

Marsupials don't adjust their thermal energetics for life in an alpine environment

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ABSTRACT

Marsupials have relatively low body temperatures and metabolic rates, and are therefore considered to be maladapted for life in cold habitats such as alpine environments. We compared body temperature, energetics and water loss as a function of ambient temperature for 4 *Antechinus* species, 2 from alpine habitats and 2 from low altitude habitats. Our results show that body temperature, metabolic rate, evaporative water loss, thermal conductance and relative water economy are markedly influenced by ambient temperature for each species, as expected for endothermic mammals. However, despite some species and individual differences, habitat (alpine vs non-alpine) does not affect any of these physiological variables, which are consistent with those for other marsupials. Our study suggests that at least under the environmental conditions experienced on the Australian continent, life in an alpine habitat does not require major physiological adjustments by small marsupials and that they are physiologically equipped to deal with sub-zero temperatures and winter snow cover.

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Introduction

Marsupials generally have a low body temperature (T_b) and basal metabolic rate (BMR) compared to placental mammals. Resting T_b is typically 2.5°C lower for marsupial compared with placental mammals, and BMR of marsupials is on average only 70% of that of equivalently-sized placental mammals.^{1–8} The consequences of this low BMR (and other physiological differences such as a lack of functional brown fat⁹) have been postulated to include inferior competition with placental mammals (especially for species occupying high-energy niches), restricted reproductive rates, and poor thermoregulatory ability.^{3,4,10–13}

McNab^{3,4,14} suggested that the limited thermogenic and reproductive capacity of marsupials results in their being excluded from energy-demanding environments or niches, such as high altitudes or latitudes, and high energy food sources. Indeed, most marsupials appear to spatially or temporally avoid cold winter conditions at high altitudes and latitudes. The North American opossum (*Didelphis virginiana*; ~1000 g) is a well-known example of a marsupial with a cold (latitudinal) distribution limit; low T_a

during winter appears to impact on their survival, and prevents their range expanding further northwards. Opossums are reliant on urbanisation in cold climates.^{5,14,15} However, as this species is recently derived from neotropical didelphids,¹⁶ it is not unexpected that T_a appears to restrict its northern distribution.

In South America, several marsupials of the families Caenolestidae, Microbiotheriidae and Didelphidae inhabit high latitudes and/or altitudes with seasonally cold conditions.^{5,14,17} For *Lestodelphes* and *Dromiciops*, the key to survival appears to be heterothermia; they use multiday torpor or hibernation to avoid seasonal climatic extremes.^{18–24} Similarly, the small (57 g) Australian mountain pygmy possum (*Burramys parvus*), a marsupial restricted to alpine habitats, also uses hibernation during winter months, remaining inactive in sheltered subnival hibernacula until more favorable environmental conditions in spring.^{25,26}

Despite the general spatial or temporal avoidance of harsh winter conditions by marsupials, some species of Australian marsupial do remain active in alpine and subalpine habitats year-round, and must be able

to physiologically cope with these harsh environmental conditions. The Australian landmass does not have extreme altitudes or very high latitudes, with the highest point, Mt Kosciuszko, being 2,228 m above sea level, and the most southerly point of Tasmania being only 43°38'37"S. Nevertheless, parts of south-eastern Australia, including the Australian Alps and the Central Highlands Bioregion of Tasmania, have alpine and sub-alpine habitats that are characterized by sub-zero temperatures and snow cover for at least 4 months of the year. For example, the Australian Alps are characterized by a mean annual temperature of 3 to 12°C, minimum average monthly temperature of -7 to -0.4°C, and snowfall for 4–6 months per year.²⁷ These alpine environments undoubtedly place considerable physiological demands on the small mammals that inhabit them.

Examining the physiology of small marsupials that are active year round in Australian alpine and sub-alpine habitats will address the question of whether these alpine marsupial species have particular physiological adaptations that enhance survival in cold, harsh climates. For the larger species (e.g. common wombats, *Vombatus ursinus*, 26 kg) thermoregulatory demand is reduced considerably by a large body mass and relatively thick insulating fur, along with behavioral adaptations such as semi-fossoriality, that buffer climatic extremes.²⁸ Moderately-sized species such as the Tasmanian devil (*Sarcophilus harrisi*; 10 kg) and quolls (*Dasyurus* spp., 525–5,500 g) presumably face a more substantial metabolic demand during the winter, but they can maintain homeothermy and do not seem to reduce activity levels during cold periods.²⁹ The eastern quoll (780 g) does however have a significantly higher BMR than other quolls, which approximates a general placental level of BMR.¹³ This elevated BMR has been attributed to its cold habitat and the necessity of increased scope for thermogenesis.

Several small species of *Antechinus* marsupial inhabit alpine and sub-alpine regions on mainland Australia. The dusky antechinus (*Antechinus swainsonii*; 53 g) and agile antechinus (*A. agilis*; 18 g) remain active in these habitats year-round, and since no dasyurid marsupial is known to hibernate,³⁰ these marsupials must be active for at least some part of each day (although they can use daily torpor^{31–34}). For these small *Antechinus*, the energetic costs of daily activity and normothermic thermoregulation in the cold alpine winter must be considerable. The aim of

our study was to compare the physiology of alpine dusky and agile antechinus with low-elevation brown antechinus (*A. stuartii*; 28 g) and yellow-footed antechinus (*A. flavipes*; 25g), to determine whether small marsupial species that are active in alpine and sub-alpine environments year round have an increased BMR or other physiological specializations to facilitate survival in cold habitats.

Methods

Wild antechinus were captured between April and June using Elliott aluminum cage traps baited with rolled oats and peanut butter, sometimes with attractants such as honey, cat food and/or sardines. Eight yellow-footed antechinus (4 males, 4 females) were captured at Dryandra Woodland, Western Australia (32° 47'S, 116° 55'E; elevation 327 m) and 8 brown antechinus (6 males, 2 females) were caught at Arthursleigh Station, near Marulan, New South Wales (34° 24'S, 149° 58'E; elevation 642 m). Seven dusky antechinus (3 males, 4 females) were measured, 2 from Werrikimbe National Park, New South Wales (31° 12'S, 152° 14'E; elevation 975 m), and 5 from Smiggins Holes, Kosciuszko National Park, New South Wales (36° 24'S, 148° 25'E; elevation 1680 m). Eight agile antechinus (6 females, 2 males) were also caught near Smiggins Holes. Antechinus were housed in large plastic crates and fed a diet of cat food, kangaroo mince and live invertebrates (mealworms, crickets), with *ad lib.* water.

Oxygen consumption (VO₂), carbon dioxide production (VCO₂) and evaporative water loss (EWL) were measured using flow-through respirometry at ambient temperatures (T_a) ranging from 10°C to 35°C. Measurements commenced within a week of capture, and the order of experimental temperatures was randomized. *Antechinus* were fasted for 24 hours before the commencement of experiments. They were measured during their inactive phase for a period of at least 8 hours under dim light, until all physiological variables became stable and minimal (except at T_a = 35°C where experiments were no longer than 6 hours to avoid excessive heat exposure and dehydration). Body mass was measured immediately before and after each respirometry experiment, and the mean was used for that experiment. Body temperature (T_b) was measured to ± 0.1°C at the conclusion of each experiment using a plastic-tipped thermocouple (connected

to a RadioSpares 611.234 thermocouple meter) inserted into the cloaca.

The respirometry system consisted of a mass flow controller (Aalborg GFC171 or Omega FMAA2412) that regulated ambient air flow (dried using Drierite) at flow rates of 400–800 mL min⁻¹, achieved using a variety of diaphragm pumps. Air passed through a 275 cm³ glass tube that served as a metabolic chamber, located in a temperature-controlled cabinet. Excurrent air passed through a temperature and humidity probe (Vaisala HMP 45A). A sub-sample (approx 100 mL min⁻¹) of excurrent air was dried (using Drierite) to measure CO₂ and O₂ levels (Sable Systems Foxbox or PA-10, or Servomex 572 or 574 O₂ analysers, and Sable Systems CA-2A or CA-10A or Leybold–Heraeus Binos-C CO₂ analysers).

Flowmeters were calibrated using a bubble flowmeter (corrected to standard temperature and pressure dry, STPD) or a Gilian Gilibrator, traceable to a national standard. Gas analysers were calibrated using room air (20.95% O₂ and 0.03 % CO₂) and a butane flame after Withers³⁵ or nitrogen (0% O₂ and CO₂) and a precision gas mix (0.53% CO₂, BOC Gases). Calibration of the RH probes (achieved by saturating air at a known temperature and then warming to T_a) was routinely confirmed using 2 points, 1% RH (dried with Drierite) and 100% RH (saturated; by breathing on the probe). The voltage outputs for O₂, CO₂, RH and T_a were recorded every 10 to 20 sec with a custom-written Visual Basic (VB ver. Six) data acquisition program (P. Withers).

Calculation of VO₂, VCO₂ and EWL was after Withers³⁵ using a custom written VB 6 data analysis program (P. Withers) for the 20 min period during which each value was steady and minimal. Respiratory exchange ratio (RER) was calculated as VCO₂/VO₂. EWL was converted to evaporative heat loss (EHL) using 2.4 J mg⁻¹ H₂O¹⁴, and MR was converted to metabolic heat production (MHP) using the oxycaloric coefficient at the measured RER for that experiment.³⁶ Wet (C_{wet}) and dry (C_{dry}) thermal conductance (J g⁻¹ h⁻¹ °C⁻¹) were calculated as MR/(T_b-T_a), and (MHP-EHL)/(T_b-T_a) respectively. Relative water economy (RWE) was calculated as MWP/EWL, where metabolic water production (MWP; ml g⁻¹ h⁻¹) was calculated from VO₂ using the measured RER for that experiment.³⁶ The point of relative water economy (PRWE) was the T_a where RWE was calculated to be 1.

All values are presented as mean ± SE, where N = number of individuals and n = number of

measurements. All eight yellow-footed antechinus were measured at all T_a (10, 15, 20, 25, 30 and 35°C), but it was not possible to measure all individuals of the other species at all T_a. For brown antechinus, N = 6 at T_a = 10 and 25°C, N = 7 at T_a = 35°C and N = 8 at T_a = 20 and 30°C. For dusky antechinus, N = 6 at 10 and 25°C and N = 7 at 15, 20, 30 and 35°C, and for agile antechinus, N = 6 at T_a = 12°C and N = 8 at T_a = 30°C. As not all individuals of each species were measured at each T_a, a conventional multivariate repeated measures analysis³⁷ was not possible, so generalized linear mixed effect models (GLMM) were used to examine T_a and habitat affects while accounting for repeated measurements of each individual as a random factor.³⁸ This was achieved with lmer³⁹ and lmerTest⁴⁰ libraries in R.⁴¹ We examined the physiological response to T_a for each species, with T_a as a fixed factor with sex and individual-nested-in-sex as random factors. T_a = 30°C (thermoneutrality) was used as the reference category to examine pair-wise fixed effects with Satterthwaite's approximations for calculation of degrees of freedom. We then compared physiological responses to T_a among species, with T_a as a polynomial function and habitat as a fixed factor, and species and individual-nested-in-species as random factors. Pair-wise species comparisons were made with the yellow-footed antechinus as the reference category.

We compared basal values (measured at T_a = 30°C) for species nested in habitat by nested ANOVA, using Statistix v 1.6, and calculated the PRWE for each species using least squares linear regression of RWE against T_a. ANCOVA was used to compare the thermolability of the 4 antechinus species, calculated as the slope of T_b against T_a from T_a = 10–30°C. For all comparisons between species, physiological variables were corrected for body mass effects using allometric scaling exponents for marsupials (0.533 for T_b, 0.737 for BMR, 0.564 for C_{wet} and C_{dry} and 0.736 for EWL), calculated from the data of Withers et al.,²³ Warnecke et al.⁴² and Pusey et al.⁴³

We compared standard T_b, BMR, standard EWL and standard C_{wet} (measured at T_a = 30°C) of our 4 species of antechinus to the 95% prediction limits⁴⁴ for the log-transformed allometric relationships for other marsupials (using the dataset of Warnecke et al.⁴² with additional data from Withers et al.,²³ Withers and Cooper,³⁷ Pusey et al.⁴³ and Tomlinson et al.,⁴⁵ using both conventional least-squares

regression and phylogenetically-informed regression, after rendering the data independent of phylogeny using autoregression^{46,47} and the phylogenetic trees of Bininda-Emonds et al.⁴⁸ and Westerman et al.⁴⁹

Results

For all species, VO_2 was lowest at $T_a = 30^\circ\text{C}$ and so we interpret this as BMR and other variables measured at $T_a = 30^\circ\text{C}$ as standard variables, to be used for interspecific comparative analyses. Body mass for standard measurements at $T_a = 30^\circ\text{C}$ did not differ between habitats (nested ANOVA, $F_{1,28} = 0.83$, $P = 0.458$) although there was a highly significant effect for body mass of species-nested-in-habitat ($F_{2,28} = 7.35$, $P = 0.003$), reflecting the clear species differences i.e. higher mass of yellow-footed (25.5 ± 1.7 g) and in particular dusky antechinus (52.7 ± 4.8 g), compared with the smaller brown (21.9 ± 1.2 g) and agile (17.4 ± 5.8 g) antechinus. Nested analyses of allometrically-corrected standard physiological variables (measured at thermoneutrality; Fig. 1) indicated that there were

no significant effects of habitat for any of the variables ($F_{1,2} \leq 9.63$, $P \geq 0.090$), although there was a species-nested-in-habitat effect for T_b ($F_{2,28} = 4.01$, $P = 0.003$).

Physiological data at all T_a , compared using GLMM, also did not reveal any significant habitat effects ($F_{1,110-132} = 0.361-3.27$; $P > 0.073$). Overall, body mass did not differ for the various T_a measurements ($t_{108-115} < 0.73$, $P > 0.462$), although individually there were significant body mass differences at some T_a for all except the yellow-footed antechinus (Table 1). Individual-nested-in-species ($\chi^2_1 = 286$, $P < 0.001$) was a highly significant determinant of body mass overall, mostly due to species (variance = 187) rather than individual-nested-in-species (variance = 64.4), as was the case for all of the individual species (Table 1).

Despite uniformly insignificant habitat effects, the overall model of T_a and habitat revealed strong T_a effects on T_b , VO_2 , VCO_2 , EWL, C_{wet} , C_{dry} and RWE ($F_{2,104-130} = 17.9-253$, $P < 0.001$; Figs 2–6), consistent with analyses for individual species (Table 1). Overall,

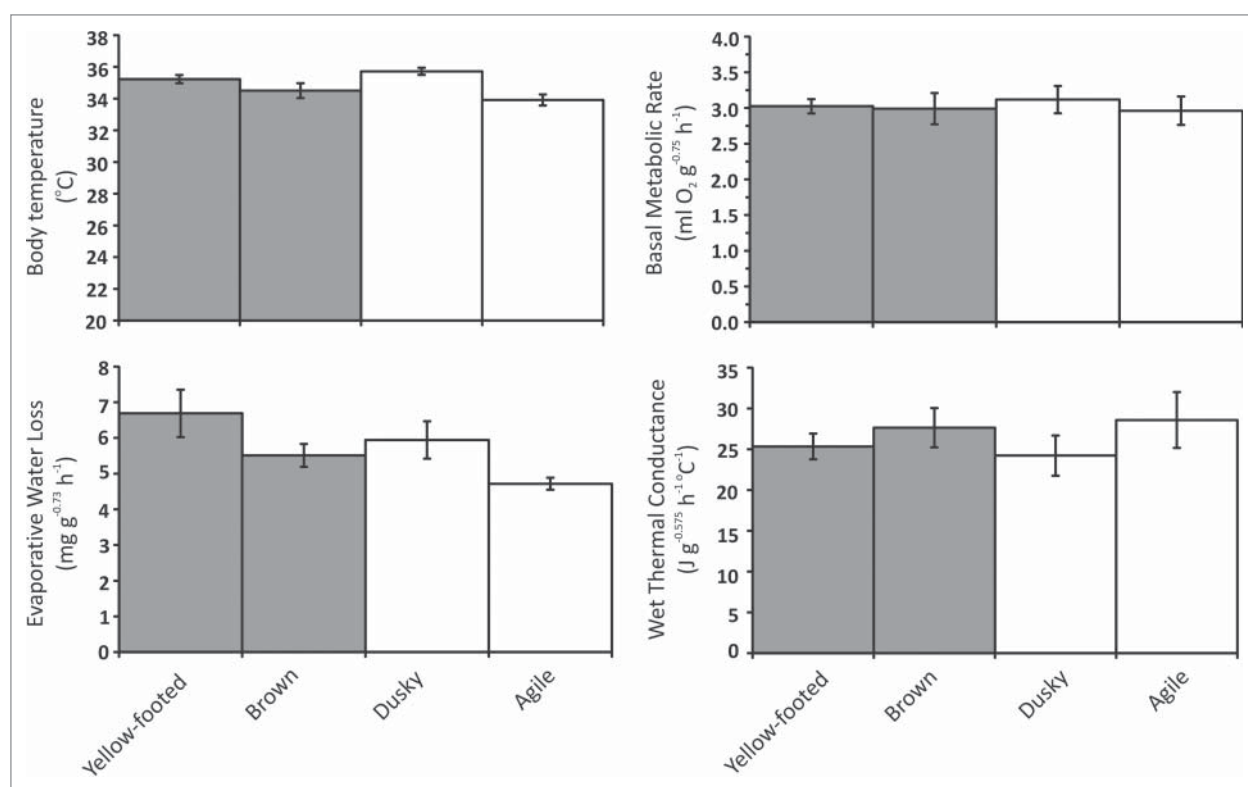


Figure 1. Comparison of standard body temperature, basal metabolic rate, standard evaporative water loss and standard wet thermal conductance for 2 low altitude antechinus (yellow-footed, *A. flavipes* and brown, *A. stuartii*; gray bars) and 2 alpine antechinus (dusky, *A. swainsonii* and agile, *A. agilis*; white bars) at thermoneutrality ($T_a = 30^\circ\text{C}$). Values are mean \pm SE, allometrically corrected by the scaling exponent for each variable (except T_b ; see text).

Table 1. Statistical summary for effects of T_a (fixed factor), and sex and individual-nested-within-sex (random factors), for 2 low elevation antechinus (yellow-footed, *A. flavipes*; brown, *A. stuartii*) and 2 alpine antechinus (dusky, *A. swainsonii*; agile, *A. agilis*), including the mean square (MS) for T_a (the model variance), the F, degrees of freedom and P for the T_a effect, T_a s that are significantly different ($P < 0.050$) from 30°C, variance for the random factors of sex and individual-nested-within-sex, and P for the random factors.

	Fixed factor T_a					Random Factors			
	MS	F	df model, residual	P	T_a s different to 30°C at $P < 0.050$	Sex variance	Animal:Sex variance	χ^2_1	P
Yellow-footed antechinus									
Mass	1.09	1.39	5,40	0.250	–	10.6	2.64	96.9	< 0.001
T_b	14.4	3.21	5,48	0.014	10	0	0	0	0.999
VO_2	6.71	50.6	5,40	< 0.001	10, 15, 20	0.025	0.014	6.36	0.012
VCO_2	15.0	33.4	5,40	< 0.001	10,15,20	0.004	0.010	2.37	0.124
EWL	7.83	9.05	5,40	< 0.001	25,35	0.085	0.096	3.78	0.052
C_{wet}	306	20.7	5,40	< 0.001	35	0	0.164	0.012	0.913
C_{dry}	75.4	22.9	5,40	< 0.001	35	0.305	0.196	3.09	0.079
RWE	0.365	22.3	5,40	< 0.001	10,15,20,25	0.001	0.007	8.18	0.004
Brown antechinus									
Mass	25.6	10.9	4,33	< 0.001	20	6.35	0	23.8	< 0.001
T_b	19.5	23.9	3,15	< 0.001	10,35	0	0.304	0.974	0.324
VO_2	8.97	46.9	4,26	< 0.001	10,20,25	0	0.039	1.04	0.308
VCO_2	3.86	12.5	4,28	< 0.001	10,20	0	0.068	1.44	0.230
EWL	4.32	7.32	4,35	< 0.001	35	0	0	0	0.999
C_{wet}	199.1	51.3	3,25	< 0.001	25,35	3.56	0	6.68	0.010
C_{dry}	81.1	36.9	3,25	< 0.001	35	2.12	0	7	0.008
RWE	0.978	59.6	4,35	< 0.001	10,20,25	0	0	0	0.999
Dusky antechinus									
Mass	70.8	2.83	5,34	0.031	15,35	153.4	71.6	60.2	< 0.001
T_b	3.93	4.85	5,34	0.002	35	0	0.144	6.11	0.013
VO_2	4.09	33.6	5,33	< 0.001	10,15,20,35	0	0.056	4.57	0.033
VCO_2	1.21	49.0	5,23	< 0.001	10,15,20,25,35	0	0.023	6.11	0.013
EWL	10.2	5.00	5,38	0.001	35	0.188	0	2.17	0.141
C_{wet}	188.4	70.9	5,33	< 0.001	10,35	0	0.311	0.596	0.440
C_{dry}	74.6	18.9	5,33	< 0.001	35	0	0.475	1.82	0.177
RWE	0.805	36.6	5,33	< 0.001	10,15,20,25	0.005	0.006	7	0.008
Agile antechinus									
Mass	3.62	14.3	1,6	0.009	12	4.81	2.36	18.2	< 0.001
T_b	0.176	0.089	1,7	0.773	—	0	0.556	0.252	0.616
VO_2	24.7	619	1,6	< 0.001	12	0	0.043	1.28	0.258
VCO_2	7.04	63.8	1,7	< 0.001	12	0	0.037	0.449	0.503
EWL	0.204	0.333	1,9	0.577	—	0	0.119	0.201	0.654
C_{wet}	46.4	17.3	1,5	0.006	12	0	3454	25.7	< 0.001
C_{dry}	23.6	11.5	1,5	0.015	12	0	1883	24	< 0.001
RWE	2.51	29.8	1,9	< 0.001	12	0	0.010	0.083	0.774

the random factor of individual-nested-within-species was highly significant for all physiological variables ($\chi^2_1 \geq 14.7$, $P < 0.001$), but the relative variance explained by species compared with individual-nested-within-species varied for the different variables; it was greater for species for T_b and C_{wet} and similar for VO_2 , while individual-nested-within-species had a greater variance than species for VCO_2 , EWL and RWE. For individual species, sex and individual effects were only apparent for metabolic rate and RWE (yellow-footed and dusky antechinus) or thermal conductance (brown and agile antechinus), and were driven by higher variance for sex compared with individual-nested-within-sex for all except the dusky antechinus (Table 1).

There were no significant T_a by habitat interactions for VO_2 , VCO_2 , EWL, C_{wet} or C_{dry} ($F_{2,104-135} = 0.16-2.40$, P

> 0.094) but there were for T_b ($F_{2,105} = 3.50$, $P = 0.034$) and RWE ($F_{2,106} = 3.73$, $P = 0.027$). The PRWE (calculated from $T_a = 10$ to 30°C) was estimated to be 15.5°C (brown antechinus), 13.7°C (agile antechinus) and 16.8°C (dusky antechinus), but for the yellow-footed antechinus the PRWE (estimated from $T_a = 15$ to 30°C) was only 6.5°C, but note that the low RWE at the lowest T_a suggests that the yellow-footed antechinus might never reach a PRWE (Fig. 6). There were no significant differences by ANCOVA ($F_{3,98} = 3.9$, $P = 0.336$) for the thermolability of the species (calculated as the slope of T_b against T_a from $T_a = 10-30$ °C); the non-alpine antechinus had thermolabilities of 0.097 ± 0.054 °C °C⁻¹ (yellow-footed antechinus) to 0.119 ± 0.039 °C °C⁻¹ (brown antechinus) and the alpine species 0.014 ± 0.029 °C °C⁻¹ (dusky antechinus) and 0.019 ± 0.052 °C °C⁻¹ (agile antechinus).

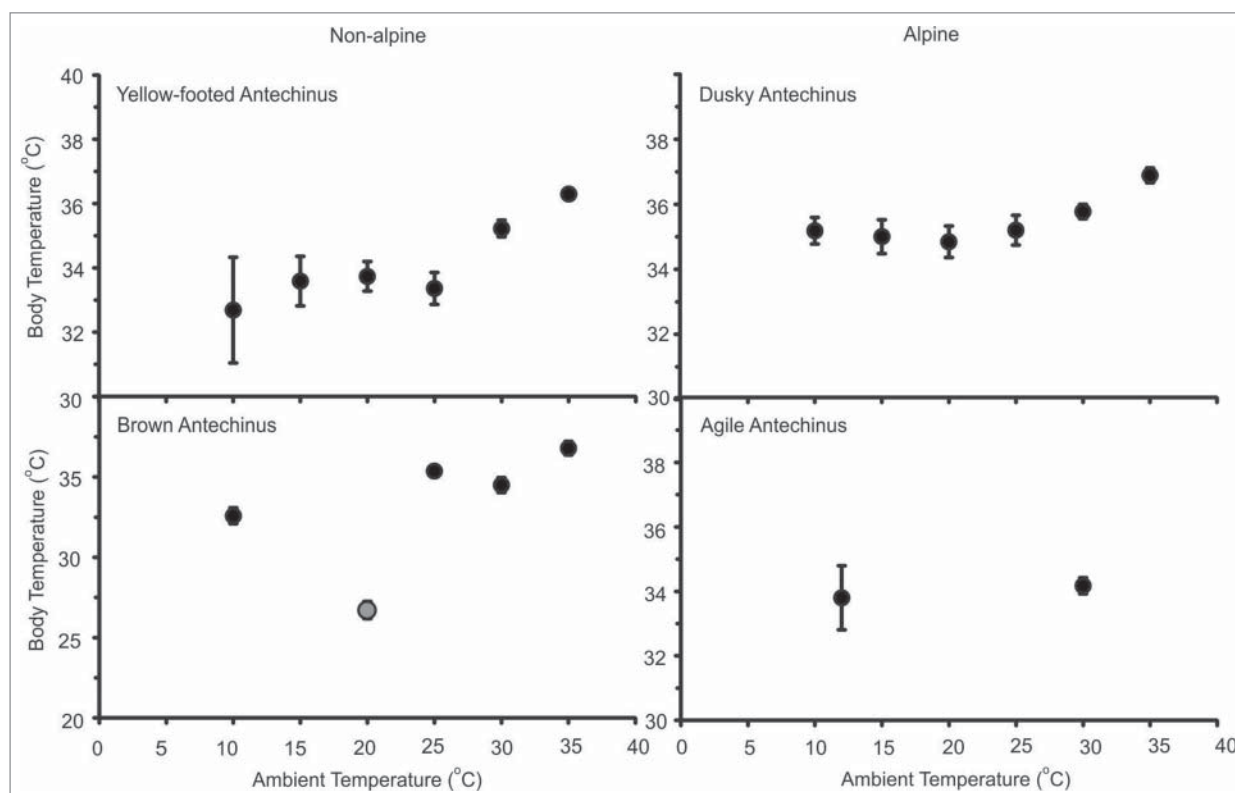


Figure 2. Body temperature for antechinus species from non-alpine (yellow-footed, *A. flavipes* and brown, *A. stuartii*) and alpine habitats (dusky, *A. swainsonii* and agile, *A. agilis*) at a range of ambient temperatures. The body temperature of the brown antechinus at an ambient temperature of 20°C is shown in gray as these individuals were torpid, so these values are not included in the analyses. Values are mean \pm SE.

Standard physiological variables (standard T_b , BMR, standard EWL and standard C_{wet}) of all 4 antechinus species conformed to those of other marsupials. Values for these variables for each species fell well inside the 95% prediction limits⁴⁴ for the marsupial allometric regressions, both before and after accounting for phylogenetic history (Fig. 7).

Discussion

We investigated possible differences between alpine and non-alpine *Antechinus* species in view of McNab's^{3,4,14} speculation that small alpine marsupials might experience thermal and energetic limitations at high altitude because of their inherently lower metabolic rates compared with equivalent-mass placental mammals. By comparing the thermal, metabolic and hygric physiology of 2 species from alpine habitats (dusky and agile antechinus) and 2 from non-alpine habitats (yellow-footed and brown antechinus), we found no evidence that alpine antechinus, active year-round in environments characterized in winter by

sub-zero temperatures and considerable snow cover, have any particular specializations of their basic physiology compared with other non-alpine antechinus, or with marsupials in general.

Sex and individual effects

Overall, there were some significant sex and individual effects on the variables we examined. Body mass of these antechinus differed with regard to species, sex and individual. Dasyurid marsupials are, in general, sexually dimorphic with respect to body mass,⁵⁰ and antechinus are no exception,^{32,51} so sex and individual differences were not unexpected. Sex differences were also apparent for a few physiological variables although for some other variables individual-nested-in-sex had a higher variance. Consistent individual differences in physiological variables have been reported for some mammals,⁵²⁻⁵⁴ although not for several species of small dasyurid (e.g., sandhill dunnart, *Sminthopsis psammophila*⁵⁵; dibbler, *Parantechinus apicalis*³⁷). There is currently no clear overall pattern

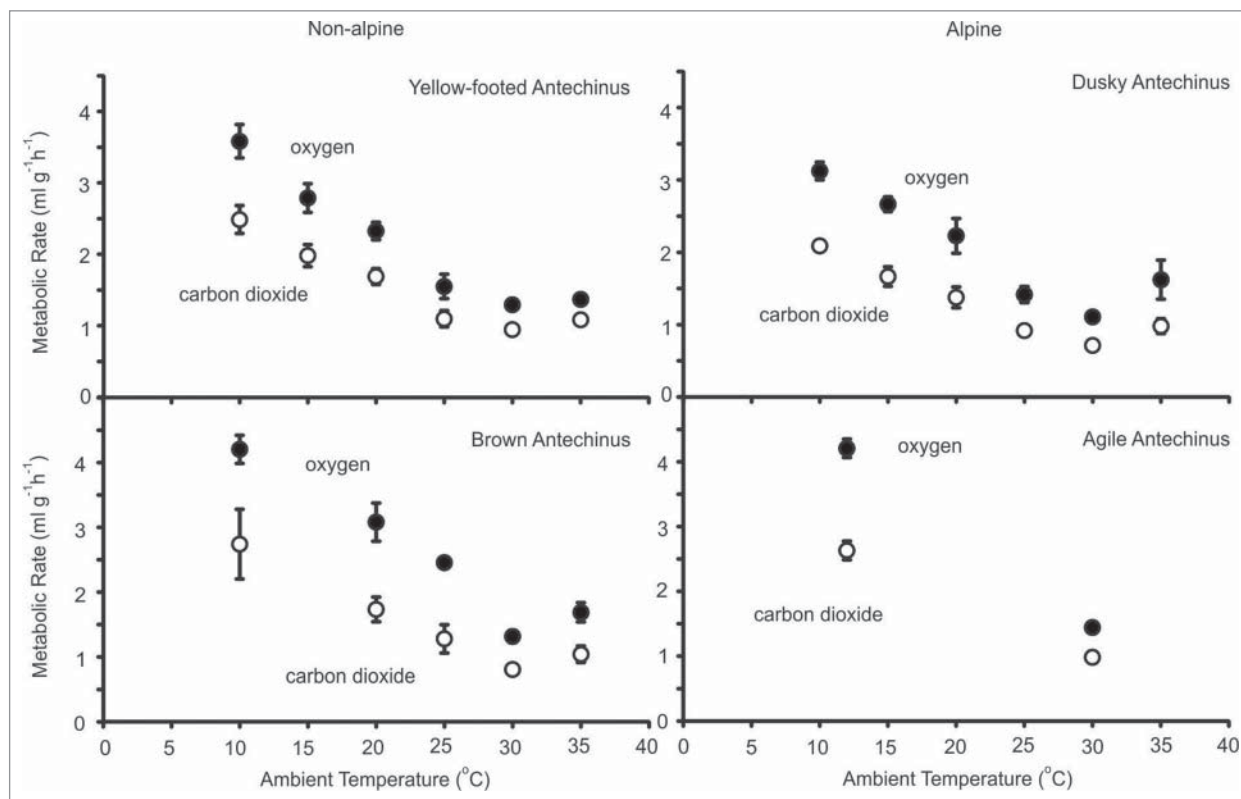


Figure 3. Metabolic rate, measured as oxygen consumption (black) and carbon dioxide production (white), for antechinus species from non-alpine (yellow-footed, *A. flavipes* and brown, *A. stuartii*) and alpine habitats (dusky, *A. swainsonii* and agile, *A. agilis*) at a range of ambient temperatures. Values are mean \pm SE.

as to the characteristics of species that do have repeatable individual differences, the specific physiological traits that differ between individuals, and potential correlating factors such as personality.^{56,57}

Body mass effects

Body mass of *Antechinus* species did differ substantially. The largest species, the dusky antechinus, was one of the alpine species. A higher body mass might be expected for an alpine mammal, just as for a high latitude mammal, in accord with Bergmann's Rule, which is generally interpreted as "mammals are bigger in colder climates," because their lower surface-to-volume ratio would reduce relative heat loss.^{14,58-61} However, Bergmann's Rule does not apply to all species or populations of mammal,⁶² and whether it is relative or absolute heat loss that is under selection pressure is arguable.⁶³ In contrast, alpine agile antechinus were not larger than the closely-related non-alpine brown antechinus, presumably reflecting their relatively recent divergence (about 6.4 MYBP⁴⁹) compared to the other antechinus species, so a mass increase is not

a requirement for marsupial survival in an alpine habitat. Analysis of body mass of marsupials accounting for their phylogenetic relationships, using autoregression, indicates that body mass was essentially as expected for the yellow-footed antechinus (phylogenetically-independent residual mass of 0.84 g), brown antechinus (0.77 g) and agile antechinus (0.62 g), but the dusky antechinus was about 18.2 g heavier than expected. Regardless of whether we interpret the high body mass of the dusky antechinus as an adaptation to an alpine environment or not, overall our study does not provide evidence that an increased body mass is characteristic of alpine antechinus.

Standard physiology of antechinus

Comparable, standardised thermal, metabolic and hygric data for antechinus are scant. Dawson and Hulbert¹ measured a T_b of 34.3°C and VO_2 of 1.00 ml O_2 g^{-1} h^{-1} for brown antechinus; this T_b is similar to that measured here (34.5°C), but the VO_2 is lower than our value of 1.3 ± 0.09 ml O_2 g^{-1} h^{-1} , likely reflecting the considerably greater body mass of brown

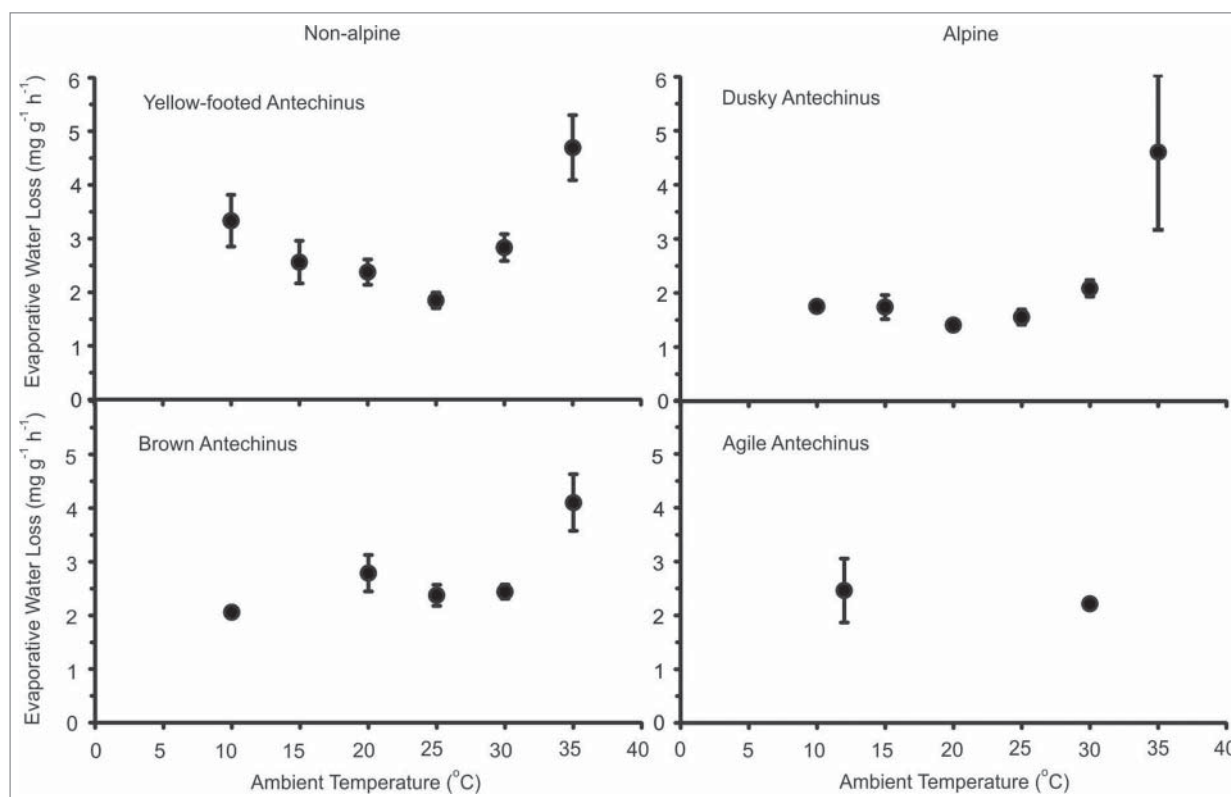


Figure 4. Evaporative water loss for antechinus species from non-alpine (yellow-footed, *A. flavipes* and brown, *A. stuartii*) and alpine habitats (dusky, *A. swainsonii* and agile, *A. agilis*) at a range of ambient temperatures. Values are mean \pm SE.

antechinus in their study (36.5 g vs 21.9 g). Wallis³¹ measured a BMR of $1.3 \text{ ml g}^{-1} \text{ h}^{-1}$ and a T_b of 35.4°C for agile antechinus (considered to be *A. stuartii* at time of measurement), similar to previous measurements for the closely-related brown antechinus and our value of $1.4 \text{ ml O}_2 \text{ g}^{-1} \text{ h}^{-1}$ for the species, despite their T_b being somewhat higher (this study 33.9°C). Chappell and Dawson⁶⁴ measured a T_b of 36.0°C and VO_2 of $0.95 \text{ ml O}_2 \text{ g}^{-1} \text{ h}^{-1}$ at $T_a = 30^\circ\text{C}$ for dusky antechinus; this is similar to our value of 35.7°C , and $1.11 \text{ ml O}_2 \text{ g}^{-1} \text{ h}^{-1}$ for somewhat smaller individuals (66.9 g vs 52.7g). Hinds and MacMillen⁶⁵ measured an EWL of $2.69 \text{ mg H}_2\text{O g}^{-1} \text{ h}^{-1}$ for 21.3 g brown antechinus at T_a of 32°C , which is slightly higher than our value of 2.4 at 30°C , and probably reflects differences in methodology that can impact on EWL measurement e.g. shorter measurement duration.^{66,67}

Habitat-related differences were not apparent among the *Antechinus* species, for any of the standard physiological variables measured at thermoneutrality. However, there were species differences for T_b . For T_b , the species-nested-in-habitat effect reflected T_b being higher for dusky (35.7°C) and yellow-footed (35.2°C) antechinus and lower for brown (34.5°C) and agile

(33.9°C) antechinus, so this seems to be a phylogenetically-related interaction, with the most closely related species being the most similar.

Ambient temperature effects

Despite a lack of a habitat effect on standard physiological variables measured at thermoneutrality for antechinus, it is possible that physiological differences between alpine and non-alpine antechinus become apparent at more extreme temperatures, outside of the thermoneutral zone. There were some significant differences between species and with different T_a but, as for standard physiological variables, there were no consistent effects of habitat for any of the physiological variables when analyzed over all the T_a s we examined. This further suggests that there are no thermal, metabolic or hygric adaptations for the small marsupials measured here that inhabit cold alpine environments compared with species from non-alpine environments. Clearly the physiological capabilities of these small marsupials are sufficient for survival in alpine environments. In particular, the close phylogenetic relationship between the agile antechinus and

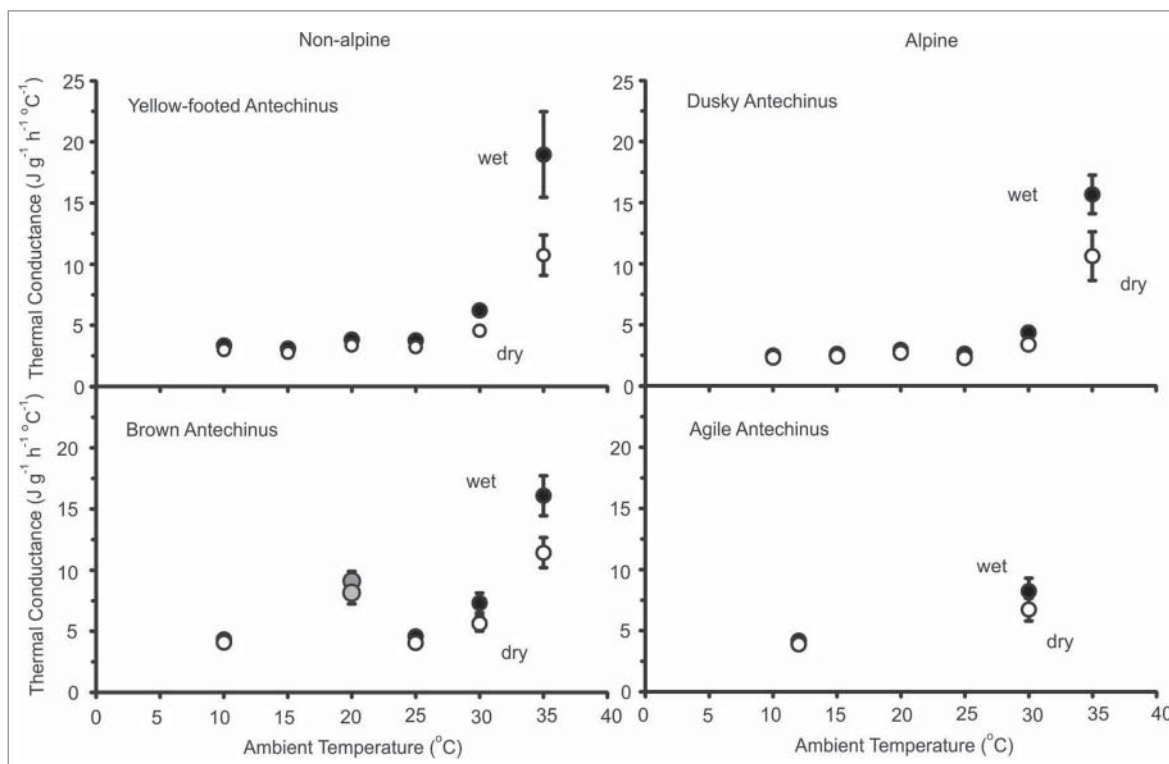


Figure 5. Wet (black) and dry (white) thermal conductance for antechinus species from non-alpine (yellow-footed, *A. flavipes* and brown, *A. stuartii*) and alpine habitats (dusky, *A. swainsonii* and agile, *A. agilis*) at a range of ambient temperatures. The thermal conductance of the brown antechinus at an ambient temperature of 20°C is shown in gray as these individuals were torpid, so these values are not included in the analyses. Values are mean \pm SE.

the brown^{49,68} provides strong evidence that no particular physiological specializations are required by alpine species to cope with the colder conditions. The only habitat-related patterns in physiological variables for the 4 *Antechinus* species were interactions between habitat and T_a for T_b and RWE.

The physiology of all 4 antechinus species conformed to the general pattern expected for endothermic mammals (and birds^{14,36,69}) with changing T_a . They all have a relatively constant T_b over a range of T_a (but with mild thermolability in the cold; see below), reflecting metabolic thermogenesis at low T_a , increased heat loss via EWL, C_{wet} and C_{dry} changes at high T_a , and RWE decreasing with higher T_a .

Although there was no statistically significant species difference by ANCOVA for thermolability, the slightly higher thermolability of non-alpine antechinus compared to the alpine species was apparently sufficient to drive the significant interaction between T_a and habitat for T_b . The thermolability of the alpine species was less than the mean thermolability of other dasyurids, of 0.064°C °C⁻¹ (ref. ⁷⁰), but thermolability of the non-alpine species was higher than the mean. While this could

be interpreted as better T_b control by species routinely exposed to low T_a , a lack of habitat differences for metabolic rate or thermal conductance suggest this is unlikely. Rather, we suggest that the significant T_a and habitat interaction for T_b may reflect environmental resource availability, particularly the predictability of this availability. For arid-habitat dasyurids, thermolability has been interpreted as a mechanism to reduce energy expenditure in an environment with limited and unpredictable food. Munn et al.⁷¹ reported that the dunnart *Sminthopsis crassicaudata* used more frequent, longer torpor bouts in response to unpredictable food availability, rather than food availability *per se*, suggesting it is predictability of resources, rather than their absolute availability, that imposes the greatest energetic constraints. Although overall abundance of food is lower for alpine antechinus in winter than for non-alpine antechinus,⁷² the relatively predictable nature of highly seasonal winter conditions means that this decline can be anticipated. Therefore, patterns of T_b thermolability with habitat may well reflect the seasonality of these habitats.

There was also a significant T_a -habitat interaction for RWE, probably reflecting the low PRWE of yellow-

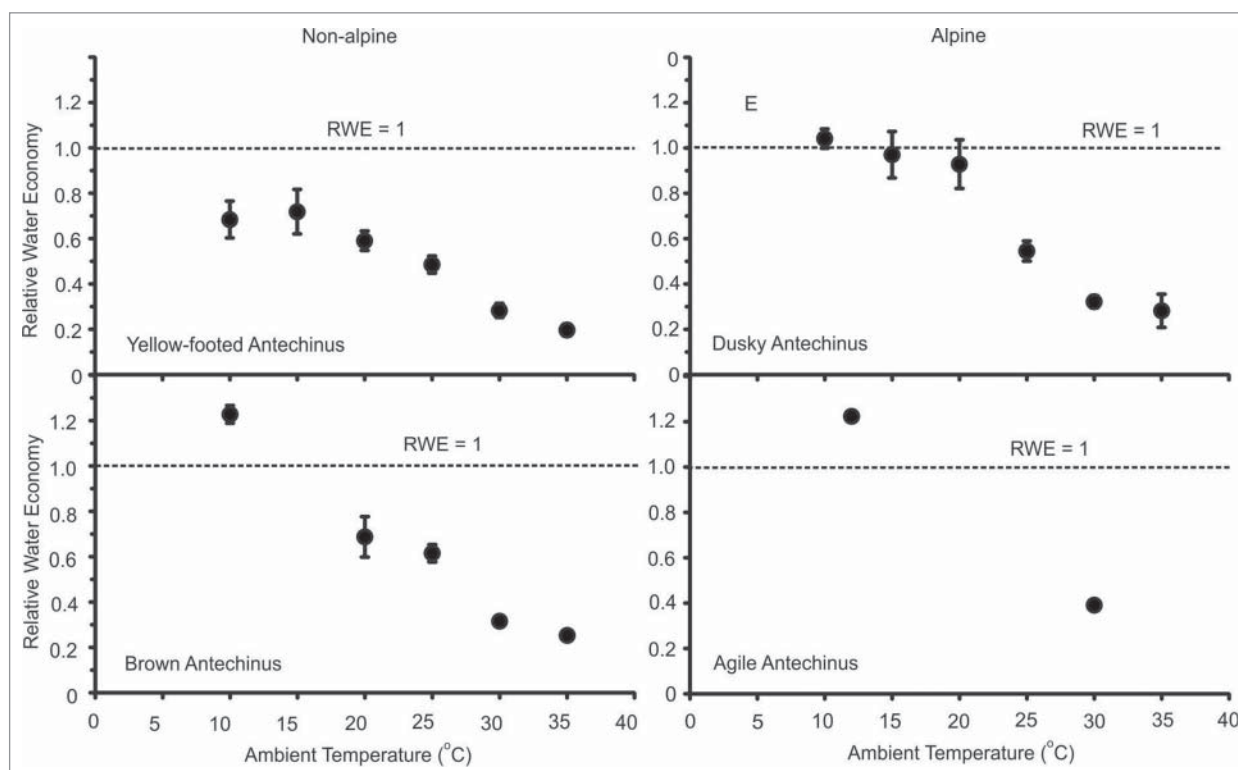


Figure 6. Relative water economy for antechinus species from non-alpine (yellow-footed, *A. flavipes* and brown, *A. stuartii*) and alpine habitats (dusky, *A. swainsonii* and agile, *A. agilis*) at a range of ambient temperatures. A dashed line indicates a relative water economy of 1, where metabolic water production equals evaporative water loss. Values are mean \pm SE.

footed antechinus compared to the other species. The PRWE is allometrically related to body mass in marsupials; $PRWE = 30.0 - 11.2 \log M^{13}$. This relationship predicts a PRWE of 14.0°C (compared to measured PRWE of 6.5°C) for yellow-footed, 15.1°C (cf 15.5°C) for brown, 10.3°C (cf 13.7°C) for dusky and 15.9°C (cf 16.8°C) for agile antechinus. There is no apparent impact on PRWE of an alpine habitat. Why the yellow-footed antechinus, being the least mesic of these species, has the poorest water economy is unclear, as RWE and particularly PRWE, is interpreted as an index of habitat aridity and water availability.^{65,73} However, the arid-zone striped-faced dunnart (*Sminthopsis macroura*) also has a poor RWE, and does not attain a PRWE,⁷⁴ so a favorable PRWE is not always associated with habitat water availability. The pattern for RWE and T_a tends to reflect patterns of EWL with T_a , which vary between species and are most different for yellow-footed antechinus.

The pattern of EWL with T_a below thermoneutrality was variable for the 4 species, as has been observed for other marsupials.^{25,55,70,74} For yellow-footed antechinus, there was a significant increase in EWL at low T_a (slope = $-0.090 \pm 0.030 \text{ mg H}_2\text{O g}^{-1} \text{ h}^{-1} \text{ }^{\circ}\text{C}^{-1}$),

but for the other 3 species, the EWL- T_a slope was not significantly different from 0 (brown antechinus, $0.033 \pm 0.035 \text{ mg H}_2\text{O g}^{-1} \text{ h}^{-1} \text{ }^{\circ}\text{C}^{-1}$; dusky antechinus, $-0.018 \pm 0.013 \text{ mg H}_2\text{O g}^{-1} \text{ h}^{-1} \text{ }^{\circ}\text{C}^{-1}$; agile antechinus, $-0.018 \pm 0.013 \text{ mg H}_2\text{O g}^{-1} \text{ h}^{-1} \text{ }^{\circ}\text{C}^{-1}$; Fig. 4). These differences presumably reflect different partitioning of total EWL into respiratory and cutaneous components. For example, increasing total EWL at low T_a for the yellow-footed antechinus may reflect a predominance of respiratory EWL, which increases in response to increased respiratory demand for gas exchange at lower T_a , possibly due to a reduced requirement for nasal counter-current heat conservation (and hence reduced water savings) in their warmer environment.

For the 3 species measured at $T_a > 30^{\circ}\text{C}$, EWL increased substantially, increasing EHL and facilitating thermoregulation when T_b approached T_a ; this is reflected by the increase in C_{wet} at 35°C . EHL ranged from 24 % (brown antechinus) to 28 % (yellow-footed antechinus) of MHP at $T_a = 30^{\circ}\text{C}$, increasing to 30% (brown antechinus) to 42% (yellow-footed antechinus) of MHP at $T_a = 35^{\circ}\text{C}$. These values are at the lower end of the range of 27.5% (dibbler, *Parantechinus apicalis*)³⁷

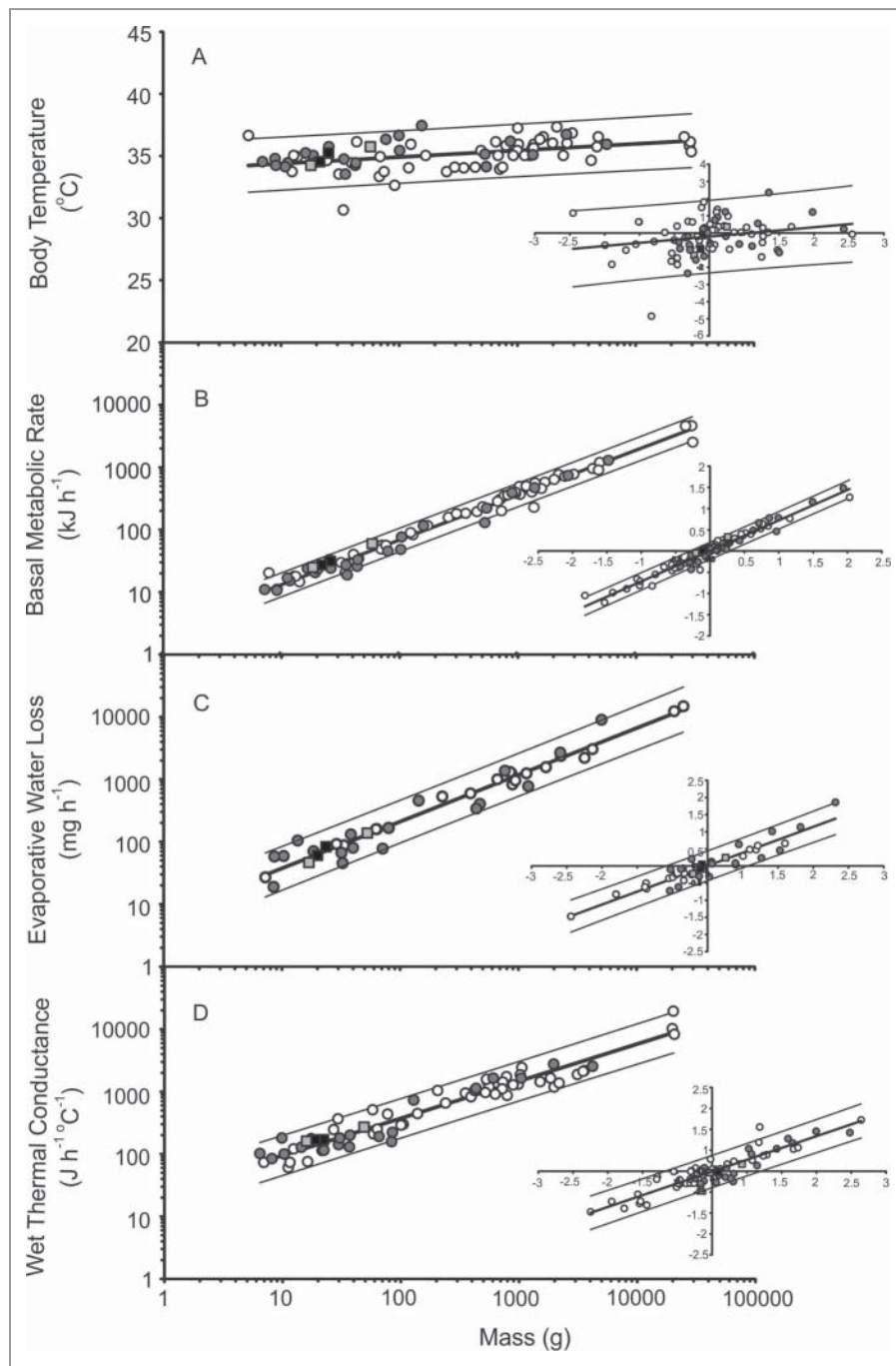


Figure 7. Comparison of body temperature, basal metabolic rate, evaporative water loss, and wet thermal conductance of 4 antechinus species with other marsupials, demonstrating that antechinus conform to allometric expectations based on data for other marsupials. Dark squares are yellow-footed, *A. flavipes*, and brown, *A. stuartii*, antechinus (non-alpine), gray squares are agile, *A. agilis*, and dusky, *A. swainsonii*, antechinus (alpine species), gray circles are dasyurid marsupials, and white circles are other marsupials. The regression lines (thick) are shown with $\pm 95\%$ prediction limits (thin). Insets are the allometric relationships for phylogenetically independent residuals; symbols are as for the conventional regressions. Marsupial data after Warnecke et al.⁴³ with additional data from Withers and Cooper³⁷ Tomlinson et al.⁴⁶, Withers et al.²³ Pusey et al.⁴⁴

to 68% (little red kaluta, *Dasykaluta rosamondae*⁷⁰) at $T_a > 30^\circ\text{C}$ for other dasyurid marsupials, presumably reflecting the generally mesic habitats of these antechinus species and therefore reduced capacity for EHL at high T_a .

Phylogenetic perspective of antechinus physiology

Before we can conclude that our data for antechinus provide evidence that the typical marsupial physiology does not prevent small marsupials from remaining active

year-round in alpine habitats, we must determine how typical the physiology of antechinus is of marsupials in general. Are antechinus somehow pre-adapted (e.g., have a high BMR) for survival in alpine environments? We therefore compared standard T_b , BMR, standard EWL and standard C_{wet} of our 4 species of antechinus to the allometric relationships for other marsupials. In comparison to other marsupials, our 4 species of antechinus have a typical physiology (Fig. 7); there is no statistical evidence that they differ significantly from allometric and phylogenetic predictions. We therefore conclude that the physiology of alpine antechinus, and antechinus in general, is typical of marsupials, and therefore marsupials do have a general physiology adequate for life in alpine environments, without any particular thermal, metabolic or hygric adaptations.

Abbreviations

ad lib.	ad libitum
ANCOVA	analysis of covariance
ANOVA	analysis of variance
BMR	basal metabolic rate
C_{dry}	dry thermal conductance
CO_2	carbon dioxide
C_{wet}	wet thermal conductance
EHL	evaporative heat loss
EWL	evaporative water loss
GLMM	generalized linear mixed model
H_2O	water
M	mass
MHP	metabolic heat production
MR	metabolic rate
MYBP	millions of years before present
N	number of individuals
n	number of measurements
O_2	oxygen
PRWE	point of relative water economy
RER	respiratory exchange ratio
RH	relative humidity
RWE	relative water economy
SE	standard error
STPD	standard temperature and pressure, dry
T_a	air temperature
T_b	body temperature
VCO_2	carbon dioxide production rate
VO_2	oxygen consumption rate

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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