RESEARCH ARTICLE

Evaporative water loss, relative water economy and evaporative partitioning of a heterothermic marsupial, the monito del monte (*Dromiciops gliroides*)

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SUMMARY

We examine here evaporative water loss, economy and partitioning at ambient temperatures from 14 to 33°C for the monito del monte (*Dromiciops gliroides*), a microbiotheriid marsupial found only in temperate rainforests of Chile. The monito's standard evaporative water loss ($2.58\,mg\,g^{-1}\,h^{-1}$ at 30°C) was typical for a marsupial of its body mass and phylogenetic position. Evaporative water loss was independent of air temperature below thermoneutrality, but enhanced evaporative water loss and hyperthermia were the primary thermal responses above the thermoneutral zone. Non-invasive partitioning of total evaporative water loss indicated that respiratory loss accounted for 59–77% of the total, with no change in respiratory loss with ambient temperature, but a small change in cutaneous loss below thermoneutrality and an increase in cutaneous loss in and above thermoneutrality. Relative water economy (metabolic water production/evaporative water loss) increased at low ambient temperatures, with a point of relative water economy of 15.4°C. Thermolability had little effect on relative water economy, but conferred substantial energy savings at low ambient temperatures. Torpor reduced total evaporative water loss to as little as 21% of normothermic values, but relative water economy during torpor was poor even at low ambient temperatures because of the relatively greater reduction in metabolic water production than in evaporative water loss. The poor water economy of the monito during torpor suggests that negative water balance may explain why hibernators periodically arouse to normothermia, to obtain water by drinking or *via* an improved water economy.

Key words: metabolic water production, ventilation, water economy, torpor, hibernation, Microbiotheria, mammal.

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INTRODUCTION

Understanding the effects of ambient temperature (T_a) on thermal and metabolic physiology is essential to understanding the thermoregulatory abilities of animals. The effects of T_a on energetics have been well studied for many endothermic mammals and birds, but effects on evaporative water loss are less well documented. For marsupials in particular, there is an extensive data set for body temperature (T_b) and metabolic rate (MR) (e.g. Dawson and Hulbert, 1970; McNab, 1984; McNab, 1986; McNab, 2005; McNab, 2008; Lovegrove, 2000; White and Seymour, 2003; White and Seymour, 2005; Withers et al., 2006) but there are considerably fewer data for total evaporative water loss (EWL_T) (e.g. Withers et al., 2006). Consequently, a clear understanding of the factors determining EWL_T, including the effects of T_a, is only now being developed for marsupials (e.g. Cooper et al., 2005; Cooper et al., 2009; Withers et al., 2006; Cooper and Cruz Neto, 2009; Cooper et al., 2010; Warnecke et al., 2010; Withers and Cooper, 2009a; Withers and Cooper, 2009b; Withers and Cooper, 2011). Understanding the pattern of change in EWL_T with T_a (or, commonly, the lack of change) requires partitioning of EWL_T into its respiratory (EWL_R) and cutaneous (EWL_C) components to understand the effects of T_a on each.

Relative water economy (RWE) is the ratio of metabolic water production (MWP) to EWL_T (MacMillen and Hinds, 1983; Hinds and MacMillen, 1986). The effect of T_a on RWE reflects the patterns of T_a on MWP and EWL_T (e.g. Bartholomew, 1972; MacMillen and Hinds, 1983; Hinds and MacMillen, 1986; Cooper et al., 2005). For normothermic endotherms, MWP typically increases at low T_a whereas EWL_T is relatively constant, hence RWE increases at low T_a. The point of relative water economy (PRWE) is the T_a where MWP=EWL_T (i.e. RWE=1), which reflects the relative water balance of a species. For heterothermic endotherms, low T_b during torpor or hibernation markedly decreases RWE (e.g. Cooper et al., 2005; Cooper et al., 2009; Withers and Cooper, 2009a; Withers and Cooper, 2009b; Warnecke et al., 2010). This is potentially important for hibernating species because maintaining water balance is one hypothesis for the necessity of periodic arousal by hibernators (Fisher and Manery, 1967; Speakman and Racey, 1989; Thomas and Cloutier, 1992; Thomas and Geiser, 1997; Humphries et al., 2003).

We examine here the basic physiology of the monito del monte ('monkey of the mountains'; *Dromiciops gliroides* Thomas 1894) with particular reference to its water balance and economy. The monito is a small, nocturnal, omnivorous marsupial that is found only in the temperate rainforests of Argentina and Chile (Greer, 1965; Marshall, 1978; Hershkovitz, 1999). Monitos shift diet seasonally, and have a remarkable acclimation of specific enzyme activities for processing fruits and insects (Cortés et al., 2011). They are social, with communal nesting and limited aggressive behaviour (Franco et al., 2011). Their dense, dark fur, reduced ear size, and

distinct pouch with only four teats suggest adaptations to a cold and low-productivity environment (Mann, 1955). This enigmatic species, the only living representative of the marsupial order Microbiotheria (Reig, 1955; Mann, 1955; Hershkovitz, 1999), is thought to be more closely related to the Australian marsupials, particularly the Australian pygmy possums, the honey possum and the feathertail glider, than other South American marsupials (Cardillo et al., 2004; Bininda-Emonds et al., 2007; Beck, 2008; Beck et al., 2008; Nilsson et al., 2010).

Early studies indicated that the monito hibernates during winter (Mann, 1955; Greer, 1965; Grant and Temple-Smith, 1987), like pygmy possums and the feathertail glider (Jones and Geiser, 1992; Geiser, 1994; Geiser, 2004). Recent studies have shown that it also uses deep daily torpor, even during summer, as well as multi-day hibernation of up to 6 days duration at T_a of 12.5 and 20°C, with T_b closely approaching T_a (Bozinovic et al., 2004; Nespolo et al., 2010). Bozinovic et al. (Bozinovic et al., 2004) measured MR over a range of T_a and concluded that the monito has a low basal metabolic rate (BMR) and similar-to-expected thermal conductance (*C*). Cortés et al. (Cortés et al., 2009) described the energetics of the monito at T_a=20°C and Nespolo et al. (Nespolo et al., 2010) further described the bioenergetics of euthermy and daily torpor at T_a=10 and 20°C. Little is known about water balance for the monito. Cortés et al. (Cortés et al., 2009) measured excurrent humidity during respirometric measurement for the monito but did not calculate its EWL_T for comparison with other species. We present here the first EWL_T data for monitos, the first value for relative water economy of a marsupial hibernator, and non-invasively partition EWL_T into cutaneous and respiratory components. We hypothesise that the monito will have a high EWL_T and poor RWE, reflecting its mesic habitat and high water content diet, and that deep torpor will reduce both EWL_T and RWE so as to compromise water balance and require periodic arousal from hibernation for rehydration.

MATERIALS AND METHODS
Study animals

Eight adult monitos, four male and four female, were captured near Valdivia, Chile (39°48′S, 73°14′W), using wire cage traps modified for placement in trees and shrubs. Traps were baited with banana (see Cortés et al., 2009) and were covered with plastic and lined with Dacron for insulation. Individuals were transported back to the laboratory at Universidad Austral de Chile on the day of capture, and maintained individually in plastic containers (45×30×20 cm) with wood shavings as bedding. They were fed a mixture of baby food (cereal and fruit consommé), honey, fruit and occasionally mealworms. Measurements were made from 30 January to 7 February 2010.

Animal capture, maintenance and experimentation were conducted under permit from Servicio Agrícola y Ganadero, and with approval of the Animal Ethics Committees of Universidad Austral Chile, University of Western Australia and Curtin University.

Respirometry measurements

We measured oxygen consumption ($\dot{V}_{O_2}$), carbon dioxide production ($\dot{V}_{CO_2}$) and EWL_T for monitos by flow-through respirometry, at T_a ranging from 14 to 33°C (order of measurements: 30, 20, 14, 33 and 25°C). Seven monitos were measured at T_a=30°C and six were measured at all other T_a. Experiments were conducted for a period of 6–10 h during the monito's rest phase (daytime). Monitos were measured at only one T_a per day, and were allowed a rest period of at least 3 days between successive measurements. To facilitate this

rest period, not all individuals were measured at each T_a. T_a was regulated to ±0.1°C using a temperature-controlled cabinet (Pi-Tec Technologies, Santiago, Chile). Body temperature was recorded *via* the cloaca at the end of experiments, and during torpor bouts (when animals had attained a constant, minimal torpid state), to ±0.1°C using an HH-25TC thermocouple meter (Omega Engineering, Stamford, CT, USA) with a fine plastic-tipped thermocouple wire.

The respirometry system consisted of a mass flow controller (GFC17, Aarlborg, Orangeburg, NY, USA, or CP7509, Cole-Palmer, Vernon Hills, IL, USA) that regulated the inlet flow rate of compressed ambient air (dried with Drierite; W. A. Hammond Drierite Co., Xenia, OH, USA) at approximately 500 ml min^{-1} at standard temperature and pressure (STPD). Metabolic chambers consisted of 188 cm^3 glass tubes with rubber stoppers; inlet and outlet ports were at opposite ends of the chambers, as were the calibration and measurement ports for ventilatory measurements (see below). Excurrent air was passed over a thin-film capacitance relative humidity (RH) probe (MNP45A, Vaisala, Helsinki, Finland) and then dried using Drierite and passed through an oxygen analyser (Foxbox-C, Sable Systems, Las Vegas, NV, USA; or 570A, Servomex, Crowborough, East Sussex, UK) and a carbon dioxide analyser (Foxbox or CA10, Sable Systems). Ambient temperature was monitored during the experiment using the Vaisala probe. Urine and faeces were produced rarely, and were quickly dried by the relatively high air flow rate. Any brief periods of increased RH resulting from urine, faeces or activity could easily be identified on the continuous EWL_T trace and were excluded from analyses.

The analog outputs of one Vaisala probe were interfaced to the Foxbox and the O_2, CO_2, RH and T_a data were logged by a PC *via* the Foxbox serial output, using a custom-written program (Visual Basic v6, Microsoft, Redmond, WA, USA). The analog outputs of the Servomex, CA-10 and Vaisala probe were interfaced to a PC *via* an A/D converter (ADC11, Pico Systems, St Neots, Cambs, UK) and logged every 20 s. $\dot{V}_{O_2}$, $\dot{V}_{CO_2}$ and EWL_T were calculated for a period of approximately 20 min when physiological variables were constant and minimal (Withers, 2001) using custom-written software (Visual Basic v6). Respiratory exchange ratio (RER) and evaporative quotient (EQ) were calculated as $\dot{V}_{CO_2}/\dot{V}_{O_2}$ and $EWL_T/\dot{V}_{O_2}$, respectively. MR was converted to MWP and metabolic heat production (MHP) using the measured RER for that experiment after Withers (Withers, 1992). EWL_T was converted to evaporative heat loss (EHL) using 2.4 J mg^{-1} H_2O (McNab, 2002). RWE was calculated as MWP/EWL_T, and the point of relative water economy (PRWE) as the T_a at which RWE=1, from the regression of RWE against T_a.

Mass flow meters were calibrated with a Gilian Gillibrator 2 (traceable to a national standard; Clearwater, FL, USA) prior to the commencement of experiments, and this calibration was confirmed during the series of experiments by volumetric replacement of water. The oxygen analysers were two-point calibrated using compressed nitrogen (0% O_2) and dry ambient air (20.95% O_2). Calibration of the CO_2 analysers was achieved using compressed nitrogen (O% CO_2) and a certified gas mix (0.011% CO_2 in nitrogen; Indura, Santiago, Chile). The calibration of the relative humidity probes was confirmed using 1% RH air (dried with Drierite to approximately 0.005 mg l^{-1}) and 100% RH air (saturated by breathing on the probe).

Ventilatory measurements

Respiratory ventilation was monitored using whole-body plethysmography (Malan, 1973). A pressure transducer (MPX2010, Motorola, Denver, CO, USA) was used to monitor chamber pressure,

and its analog output was interfaced to a PC using an ADC11 A/D converter and the values logged using PicoLogger software (Pico Systems). A volume of air (0.05 ml) was repeatedly (approximately five times for each experiment) injected into the system before removing the animal from the chamber at the end of each experiment. The washout characteristics of these calibration pulses were used to convert ventilatory pressure changes to pressures for a virtual closed system, by numerical integration (Szewczak and Powell, 2003). These 'closed-system' data were then analysed for respiratory frequency ($\dot{f}_R$; min^{-1}) and tidal volume (V_T; ml) after Malan (Malan, 1973) using custom-written software (Visual Basic v6). Respiratory minute volume ($\dot{V}_I$; ml min^{-1}) was calculated as $f_R \times V_T$ and oxygen extraction efficiency (E_{O_2}) was calculated at the time of breathing measurements as $E_{O_2}=100\times(\dot{V}_{O_2}/\{[(0.2095+F_{E_{O_2}})/2]\times\dot{V}_I\})$, where $F_{E_{O_2}}$ is the mean O_2 fraction of excurrent air. Ventilatory variables are presented as body temperature and pressure saturated (BTPS), but E_{O_2} was calculated from $\dot{V}_I$ converted to STPD.

Partitioning of evaporative water loss

We partitioned EWL_T using an iterative mathematical model that calculated respiratory and cutaneous evaporative water loss components at each T_a. EWL_R (mg g^{-1} h^{-1}) was calculated from $\dot{V}_I$, based on expired humidity (assumed to be 100%) and temperature (T_{exp}; °C), and inspired (chamber) humidity (RH$_{ins}$) and temperature (T_a; °C): $EWL_R=(60/M)(\dot{V}_I/1000)[\chi_{Texp}-(\chi_{Ta}RH_{ins}/100)]$, where M is mass and χ is the water vapour density for inspired air (χ_{Ta}) and expired air (χ_{Texp}) at the appropriate temperature [χ was calculated after Parrish and Putnam (Parrish and Putnam, 1977)]. EWL_C (mg g^{-1} h^{-1}) was calculated from skin temperature (T_{skin}; °C), cutaneous evaporative resistance (R_{skin}; s cm^{-1}) and body surface area [A_s; calculated as $10M^{0.67}$ cm^2 (Dawson and Hulbert, 1970; Walsberg and King, 1978)]: $EWL_C=(3.6/R)(A_s/M)(\chi_{Tskin}-(\chi_{Ta}RH_{ins}/100))/R_{skin}$, where χ_{Tskin} is the water vapour density for air at skin temperature (100% RH) and χ_{Ta} is the water vapour density for air at ambient temperature/humidity. The values of T_{skin}, R_{skin} and T_{exp} that minimised the difference between calculated EWL_T (EWL_R+EWL_C) and measured EWL_T were determined by iteratively calculating and summing EWL_R and EWL_C, for various combinations of T_{skin}, R_{skin} and T_{exp}. T_{exp} was varied in steps of 0.01°C from 5°C less than T_a up to T_b (e.g. Jackson and Schmidt-Nielsen, 1964; Schmidt-Nielsen et al., 1970; Collins et al., 1971; Schmid, 1976; Hill, 1978), T_{skin} in steps of 0.01°C from T_a to T_b (e.g. Bartholomew, 1956; Edwards and Haines, 1978; Webster and Campbell, 1985; Dausmann, 2005) and R_{skin} in steps of 1 s cm^{-1} from 100 to 1000 s cm^{-1} (e.g. Webster and Campbell, 1985; Withers, 1992) (see Discussion). Estimation of EWL_C is dependent on the interaction between T_{skin} and R_{skin} (i.e. a decrease in T_{skin} can counterbalance a decrease in R_{skin} at constant EWL_C), so estimation of these parameters is expected to be variable but not to affect the partitioning of EWL_T into EWL_R and EWL_C. The sensitivity of the mathematical partitioning model to potential errors in the iteratively determined T_{skin}, T_{exp} and R_{skin} was examined at each T_a by fixing values for two of these variables at the calculated value and determining the effect of varying the third variable on the partitioning calculation.

Statistics

Statistics were calculated in Excel (Microsoft) using v1.8 of statistiXL (www.statistixl.com) and spreadsheet formulae (Zar, 1999). Effects of T_a on physiological variables were investigated for normothermic monitos using least squares regression and one-way ANOVA with a priori contrasts (Cohen, 2008; Withers and

Cooper, 2011). Two-way ANOVA was used to compare values for torpid and normothermic monitos. Percentage data were arcsin transformed prior to analysis. All values are presented as means ± s.e.m. Sample size (N) is the number of individuals, and is provided where it differs from that stated previously (e.g. not all individuals entered torpor, so N is less than the stated sample size for some variables); n is the total number of measurements.

RESULTS

Body mass of monitos increased over the study period, resulting in significant differences in mass as successive T_a were measured ($F_{4,26}=11.6$, $P<0.001$). Body mass ranged from 24.9±1.00 g at $T_a=30$°C to 36.4±2.37 g at $T_a=20$°C.

Monitos remained normothermic at $T_a\geq30$°C, but entered torpor [defined as a $\dot{V}_{O_2}$ of <75% of BMR (Geiser and Baudinette, 1988)] at other T_a (Fig. 1). Body temperature of normothermic monitos was significantly and linearly related to T_a (linear polynomial contrast $F_{1,26}=41.9$, $P<0.001$), ranging from 30.1±1.44°C at $T_a=14$°C to 37.2±0.35°C at $T_a=33$°C. Thermoneutral T_b (at $T_a=30$°C; see below) was 34.6±0.34°C. Body temperature of torpid monitos
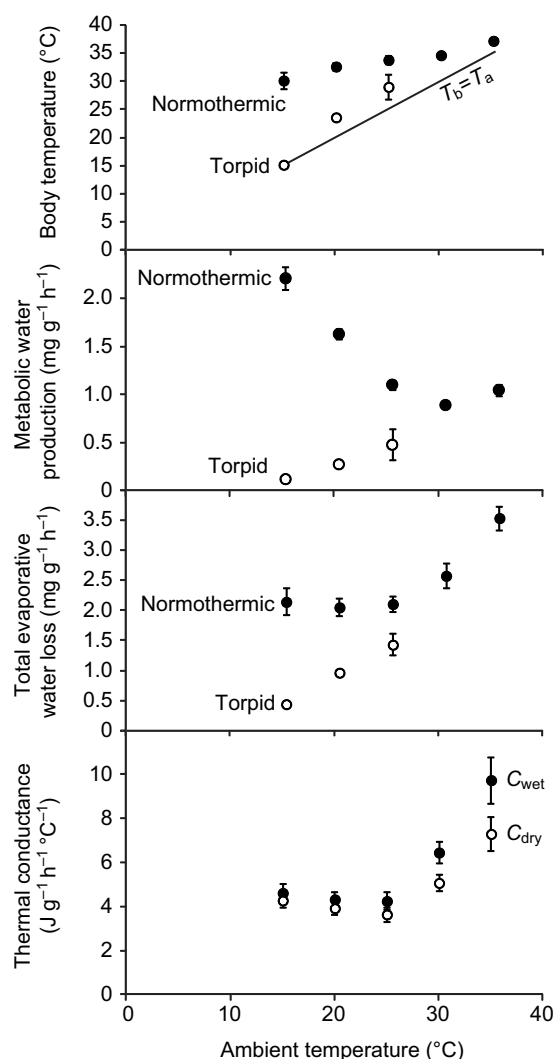

Fig. 1. Body temperature (T_b), metabolic water production, total evaporative water loss, and wet (C_{wet}) and dry (C_{dry}) thermal conductance of monitos. Values are means ± s.e.m. T_a, ambient temperature.

decreased with T_a ($F_{2,24}$=38.9, P<0.001) and was significantly lower compared with euthermic monitos ($F_{1,24}$=139, P<0.001) at T_a=14°C (15.2±0.31°C, N=5), 20°C (23.6±0.38°C, N=5) and 25°C (29.0±2.20°C, N=2).

Metabolic water production of normothermic monitos closely mirrored both $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$, as there was no effect of T_a on RER ($F_{1,26}$=0.10, P=0.749), which was 0.91±0.014 (N=8, n=31) over all T_a. The lowest MR of 1.42±0.052 ml O_2 g^{-1} h^{-1} at T_a=30°C, which we consider to be BMR, corresponds to a MWP of 0.89±0.04 mg H_2O g^{-1} h^{-1}. MWP was strongly influenced by T_a ($F_{4,26}$=61.9, P<0.001), increasing to 2.2±0.12 mg H_2O g^{-1} h^{-1} at T_a=14°C (Fig. 1). There was a significant linear relationship for MWP at T_a=14 to 30°C ($F_{1,21}$=8.8, P<0.001), and MWP increased to 1.05±0.06 mg H_2O g^{-1} h^{-1} at T_a=33°C (significant linear and quadratic contrasts for T_a=14 to 33°C; $F_{1,26}$≥24.5, P<0.001). MWP of torpid monitos decreased with T_a ($F_{2,18}$=9.99, P<0.001), and was significantly lower when torpid than normothermic at the same T_a ($F_{1,28}$=531, P<0.001). There was a significant interaction ($F_{2,28}$=69.1, P<0.001) between torpor and T_a effects on MWP, with MWP decreasing more below normothermia at lower T_a (Fig. 1).

Standard EWL_T (at thermoneutrality, 30°C) was 2.6±0.21 mg H_2O g^{-1} h^{-1}. There was an overall effect of T_a on EWL_T ($F_{4,26}$=10.8, P<0.001; Fig. 1). EWL_T did not change between T_a of 14 and 20°C (no significant reverse Helmert, linear or quadratic contrasts; $F_{1,15}$≤0.871, P≥0.739) but increased at T_a=30°C ($F_{1,26}$=5.25, P=0.030) and 33°C ($F_{1,26}$=38.5, P<0.001) and linearly from 25 to 33°C ($F_{1,16}$=259, P<0.001). EWL_T of torpid monitos decreased with T_a ($F_{2,28}$=4.94, P=0.015) and was significantly lower compared with normothermic monitos at the same T_a ($F_{1,28}$=85.0, P<0.001). The significant interaction between torpor and T_a effects on EWL_T ($F_{2,28}$=5.72, P=0.008) indicated that EWL_T during torpor decreased more below normothermia at lower T_a, with absolute water savings resulting from torpor of 0.68 mg H_2O g^{-1} h^{-1} at 25°C to 1.70 mg H_2O g^{-1} h^{-1} at 14°C.

Wet and dry thermal conductances of monitos were influenced by T_a (C_{wet}, $F_{4,26}$=15.1, P<0.001; C_{dry}, $F_{4,26}$=10.3, P<0.001; Fig. 1). They were constant at T_a from 14 to 25°C (C_{wet}, $F_{1,15}$≤0.503, P≥0.489; C_{dry}, $F_{1,15}$≤1.98, P≥0.180), but there were significant linear and quadratic components for T_a=14 to 35°C (C_{wet}, $F_{1,26}$≥21.2, P<0.001; C_{dry}, $F_{1,26}$≥18.1, P<0.001), reflecting the curvilinear increase in C_{wet} and C_{dry} at the higher T_a.

In thermoneutrality, f_R was 52.0±6.95 min^{-1}, V_T was 0.50±0.088 ml, and $\dot{V}_I$ was 24.8±8.77 ml min^{-1}. There were significant T_a effects for all ventilatory variables ($F_{4,26}$≥0.367, P≤0.017; Fig. 2). f_R decreased markedly with increasing T_a, as demonstrated by a significant linear contrast ($F_{1,26}$=11.6, P=0.002), with no quadratic effect (P=0.071). V_T did not differ between T_a=14 and 25°C, but decreased at 30°C (reverse Helmert contrast, P>0.546 and 0.003, respectively); V_T did not differ between T_a=30 and 33°C (Helmert contrast, P=0.741). $\dot{V}_I$ decreased linearly with increasing T_a ($F_{1,26}$=30.0, P<0.001), with no significant quadratic effect (P=0.497). E_{O_2} increased with T_a ($F_{1,26}$=7.21, P=0.012), from 12.8% at 14°C to 16.4% at 33°C.

EWL_R from the iterative model (Fig. 3) ranged from 1.72±0.31 mg g^{-1} h^{-1} at T_a=14°C to 1.63±0.09 mg g^{-1} h^{-1} at T_a=30°C and 2.13±0.25 mg g^{-1} h^{-1} at T_a=33°C, but there was no significant change between T_a=14 and 33°C (polynomial and reverse Helmert contrasts, $F_{4,26}$=0.762, P=0.599); the increase in EWT_R at T_a=33°C was not significant (P=0.101). EWL_C increased slightly but significantly from 0.43±0.01 mg g^{-1} h^{-1} at T_a=14°C to 0.47±0.01 mg g^{-1} h^{-1} at T_a=20°C (linear contrast, $F_{1,15}$=6.28, P=0.024), and increased further to 0.95±0.01 mg g^{-1} h^{-1} at T_a=30°C
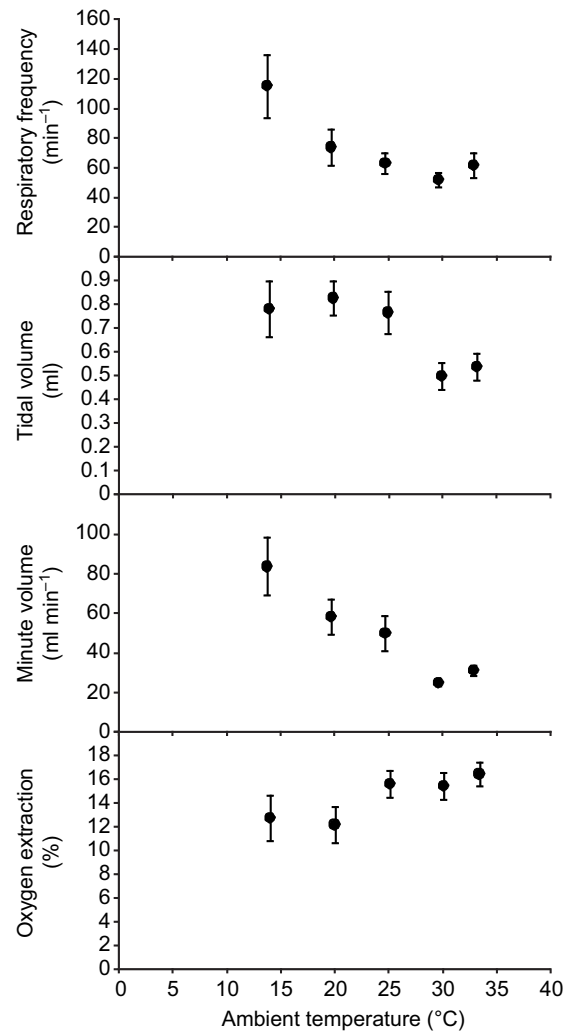

Fig. 2. Respiratory frequency, tidal volume, minute volume and oxygen extraction for normothermic monitos. Values are means ± s.e.m.

(reverse Helmert contrast, $F_{1,26}$=1288, P<0.001) and 1.41±0.06 mg g^{-1} h^{-1} at 33°C (reverse Helmert contrast, $F_{1,26}$=1288, P<0.001), with linear ($F_{1,26}$=3179, P<0.001) and quadratic ($F_{1,26}$=1167, P<0.001) components from T_a=14 to 33°C. EWL_R was 77±5% of calculated EWL_T at T_a=14°C, and decreased to 63% at T_a=30°C and 59±2% at 33°C, with significant linear ($F_{1,26}$=30.3, P<0.001) and quadratic ($F_{1,26}$=9.84, P=0.004) components. Correspondingly, EWL_C was approximately 23% of calculated EWL_T at T_a=14°C, and increased to 41% at T_a=33°C. T_{exp} increased from 13.3°C at T_a=14°C to 16.4, 24.5, 29.5 and 34.2°C at successively higher T_a values. T_{skin} increased from 22.8°C at T_a=10°C to 27.6, 30.1, 30.8 and then 36.0°C at successively higher T_a values. R_{skin} was high at the low T_a values (506, 914 and 659 s cm^{-1} at T_a=14, 20 and 25°C, respectively), and declined to 381 and 311 s cm^{-1} at T_a=30 and 33°C, respectively.

The sensitivity analysis of our EWL partitioning model indicated that the % EWL_R increased with T_{exp}. Increasing T_{exp} from T_a–5°C to T_b resulted in an EWL_R ranging from 53–97% at T_a=14°C to 42–71% at T_a=33°C. The maximum error in our estimated % EWL_R resulting from under/overestimation of T_{exp} would range from approximately –24 to +21%. For example, at a T_a of 14°C, a T_{exp} of 9°C rather than the calculated 13.3°C would decrease EWL_R from
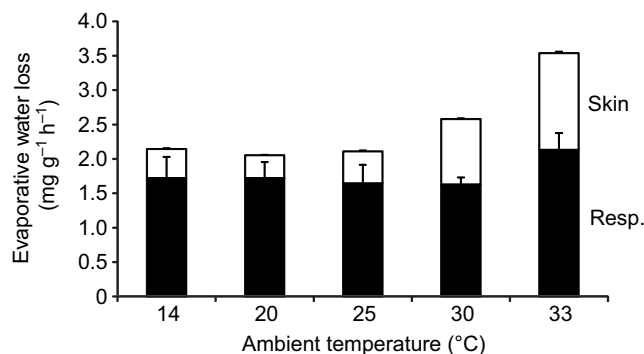
Fig. 3. Partitioning of total evaporative water loss into respiratory and skin components for normothermic monitos. Values are means + s.e.m.

Fig. 4. Evaporative quotient (EWL$_T$/MR) and relative water economy (MWP/EWL$_T$) of normothermic and torpid monitos. Asterisk indicates the point of relative water economy (MWP=EWL$_T$). Values are means ± s.e.m.

77 to 53% of EWL$_T$, and a T_{exp} of 30.1°C (T_b) would increase EWL$_R$ to 97% of EWL$_T$. At a T_a of 33°C, a T_{exp} of 28°C rather than the calculated 34.2°C would decrease EWL$_R$ from 60 to 42% of EWL$_T$, and a T_{exp} of 37.2°C (T_b) would increase EWL$_R$ to 71% of EWL$_T$. The % EWL$_R$ decreased with higher T_{skin}. Increasing T_{skin} from T_a to T_b resulted in a % EWL$_R$ ranging from 87–66% at T_a=14°C to 69–55% at 33°C. The maximum error in our estimated % EWL$_R$ would range from approximately –12 to +11%. For example, at a T_a of 14°C, a T_{skin} of 14°C rather than the calculated 22.8°C would increase EWL$_R$ to 87% from 77% of EWL$_T$, and a T_{skin} of 30.1°C (T_b) would decrease EWL$_R$ to 66% of EWL$_T$. At a T_a of 33°C, a T_{skin}=T_a rather than the calculated 36.0°C would increase EWL$_R$ to 69% from 60% of EWL$_T$, and a T_{skin}=37.2°C (T_b) would decrease EWL$_R$ to 55% of EWL$_T$. The % EWL$_R$ increased with higher R_{skin}. Increasing R_{skin} from 100 to 1000 s cm^{-1} resulted in an EWL$_R$ ranging from 48–82% at T_a=14°C to 28–84% at 33°C. The maximum error in our estimated % EWL$_R$ would range from approximately –42 to +24%. For example, at a T_a of 14°C, an R_{skin} of 100 s cm^{-1} rather than the calculated 506 s cm^{-1} would decrease EWL$_R$ from 77 to 48% of EWL$_T$, and an R_{skin} of 1000 s cm^{-1} would increase EWL$_R$ to 82% of EWL$_T$. At a T_a of 33°C, an R_{skin} of 100 s cm^{-1} rather than the calculated 311 s cm^{-1} would decrease EWL$_R$ from 60% to 28% of EWL$_T$, and an R_{skin} of 1000 s cm^{-1} would increase EWL$_R$ to 84% of EWL$_T$.

There was an effect of T_a on EQ, which increased from 0.61±0.06 at T_a=14°C to 2.14±0.11 at T_a=33°C (Fig. 4). The pattern of T_a response for RWE was described by significant linear ($F_{1,26}$=142, P<0.001) and quadratic ($F_{1,26}$=6.57, P=0.0165) contrasts over the T_a range, but from 14 to 30°C was only linear ($F_{1,21}$=91.2, P<0.001). The PRWE was calculated to be 15.4°C, based on T_a values from 14 to 30°C: RWE=1.70±0.107–0.046±0.005T_a (r^2=0.811, $F_{1,23}$=98.4, P<0.001). RWE of torpid monitos was lower than for normothermic individuals ($F_{2,28}$=4.67, P=0.018) and was significantly affected by T_a ($F_{1,28}$=87.7, P<0.001), with a significant interaction ($F_{2,28}$=18.6, P<0.001) indicating a greater difference between torpid and normothermic RWE at lower T_a. Torpid monitos did not reach a PRWE at low T_a.

DISCUSSION

Thermal, metabolic and hygric responses of monitos to variation in T_a were typical of endotherms in general and other marsupials specifically. The increase in MR with decreasing T_a for thermoregulation directly increased MWP (as RER remained constant). In contrast, EWL$_T$ remained constant below thermoneutrality, as has been demonstrated for many other
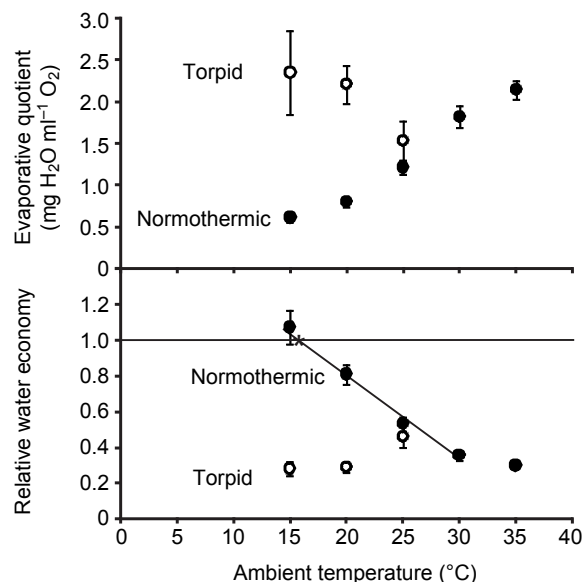
marsupials (e.g. Hinds and MacMillen, 1986; Cooper and Withers, 2002; Larcombe, 2006; Withers and Cooper, 2009a; Withers and Cooper, 2009b). Monitos were thermolabile during normothermia over the 14–33°C T_a range investigated (Fig. 1), with a $\Delta T_b / \Delta T_a$ of 0.32±0.050. The small standard errors for T_b suggest that this thermolability did not reflect an inability to thermoregulate precisely. Rather, thermolability seems to be a strategy to conserve energy at low T_a. For example, MR would have been approximately 4.51 ml O$_2$ g^{-1} h^{-1} at T_a=14°C if T_b had been maintained at thermoneutral T_b=34.6°C (calculated assuming a constant C_{wet}) rather than the actual 3.53 ml O$_2$ g^{-1} h^{-1}; this is an energy saving of 22%. Thermolability also reduces both MWP and EWL, and lowers RWE, although the effect is small because the small change in T_b has nearly equivalent effects on MWP and EWL$_T$. For example, the RWE is estimated to be 1.11 at T_a=14°C if T_b was maintained at 34.6°C, whereas RWE is 1.07 at the actual T_b of 30.1°C. The increase in EWL$_T$ at T_a within and above the thermoneutral zone suggests that monitos become heat stressed at relatively low T_a, reflecting their cool, mesic habitat and the low fitness advantage of water conservation for this species. EWL$_T$ had increased by 22% in thermoneutrality compared with T_a=25°C, and increased further at 33°C by 68%. However, this, even combined with an increased C_{dry}, was insufficient to prevent a hyperthermia of 3.4°C at T_a=33°C (compared with T_b at thermoneutrality).

Standard EWL$_T$ (at 30°C) of monitos conformed to that for other marsupials, being 109% of that predicted for an equivalently sized marsupial [using the minimum variance unbiased estimate (Hayes and Shonkwiler, 2006; Hayes and Shonkwiler, 2007); data set from Warnecke et al. (Warnecke et al., 2010)]. The monitos' EWL$_T$ fell well inside the 95% prediction limits for the log-transformed allometric regression for marsupial EWL$_T$, both before and after accounting for phylogenetic history *via* autoregression (Cheverud and Dow, 1985; Rohlf, 2001) using the mammal 'supertree' (Bininda-Emonds et al., 2007). Two other South American marsupials, the gracile mouse opossum [*Gracilinanus agilis* (Cooper

et al., 2009)] and the woolly mouse opossum [*Micoureus paraguayanus* (Cooper et al., 2010)], also conform closely to the EWL_T allometric relationship for Australian marsupials, as do the monito's presumed Australian relatives, the honey possum [*Tarsipes rostratus* (Cooper and Cruz Neto, 2009)] and the pygmy possum [*Cercartetus nanus* (Bartholomew and Hudson, 1962)], reflecting the conservative physiology of marsupials.

There was no effect of T_a on EWL_T from 14 to 25°C, but EWL_T increased at T_a=30 and 33°C. Little is currently known about the relative contribution of EWL_R and EWL_C components for mammals, particularly below thermoneutrality. Physical partitioning methodologies potentially limit the animal's natural posture and prevent postural changes as a response to varying T_a, they don't completely account for non-respiratory components in estimates of EWL_R (e.g. skin or ocular EWL may be included) and they presumably result in some stress to the animal that might modify both MR and EWL_T (e.g. Tracy and Walsberg, 2000). Indeed, simply handling an animal to place it in a metabolic chamber has a significant, measurable effect on physiological variables, in particular EWL_T, for a considerable period (Hayes et al., 1992; Cooper and Withers, 2009; Page et al., 2011). Invasive partitioning techniques are therefore likely to elevate both EWL_R and EWL_C, and possibly influence the partitioning of EWL_T.

To overcome these issues, we used a non-invasive mathematical modelling approach to partition the EWL_T of monitos into EWL_R and EWL_C, and explain the pattern of EWL_T below thermoneutrality. EWL_R has previously been calculated for unrestrained animals from respiratory ventilation using measured or assumed expired air temperature (e.g. Chew and White, 1960; Withers et al., 1979; Withers and Williams, 1990; Thomas and Cloutier, 1992; Thomas and Geiser, 1997; Withers and Cooper, 2011); EWL_C can then be calculated by difference. The iterative model that we used to solve for the best estimates of EWL_R and EWL_C, as we did not measure T_{exp} for monitos, found EWL_R to be the major component of EWL_T at all T_a. Our estimate of 62% EWL_R at a thermoneutral T_a=30°C is lower than that reported for deer mice [*Peromyscus maniculatus*; 88% (Chew, 1955)], similar to that reported for the numbat [*Myrmecobius fasciatus*; 65% at 25°C (Cooper, 2003)] and house mice and deer mice [*Mus musculus* and *Peromyscus maniculatus*; 59–60% (Edwards and Haines, 1978) and 54% (Chew and Dammann, 1961)], and higher than that for Merriam's kangaroo rat [*Dipodomys merriami*; 38–44% (Tracy and Walsberg, 2000)], the hopping mouse [*Notomys alexis*; 40% (Withers et al., 1979)] and the pallid bat [*Antrozous pallidus*; 22% at 26°C (Chew and White, 1960)]. These species differences could reflect differing experimental methodologies (invasive and potentially disturbed/stressed *versus* non-invasive and basal, and iterative modelling to partition EWL_T), or species differences due to particular morphological or environmental selective pressures on EWL_R and EWL_C, hence the partitioning of EWL_T. Both the kangaroo rat and hopping mouse inhabit arid environments, and a low EWL_R by enhanced counter-current heat and water exchange might explain their high % EWL_C. The large, naked surface area of the wing membranes of bats presumably explains their relatively low EWL_R and high EWL_C. Further measurements of EWL_T partitioning for other species, and a critical evaluation of differing methodologies, seems a fruitful direction for future research.

In the present study, the EWL_R of the monito increased slightly (but not significantly; *P*=0.101) at T_a=33°C, whereas EWL_C increased substantially at T_a=30 and 33°C (Fig. 3). Non-respiratory EWL thus appears to be the primary avenue for the monito to enhance thermoregulatory heat loss at high T_a, although it is still

only 37–41% of EWL_T at T_a=30 and 33°C. EWL_T and EWL_R are constant at low T_a, with EWL_R ranging from 76 to 83% of EWL_T. EWL_C increases slightly from T_a=14 to 25°C, but is only 17 to 24% of EWT_L. Thus, partitioning of EWL_T into EWL_R and EWL_C suggests that the relatively constant EWL_T from T_a=14 to 25°C reflects a fairly constant EWL_R and EWL_C. The constancy of EWL_R is despite the increase in $\dot{V}_I$ at lower T_a (Fig. 2), reflecting the decrease in T_{exp} at lower T_a. The near constancy of EWL_C is expected, based on the relatively small change in saturation water vapour pressure between 14 and 25°C.

Our modelling of EWL_R and EWL_C suggests that T_{exp} was generally similar to T_a, although it was calculated to be 3.6°C less than T_a at 20°C. T_{exp} less than or equal to T_a has been reported for some other small mammals, e.g. kangaroo rats [*Dipodomys merriami* (Jackson and Schmidt-Nielsen, 1964; Schmidt-Nielsen et al., 1970; Collins et al., 1971)], house and deer mice [*Mus musculus*, *Peromyscus maniculatus* (Edwards and Haines, 1978)] and hopping mice [*Notomys alexis* (Withers et al., 1979)]. A low T_{exp} would reduce the EWL_T at low T_a, explaining the relatively constant respiratory component to EWL_T (Fig. 3) despite the increase in $\dot{V}_I$ at low T_a (Fig. 2). T_{skin} was considerably higher than T_a and lower than T_b at low T_a, and approached T_b at higher T_a, a pattern consistent with measurements for other furred species (e.g. Bartholomew, 1956; Edwards and Haines, 1978). Our calculated values for R_{skin} ranged from over 500 s cm^{-1} at T_a<30°C to 300–400 s cm^{-1} at 30 and 33°C. Estimates of R_{skin} from other studies at T_a=25–30°C range from approximately 100 to 300 s cm^{-1} (Chew, 1955; Chew and Dammann, 1961; Edwards and Haines, 1978; Withers et al., 1979; Tracy and Walsberg, 2000). However, simultaneous estimation of T_{skin} and R_{skin} in our iterative model is problematic because of their interacting effect on EWL_C, so our slightly high estimates of R_{skin} might reflect an overestimation of T_{skin}.

The RWE of normothermic monitos increased at lower T_a, reflecting the pattern of increased MWP but constant EWL_T. This pattern of RWE increasing at lower T_a is consistent with that observed for other marsupials (e.g. Cooper and Cruz-Neto, 2009; Cooper et al., 2009; Cooper et al., 2010; Warnecke et al., 2010; Withers and Cooper, 2009a; Withers and Cooper, 2009b) and placentals (e.g. MacMillen and Hinds, 1983; Hinds and MacMillen, 1986), resulting in a PRWE where MWP=EWL_T. The PRWE of 15.4°C for monitos conforms closely to the PRWE of other marsupials [summary data (Cooper and Withers, 2010), with additional data (Warnecke et al., 2010; Schmidt, 2011)], reflecting its mesic environment and thus little requirement for water conservation during normothermia. The PRWE for the related honey possum [17°C (Cooper and Cruz-Neto, 2009)] was higher than that for the monito, reflecting its smaller (5.4 g) body mass, but the monito had a higher PRWE than the similarly sized (29 g) South American gracile mouse opossum [12°C (Cooper et al., 2009)] and the larger (69 g) woolly mouse opossum [11.3°C (Cooper et al., 2010)] despite its very mesic environment. Further data on the PRWE of South American didelphid marsupials are required to interpret patterns in PRWE, but it does appear that they have less favourable PRWEs than marsupials with an Australian derivation, regardless of current distribution and habitat.

During torpor, EWL_T of the monito was reduced to as little as 21% of normothermic EWL_T, which is typical of other mammalian hibernators. Torpid gerbils (*Gerbillus pusillus*) reduce EWL_T to 27% of normothermic values (Buffenstein, 1985) and a variety of bat species reduce EWL_T during torpor to 14–50% of the normothermic value (Carpenter, 1969; Morris et al., 1994; Hosken and Withers, 1997; Hosken and Withers, 1999). These absolute water savings

are generally higher than those measured previously for marsupials that enter torpor daily but do not hibernate [maximum reductions of 32 to 69% of normothermic values (Cooper et al., 2005; Cooper et al., 2009; Withers and Cooper, 2009a; Withers and Cooper, 2009b; Warnecke et al., 2010)], presumably because the lower T_b of hibernators compared with torpidators (e.g. Geiser and Ruf, 1995) increases their absolute hygric and energetic savings.

Torpor has a more profound effect on MWP than EWL_T, with the decrease in MR associated with torpor reducing MWP to as little as 6% of normothermic values. The consequence of this greater reduction in MWP than EWL_T during torpor is a much less favourable RWE. This pattern for monitos is consistent with other marsupials and also placental mammals (e.g. MacMillen, 1965; Buffenstein, 1985; Cooper et al., 2005; Withers and Cooper, 2009a; Withers and Cooper, 2009b; Warnecke et al., 2010), where torpor provides an absolute water savings but reduces RWE. As MWP and EWL_T are the primary avenues of water gain and loss during torpor, the unfavourable RWE during long periods of torpor will presumably result in a long-term negative water balance and could limit torpor duration for hibernators. For example, at T_a=15°C, the RWE of torpid monitos is approximately 0.28, which corresponds to an MWP of 96 mg day^{-1} (for a 30 g individual with a torpid MR of 0.21 ml O$_2$ g^{-1} h^{-1}) and an EWL_T of approximately 0.444 mg g^{-1} h^{-1}, or 320 mg day^{-1}. The differential of –224 mg day^{-1} is equivalent to a 5% mass loss per week, which is not insignificant [torpid *Myotis lucifugus* lose approximately 4.3% of lean body mass before arousing (Kallen, 1964)]. Torpor bout duration increases for monitos with decreasing T_a to 5 days at T_a=12.5°C in winter (Bozinovic et al., 2004), which is consistent with our prediction of a torpor duration of approximately 1 week based on a critical EWL_T of 5% body mass. Thus, our findings for the monito support the idea that the periodic arousals characteristic of hibernators may be at least in part a consequence of water imbalance and the need to drink (Fisher and Manery, 1967; Speakman and Racey, 1989; Thomas and Cloutier, 1992; Thomas and Geiser, 1997).

LIST OF SYMBOLS AND ABBREVIATIONS

A_s	body surface area (cm^2)
BMR	basal metabolic rate (ml O$_2$ g^{-1} h^{-1})
BTPS	body temperature and pressure, saturated
C	thermal conductance (J g^{-1} h^{-1} °C^{-1})
C_{dry}	dry thermal conductance (J g^{-1} h^{-1} °C^{-1})
C_{wet}	wet thermal conductance (J g^{-1} h^{-1} °C^{-1})
E_{O_2}	oxygen extraction efficiency (%)
EHL	evaporative heat loss (J g^{-1} h^{-1})
EQ	evaporative quotient (mg H$_2$O ml^{-1} O$_2$)
EWL_C	cutaneous evaporative water loss (mg g^{-1} h^{-1})
EWL_R	respiratory evaporative water loss (mg g^{-1} h^{-1})
EWL_T	total evaporative water loss (mg g^{-1} h^{-1})
$F_{E_{O_2}}$	fractional excurrent oxygen concentration
f_R	respiratory rate (breaths min^{-1})
M	body mass
MHP	metabolic heat production (J g^{-1} h^{-1})
MR	metabolic rate (ml O$_2$ g^{-1} h^{-1})
MWP	metabolic water production (mg H$_2$O g^{-1} h^{-1})
N	sample size for individuals
n	total sample size for measurements
PRWE	point of relative water economy (°C)
R_{skin}	evaporative resistance of the skin (s cm^{-1})
RER	respiratory exchange ratio (ml CO$_2$ ml^{-1} O$_2$)
RH	relative humidity (%)
RH_{ins}	inspired relative humidity (%)
RWE	relative water economy (dimensionless)
STPD	standard temperature and pressure, dry
T_a	ambient temperature (°C)
T_b	body temperature (°C)
T_{exp}	expired air temperature (°C)
T_{skin}	skin temperature (°C)
$\dot{V}_I$	respiratory minute volume (ml min^{-1})
V_T	tidal volume (ml)
$\dot{V}_{CO_2}$	carbon dioxide production rate (ml CO$_2$ g^{-1} h^{-1})
$\dot{V}_{O_2}$	oxygen consumption rate (ml O$_2$ g^{-1} h^{-1})
χ	absolute water vapour density (mg cm^{-3})

REFERENCES

Bartholomew, G. A. (1956). Temperature regulation in the macropod marsupial, *Setonix brachyurus*. *Physiol. Zool.* **29**, 26-40.

Bartholomew, G. A. (1972). The water economy of seed-eating birds that survive without drinking. *Proc. XVth Int. Ornithol. Congr.* **15**, 237-254.

Bartholomew, G. A. and Hudson, J. W. (1962). Hibernation, aestivation, temperature regulation, evaporative water loss, and heart rate of the pygmy possum, *Cercartetus nanus*. *Physiol. Zool.* **29**, 94-107.

Beck, R. M. D. (2008). A dated phylogeny of marsupials using a molecular supermatrix and multiple fossil constraints. *J. Mammal.* **89**, 175-189.

Beck, R. M. D., Godthelp, H., Weisbecker, V., Archer, M. and Hand, S. J. (2008). Australia's oldest marsupial fossils and their biogeographical implications. *PLoS ONE* **3**, e1858.

Bininda-Emonds, O. R. P., Cardillo, M., Jones, K. E., MacPhee, R. D. E., Beck, R. M. D., Grenyer, R., Price, S. A., Vos, R. A., Gittleman, J. L. and Purvis, A. (2007). The delayed rise of present-day mammals. *Nature* **446**, 507-512.

Bozinovic, F., Ruiz, G. and Rosenmann, M. (2004). Energetics and torpor of a South American "living fossil", the microbiotheriid *Dromiciops gliroides*. *J. Comp. Physiol. B* **174**, 293-297.

Buffenstein, R. (1985). The effect of starvation, food restriction and water deprivation on thermoregulation and average daily metabolic rates in *Gerbillus pusillus*. *Physiol. Zool.* **58**, 320-328.

Cardillo, M., Bininda-Emonds, O. R. P., Boakes, E. and Purvis, A. (2004). A species-level phylogenetic supertree of marsupials. *J. Zool.* **264**, 11-31.

Carpenter, R. E. (1969). Structure and function of the kidney and the water balance of desert bats. *Physiol. Zool.* **42**, 288-302.

Cheverud, J. M. and Dow, M. M. (1985). An autocorrelation analysis of genetic variation due to lineal fission in social groups of rhesus macaques. *Am. J. Phys. Anthropol.* **67**, 113-121.

Chew, R. M. (1955). The skin and respiratory losses of *Peromyscus maniculatus sonoriensis*. *Ecol.* **36**, 463-467.

Chew, R. M. and Dammann, A. E. (1961). Evaporative water loss of small vertebrates, as measured with an infrared analyzer. *Science* **133**, 384-385.

Chew, R. M. and White, H. E. (1960). Evaporative water losses of the pallid bat. *J. Mammal.* **41**, 452-458.

Cohen, B. H. (2008). *Explaining Psychological Statistics*. Hoboken, NJ: John Wiley & Sons.

Collins, J. C., Pilkington, T. C. and Schmidt-Nielsen, K. (1971). A model of respiratory heat transfer in a small mammal. *Biophys. J.* **11**, 886-914.

Cooper, C. E. (2003). Physiological specialisations of the numbat, *Myrmecobius fasciatus* Waterhouse 1836 (Marsupialia: Myrmecobiidae): a unique termitivorous marsupial. PhD thesis, Department of Zoology, University of Western Australia, Perth.

Cooper, C. E. and Cruz-Neto, A. P. (2009). Metabolic, hygric and ventilatory physiology of a hypermetabolic marsupial, the honey possum (*Tarsipes rostratus*). *J. Comp. Physiol. B* **179**, 773-781.

Cooper, C. E. and Withers, P. C. (2002). Metabolic physiology of the numbat (*Myrmecobius fasciatus*). *J. Comp. Physiol. B* **172**, 669-675.

Cooper, C. E. and Withers, P. C. (2009). Effects of measurement duration on the determination of basal metabolic rate and evaporative water loss of small marsupials: how long is long enough? *Physiol. Biochem. Zool.* **82**, 438-446.

Cooper, C. E. and Withers, P. C. (2010). Comparative physiology of Australian quolls (*Dasyurus*; Marsupialia). *J. Comp. Physiol. B* **180**, 857-868.

Cooper, C. E., McAllan, B. M. and Geiser, F. (2005). Effect of torpor on the water economy of an arid-zone marsupial, the stripe-faced dunnart (*Sminthopsis macroura*). *J. Comp. Physiol. B* **175**, 323-328.

Cooper, C. E., Withers, P. C. and Cruz-Neto, A. P. (2009). Metabolic, ventilatory, and hygric physiology of the gracile mouse opossum (*Gracilinanus agilis*). *Physiol. Biochem. Zool.* **82**, 153-162.

Cooper, C. E., Withers, P. C. and Cruz-Neto, A. P. (2010). Metabolic, ventilatory and hygric physiology of a South American marsupial, the long-furred woolly mouse opossum. *J. Mammal.* **91**, 1-10.

Cortés, P., Quijano, S. A. and Nespolo, R. F. (2009). Bioenergetics and inter-individual variation in physiological capacities in a relict mammal – the monito del monte (*Dromiciops gliroides*). *J. Exp. Biol.* **212**, 297-304.

Cortés, P. A., Franco, M., Sabat, P., Quijano, S. A. and Nespolo, R. F. (2011). Bioenergetics and intestinal phenotypic flexibility in the microbiotherid marsupial

(*Dromiciops gliroides*) from the temperate forest in South America. *Comp. Biochem. Physiol.* **160**, 117-124.

Dausmann, K. H. (2005). Measuring body temperature in the field – evaluation of external vs. implanted transmitters in a small mammal. *J. Therm. Biol.* **30**, 195-202.

Dawson, T. J. and Hulbert, A. J. (1970). Standard metabolism, body temperature, and surface areas of Australian marsupials. *Am. J. Physiol.* **218**, 1233-1238.

Edwards, R. M. and Haines, H. (1978). Effects of ambient water vapour pressure and temperature on evaporative water loss in *Peromyscus maniculatus* and *Mus musculus*. *J. Comp. Physiol.* **128**, 177-184.

Fisher, K. C. and Manery, J. F. (1967). Water and electrolyte metabolism in heterotherms. In *Mammalian Hibernation*, Vol. 3 (ed. K. C. Fisher, A. R. Dawe, C. P. Lyman, E. Schönbaum and F. E. South), pp. 235-279. Edinburgh: Oliver and Boyd.

Franco, M., Quijano, A. and Soto-Gamboa, M. (2011). Communal nesting, activity patterns, and population characteristics in the near-threatened monito del monte, *Dromiciops gliroides*. *J. Mammal.* **92**, 994-1004.

Geiser, F. (1994). Hibernation and daily torpor in marsupials: a review. *Aust. J. Zool.* **42**, 1-16.

Geiser, F. (2004). Metabolic rate and body temperature reduction during hibernation and daily torpor. *Annu. Rev. Physiol.* **66**, 239-274.

Geiser, F. and Baudinette, R. V. (1988). Daily torpor and thermoregulation in the small dasyurid marsupials *Planigale gilesi* and *Ningaui yvonneae*. *Aust. J. Zool.* **36**, 473-481.

Geiser, F. and Ruf, T. (1995). Hibernation versus daily torpor in mammals and birds: physiological variables and classification of torpor patterns. *Physiol. Zool.* **68**, 935-966.

Grant, T. R. and Temple-Smith, P. D. (1987). Observations on torpor in the small marsupial *Dromiciops australis* (Marsupialia: Microbiotheriidae) from Southern Chile. In *Possums and Opossums: Studies in Evolution* (ed. M. Archer), pp. 273-277. Sydney: Surrey and Beatty and Royal Zoological Society of New South Wales.

Greer, J. K. (1965). *Mammals of Malleco Province Chile*. Publications of the Museum, Michigan State University, Biological Series **3**, 53-151.

Hayes, J. P. and Shonkwiler, J. S. (2006). Allometry, antilog transformations, and the perils of prediction on the original scale. *Physiol. Biochem. Zool.* **79**, 665-674.

Hayes, J. P. and Shonkwiler, J. S. (2007). Erratum: allometry, antilog transformations, and the perils of prediction on the original scale. *Physiol. Biochem. Zool.* **80**, 556.

Hayes, J. P., Speakman, J. R. and Racey, P. A. (1992). Sampling bias in respirometry. *Physiol. Zool.* **65**, 604-619.

Hershkovitz, P. (1999). Dromiciops gliroides *Thomas, 1894, Last of the Microbiotheria (Marsupialia), With a Review of the Family Microbiotheriidae*. Fieldiana Zoology new series, no. 93. Chicago, IL: Field Museum of Natural History.

Hill, R. W. (1978). Exhalant air temperatures in the Virginia opossum. *J. Therm. Biol.* **3**, 219-221.

Hinds, D. S. and MacMillen, R. E. (1986). Scaling of evaporative water loss in marsupials. *Physiol. Zool.* **59**, 1-9.

Hosken, D. J. and Withers, P. C. (1997). Temperature regulation and metabolism of an Australian bat, *Chalinolobus gouldii* (Chiroptera: Vespertilionidae) when euthermic and torpid. *J. Comp. Physiol. B* **167**, 71-80.

Hosken, D. J. and Withers, P. C. (1999). Metabolic physiology of normothermic and torpid lesser long-eared bats, *Nyctophilus geoffroyi* (Chiroptera: Vespertilionidae). *J. Mammal.* **80**, 42-52.

Humphries, M. M., Thomas, D. W. and Kramer, D. L. (2003). The role of energy availability in Mammalian hibernation: a cost-benefit approach. *Physiol. Biochem. Zool.* **76**, 165-179.

Jackson, D. C. and Schmidt-Nielsen, K. (1964). Countercurrent heat exchange in the respiratory passages. *Proc. Natl. Acad. Sci. USA* **51**, 1192-1197.

Jones, C. J. and Geiser, F. (1992). Prolonged and daily torpor in the feathertail glider, *Acrobates pygmaeus* (Marsupialia: Acrobatidae). *J. Zool.* **227**, 101-108.

Kallen, F. C. (1964). Some aspects of water balance in the hibernating bat. *Ann. Acad. Sci. Fenn. Ser. 4* **71**, 257-267.

Larcombe, A. (2006). Comparative metabolic, thermoregulatory and ventilatory physiology of bandicoots (Peramelidae). PhD thesis, Department of Zoology, University of Western Australia, Perth.

Lovegrove, B. G. (2000). The zoogeography of mammalian basal metabolic rate. *Am. Nat.* **156**, 201-219.

MacMillen, R. E. (1965). Aestivation in the cactus mouse, *Peromyscus eremicus*. *Comp. Biochem. Physiol.* **16**, 227-248.

MacMillen, R. E. and Hinds, D. S. (1983). Water regulatory efficiency in heteromyid rodents: a model and its application. *Ecology* **64**, 152-164.

Malan, A. (1973). Ventilation measured by body plethysmography in hibernating mammals and in poikilotherms. *Respir. Physiol.* **17**, 32-44.

Mann, G. F. (1955). Monito del monte *Dromiciops australis* Philippi. *Invest. Zool. Chil.* **2**, 159-166.

Marshall, L. G. (1978). *Dromiciops australis*. *Mamm. Species* **99**, 1-5.

McNab, B. K. (1984). Physiological convergence amongst ant-eating and termite-eating mammals. *J. Zool.* **203**, 485-510.

McNab, B. K. (1986). Food habits, energetics, and the reproduction of marsupials. *J. Zool.* **208**, 595-614.

McNab, B. K. (2002). *The Physiological Ecology of Vertebrates: A View From Energetics*. Ithaca, NY: Cornell University Press.

McNab, B. K. (2005). Uniformity in the basal metabolic rate of marsupials: its causes and consequences. *Rev. Chil. Hist. Nat.* **78**, 183-198.

McNab, B. K. (2008). An analysis of the factors that influence the level and scaling of mammalian BMR. *Comp. Biochem. Physiol.* **151**, 5-28.

Morris, S., Curtin, A. L. and Thompson, M. B. (1994). Heterothermy, torpor, respiratory gas exchange, water balance and the effect of feeding in Gould's long-eared bat *Nyctophilus gouldi*. *J. Exp. Biol.* **197**, 309-335.

Nespolo, R. F., Verdugo, C., Cortés, P. A. and Bacigalupe, L. D. (2010). Bioenergetics of torpor in the microbiotherid marsupial, monito del monte (*Dromiciops gliroides*): the role of temperature and food availability. *J. Comp. Physiol. B* **180**, 767-773.

Nilsson, M. A., Churakov, G., Sommer, M., Tran, N. V., Zemann, A., Brosius, J. and Schmitz, J. (2010). Tracking marsupial evolution using archaic genomic retroposon insertions. *PLoS Biol.* **8**, e1000436.

Page, A. J., Cooper, C. E. and Withers, P. C. (2011). Effects of experiment start time and duration on measurement of standard physiological variables. *J. Comp. Physiol.* **181**, 657-665.

Parish, O. O. and Putnam, T. W. (1977). Equations for the determination of humidity from dewpoint and psychrometric data. NASA Technical Note, NASA TN D-8401.

Reig, O. A. (1955). Noticia preliminary sobre la presencia de microbiotherinos vivientes en la fauna Sudamericana. *Invest. Zool. Chil.* **2**, 121-130.

Rohlf, F. J. (2001). Comparative methods for the analysis of continuous variables: geometric interpretations. *Evolution* **55**, 2143-2160.

Schmid, W. D. (1976). Temperature gradients in the nasal passage of some small mammals. *Comp. Biochem. Physiol.* **54A**, 305-308.

Schmidt, S. (2011). Ecological physiology of macropod marsupials. PhD thesis, School of Animal Biology, University of Western Australia, Perth.

Schmidt-Nielsen, K., Hainsworth, F. R. and Murrish, D. E. (1970). Counter-current heat exchange in the respiratory passages: effect on water and heat balance. *Respir. Physiol.* **9**, 263-276.

Speakman, J. R. and Racey, P. A. (1989). Hibernal ecology of the pipistrelle bat: energy expenditure, water requirements and mass loss, implications for survival and the function of winter emergence flights. *J. Anim. Ecol.* **58**, 797-813.

Szewczak, J. M. and Powell, F. L. (2003). Open-flow plethysmography with pressure-decay compensation. *Respir. Physiol. Neurobiol.* **134**, 57-67.

Thomas, D. W. and Cloutier, D. (1992). Evaporative water loss by hibernating little brown bats, *Myotis lucifugus*. *Physiol. Zool.* **65**, 443-456.

Thomas, D. W. and Geiser, F. (1997). Periodic arousals in hibernating mammals: is evaporative water loss involved? *Funct. Ecol.* **11**, 585-591.

Tracy, R. L. and Walsberg, G. E. (2000). Prevalence of cutaneous evaporation in Merriam's kangaroo rat and its adaptive variation at the subspecific level. *J. Exp. Biol.* **203**, 773-781.

Walsberg, G. E. (1978). The relationship of the external surface area of birds to skin surface area and body mass. *J. Exp. Biol.* **76**, 185-189.

Warnecke, L., Cooper, C. E., Geiser, F. and Withers, P. C. (2010). Environmental physiology of a small marsupial inhabiting arid floodplains. *Comp. Biochem. Physiol.* **157**, 73-78.

Webster, M. D. and Campbell, G. S. (1985). Cutaneous resistance to water-vapor diffusion in pigeons and the role of the plumage. *Physiol. Zool.* **58**, 58-70.

White, C. R. and Seymour, R. S. (2003). Mammalian basal metabolic rate is proportional to body mass$^{2/3}$. *Proc. Natl. Acad. Sci. USA* **100**, 4046-4049.

White, C. R. and Seymour, R. S. (2005). Allometric scaling of mammalian metabolism. *J. Exp. Biol.* **208**, 1611-1619.

Withers, P. C. (1992). *Comparative Animal Physiology*. Philadelphia, PA: Saunders College Publishing.

Withers, P. C. (2001). Design, calibration and calculation for flow-through respirometry systems. *Aust. J. Zool.* **49**, 445-461.

Withers, P. C. and Cooper, C. E. (2009a). Thermal, metabolic, hygric and ventilatory physiology of the sandhill dunnart (*Sminthopsis psammophila*; Marsupialia, Dasyuridae). *Comp. Biochem. Physiol.* **153**, 317-323.

Withers, P. C. and Cooper, C. E. (2009b). Thermal, metabolic, and hygric physiology of the little red kaluta, *Dasykaluta rosamondae* (Dasyuromorphia: Dasyuridae). *J. Mammal.* **90**, 752-760.

Withers, P. C. and Cooper, C. E. (2011). Using a priori contrasts for multivariate repeated-measures ANOVA to analyze thermoregulatory responses of the dibbler (*Parantechinus apicalis*; Marsupialia, Dasyuridae). *Physiol. Biochem. Zool.* **84**, 514-521.

Withers, P. C. and Williams, J. B. (1990). Metabolic and respiratory physiology of an arid-adapted Australian bird, the Spinifex pigeon. *Condor* **92**, 961-969.

Withers, P. C., Lee, A. K. and Martin, R. W. (1979). Metabolism, respiration and evaporative water loss in the Australian hopping mouse *Notomys alexis* (Rodentia: Muridae). *Aust. J. Zool.* **27**, 195-204.

Withers, P. C., Cooper, C. E. and Larcombe, A. N. (2006). Environmental correlates of physiological variables in marsupials. *Physiol. Biochem. Zool.* **79**, 437-453.

Zar, J. (1999). *Biostatistical Analysis*. Upper Saddle River, NJ: Prentice Hall.