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

Philip C. Withers1,2,*, Christine E. Cooper1,2 and Roberto F. Nespolo3

1School of Animal Biology M092, University of Western Australia, Crawley, WA 6009, Australia, 2Department of Environment and Agriculture, Curtin University, PO Box U1987, Perth, WA 6845, Australia and 3Instituto de Ecología y Evolución, Universidad Austral de Chile, Casilla 567, Valdivia, Chile

*Author for correspondence (philip.withers@uwa.edu.au)

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.

Received 19 January 2012; Accepted 19 April 2012

INTRODUCTION
Understanding the effects of ambient temperature (Tₐ) on thermal and metabolic physiology is essential to understanding the thermoregulatory abilities of animals. The effects of Tₐ 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 (T₉) 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 (EWLT) (e.g. Withers et al., 2006). Consequently, a clear understanding of the factors determining EWLT, including the effects of T₉, 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 EWLT with T₉ (or, commonly, the lack of change) requires partitioning of EWLT into its respiratory (EWLR) and cutaneous (EWLC) components to understand the effects of T₉ on each.

Relative water economy (RWE) is the ratio of metabolic water production (MWP) to EWLT (MacMillen and Hinds, 1983; Hinds and MacMillen, 1986). The effect of T₉ on RWE reflects the patterns of T₉ on MWP and EWLT (e.g. Bartholomew, 1972; MacMillen and Hinds, 1983; Hinds and MacMillen, 1986; Cooper et al., 2005). For normothermic endotherms, MWP typically increases at low T₉ whereas EWLT is relatively constant, hence RWE increases at low T₉. The point of relative water economy (PRWE) is the T₉ where MWP=EWLT (i.e. RWE=1), which reflects the relative water balance of a species. For heterothermic endotherms, low T₉ 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 (Cortez 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 (\( \alpha \)). Cortés et al. (Cortés et al., 2009) described the energetics of the monito at \( T_a=20^\circ C \) and Nespolo et al. (Nespolo et al., 2010) further described the bioenergetics of euthermic 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 ecorrent humidity during respirometric measurement for the monito but did not calculate its EWLT for comparison with other species. We present here the first EWLT data for monitos, the first value for relative water economy of a marsupial hibernator, and non-invasively partition EWLT into cutaneous and respiratory components. We hypothesise that the monito will have a high EWLT and poor RWE, reflecting its mesic habitat and high water content diet, and that deep torpor will reduce both EWLT 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 \( 39^\circ 48^\prime S, 73^\circ 14^\prime W \), 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 \( 45 \times 30 \times 20 \) 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 \( V_{O2} \), carbon dioxide production \( V_{CO2} \) and EWLT 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 \( T_a=30^\circ C \) and six were measured at all other \( T_a \). Experiments were conducted for a period of 6–10h 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 \( \pm 0.1^\circ \)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 \( \pm 0.1^\circ \)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\(^2\) 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). Ecorrent air was passed over a thin-film capacitance relative humidity (RH) probe (MNPN45A, 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 EWLT 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 20s. \( V_{O2}, V_{CO2} \) and EWLT 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 \( V_{CO2}/V_{O2} \) and EWLT/\( V_{O2} \), respectively. MR was converted to MWP and metabolic heat production (MHP) using the measured RER for that experiment after Withers (Withers, 1992). EWLT was converted to evaporative heat loss (EHL) using 2.4 J mg\(^{-1} \)H\(_2\)O (McNab, 2002). RWE was calculated as MWP/EWLT, 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 Gillan 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 (0% \( CO_2 \)) 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 ($f_R; \text{min}^{-1}$) and tidal volume ($V_T; \text{ml}$) after Malan (Malan, 1973) using custom-written software (Visual Basic v6). Respiratory minute volume ($V_e; \text{ml min}^{-1}$) was calculated as $f_R \times V_T$ and oxygen extraction efficiency ($E_{O2}$) was calculated at the time of breathing measurements as $E_{O2}=100 \times (V_{O2,\text{exp}} - (0.2095 + F_{EO2})/2) \times V_{e}$, where $F_{EO2}$ is the mean O2 fraction of excurrent air. Ventilatory variables are presented as body temperature and pressure saturated (BTPS), but $E_{O2}$ was calculated from $V_e$ 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 $V_e$, based on expired humidity (assumed to be 100%) and temperature ($T_{\text{exp}}$; °C), and inspired (chamber) humidity (RH$_{\text{ins}}$) and temperature ($T_{a}$; °C): EWL$_R=\{60/M\} (V_e/1000) [\chi_{\text{exp}} - (\chi_{Ta} \times RH_{\text{ins}}/100)]$, where $M$ is mass and $\chi$ is the water vapour density for inspired air ($\chi_{Ta}$) and expired air ($\chi_{\text{exp}}$) at the appropriate temperature [$\chi$ was calculated after Parrish and Putnam (Parrish and Putnam, 1977)].

EWL$_C$ (mg g$^{-1}$ h$^{-1}$) was calculated from skin temperature ($T_{\text{skin}}$; °C), cutaneous evaporative resistance ($R_{\text{skin}}$; s cm$^{-1}$) and body surface area [$A_s$; calculated as $10 A^{0.67}$ cm$^2$ (Dawson and Hulbert, 1970; Walsberg and King, 1978)]; EWL$_C=(3.6/R_s) (A_s/M) (T_{\text{skin}} - (\chi_{\text{skin}} \times RH_{\text{ins}}/100))/R_{\text{skin}}$ where $\chi_{\text{skin}}$ is the water vapour density for air at skin temperature (100% RH) and $\chi_{Ta}$ is the water vapour density for air at ambient temperature/humidity. The values of $T_{\text{skin}}$, $R_{\text{skin}}$ and $T_{\text{exp}}$ 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 $T_{\text{skin}}$, $R_{\text{skin}}$ and $T_{\text{exp}}$. $T_{\text{exp}}$ 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), $T_{\text{skin}}$ in steps of 0.01°C from $T_a$ to $T_b$ (e.g. Bartholowem, 1956; Edwards and Haines, 1978; Webster and Campbell, 1985; Dausmann, 2005) and $R_{\text{skin}}$ in steps of 1 cm$^{-1}$ from 100 to 1000 cm$^{-1}$ (e.g. Webster and Campbell, 1985; Withers, 1992) (see Discussion). Estimation of EWL$_C$ is dependent on the interaction between $T_{\text{skin}}$ and $R_{\text{skin}}$ (i.e. a decrease in $T_{\text{skin}}$ can counterbalance a decrease in $R_{\text{skin}}$, at constant EWL$_R$), 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 $T_{\text{skin}}$, $T_{\text{exp}}$ and $R_{\text{skin}}$ 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 statistixXL (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 $T_a=30^\circ C$ to 36.4±2.37 g at $T_a=20^\circ C$.

Monitos remained normothermic at $T_a\geq30^\circ C$, but entered torpor [defined as a $V_{O2}$ 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 $T_a=14^\circ C$ to 37.2±0.35°C at $T_a=33^\circ C$. Thermoneutral $T_b$ (at $T_a=30^\circ C$; see below) was 34.6±0.34°C. Body temperature of torpid monitos

---

**Fig. 1.** Body temperature ($T_a$), metabolic water production, total evaporative water loss, and wet ($C_{\text{wet}}$) and dry ($C_{\text{dry}}$) thermal conductance of monitos. Values are means ± s.e.m. $T_a$, ambient temperature.
decreased with \( T_a \) (\( F_{2,2a}=38.9, P<0.001 \)) and was significantly lower compared with euthermic monitos (\( F_{1,2a}=139, P<0.001 \)) at \( T_a=14^\circ \)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 \( V_{O2} \) and \( V_{CO2} \), as there was no effect of \( T_a \) on RER (\( F_{1,2a}=0.10, P=0.749 \)), which was 0.91±0.014 (\( N=8, n=31 \)) over all \( T_a \). The lowest MR of 1.42±0.052 ml O² g⁻¹ h⁻¹ at \( T_a=30^\circ \)C, which we consider to be BMR, corresponds to a MWP of 0.89±0.044 mg H₂O g⁻¹ h⁻¹. MWP was strongly influenced by \( T_a \) (\( F_{2,2a}=61.9, P<0.001 \)), increasing to 2.2±0.12 mg H₂O g⁻¹ h⁻¹ at \( T_a=14^\circ \)C (Fig. 1). There was a significant linear relationship for MWP at \( T_a=14^\circ \) to 30°C (\( F_{1,2a}=8.8, P<0.001 \)), and MWP increased to 1.05±0.06 mg H₂O g⁻¹ h⁻¹ at \( T_a=33^\circ \)C (significant linear and quadratic contrasts for \( T_a=14^\circ \) to 33°C; \( F_{1,2a}=24.5, P<0.001 \)). 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 \( T_a \) (\( F_{1,2a}=53.1, P<0.001 \)). There was a significant interaction (\( F_{2,2a}=69.1, P<0.001 \)) between torpor and \( T_a \) effects on MWP, with MWP decreasing more below normothermia at lower \( T_a \) (Fig. 1).

Standard EWLₜ (at thermoneutrality, 30°C) was 2.6±0.21 mg H₂O g⁻¹ h⁻¹. There was an overall effect of \( T_a \) on EWLₜ (\( F_{3,2a}=10.8, P<0.001 \); Fig. 1). EWLₜ did not change between \( T_a \) of 14 and 20°C (no significant reverse Helmert, linear or quadratic contrasts; \( F_{1,2a}=0.871, P=0.397 \)) but increased at \( T_a=30^\circ \)C (\( F_{1,2a}=5.25, P=0.030 \)) and 33°C (\( F_{1,2a}=38.5, P<0.001 \)) and linearly from 25 to 33°C (\( F_{1,2a}=25.9, P<0.001 \)). EWLₜ of torpid monitos decreased with \( T_a \) (\( F_{2,2a}=4.94, P=0.015 \)) and was significantly lower compared with normothermic monitos at the same \( T_a \) (\( F_{1,2a}=85.0, P<0.001 \)). The significant interaction between torpor and \( T_a \) effects on EWLₜ (\( F_{2,2a}=5.72, P=0.008 \)) indicated that EWLₜ during torpor decreased more below normothermia at lower \( T_a \), with absolute water savings resulting from torpor of 0.68 mg H₂O g⁻¹ h⁻¹ at 25°C to 1.70 mg H₂O g⁻¹ h⁻¹ at 14°C.

Fig. 2. Respiratory frequency, tidal volume, minute volume and oxygen extraction for normothermic monitos. Values are means ± s.e.m.
77 to 53% of EWL_T, and a T_a of 30.1°C (T_a) 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 by 60 to 42% of EWL_T, and a T_exp of 37.2°C (T_a) would increase EWL_R to 71% of EWL_T. The % EWL_R decreased with higher T_s. Increasing T_s 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_s of 14°C rather than the calculated 22.8°C would increase EWL_R to 87% from 77% of EWL_T, and a T_s of 30.1°C (T_a) would decrease EWL_R to 66% of EWL_T. At a T_a of 33°C, a T_s of 36°C would increase EWL_R to 69% from 60% of EWL_T, and a T_s of 37.2°C (T_a) would decrease EWL_R to 55% of EWL_T. The % EWL_R increased with higher T_s. Increasing R_s from 100 to 1000 cm⁻¹ resulted in an EWL_R ranging from 48–82% at T_a=14°C to 58–84% 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_s of 100 cm⁻¹ rather than the calculated 506 cm⁻¹ would decrease EWL_R from 77 to 84% of EWL_T, and an R_s of 1000 cm⁻¹ would increase EWL_R to 82% of EWL_T. At a T_a of 33°C, an R_s of 100 cm⁻¹ rather than the calculated 311 cm⁻¹ would decrease EWL_R from 60 to 28% of EWL_T, and an R_s of 1000 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 (F₁,₂₅=142, P<0.001) and quadratic (F₁,₂₅=6.57, P=0.0165) contrasts of T_a range, but from 14 to 30°C was only linear (F₁,₂₅=91.2, P<0.001). The PRWE was calculated to be 15.4°C, based on a range, but from 14 to 30°C was only linear (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, 2009b; Withers and Cooper, 2009b). Monitos were thermolabile during normothermia over the 14–33°C T_a range investigated (Fig. 1), with a ΔT_a/ΔTₜ of 0.32±0.050. The small standard errors for T_s 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 O₂ g⁻¹ h⁻¹ at T_a=14°C if T_b had been maintained at thermoneutral Tₜ=34.6°C (calculated assuming a constant Cₜₚₐ) rather than the actual 3.53 ml O₂ 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 EWL 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 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 Cₜₚₐ, 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

---

**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 (EWLₚ/MR) and relative water economy (MWP/EWLₚ) of normothermic and torpid monitos. Asterisk indicates the point of relative water economy (MWP→EWLₜ). Values are means ± s.e.m.
et al., 2009]) and the woolly mouse opossum [Monodelphis domestica (Cooper et al., 2010)], also conform closely to the EWL_T allometric relationship for Australian marsupials, as do the montito’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 for unrestrained animals from particular EWLT, for a considerable period (Hayes et al., 1992; Cooper and Withers, 2009; Page et al., 2011). Invassive partitioning techniques are therefore likely to elevate both EWL_R and EWL_C, and possibly influence the partitioning of EWLT.

To overcome these issues, we used a non-invasive mathematical modelling approach to partition the EWLT of monitos into EWL_R and EWL_C, and explain the pattern of EWLT below thermoneutrality. EWL_R has previously been calculated for unrestrained animals from respiratory ventilation measured using assumed air temperature (e.g. Chew and White, 1960; Cooper et al., 2009; Page et al., 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 EWLT 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 [Peromysculus 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 EWLT), or species differences due to particular morphological or environmental selective pressures on EWL_R and EWL_C, hence the partitioning of EWLT. 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 EWLT 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 montito 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 montito 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_T. Thus, partitioning of EWLT 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 $V_i$ 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 EWLT at low $T_a$, explaining the relatively constant respiratory component to EWLT (Fig. 3) despite the increase in $V_i$ at lower $T_a$ (Fig. 2). $T_{skin}$ was considerably higher than $T_a$ and lower than $T_L$ at low $T_a$, and approached $T_a$ 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 cm$^{-1}$ at $T_a=30°C$ to 300–400 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 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 EWLT, 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 EWLT. 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 placental species (e.g. MacMillen and Hinds, 1983; Hinds and MacMillen, 1986), resulting in a PRWE where MWP=EWLT. 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 montito, reflecting its smaller (5.4 g) body mass, but the montito 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, EWLT of the montito was reduced to as little as 21% of normothermic EWLT, which is typical of other mammalian hibernators. Torpid gerbils (Gerbillus pusillus) reduce EWLT to 27% of normothermic values (Buffenstein, 1985) and a variety of bat species reduce EWLT 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 hygic and energetic savings.

Torpor has a more profound effect on MWP than EWLT, 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 EWLT 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 EWLT 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 without drinking. The differential of –224 (or 320) mg g$^{-1}$ 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 EWLT 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$^{-1}$)
- $C_w$: wet thermal conductance (J g$^{-1}$ h$^{-1}$ C$^{-1}$)
- $E_{O_2}$: oxygen extraction efficiency (%)
- EHL: evaporative heat loss (J g$^{-1}$ h$^{-1}$)
- $F_{co_2}$: fractional excurrent oxygen concentration
- $f_R$: respiratory rate (breath 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)
- $R_{e_in}$: evaporative resistance of the skin (s cm$^{-1}$)
- RER: respiratory exchange ratio (ml CO$_2$ ml$^{-1}$ O$_2$)
- RH: relative humidity (%)
- RH$_{in}$: inspired relative humidity (%)
- RWE: relative water economy (dimensionless)
- STPD: standard temperature and pressure, dry
- $T_{exp}$: expired air temperature (°C)
- $T_{skin}$: skin temperature (°C)
- $V_1$: respiratory minute volume (ml min$^{-1}$)
- $V_T$: tidal volume (ml)
- $V_{CO_2}$: carbon dioxide production rate (ml CO$_2$ g$^{-1}$ h$^{-1}$)
- $V_{O_2}$: oxygen consumption rate (ml O$_2$ g$^{-1}$ h$^{-1}$)
- $x$: absolute water vapour density (mg cm$^{-3}$)

### ACKNOWLEDGEMENTS

We thank Pablo Cortés and Marcela Franco for their enthusiastic help throughout the study, and the anonymous referees for their useful comments.

### REFERENCES
