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Reexamining Echidna Physiology: The Big Picture for *Tachyglossus aculeatus acanthion*

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ABSTRACT

The early divergence of monotremes and therian mammals has resulted in considerable interest in the comparative physiology of the short-beaked echidna (*Tachyglossus aculeatus*), the most common and widespread living monotreme. However, there are many and varied interpretations of its physiology, reflecting the many and varied studies, limitations and uncertainties of aspects of some previous studies, and potential differences between the various subspecies. Consequently, we thoroughly examine here the standardized physiology of the most widely distributed subspecies of short-beaked echidna (*T. aculeatus acanthion*) over a wide range of ambient temperatures to definitively assess its physiology in a comparative context. We conclude that the low and variable body temperature of the short-beaked echidna is physiologically "primitive," but it also reflects adaptation to its myrmecophagous niche. Other aspects of its physiology are more typically mammalian. A low metabolic rate reflects its low body temperature, and ventilatory variables are matched to accommodate a modest gas exchange requirement. Thermal conductance is typical for a mammal of equivalent mass. In contrast to previous studies, we demonstrate that short-beaked echidnas can enhance evaporative water loss above thermoneutrality, like other mammals, with a similar capacity for evaporative heat loss. Cooling of their nasal blood sinus with nasal mucous may contribute to this enhanced evaporative cooling. Their capacity to evaporatively cool explains how their distribution can include habitats where ambient temperature, even in shelters, exceeds their supposed critical thermal limit.

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Introduction

The lineages of modern monotremes and modern therian mammals diverged about 166 million years ago (Bininda-Emonds et al. 2007), and in their anatomy and reproductive biology, monotremes show many characteristics of basal mammals not shared with therians (e.g., egg laying). Due to this, monotremes have been of great interest to comparative physiologists hoping to gain insights into the evolution of mammalian physiology. There are five extant monotreme species (Strahan and Conder 2007; Nicol 2015): the platypus (*Ornithorhynchus anatinus*), the short-beaked echidna (*Tachyglossus aculeatus*), and three species of long-beaked echidna (*Zaglossus attenboroughi*, *Zaglossus bartoni*, and *Zaglossus bruijni*). The ubiquitous distribution of the short-beaked echidna throughout Australia and its accessibility for scientific research have made this species a focus for studies of monotreme physiology. The many and varied physiological studies of the short-beaked echidna, conducted over a long period of time using a variety of methodologies, have resulted in a confusing array of interpretations and conclusions concerning the primitiveness or otherwise of its physiology (Brice 2009). Added to this confusion is the recognition of five subspecies of short-beaked echidna, distinguished by geographical distribution and anatomical characteristics, which appear to also vary physiologically (Augee 1978; Nicol 2015). We aim here to provide the only complete analysis to date of the standard thermal, metabolic, ventilatory, and hygric physiology of a short-beaked echidna over a wide range of ambient temperatures (T_a; 10°–32.5°C), by measuring one subspecies, *Tachyglossus aculeatus acanthion*, sourced from a single population. *Tachyglossus a. acanthion* is the most widely distributed subspecies, occurring throughout much of the Australian arid zone, whereas most previous physiological studies of echidnas have been for various mesic-zone subspecies.

Short-beaked echidnas have a low body temperature (T_b) and basal metabolic rate (BMR) at thermoneutrality, but the extent of their thermoregulatory capability over a wide range of T_a, and how this reflects their evolutionary history and prototherian phylogenetic position, remains unclear (Brice 2009). Early physiological studies (de Miklouho Maclay 1883; Sutherland 1897) suggested that short-beaked echidnas are physiologically "primitive" due to their low and variable T_b, reflecting an intermediate position between the "lower" reptiles and "higher" mammals. Martin (1903) measured the short-beaked echidna

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over a range of T_a and also concluded that they are poor thermoregulators, with a low T_b that fluctuates by up to 10°C from T_a of 5°–35°C, with higher T_a resulting in death. Robinson (1954), McMurchie and Raison (1975), and Augee (1976) also subsequently concluded that short-beaked echidnas are poor homeotherms, unable to maintain a constant T_b over fluctuating T_a. However, Schmidt-Nielsen et al. (1966) concluded that echidnas are generally very effective thermoregulators, especially at low T_a, as did McNab (1984); echidnas maintained T_b within the usual range (30°–31°C) even at T_a as low as 0°C, although T_b increased by several degrees when exposed to high T_a (30°C or above).

Dawson and Hulbert (1970) argued that the pattern of metabolism/endothermy/homeothermy of mammals is a distinct jump from the reptilian pattern, and differences between monotremes, marsupials, and placentals need not reflect phylogenetic patterns. However, Dawson et al. (1979, p. 511) interpreted the low BMR of echidnas (both *Tachyglossus* and *Zaglossus*) and to a lesser extent platypus as "support [of] the idea of a gradual evolution in metabolic capability," as did Grigg et al. (2004), who suggested that the pattern of endothermy of short-beaked echidnas may be a useful model for advanced protoendotherms, representing one stage in the evolution of endothermy. Lovegrove (2012*a*) has argued that the monotremes are basoendotherms, fitting within his scheme of the evolution of endothermy along a plesiomorphic-apomorphic continuum.

Contributing further to the notion that echidnas have a primitive physiology is the fact that they seem unable to enhance evaporative cooling at high T_a. They apparently lack sweat glands (Martin 1903; Schmidt-Nielsen et al. 1966; Augee 1976), and Martin (1903) concluded that they lack vasomotor adjustments (e.g., vasoconstriction, vasodilation). Augee (1976) suggested that (anesthetized) echidnas increase ventilation but do not pant in the heat. In contrast, both long-beaked echidnas and platypus sweat to increase their evaporative heat loss (EHL; Augee 1976; Dawson et al. 1978), suggesting that if short-beaked echidnas lack sweat glands, then this is a derived characteristic. As EHL is the only mechanism available for an animal to lose heat when environmental temperature is greater than T_b, the echidna's inability to enhance EHL would indicate a very limited thermoregulatory capacity and necessitate inactivity and behavioral avoidance during periods of high T_a (Martin 1903; Robinson 1954). However, despite apparent lethal effects of moderately high T_a in the laboratory (35°C; Martin 1903; Augee 1976), the T_a of echidnas' daytime shelters can exceed what has been reported as their lethal T_a, so these echidnas must presumably have some physiological mechanism to tolerate high T_a in the wild (Brice et al. 2002*a*).

Previous studies of short-beaked echidnas have reported a respiratory frequency (f_R) lower than that of marsupials; this f_R did not vary greatly over a range of T_a, except at high T_a, where it increased (Martin 1903; Robinson 1954; Parer and Hodson 1974; Bech et al. 1992; Frappell et al. 1994). Tidal volume (V_T) and minute volume (V_I) values have been variable between studies. Parer and Hodson (1974) found that both were considerably lower for echidnas than other mammals of a similar body size, presumably reflecting their low T_b and BMR. Bech et al. (1992) suggest that an especially low V_T results in a low ventilatory requirement. Bentley et al. (1967), however, reported higher values for V_T and V_I, within the expected range for mammals, and both Bentley et al. (1967) and Frappell et al. (1994) calculated an oxygen extraction (EO_2) and ventilatory requirement comparable to that of other mammals.

Clearly, there are considerable differences in the interpretation of the physiology of the short-beaked echidna, and so we lack a definitive understanding of the "standard" physiology of this species. Currently, it is necessary to piece together an overall picture of standardized echidna thermoregulatory physiology from a variety of disparate studies of different subspecies that potentially differ physiologically (e.g., Augee 1978; Nicol 2015). Furthermore, as McKechnie and Wolf (2004, p. 509) point out, "the validity of the conclusions . . . remains strongly dependent on data quality." Existing studies sometimes have very low sample sizes, limited or unclear experimental design, experimental aims other than measurement of standard variables, measurement of only a few physiological variables, and/or only one or a few T_a, unclear measurement conditions, and, in some cases, methodological issues such as short measurement duration or physical and chemical restraint that violate the requirements for standard physiological measurement (see McNab 1997; Cooper and Withers 2009). We therefore present here a comprehensive assessment of the standardized physiology, over a range of T_a, for the most widely distributed subspecies of short-beaked echidna, to allow robust reevaluation of the various interpretations of echidna physiology in a comparative context. Specifically, we examine how the evolutionary history and ecological niche of the short-beaked echidna impact a suite of standard physiological variables and test the various hypotheses concerning echidna physiology raised in the literature. Are they really physiologically primitive, or do their thermal, metabolic, and hygric physiological capabilities more closely approximate those of other mammals?

Methods

Seven reproductively mature short-beaked echidnas (*Tachyglossus aculeatus acanthion*) were captured by hand at Dryandra Woodland (31°46′S, 117°1′E), 170 km southeast of Perth, Western Australia. The echidnas were housed in a custom-built outdoor enclosure at Curtin University, Bentley (32°0′S, 115°53′E), where they experienced natural weather variation and photoperiod. Echidnas were maintained on a diet consisting of tinned cat food, flaky bran, insectivore powder (Wambaroo), and primate omnivore and leaf-eater powders (Specialised Feeds), blended with water to make a smooth mash. Fresh drinking water was provided ad lib. All the echidnas were housed together in a large (10 m × 9 m) enclosure, except the night preceding experimentation, when one or two echidnas were moved into a small enclosure (approximately 10 m × 1 m) and fasted to ensure that they were postabsorptive. Four of the echidnas had been housed in captivity for several months before experimentation, while three of the echidnas were captured, measured, and released back into the wild within a week.

Experiments were in late summer, with measurements made from approximately 0500 to 1400 hours, which is the echidnas'

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inactive phase, at T_a of 10°, 15°, 20°, 25°, 27.5°, 30°, and 32.5°C, measured in random order. Metabolic rate (MR) was measured as oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$), simultaneously with evaporative water loss (EWL), using open-flow respirometry. Each echidna was weighed and then placed in a 32-L metabolic chamber located inside a constant-temperature room. Air (dried using Drierite) flowed through the chamber at a constant rate of 11.5–12.8 L min^{-1}, regulated by an Aalborg GFC37 mass flow controller. Excurrent air passed over a Vaisala MNP45A thin-film capacitance relative humidity (RH)/T_a probe, with a subsample flowing through a column of Drierite before O_2 and CO_2 were measured with a Sable Systems Foxbox-C. The analog outputs of the RH/T_a probe were interfaced via the analog inputs of the Foxbox to a PC running a custom-written Visual Basic (VB) program (P. Withers) that logged the serial data from the Foxbox every 10 s.

Baseline values of background CO_2, O_2, and H_2O vapor were recorded for at least 30 min before and after each experiment, as well as for 15–20 min at least once during experimentation to account for any O_2 baseline drift due to T_a and pressure changes. $\dot{V}O_2$, $\dot{V}CO_2$, and EWL were calculated after Withers (2001), using a custom-written VB program. Minimal values were calculated for a 20-min period where the echidna was resting and inactive and the values had become minimal and steady. Immediately after removal from the chamber, T_b was measured using an Omega-HH25TC meter with a plastic-tipped thermocouple inserted approximately 6 cm into the cloaca.

The mass flow meters were calibrated using a Gilian Gilibrator 2 (Sensidyne, Honeywell), traceable to a national standard. The oxygen analyzers were two-point calibrated using compressed nitrogen (0% O_2) and dry ambient air (20.95% O_2). A certified gas mix (0.53% CO_2; BOCS, Perth, Western Australia) and compressed nitrogen (0% CO_2) were used to calibrate the CO_2 analyzers. The calibration of the RH probes (achieved by bubbling incurrent air through water at various temperatures and then warming it to a known T_a, hence RH) was routinely confirmed using 1% RH air (dried using Drierite to approximately 0.005 mg L^{-1}) and 100% RH air (saturated by breathing on the probe).

Ventilatory data were measured at each temperature using whole-body plethysmography (Malan 1973; Withers 1977; Cooper and Withers 2004*b*), simultaneous with MR and EWL. A custom-built MPX2010 transducer detected pressure changes due to warming and humidifying of inspired air. A Pico ADC11 A/D converter monitored the voltage output from the pressure transducer, which was recorded by a computer every 10 ms for the duration of the experiment (starting approximately 2 h after placing the animal in the chamber), using Pico Logger software. Ventilatory variables (f_R, breaths min^{-1}; V_T, mL; V_I, mL min^{-1}; and EO_2, %) were calculated after Malan (1973) and Cooper and Withers (2004*b*), using the average of several minutes where the animal was breathing most steadily and consistently and MR was minimal. The open-system plethysmography traces were mathematically converted to a closed system to account for the time course of breaths and calibrated by injecting 3 mL of air into the chamber with a syringe, after Szewczak and Powell (2003). Ventilatory variables were calculated using a custom-written VB program (P. Withers).

Respiratory exchange ratio (RER) was calculated as $\dot{V}CO_2/\dot{V}O_2$ and was used to determine the conversion of O_2 and CO_2 to joules after Withers (1992). Wet thermal conductance (C_{wet}; J g^{-1} h^{-1} °C^{-1}) was calculated as MR (joules)/($T_b - T_a$); C_{dry} was calculated as [MR (joules) − EHL (joules)]/($T_b - T_a$). EHL (J g^{-1} h^{-1}) was calculated from EWL assuming 2.4 kJ g^{-1} (McNab 2002). Metabolic water production (MWP; J g^{-1} h^{-1}) was calculated after Withers (1992), based on the measured RER. Relative water economy (RWE) was calculated as MWP/EWL, with the point of relative water economy (PRWE) being the T_a at which water balance was achieved (i.e., RWE = 1).

All values are presented as mean ± standard error, with N being the number of individuals and n the number of measurements. As not all individuals were measured at each T_a, linear mixed-effect models were used to examine T_a while accounting for repeated measurements of each individual (Crawley 2007). We used lmer (Bates et al. 2014) and lmerTest (Kuznetsova et al. 2014) libraries in R (R Core Team 2014), with Satterthwaite's approximations for calculation of degrees of freedom. T_a was a fixed factor, individual was a random variable in each model, and T_a = 27.5°C (thermoneutrality) was used as the reference category to examine pairwise fixed effects of T_a.

BMR, T_b, EWL, C_{wet}, and ventilatory variables were compared to values for other mammals using linear regressions of log_{10}-transformed variables (except T_b). Data for comparative analyses were obtained from McNab (2008) for BMR, from White and Seymour (2004) for T_b, from Van Sant et al. (2012) for EWL, and from Bradley and Deavers (1980) for C_{wet}. Ventilatory variables were compared to the combined data sets of Casey et al. (1979) and Cooper et al. (2010), with additional data from Darden (1972), Arieli and Ar (1979), Chappell (1985, 1992), Schlenker (1985), Chappell and Roverud (1990), Cooper and Withers (2010, 2014), Mella et al. (2010, 2016), Warnecke et al. (2010), Withers et al. (2012), Pusey et al. (2013), and Withers and Cooper (2009). Comparative analyses were made using both conventional linear regression and autoregression (Cheverud and Dow 1985; Rohlf 2001) to account for phylogenetic history, based on the mammal supertree of Bininda-Emonds et al. (2007). Autoregression renders a single trait (X or Y) independent of phylogeny by determining the residuals between the original trait and the phylogenetically predicted trait. These phylogenetically independent residuals are calculated by maximizing the correlation between the original trait and the trait premultiplied by a constant (ρ; determined by an iterative procedure, using the maximum-likelihood function, and constrained to the range of the reciprocals of the negative and positive eigenvalues for $\mathbf{W}$ closest to 0) and a matrix representing the phylogenetic structure ($\mathbf{W}$; determined from a distance matrix constructed from the mammal supertree with the diagonal set to 1). The residuals (ϵ) from this relationship are then the phylogenetically independent component of Y (or X), that is, $Y = \rho \mathbf{W} Y + \epsilon$ (Cheverud and Dow 1985; Rohlf 2001; Withers et al. 2006). We calculated phylogenetically independent residuals separately for body mass (X) and various physiological variables

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(Y) and then regressed the Y-trait residuals against the mass residuals to obtain the phylogenetically corrected allometric relationship. Individual species (short- and long-beaked echidnas and the platypus) were assessed as to their conformity to the relationship by examining their residuals relative to this relationship. There are several advantages to this approach; first, it does not assume any particular model of evolution (it is simply a mathematical "fit" of the data to the phylogenetic tree), and, second (and importantly for our study), it allows for X and Y traits to be plotted after phylogenetic correction, so that species-specific residuals can be compared to the 95% prediction limits for the phylogenetically independent allometric regression and therefore a species' conformity to that relationship can be assessed statistically (see Withers et al. 2006 for a comparison of phylogenetic approaches). We used these 95% prediction limits to determine whether echidnas conformed to the conventional and phylogenetically independent allometric relationships after Cooper and Withers (2006) and compared our data for echidnas to allometrically predicted values that were anti–log transformed using the maximum variance unbiased estimate (Hayes and Shonkwiler 2006, 2007). StatistiXL (ver. 1.6), SPSS (v17 for Windows), and custom-written Excel macros and VB (VB 6) programs (P. Withers) were used for statistical analyses.

Results

The mean body mass of short-beaked echidnas over all experiments ($N = 7$, $n = 42$) was 3.53 ± 0.068 kg, ranging from 2.77 to 4.72 kg. Echidnas rested quietly in the metabolic chamber during experimentation. They adjusted their posture at different T_a. In their thermoneutral zone (TNZ), echidnas rested on their feet, loosely curled. At low T_a, they rested tightly curled on their feet, often noticeably shivering. At the highest T_a, they sprawled out across the chamber, lying flat on their stomach with limbs and beak outstretched. Echidnas were also observed blowing bubbles from their nostrils at high T_a; expired air would form a mucous bubble on the tip of the snout, which would break, creating a moist layer across this highly vascular region.

Standard Thermal, Metabolic, and Hygric Variables

T_b ranged from 23.9° ± 0.72°C at T_a = 10°C to 32.0° ± 0.24°C at T_a = 32.5°C (fig. 1*A*; table 1), being significantly influenced by T_a ($F_{6,36} = 59.8$, $P < 0.001$). T_b was significantly different from that at T_a = 27.5°C (29.5° ± 0.32°C) at T_a = 10°, 15°, 20°, and 32.5°C ($t \geq 4.45$, $P < 0.001$). There were no significant individual differences in T_b ($\chi^2_1 = 1.83$, $P = 0.176$).

MR, both $\dot{V}O_2$ and $\dot{V}CO_2$, was significantly influenced by T_a ($F_{6,36} = 10.1$, $P < 0.001$; $F_{6,36} = 9.27$, $P < 0.001$, respectively; fig. 1*B*). $\dot{V}O_2$ ranged from 0.083 ± 0.010 mL O_2 g^{-1} h^{-1} at T_a = 27.5°C to 0.25 ± 0.045 mL O_2 g^{-1} h^{-1} at T_a = 10°C and was significantly higher at T_a = 10°, 15°, and 20°C than at T_a = 27.5°C. We consider the lowest MR, at T_a = 27.5°C, to be BMR (table 1). There were significant individual differences in $\dot{V}O_2$ ($\chi^2_1 = 7.3$, $P = 0.007$) but not $\dot{V}CO_2$ ($\chi^2_1 = 2.0$, $P = 0.200$).

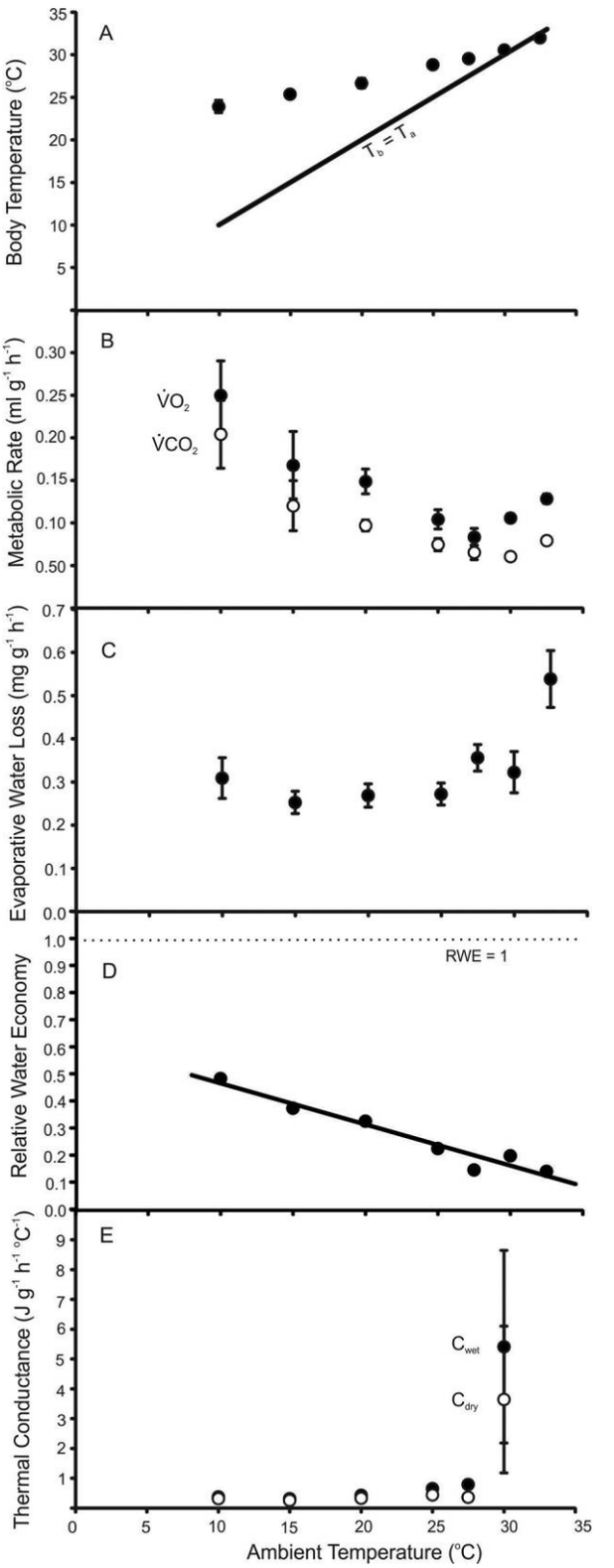

Figure 1. Body temperature (T_b; *A*), metabolic rate (*B*; filled circles, mL O_2 g^{-1} h^{-1}; open circles, mL CO_2 g^{-1} h^{-1}), evaporative water loss (*C*), relative water economy (RWE; *D*), and wet (C_{wet}; filled circles) and dry (C_{dry}; open circles) thermal conductance (*E*) of the short-beaked echidna (*Tachyglossus aculeatus acanthion*). Values are mean ± SE; $N = 7$, $n = 6$. T_a = ambient temperature.

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Table 1: Standard physiological (measured at ambient temperature [T_a] = 27°C) and respiratory (measured at T_a = 25°C) variables for the West Australian short-beaked echidna (*Tachyglossus aculeatus acanthion*)

Variables	Mean	SE
Physiological variables:		
T_b (°C)	29.5	.32
C_{wet} (J g^{-1} h^{-1} °C^{-1})	.789	.117
C_{dry} (J g^{-1} h^{-1} °C^{-1})	.359	.066
BMR (mL O_2 g^{-1} h^{-1})	.083	.010
BMR (mL CO_2 g^{-1} h^{-1})	.065	.009
EWL (mg H_2O g^{-1} h^{-1})	.356	.031
Respiratory variables:		
f_R (breaths min^{-1})	5.1	.3
V_T (mL)	48.0	6.56
V_I (mL min^{-1})	251	41.7
EO_2 (%)	21.4	2.38

Note. T_b = body temperature; C_{wet} and C_{dry} = wet and dry thermal conductance, respectively; BMR = basal metabolic rate; EWL = evaporative water loss; f_R = respiratory frequency; V_T = tidal volume; V_I = minute volume; and EO_2 = oxygen extraction. $N = 7$, $n = 6$.

There was no overall effect of T_a on RER ($F_{6,36} = 2.13$, $P = 0.073$), but RER ranged from 0.62 ± 0.025 at T_a = 30°C to 0.84 ± 0.144 at T_a = 27.5°C.

T_a had a significant effect on EWL ($F_{6,36} = 7.34$, $P < 0.001$; fig. 1*C*). EWL was relatively constant at $T_a \leq 30$°C (mean EWL = 0.33 ± 0.020 mg H_2O g^{-1} h^{-1}; table 1) but increased significantly at T_a = 32.5°C to 0.54 ± 0.066 mg H_2O g^{-1} h^{-1} ($P < 0.001$). There were no significant individual differences in EWL ($\chi^2_1 = 0.432$, $P = 0.511$). RWE ranged from 0.145 ± 0.015 at T_a = 32.5°C to 0.487 ± 0.075 at T_a = 10°C (fig. 1*D*), with a significant T_a effect ($F_{6,37} = 14.0$, $P < 0.001$). The inverse linear relationship RWE = $-0.0152T_a + 0.621$ ($R^2 = 0.62$, $P < 0.001$) extrapolates to a PRWE of −24.9°C.

Both C_{wet} and C_{dry} were relatively constant below T_a = 30°C (fig. 1*E*). There was no statistically significant effect of T_a (10°–30°C) on either C_{wet} ($F_{6,42} = 0.899$, $P = 0.505$) or C_{dry} ($F_{6,42} = 0.867$, $P = 0.527$). At thermoneutrality (T_a = 27.5°C), C_{wet} was 0.789 ± 0.117 J g^{-1} h^{-1} °C^{-1} and C_{dry} was 0.359 ± 0.066 J g^{-1} h^{-1} °C^{-1} (table 1). At T_a = 32.5°C, the calculation of conductance was unreliable, as T_b approximated T_a.

Ventilatory Physiology

Ventilation was measured only at $T_a \leq 25$°C, as above this temperature T_b approximated T_a and pressure changes from warming and humidifying inspired air could not be reliably detected. We consider that these ventilatory variables at T_a = 25°C are sufficiently close to basal to be used in comparison with those of other mammalian species (MR at T_a = 25°C was statistically indistinguishable from the BMR measured at T_a = 27.5°C; $t_{38} = 0.735$, $P = 0.467$). The f_R changed significantly ($F_{3,24} = 4.39$, $P = 0.013$; fig. 2*A*) from 5.1 ± 0.3 breaths min^{-1} at T_a = 25°C to 10.9 ± 2.5 breaths min^{-1} at T_a = 10°C ($t_{24} = 3.17$, $P = 0.004$). There was no overall T_a effect on V_T ($F_{3,18} = 1.24$, $P = 0.324$; fig. 2*B*), which ranged from 48.0 ± 6.56 mL at T_a = 25°C to 63.2 mL at T_a = 10°C. T_a did, however, have a significant influence on V_I ($F_{3,24} = 6.00$, $P = 0.003$; fig. 2*C*). As for f_R, V_I was significantly higher at T_a = 10°C (621 ± 213.2 mL min^{-1}) than at T_a = 25°C

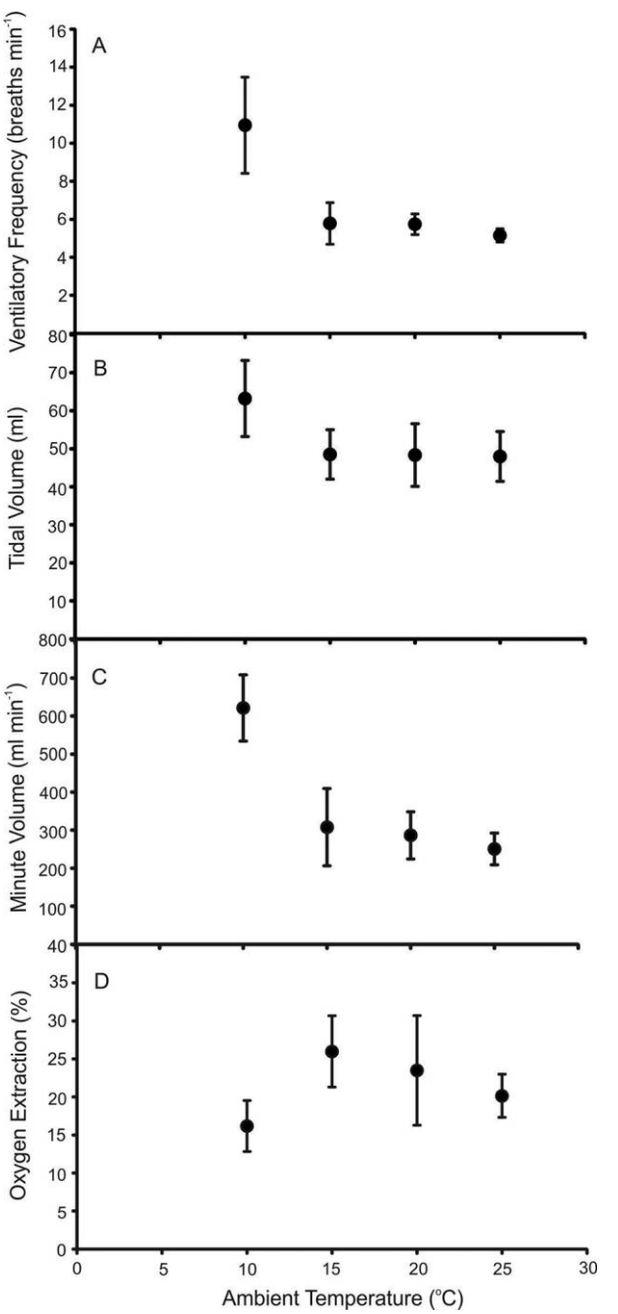

Figure 2. Ventilatory parameters of the short-beaked echidna (*Tachyglossus aculeatus acanthion*) at ambient temperatures of 10°–25°C. *A*, Ventilatory frequency; *B*, tidal volume; *C*, minute volume; and *D*, oxygen extraction. Values are mean ± SE; $N = 7$, $n = 6$.

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(251 ± 41.7 mL min^{-1}; $P = 0.001$). EO_2 was independent of T_a ($F_{3,18} = 1.64$, $P = 0.216$; fig. 2*D*), with an overall mean for all echidnas at all T_a ($N = 7$, $n = 24$) of 21.4% ± 2.38%. The only significant individual difference for respiratory variables was for EO_2 ($\chi^2_1 = 5.2$, $P = 0.020$).

Comparisons with Other Mammals

The thermoneutral T_b of the short-beaked echidna measured here of 29.5°C was significantly lower (by 7.2°C) than that of a generalized mammal (fig. 3*A*). The BMR was significantly lower than that of a generalized mammal (22% of the allometrically predicted value; fig. 3*B*), falling below the 95% prediction limits both before and after accounting for phylogeny. EWL statistically conformed to that of other mammals, both before and after considering phylogeny, despite being only 48% of the allometrically predicted value (fig. 3*C*). C_{wet} of echidnas was 127% of the predicted value for a mammal of their size but was within the 95% prediction limits for the allometric relationship for C_{wet}, both before and after accounting for phylogeny (fig. 3*D*). The f_R of echidnas was statistically lower than that of other mammals (only 20% of predicted), as was their V_I, which was only 33% of predicted. The V_T (121% of predicted) and EO_2 (1% lower), however, statistically conformed to the respective allometric relationships for other mammals, falling well within the 95% prediction limits.

Discussion

Our study of the thermal, metabolic, hygric, and ventilatory physiology for *Tachyglossus aculeatus acanthion* provides a more complete picture of the basic physiology of the short-beaked echidna than any previous study. We conclude that its low and thermolabile T_b reflects both a phylogenetic and adaptive effect, and other physiological variables are as expected for a mammal with its T_b and MR. We use our findings to assess previous disparate conclusions concerning the physiology of this species.

Basal Physiology

Our measured basal T_b of 29.5°C for Western Australian echidnas was 0.6°–2.9°C lower than other basal T_b estimates for this species (at T_a between 23° and 30°C; Martin 1903; Schmidt-Nielsen et al. 1966; Augee 1976; Dawson et al. 1979; Frappell et al. 1994). It is unclear whether this reflects differences between subspecies (e.g., a lower T_b of the Western Australian *T. a. acanthion* as an adaptation to its more arid environment; Withers et al. 2006) or whether it is a consequence of varying methodology between studies; our long measurement durations, consideration of circadian minima, and postabsorptive condition ensured that our echidnas were truly basal before T_b measurement (see Cooper and Withers 2009; Page et al. 2011; Connolly and Cooper 2014). Indeed, Nicol and Andersen (2003) suggested that posture of echidnas in some previous physiological studies indicated defensive, vigilant animals likely to have an elevated T_b (and other associated physiological variables). Waugh et al. (2006) observed a decrease in T_b of 2.2°C over 5 h for echidnas placed in a metabolic chamber (despite their being able to bury in a substrate that would reduce heat loss), reinforcing the need for long experimental duration to attain thermal equilibrium by the time of measurement.

Despite differences between studies in T_b, our low value for *T. a. acanthion* confirms the general conclusion that basal T_b of short-beaked echidnas is low compared to that of other mammals (e.g., Clarke and Rothery 2008; Lovegrove 2012*b*), in our case 7.2°C lower than expected for a mammal of similar size. Other monotremes also have a low T_b, for example, 31.7°C for long-beaked echidnas (*Zaglossus bruijni*; Dawson et al. 1978) and 32.0°C for platypus (Grant and Dawson 1978; fig. 3*A*). This suggests that a low T_b is indeed a plesiomorphic mammalian trait. Lovegrove (2012*b*) predicted that the T_b of ancestral mammals was 34.2°C, but all monotremes and particularly short-beaked echidnas have a T_b lower than this. The short-beaked echidna's low T_b, even after accounting for its phylogenetic history, is consistent with adaptation to its low-energy myrmecophagous lifestyle, semifossoriality, and being spinous ("armored"); all of these characteristics are associated with low T_b in other mammals (e.g., McNab 1984; Lovegrove 2001; Cooper and Withers 2002).

The low BMR that we measured here for *T. a. acanthion* (0.083 mL O_2 g^{-1} h^{-1}) supports previous conclusions for short-beaked echidnas. Our value is even lower (one-sample *t*-tests; $P \leq 0.005$) than that reported for short-beaked echidnas from previous studies (range, 0.132–0.217 mL O_2 g^{-1} h^{-1}; mean, 0.163 mL O_2 g^{-1} h^{-1}; Martin 1903; Schmidt-Nielsen et al. 1966; Augee 1976; Dawson et al. 1979; McNab 1984; Bech et al. 1992; Frappell et al. 1994), except that of Waugh et al. (2006) of 0.108 mL O_2 g^{-1} h^{-1} ($t_5 = 2.45$, $P = 0.058$). Approximately 80% of the difference between our estimate of BMR and these varying estimates for the species can be accounted for by differences in T_b (i.e., increased BMR is related to a higher T_b). The remaining 20% is most likely either due to adaptive differences between subspecies and/or measurement variation reflecting differing technology and methodology. Indeed, the only previous estimate of echidna MR to statistically conform to our measurement is also for *T. a. acanthion* (Waugh et al. 2006), although they measured buried echidnas. However, Augee (1978) measured three echidna subspecies (*acanthion*, *aculeatus*, and *setosus*) from disparate climatic regions (central Queensland, eastern Victoria, and Tasmania) and obtained near-identical BMR for each of the three (although higher than our *acanthion* value) but differences in MR at lower T_a for the subspecies. The potential for geographic variation in echidna metabolic physiology merits further study at a range of T_a and using consistent methodology to compare between subspecies.

The low BMR of short-beaked echidnas that we confirm here has commonly been interpreted as a plesiomorphic characteristic of prototherians (e.g., protoendotherms; Grigg et al. 2004; Lovegrove 2012*a*), but it has also been considered to be a derived character related to their overall low-energy lifestyle (Brice 2009).

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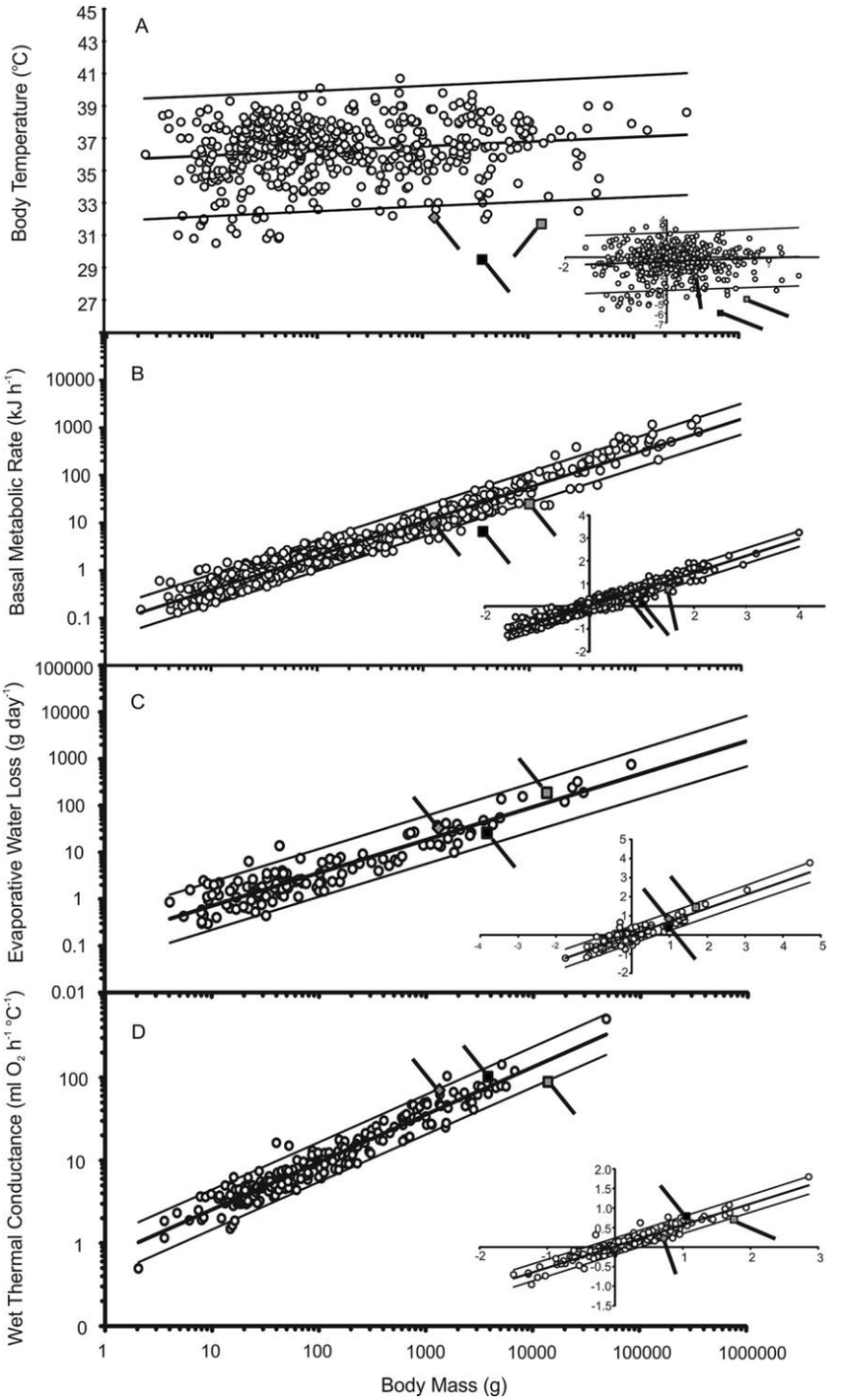

Figure 3. Allometric relationships for standard body temperature (*A*; data from White and Seymour 2004), basal metabolic rate (*B*; data from McNab 2008), evaporative water loss (*C*; data from Van Sant et al. 2012), and wet thermal conductance (*D*; data from Bradley and Deavers 1980) of marsupial and placental mammals (white circles), with the regression line (thick line) and 95% prediction limits (thin line). The short-beaked echidna (*Tachyglossus aculeatus acanthion*; this study) is shown with a black square, the long-beaked echidna (*Zaglossus bruijni*; Dawson et al. 1978) with a gray square, and the platypus (*Ornithorhynchus anatinus*; Grant and Dawson 1978) with a gray diamond. Insets are the allometric relationships for phylogenetically independent autoregression residuals.

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Short-beaked echidnas are primarily myrmecophagous; their ant/termite diet has a low energy density, poor digestibility, and low-energy feeding behavior (McNab 1984; Redford and Dorea 1984; Cooper and Withers 2004*a*), and a low BMR is characteristic of myrmecophagous mammals in general (Cooper and Withers 2002). Short-beaked echidnas are also armored, with a dense dorsal covering of sharp spines, and semifossorial; both of these characteristics are also associated with a low BMR (McNab 1979, 2008; Lovegrove 2000, 2001). In addition, echidnas use both short-term torpor and long-term hibernation (e.g., Augee and Ealey 1968; Brice et al. 2002*b*; Nicol and Andersen 2002), and a low BMR is also a characteristic of heterothermic mammals (McNab 1970; Cooper and Geiser 2008). This idea of an adaptively low BMR for short-beaked echidnas (and to a lesser extent the long-beaked echidna) is supported by the considerably higher BMR of the aquatic platypus (Grant and Dawson 1978), which statistically conforms to that of other mammals (fig. 3*B*), probably as a consequence of its semiaquatic lifestyle. After accounting for phylogeny, both the platypus and the long-beaked echidna conformed to the BMR of other mammals, but the short-beaked echidna was still significantly low, further suggesting adaptive reduction in BMR.

Despite these two contrasting hypotheses (ecology, phylogeny) accounting at least in part for the low BMR of short-beaked echidnas, their low BMR also reflects their low T_b. BMR increases about 10% for every 1°C increase in T_b (if Q_{10} for MR is about 2.5; Guppy and Withers 1999). Dawson and Hulbert (1970) and Dawson (1973) concluded that using Q_{10} to "correct" the BMR of monotremes, marsupials, and placental mammals to a common T_b (38°C) accounted for observed differences in BMR between these groups, although Dawson et al. (1979) concluded that Q_{10} correction of BMR did not fully explain these differences. However, a Q_{10}-based correction of BMR also changes thermal conductance (C), which is not necessarily realistic; insulation would not change just because the T_b set point is higher. We therefore corrected for the effect of T_b on BMR with a constant conductance correction method, using the formula $C_{wet} = MR_{(1)}/(T_{b(1)} - T_a) = MR_{(2)}/(T_{b(2)} - T_a)$, where C_{wet} is our measured wet thermal conductance (0.789 ± 0.117 J g^{-1} h^{-1} °C^{-1}), $MR_{(1)}$ is our measured BMR (0.083 mL O_2 g^{-1} h^{-1}) at the actual $T_{b(1)}$ of 29.5°C and thermoneutral T_a of 27.5°C, and $MR_{(2)}$ is the predicted BMR at a $T_{b(2)}$ of 35.5° or 38°C. Our calculations support the conclusions of Dawson and Hulbert (1970) and Dawson (1973); if short-beaked echidnas had the same T_b as a generalized marsupial or placental mammal (35.5° or 38°C, respectively), then they would have a BMR of 0.31 or 0.41 mL O_2 g^{-1} h^{-1}, respectively, both of which conform closely to allometric predictions (fig. 4). Therefore, we interpret the low BMR of short-beaked echidnas not as a primitive characteristic indicative of poor metabolic capacity or as an energy-conserving adaptation to a low-energy ecological niche, as previously proposed, but simply as a consequence of their low T_b—albeit their low T_b is likely both a plesiomorphic character and an adaptive character.

In terms of the respiratory physiology of the short-beaked echidna, we confirmed the previous observations of Bech et al. (1992) that a very low f_R accounts for the low basal V_I, with basal V_T conforming closely to that of other mammals. Our values for f_R do not differ from those of Bech et al. (1992; 6 min^{-1}) or Waugh et al. (2006; 4.6 min^{-1}), but other studies have reported significantly higher f_R (7–17.5 min^{-1}; one-sample t-test, $t_5 > 5.38$, $P < 0.003$; Bentley et al. 1967; Augee et al. 1971; Parer and Hodson 1974; Frappell et al. 1994; Nicol and Andersen 2003). Our V_T measurement is similar to that of Frappell et al. (1994; 44.8 mL), but other studies report significantly lower values (19–24 mL; $t_5 > 3.21$, $P < 0.024$; Bentley et al. 1967; Parer and Hodson 1974; Bech et al. 1992). These differences in ventilatory variables are most likely due to methodological differences such as short measurement durations, measurement of awake or buried animals, and the use of restraints and/or tactile

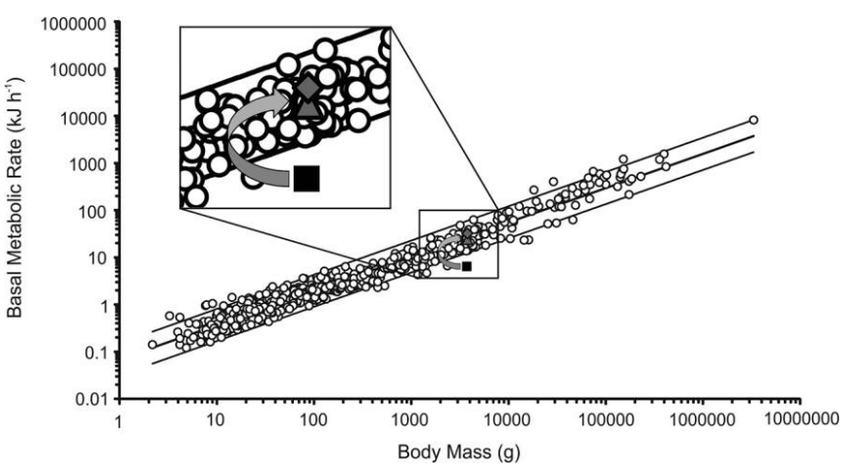

Figure 4. Basal metabolic rate for the short-beaked echidna (*Tachyglossus aculeatus acanthion*; black square) adjusted from its body temperature of 29.5°C to a typical marsupial body temperature (T_b) of 35.5°C (gray triangle) and a typical placental T_b of 38°C (gray diamond) compared to the allometric relationship for mammals (white circles; data from fig. 1), with the 95% prediction limits.

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stimuli. These may lead to hyperventilation, with elevated f_R and reduced V_T (Larcombe 2002; Cooper and Withers 2004*b*; Waugh et al. 2006).

Bech et al. (1992) measured a lower than expected V_T in their study and in addition found a low f_R, resulting in a very low V_I and a lower ventilatory requirement ($V_I/\dot{V}O_2 = 17.9$) than expected for an equivalent-sized mammal ($V_I/\dot{V}O_2 = 34$). Our results here ($V_I/\dot{V}O_2 = 31.1$) are similar to those of Frappell et al. (1994; $V_I/\dot{V}O_2 = 37.1$), with a low V_I proportionate to the low BMR, so that EO_2 of short-beaked echidnas in this study is the same as for other mammals.

Low T_a *Physiology*

Our results support early observations that under resting conditions, nonreproductive short-beaked echidnas are very thermolabile (e.g., Martin 1903; Robinson 1954; McMurchie and Raison 1975; Augee 1976); T_b varies considerably over a wide range of T_a (fig. 1*A*), unlike the relative constancy expected for an endothermic mammal. For thermoconformers, T_b follows T_a quite closely (T_b/T_a slope ~1), whereas effective thermoregulators maintain T_b near constant regardless of T_a and T_b/T_a slope ~0 (Withers 1992). The overall T_b/T_a slope of 0.35 for our short-beaked echidnas, although closer to that of effective thermoregulators (0) than thermoconformers (1), nevertheless suggests that echidnas are less effective thermoregulators than other mammals, at least when inactive and nonreproductive. Indeed, the thermolability below thermoneutrality of a 3.5-kg echidna of 0.282 °C °C^{-1} is much greater than for various small heterothermic dasyurid marsupials (0.064 °C °C^{-1}; Pusey et al. 2013). Nevertheless, they are clearly able to maintain a considerable T_b-T_a differential.

Is the short-beaked echidna's thermolability a plesiomorphic or derived trait? Both the smaller (0.7-kg) semiaquatic platypus and the larger (13.6-kg) long-beaked echidna have limited or no thermolability at low T_a (Dawson et al. 1978; Grant and Dawson 1978), suggesting that the short-beaked echidna's thermolability is probably not a plesiomorphic monotreme trait but possibly an energy-conserving adaptation to their low-energy niche. Thermolability results in substantial energy savings for short-beaked echidnas (in addition to that from their already low T_b). By reducing T_b by 5.6°C at $T_a = 10$°C from their basal value, MR is only 0.25 mL O_2 g^{-1} h^{-1} compared with 0.36 mL O_2 g^{-1} h^{-1} calculated if T_b were maintained at 29.5°C; this represents a 30% (0.11 mL O_2 g^{-1} h^{-1}) energy saving. Martin (1903) also noted that echidnas saved energy by their "imperfect homeothermism." Energy conservation by thermolability is consistent with an overall low-energy physiology being an adaptation to a myrmecophagous, armored, semifossorial niche. However, thermolability is not a general characteristic of the various myrmecophagous mammals measured by McNab (1984; but note that the short-beaked echidnas measured in his study were not thermolabile either) or the termitivorous numbat (Cooper and Withers 2002) or aardwolf (Anderson et al. 1997). Interestingly, female short-beaked echidnas incubating eggs and young have a very closely regulated T_b, suggesting that they are capable of more precise homeothermy when required (Beard et al. 1992; Beard and Grigg 2000; Nicol and Andersen 2006), as observed for other thermolabile mammals (see Farmer 2000). More precise homeothermy during reproduction presumably enhances the development of embryos and young, and, as such, parental care has been proposed as a driver of the evolution of endothermy (Farmer 2000).

The increased thermoregulatory O_2 demand of echidnas at low T_a (like other endotherms) is accommodated mainly by increasing f_R to increase V_I; V_T remains relatively constant. This pattern of respiratory accommodation is surprising considering their moderate body mass; generally, small species increase f_R while larger species increase V_T. Ventilatory data for the other species of monotreme at a range of T_a are required to unequivocally determine whether this $\Delta f_R/\Delta V_T$ pattern for short-beaked echidnas is a general characteristic of monotremes compared with placental and marsupial mammals. Ventilatory variables have been measured for normoxic platypus only at thermoneutrality, but they do respond to hypoxia by increasing V_T rather than f_R (Frappell 2003).

For short-beaked echidnas, RWE (MWP/EWL) increases linearly with decreasing T_a (fig. 1*D*), as is generally observed for mammals (Cooper et al. 2005). Their PRWE of about −24.9°C is very low (and this calculation of PRWE requires a considerable T_a extrapolation). Generally, PRWE is dependent on body mass, with smaller mammals having higher values (Cooper et al. 2005), so echidnas would be expected to have a reasonably poor PRWE (about 0°C) due to their size (Pusey et al. 2013), although arid-adapted mammals have a higher PRWE than those from more mesic environments (MacMillen and Hinds 1983; MacMillen 1990). The very low PRWE of echidnas is also a consequence of their low-energy physiology and myrmecophagous diet. Echidnas have a low MR (and thus MWP) but a normal mammalian EWL, so their RWE is low. A termite diet has a high water content and a relatively low energy return (Cooper and Withers 2004*a*), which also lowers RWE. Consequently, it is unsurprising that *T. a. acanthion* has a poor RWE despite its predominately arid-zone distribution.

High T_a *Physiology*

Heat-challenged mammals typically increase heat loss by various nonevaporative (e.g., vasodilation, posture) and evaporative (e.g., panting, licking, sweating, insensible) avenues. However, previous studies have suggested that short-beaked echidnas do not pant, lick, or sweat (Robinson 1954; Schmidt-Nielsen et al. 1966; Augee 1976) or use vasomotor adjustments (Martin 1903) to enhance heat loss at high T_a, despite evidence that both the platypus and *Zaglossus* do increase EWL for thermoregulation (Dawson et al. 1978; Grant and Dawson 1978). We found here that EWL of short-beaked echidnas does unequivocally increase at high T_a (fig. 1*C*), as is typical of other mammals, but we could not accurately quantify any changes in C_{wet} or C_{dry} at high T_a due to the close approximation of T_a and T_b.

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The EHL of short-beaked echidnas at $T_a = 32.5°C$ was 1.29 J g^{-1} h^{-1}, equivalent to half (51%) of an echidna's metabolic heat production at that T_a and considerably more than the 33% observed by Schmidt-Nielsen et al. (1966) for echidnas at T_a of up to 34°C. It is possible that our arid-habitat subspecies has a greater propensity for EHL or that differing measurement techniques may account for these differences. Our measurement for EHL is at the upper end of the range of 25% (*Pseudomys hermannsburgensis*; MacMillen et al. 1972) to 56% (*Macropus rufus*; Dawson et al. 2000) for a variety of arid-habitat Australian mammals at T_a approaching T_b, including the termitivorous marsupial numbat (*Myrmecobius fasciatus*; 45%; Cooper and Withers 2002) from the same study location as our short-beaked echidnas. Therefore, the echidna's capacity for dissipating metabolic heat by EHL appears comparable to other mammals inhabiting similar environments.

It is not clear how our short-beaked echidnas increase EWL at high T_a. Augee (1976) reported that they do not sweat or pant (although he did note an increase in V_I for lightly anesthetized echidnas after a short exposure to heat); however, this is not necessarily plesiomorphic or thermoregulatorily limiting. Various other mammals lack sweat glands but are effective thermoregulators in the heat; for example, rodents do not have sweat glands but can increase EWL at high T_a (MacMillen and Lee 1970; MacMillen et al. 1972; Barker et al. 2012), and at least in some species this reflects increased insensible cutaneous EWL (Tracy and Walsberg 2000). Dawson et al. (1978) reported that *Zaglossus* increases EHL at high T_a, by possibly increasing insensible EWL and sweating, and the platypus can sweat (Augee 1976; Grant and Dawson 1978), so short-beaked echidnas might also increase insensible heat loss and/or sweat. We never observed our echidnas to lick or salivate during measurements, but at high T_a, air expired from the nostrils formed bubbles of nasal mucous that broke and moistened the tip of the snout, which contains a blood sinus. This bubble blowing by echidnas presumably enhances EHL in a similar way to the salivation and licking by kangaroos of their highly vascular forearms (Dawson 1995), as well as its normal role of facilitating electrosensory reception (Proske et al. 1998).

Although many previous studies have concluded that short-beaked echidnas rely on behavioral adaptations and low heat production to survive thermal extremes (Martin 1903; Robinson 1954; Griffiths 1968, 1978; Augee 1976), some more recent studies have suggested that enhanced physiological heat loss is necessary to avoid lethal T_b at high T_a. For example, Wilkinson et al. (1998) and Brice et al. (2002*a*) noted that echidnas were commonly found in logs during summer, when temperatures varied as much as shaded outside T_a and often rose above 35°C for 10 h (the previously reported lethal T_a) and reached 42°C in hot weather (Brice et al. 2002*b*). When T_a exceeds T_b, as observed by Brice et al. (2002*b*) for echidnas in logs in summer, evaporative cooling is the only mechanism available for heat dissipation, so it is significant that we confirm here that echidnas can physiologically enhance EWL at high T_a.

The echidna's presumed low environmental heat tolerance must also be considered in perspective with its low T_b and therefore increased propensity for heat gain in hot environments. The reported lethal T_b of 38°C for echidnas is some 8.5° C above their thermoneutral T_b; for other mammals, lethal T_b (typically 42°–43°C) exceeds normothermic T_b (37°C) by only 5°–6°C (Adolph 1947). So, the low T_b of echidnas means that their upper lethal temperature is expected to be comparatively low and is not indicative of poor thermoregulatory ability. Indeed, echidnas have considerable thermal tolerance of high environmental T_a of ≥35°C for long periods in the field (Brice et al. 2002*a*).

Conclusion

We conclude that most aspects of the physiology of the short-beaked echidna (*Tachyglossus aculeatus acanthion*) are similar to those of other mammals, after their low T_b is accounted for. Although the low T_b of short-beaked echidnas might be considered a plesiomorphic trait, there is likely an additional reduction in T_b associated with the echidna's ecological niche (myrmecophagous, spiny armored, semifossorial). Their low MR reflects their low T_b, their lower than expected respiratory ventilation is sufficient to accommodate their modest gas exchange requirements, and their thermal conductance is as expected. Short-beaked echidnas are flexible thermoregulators, being considerably thermolabile over a range of T_a, but it is unclear whether this is a plesiomorphic trait or whether it is derived and reflects their low-energy myrmecophagous niche. In contrast to previous studies, our findings indicate that echidnas increase EWL above thermoneutrality. Cooling of their nasal blood sinus with nasal mucus may contribute to this enhanced evaporative cooling, along with increased respiratory EWL and presumably also cutaneous EWL. This capacity for evaporative cooling explains how their distribution can include hot environments, where T_a even in daytime shelters would exceed their critical thermal limit.

Acknowledgments

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