult thermoregulators. There was no significant difference between juvenile and adult thermoconformers in the time they spent torpid ($F_{10,2} = 1.18$, $P = 0.186$); however, juveniles used 86% less energy during torpor ($F_{10,2} = 31.55$, $P < 0.001$). Juvenile thermoregulators were torpid, on average, for 1 h longer than adult thermoregulators ($F_{8,2} = 6.58$, $P = 0.024$) and used less than 25% of the energy ($F_{8,2} = 39.46$, $P = 0.001$).

Endogenous Rewarming. Juvenile thermoconformers spent more time (mean time of the whole rewarming phase: thermoconformers, 1.6 ± 0.1 h; thermoregulators, 0.9 ± 0.1 h) and used more energy during rewarming from torpor (thermoconformers, 3.29 ± 0.58 kJ; thermoregulators, 2.20 ± 0.27 kJ) than juvenile thermoregulators. Adult thermoconformers also spent more time rewarming (mean time of the whole rewarming phase: thermoconformers, 1.3 ± 0.3 h; thermoregulators, 0.8 ± 1.1 h) and therefore used more energy during the rewarming phase (thermoconformers, 5.0 ± 1.0 kJ; thermoregulators, 2.9 ± 0.9 kJ) than adult thermoregulators. Juvenile and adult thermoconformers spent a similar amount of time rewarming from torpor ($F_{10,2} = 6.25$, $P = 0.058$) but differed significantly in the energetic cost of rewarming ($F_{10,2} = 18.14$, $P < 0.001$), with adult thermoconformers using 33% more energy. Juvenile and adult thermoregulators also spent a similar amount of time rewarming from torpor ($F_{8,2} = 4.22$, $P = 0.188$) but differed significantly in the energetic cost of rewarming ($F_{8,2} = 28.68$, $P = 0.001$), with adult thermoregulators using 27% more energy during the rewarming phase.

Activity. Juvenile thermoconformers spent more time active (thermoconformers, 6.2 ± 0.7 h; thermoregulators, 2.1 ± 0.5 h) and used more energy while active (thermoconformers, 12.76 ± 1.39 kJ; thermoregulators, 4.12 ± 1.04 kJ) than juvenile thermoregulators. However, juvenile thermoregulators spent more time (thermoregulators, 11.9 ± 0.3 h; thermoconformers, 5.0 ± 0.6 h) and energy (thermoregulators, 16.25 ± 1.20 kJ; thermoconformers, 6.32 ± 1.03 kJ) resting. There was no significant difference between the time or energy that adult thermoconformers and thermoregulators spent in activity. While juvenile and adult thermoconformers ($F_{10,2} = 1.01$, $P = 0.198$) and thermoregulators ($F_{8,2} = 6.11$, $P = 0.053$) spent similar amounts of time active, the energy used while active did differ, with juvenile thermoconformers using nearly half the energy as adult thermoconformers ($F_{10,2} = 25.50$, $P = 0.005$) and juvenile thermoregulators using less than one-third the energy as adult thermoregulators ($F_{8,2} = 40.21$, $P < 0.001$).

Average Daily Metabolic Rate. The ADMR of juvenile thermoconformers was significantly lower than that of juvenile thermoregulators ($F_{10,2} = 3.86$, $P = 0.039$), with juvenile thermoconformers using 23.49 ± 1.36 kJ d^{-1} and juvenile thermoregulators using 25.28 ± 1.42 kJ d^{-1}—that is, approximately 7% more energy than juvenile thermoconformers each day. The ADMR of adult thermoconformers was significantly lower than that of adult thermoregulators ($F_{8,2} = 20.99$, $P = 0.006$), with adult thermoconformers using 41.2 ± 2.8 kJ d^{-1} and adult thermoregulators using 49.3 ± 2.9 kJ d^{-1}—that is, approximately 17% more energy than adult thermoconformers each day. Adult thermoconformers had an ADMR that was nearly twice that of juvenile thermoconformers ($F_{10,2} = 42.53$, $P < 0.001$), and adult thermoregulators also used twice as much energy per day as juvenile thermoregulators ($F_{8,2} = 38.98$, $P < 0.001$).

Discussion

Our study demonstrates for the first time that the cooling rate during torpor entry influences the subsequent temporal and thermoenergetic aspects of a torpor bout and therefore how much energy is saved during torpor, with thermoconformers having deeper and longer torpor bouts than thermoregulators. However, our data only partially support our prediction of universal thermoconformation in growing young, as during development juveniles should choose the energetically cheapest and most beneficial method of torpor entry to save as much energy as possible to incorporate into growth. Thus, the observation of some growing juvenile dunnarts thermoregulating during torpor entry was surprising.

Once juvenile dunnarts could rewarm from torpor endogenously, those with a lower BM typically thermoconformed during torpor entry. This is likely due to the smaller juvenile thermoconformers cooling twice as fast as juvenile thermoregulators because of their smaller BM and higher normothermic thermal conductance increasing heat loss (Bradley and Deavers 1980; Snyder and Nestler 1990). Most juveniles larger than 13.5 g thermoregulated during torpor entry, and by 15 g, the majority of juveniles did not use torpor at all. The frequency of torpor was further reduced in the adults, and somewhat surprisingly, among those that did use torpor, 50% thermoconformed and 50% thermoregulated during torpor entry. The adults in our study were of a similar BM and age, and there was no effect of sex on any variable, so we are unable to explain this pattern. All adults were housed separately with ad lib. food, so they had access to the same resources without competition. Because food intake was not measured, it is possible that adults who thermoconformed during torpor entry did not eat as much food on the days leading up to measurements (Munn et al. 2010). Juvenile and adult thermoregulators regulated against the decrease in T_{sub} during torpor entry by increasing MR and slowing the cooling rate and therefore used at least 40% more energy during the cooling phase than thermoconformers. However, in both thermoconforming and thermoregulating adults, $cool_{max}$ was high and similar to that in juvenile thermoconformers weighing 10–12 g. This suggests that the reduction in cooling rate and the expression of torpor during growth are reversed once animals become adults (Geiser 2021).

The rate of cooling during torpor entry influenced the subsequent depth and duration of the torpor bout and therefore the amount of energy saved during torpor, with thermoconformers having T_{sub}'s that were 4°C lower and TBDs that were 40% longer than those of thermoregulators. Because the T_{sub} and the lowest $T_{sub} - T_a$ differential of torpid thermoconformers were lower, the TMR was also 50% lower in thermoconformers than in thermoregulators (Geiser 2021). Although these patterns were similar in the adult dunnarts, they were not as pronounced because of their larger BM. TBD is influenced by the timing of arousal from torpor. While the gradual depletion of metabolites may prompt periodic arousals in hibernators, the rewarming from daily torpor in daily heterotherms is likely prompted by a circadian rhythm and readiness for foraging, especially in nocturnal mammals such as the dunnart (Malan 2010; Ruf and Geiser 2015; Ruf et al. 2021).

During the arousal phase, the mass-specific MR during endogenous rewarming of juvenile thermoconformers and thermoregulators of comparable body sizes was similar. Because thermoconformers had deeper torpor bouts and therefore had to increase their T_b by more, rewarming from torpor took twice the time and cost 33% more energy in thermoconformers than in thermoregulators. Upon rewarming, the torpor bouts of most heterotherms include an overshoot in MR. This overshoot reflects the excess heat required to regain a normothermic T_b (Tucker 1965; Snyder and Nestler 1990; Ellison and Skinner 1992). While necessary to raise T_b, a large overshoot will significantly decrease the energy saved from torpor, especially if the torpor bout is relatively short (Frey 1991). Because the thermoconformers in our study had to rewarm from a lower T_{sub}, it is likely that they also had a larger MR overshoot than the thermoregulators.

While the deeper torpor bout due to thermoconforming during torpor entry did incur greater rewarming costs, the ADMR of both juvenile and adult thermoconformers was still low even though they were active for more than twice as long as thermoregulators and spent less than half the time resting. Therefore, the energetic costs of rewarming were more than offset by thermoconforming during torpor entry, a low TMR, and long torpor bouts. The reduced ADMR of juvenile thermoconformers due to pronounced torpor equates to approximately 2 kJ d^{-1}, which can be incorporated into growth or energy storage (Dawson and Hulbert 1970; Giroud et al. 2014). To put this into perspective, approximately 4–17 kJ are stored in the tail of adults in this species (Hume 1982). In the wild, dunnarts mainly eat insects and spiders, and depending on the type of food eaten, each night they will eat as much as they weigh. While there are ~16 kJ in 1 g of crickets, the animals in our study were fed a diet of energy-rich cat food and therefore typically ate less than they would in the wild (Stannard et al. 2014).

As expected, our laboratory-obtained ADMR values were lower than those measured in the field (Nagy et al. 1988). The daily field MR (FMR) of the dunnarts has been measured during spring as 68.7 kJ d^{-1} for adults (~17 g) and 29.2 kJ d^{-1} for juveniles (~6 g; Nagy et al. 1988). Our ADMR values, including periods of torpor, were approximately 22 kJ d^{-1} for juveniles and 45 kJ d^{-1} for adults, and other laboratory adult ADMR values (45.8 kJ d^{-1}; Warnecke et al. 2008) are similar to those obtained from our study. When calculating FMR, it is rarely possible to obtain a complete measurement of the animal's activity levels and the extent of torpor use. The very high (compared to laboratory measurements) FMR indicates that activity periods were long or that torpor use was limited or absent. Measurements of field ADMR for dunnarts (Nagy et al. 1988) were conducted during the Southern Hemisphere spring, a time of intense reproductive effort for the species (Morton 1978). Although torpor is often used during reproduction (Geiser and Brigham 2012), this high FMR indicates that it was not used in this instance. However, the FMR of 29.2 kJ d^{-1} in juveniles weighing ~6 g (Nagy et al. 1988) is interesting because it is slightly higher than our laboratory-obtained ADMR in laboratory juveniles weighing ~13 g and perhaps represents the high energetic cost of growth and development.

Young dunnarts become too big for the pouch early in their development and are often left in the nest while the mother forages for food (Tyndale-Biscoe and Renfree 1987). Even while huddling, the T_b of very young marsupials can still decrease to less than 5°C (Soderquist 1993) while the mother makes short trips to forage, indicating that huddling may not be enough to help animals maintain a high T_b (Geiser et al. 1986). As the young animals begin to forage independently, they need to spend long periods of time active. Younger and smaller thermoconformers therefore need longer torpor bouts to offset the costs of activity (Lyman et al. 1982). While the larger thermoregulators have shorter torpor bouts, they are active for less time (Lyman et al. 1982; Geiser and Ruf 2023) and have more time for rest, but ADMR is still high as

a result of maintaining a high normothermic T_b. In comparison, thermoconformers use more energy during activity, but with faster cooling rates and longer, deeper torpor bouts, ADMR remains low (Génin and Perret 2003; Christian and Geiser 2007; Giroud et al. 2008; Schubert et al. 2010).

While thermoconforming during torpor is energetically cheaper than thermoregulating and leads to a deeper and longer torpor bout, rewarming from a deep torpor bout is more expensive than rewarming from a shallow one. This rewarming cost is greater if passive rewarming is not possible, and active rewarming can offset the energetic benefits from using torpor to some extent (Geiser and Drury 2003; Mckechnie and Wolf 2004; Warnecke et al. 2008). The benefits of thermoregulating during torpor entry must outweigh the costs; for example, as running speed is a function of T_b during torpor (Rojas et al. 2012), maintaining a relatively high T_b may permit coordinated movement to escape a predator (Warnecke et al. 2008), meaning that thermoregulating torpid animals will have better coordination and a greater awareness of their environment (Geiser and Brigham 2012; Rojas et al. 2012; Stawski et al. 2015). Therefore, very deep torpor bouts may not always be desirable unless necessary, such as when environmental temperatures are very low and the cost of normothermic thermoregulation outweighs the potential costs of rewarming from both deep and shallow torpor bouts.

Other observed disadvantages associated with very deep torpor bouts include stress on the heart during arousal (Wiersma et al. 2018), sleep debt (Schmidt 2014; Royo et al. 2019), reduced immune function (Pendergast et al. 2002), possibility of brain damage (Giroud et al. 2021; Chmura et al. 2022), metabolic imbalances (Ruf et al. 2021), and shortening of telomeres due to oxidative stress when rewarming from torpor (Carey et al. 2000; Humphries et al. 2003; Wei et al. 2018; Nowack et al. 2019; de Wit et al. 2023; Ruf and Bieber 2023). Nearly all this research has been conducted on hibernators (those heterotherms that use multiday bouts of torpor interspersed with periods of arousal to normothermic T_b's), and very little work has been done on the disadvantages of deep torpor bouts in daily heterotherms, such as the dunnart. However, the costs associated with the deep torpor bouts of hibernators may provide clues as to why daily heterotherms choose shallower torpor bouts where possible to balance the energetic savings with the physiological costs.

Conclusion

The rate of cooling during torpor entry drives torpor patterns in the dunnart and therefore influences how much energy an animal can save by using torpor. Cooling rapidly during torpor entry is advantageous because it leads to a deeper and longer torpor bout, but a greater energy output may be required to rewarm from a lower T_b. In contrast, while thermoregulating during torpor entry may not allow an animal to save as much energy during torpor, it may allow them to stay alert for longer and not spend as much energy on endogenous rewarming. Because smaller and younger juveniles thermoconformed during torpor entry and had a lower ADMR, thermoconforming can help an animal save more energy that can be allocated to growth.

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