

Allometry of evaporative water loss in marsupials: implications of the effect of ambient relative humidity on the physiology of brushtail possums (*Trichosurus vulpecula*)

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SUMMARY

To better understand the effects of ambient relative humidity (RH) on physiological variables and the implications of RH-correcting evaporative water loss (EWL) data for marsupials, we examined the effect of RH on EWL, body temperature (T_b), metabolic rate (MR) and thermal conductance (C) of the brushtail possum (*Trichosurus vulpecula*), a medium-sized marsupial. Correcting EWL data for 27 species of marsupial for water vapour pressure deficit (ΔWVP) in the chamber during measurement significantly increased, rather than decreased, the variability of the allometric relationship for EWL. For the brushtail possum, both ambient temperature (T_a) and RH significantly affected EWL. At $T_a=25^\circ\text{C}$, EWL was independent of RH at $\leq 63\%$ RH, but decreased linearly at higher RH values. At $T_a=30^\circ\text{C}$, EWL was significantly related to RH from 26% to 92% RH. There was a significant effect of T_a on T_b and dry thermal conductance (C_{dry} ; higher at 30°C), but no effect of RH. For MR and wet thermal conductance (C_{wet}) there was a significant effect of T_a (MR higher and C_{wet} lower at 25°C), and RH at $T_a=30^\circ\text{C}$ (MR higher and C_{wet} lower at the lowest RH) but not at 25°C . Our results indicate that brushtail possums do not necessarily show the linear relationship between ambient RH and EWL expected for an endotherm, possibly because of behavioural modification of their immediate microclimate. This may account for the failure of WVP deficit correction to improve the allometric EWL relationship for marsupials. Chamber RH is an important environmental factor to be considered when measuring standard physiological variables such as MR and C_{wet} .

Key words: evaporative water loss, respirometry, relative humidity, marsupial, brushtail possum, methodology, water vapour pressure deficit

INTRODUCTION

Evaporative water loss (EWL) is an important aspect of a terrestrial animal's physiology, as it is a substantial component of the water budget and also the primary means of dissipating body heat at high ambient temperature (T_a). Water evaporates from the skin surface as cutaneous water loss, and from the respiratory surfaces as respiratory water loss. The relative contributions of these two avenues to the animal's total EWL varies with species, activity, morphology and physiology, e.g. counter-current heat and water exchange structures in the nasal passages, and presence or absence of cutaneous sweat glands (e.g. Hulbert and Rose, 1972; Hulbert and Dawson, 1974; Dawson, 1969; Dawson et al., 1969; Dawson, 1973; MacMillen and Hinds, 1983; Bell et al., 1983; Hinds and MacMillen, 1986; Schmidt-Nielsen et al., 1970).

Total EWL (EWL hereafter refers to total EWL unless otherwise stated) is in turn affected by factors that influence cutaneous and/or respiratory water loss, including activity, and a number of environmental parameters including water vapour pressure (WVP) and T_a . The principal environmental factor determining EWL is the water vapour pressure deficit (ΔWVP) between an animal and ambient air (Christian, 1978). High ambient relative humidity, RH (small ΔWVP), retards EWL, whereas low RH (large ΔWVP) enhances EWL (Lasiewski et al., 1966; Proctor and Studier, 1970). Thus animals should have higher EWL under drier ambient conditions. This relationship between EWL and ambient WVP is

generally inverse and linear for small animals including birds (Lasiewski et al., 1966), rodents (Baudinette, 1972; Christian, 1978; Edwards and Haines, 1978) and bats (Proctor and Studier, 1970; Webb et al., 1995). There is also in theory an influence of ambient RH on metabolic rate, as reduced evaporative heat loss due to high RH and low WVP will decrease wet thermal conductance (C_{wet}) and thus might reduce metabolic heat production and/or increase body temperature (T_b) and dry thermal conductance (C_{dry}). This is of particular importance at T_a values above the upper critical temperature (T_{uc}) of the thermoneutral zone where an endotherm must dissipate much or even all of its metabolic heat production by evaporative heat loss. A high ambient RH may therefore reduce or prevent heat loss, having dire thermoregulatory consequences. There is, however, little evidence of a relationship between RH and metabolic rate (MR) and/or T_b below T_{uc} (Proctor and Studier, 1970; Baudinette, 1972; Ewing and Studier, 1973; Kay, 1975).

The standard technique for studying metabolism and EWL of endotherms in the laboratory is flow-through respirometry, where dry air flows through a metabolic chamber containing an animal, and the gas composition (water vapour, oxygen and/or carbon dioxide) of the excurrent air is measured (e.g. Withers, 2001). However, the flow rate together with the T_a and EWL of the animal will determine the RH within the metabolic chamber, as the water evaporated from the animal will mix with incoming dry air and humidify the chamber air (Lasiewski et al., 1966). This

humidification will potentially reduce EWL and evaporative heat loss (and thus affect T_b and/or MR). Low flow rates are often used in flow-through respirometry to maximise the O_2/CO_2 differential between incurrent and excurrent air to ~1% (e.g. Willis and Cooper, in press) but may not be optimal in terms of chamber washout and the maintenance of a low ambient RH within the chamber, especially at high T_a (Lasiewski et al., 1966). Chamber RH is often not considered in flow-through respirometry experiments (despite its potential to influence MR and EWL, particularly at high T_a). The effect of ambient RH on EWL makes it difficult to (1) compare EWL and possibly MR between different T_a values within a single study, since ambient RH changes with T_a , and (2) compare 'standard' values, e.g. basal metabolic rate (BMR) and standard EWL, between studies (e.g. Cooper and Withers, 2002; Cooper et al., 2005) because ambient RH differs with varying flow rate and T_a . Some authors have attempted to correct the EWL of birds and mammals for the effect of ambient RH (e.g. Salt, 1964; Lasiewski et al., 1966; Coulombe, 1970; Larcombe et al., 2003; Larcombe, 2004; Larcombe and Withers, 2006; Larcombe et al., 2006) but to do this effectively the relationship between EWL and RH must be well understood.

For marsupials, there is a strong allometric effect on EWL (Withers et al., 2006); $\log_{10}EWL=0.96+0.68\log_{10}M$ with $R^2=0.95$, i.e. body mass (M , in g) explains 95% of the variability in EWL ($mg\ h^{-1}$). Presumably some of the remaining variability is explained by differences in measurement technique (gravimetric vs hygrometric) and respirometry conditions (flow rate and chamber ambient RH). To better understand the allometry of EWL in marsupials and sources of variability in the data, we examined here the effects of correcting EWL for ambient ΔWVP . To interpret the implications of RH correction on the EWL data for marsupials, we measured the effect of ambient RH on the EWL (and T_b , MR, C_{wet} and C_{dry}) of a medium-sized marsupial, the brushtail possum (*Trichosurus vulpecula* Kerr 1792).

MATERIALS AND METHODS

Allometry of EWL

EWL data were obtained from the literature for 27 species of marsupial (Bartholomew and Hudson, 1962; Cooper and Withers, 2002; Cooper et al., 2005; Cooper et al., in press; Dawson, 1969; Dawson, 1973; Dawson and Bennett, 1978; Dawson and Degraïbrielle, 1973; Dawson et al., 1969; Hinds and MacMillen, 1986; Hudson and Dawson, 1975; Larcombe, 2004; Withers, 1992a; Withers et al., 1990). These data were analysed to determine the allometry of EWL, and to examine the effect of correction for chamber RH on the allometric relationship for marsupials. The chamber WVP (in Torr; 1 Torr \approx 133 Pa) for each species was obtained from the data source or was calculated from flow rate, T_a and EWL. EWL ($mg\ h^{-1}$) was corrected for the ΔWVP [calculated as $\Delta WVP=WVP_{sat}-WVP_{chamber}$, where WVP_{sat} is WVP saturation at T_a (Coulombe, 1970; Edwards and Haines, 1978)] to units of $mg\ H_2O\ h^{-1}\ Torr^{-1}$. Allometric relationships for log-transformed uncorrected EWL data ($mg\ H_2O\ h^{-1}$), and EWL corrected for ΔWVP ($mg\ H_2O\ h^{-1}\ Torr^{-1}$), were determined by least squares linear regression, and the variance ratio test (Zar, 1999) was used to test for differences in remaining variance for uncorrected and corrected allometries, using the regression residual mean square errors.

Brushtail possums

Six adult male brushtail possums were captured at Mount Caroline Nature Reserve (31°47'S, 117°38'E), near Quairading, Western Australia. The possums were housed at Curtin University in large outdoor enclosures, where they experienced natural weather and

photoperiod for Perth, Western Australia. Possums were fed rabbit and guinea pig pellets, fruit, vegetables, cheese and *Eucalyptus* leaves, with *ad libitum* water. Experiments were conducted from November to January 2007/2008.

Metabolic rate (oxygen consumption, \dot{V}_{O_2} ; $ml\ O_2\ g^{-1}\ h^{-1}$), carbon dioxide production (\dot{V}_{CO_2} ; $ml\ CO_2\ g^{-1}\ h^{-1}$) and EWL ($mg\ H_2O\ g^{-1}\ h^{-1}$) were determined by flow-through respirometry. The respirometry system consisted of a mass flow controller, either an Aalborg GFC171 (Orangeburg, NY, USA) or an Omega FMA-A2412 (Stamford, CT, USA) that regulated the flow of compressed air through a Perspex metabolic chamber (8000 cm^3) at a rate of 2300–3000 $ml\ min^{-1}$. The metabolic chamber was located inside a controlled temperature cabinet or room. Excurrent air passed over a thin film capacitance RH/ T_a sensor (Vaisala HMP 45A, Helsinki, Finland), then through a column of Drierite (W. A. Hammond Drierite Co. Ltd, Xenia, OH, USA), an oxygen analyser (Servomex OA184 or 572, Crowborough, East Sussex, UK), and finally a carbon dioxide analyser (Qubit S153, Kingston, Ontario, Canada; or Sable Systems CA-2A, Las Vegas, NV, USA). The gas analysers were interfaced to a PC via a RS232 serial port using Brymen (Taipei, Taiwan) multimeters (BM202 for RH, CO_2 and T_a ; TBM859CF for O_2) or a Pico Technology ADC11 analog-to-digital converter (St Neots, Cambs, UK). Custom-written data acquisition software (using Visual Basic v6, Microsoft, Redmond, WA, USA) was used to record O_2 , CO_2 , RH and T_a every 10–20 s throughout the experimental period. The O_2 analysers were 2-point calibrated using compressed N_2 (0% O_2) and dry ambient air (20.95% O_2); the CO_2 analysers were calibrated with compressed N_2 (0% CO_2) and a certified gas mix (0.53% CO_2 ; BOC, Perth, Western Australia).

The humidity of the incurrent air was controlled by bubbling it through an aerator in water at a specified temperature (Ewing and Studier, 1973; Christian, 1978). The temperature of the water was regulated by a Techne Tempette TE-8A heater (Duxford, Cambs, UK) and a FST LC-20 liquid cooler (Stone Ridge, NY, USA) and was varied to provide inlet RH values of 5–11%, 25%, 50%, 75% and 85%. Factory calibration of the RH probes was confirmed by comparing actual and theoretical RH during initial and final baseline measurements. The actual RH within the chamber with a possum was higher than the incurrent RH due to mixing of incurrent air and chamber air containing water evaporated from the animal (Lasiewski et al., 1966). Excurrent RH values were considered to be indicative of chamber RH. All six possums were measured at five RH values for thermoneutral temperatures of 25°C and 30°C (Dawson, 1969) (C.E.C. and P.C.W., unpublished data).

Baseline values for background O_2 , CO_2 and RH were measured for at least 30 min before and after experiments. All measurements were conducted for post-absorptive (fasted for at least 24 h) possums during their inactive phase (daytime) for a period of 6–9 h, during which \dot{V}_{O_2} , \dot{V}_{CO_2} and EWL invariably reached a constant and minimal value for at least 20 min. A possum was removed from its enclosure in the morning, weighed to the nearest $\pm 1g$, and placed in the metabolic chamber. The possum was observed in the chamber during experiments without disturbance with a Swann Max-IP-cam camera under infrared light (Richmond, Victoria, Australia). At the conclusion of the experiment, the possum was removed from the chamber and its T_b measured with a plastic-tipped thermocouple inserted 3 cm into the cloaca and/or an Omron MC-510 (Singapore) infrared thermometer via the ear canal (the two methods recorded identical T_b values when used simultaneously). The possum was then reweighed and returned to its enclosure.

\dot{V}_{O_2} , \dot{V}_{CO_2} and EWL were calculated (Withers, 2001) using a custom-written data analysis program (Visual Basic v6). For each individual possum, the minimum 20 min mean \dot{V}_{O_2} , \dot{V}_{CO_2} and EWL were determined for the experiment. \dot{V}_{CO_2} data are not presented here, as they mirror \dot{V}_{O_2} , but CO_2 was measured to enable accurate calculation of \dot{V}_{O_2} without use of a CO_2 scrubber (Withers, 2001), and the conversion of \dot{V}_{O_2} to J using the measured respiratory exchange ratio (RER) for calculation of thermal conductance. C_{wet} ($Jg^{-1}h^{-1}^{\circ}C^{-1}$) was calculated separately for each experiment as $MHP/(T_b-T_a)$, with metabolic heat production (MHP) calculated from MR using the oxycalorific coefficient [$JmlO_2^{-1}$ (Withers, 1992b)]. C_{dry} ($Jg^{-1}h^{-1}^{\circ}C^{-1}$) was calculated as $(MHP-EHL)/(T_b-T_a)$, with evaporative heat loss (EHL; Jh^{-1}) determined from EWL from the latent heat of vaporisation [$2.4JmgH_2O^{-1}$ (McNab, 2002)].

Body mass of a possum for a particular measurement was calculated as the mean of masses before and after an experiment. The effects of ambient RH and T_a on \dot{V}_{O_2} , EWL, T_b , C_{wet} and C_{dry} were determined by linear least squares regression, and ANOVA with Student–Newman–Keuls (SNK) *post hoc* tests. Values are presented as means \pm s.e.m. ($N=6$) unless otherwise stated. All statistical analyses were accomplished using *statistix* V1.7 (Nedlands, Western Australia).

RESULTS

Allometric relationship for marsupial EWL

There was a highly significant positive linear regression between $\log M$ (g) and $\log EWL$ ($mgH_2O h^{-1}$), $\log EWL = 0.679(\pm 0.029)\log M + 0.931(\pm 0.078)$, for 27 species of marsupial ($F_{1,25}=532$, $P<0.001$, $R^2=0.955$, residual mean square=0.023; Fig. 1). Calculated WVP within the metabolic chamber for each marsupial species during measurement ranged from 1.3 to 22.5 Torr (Table 1). The relationship between $\log M$ and $\log EWL$ corrected for ΔWVP ($mgH_2O h^{-1} Torr^{-1}$), $\log EWL = 0.745(\pm 0.045)\log M - 0.605(\pm 0.119)$, was also highly significant ($F_{1,25}=276$, $P<0.001$, $R^2=0.917$, residual

Table 1. Summary of body mass, evaporative water loss (EWL), water vapour pressure (WVP) within the metabolic chamber during measurement and evaporative water loss per water vapour pressure deficit (EWL/ ΔWVP) for marsupials

Species	Body mass (g)	EWL ($mgH_2O h^{-1}$)	WVP (Torr)	EWL/ ΔWVP ($mgH_2O h^{-1} Torr^{-1}$)
<i>Planigale maculata</i> ¹	9.2	50.6	4.5	1.85
<i>Tarsipes rostratus</i> ²	10	46	1.6	1.35
<i>Sminthopsis crassicaudata</i> ¹	14.6	91.3	2.5	3.11
<i>Sminthopsis macroura</i> ³	20	36.2	2.1	1.228
<i>Antechinus stuartii</i> ¹	21.3	96.5	2.5	3.29
<i>Antechinomys laniger</i> ¹	24.3	98.4	2.5	3.35
<i>Gracilinanus agilis</i> ⁴	30.7	78.6	2.7	2.70
<i>Pseudantechinus macdonnellensis</i> ¹	41.8	115	3.5	4.04
<i>Cercartetus nanus</i> ⁵	70	60.2	2.6	2.059
<i>Dasyercus cristicaudata</i> ¹	88	146	3.5	5.15
<i>Dasyroides byrnei</i> ¹	88.5	146	4.5	5.34
<i>Phascogale tapoatafa</i> ¹	159.7	404	16.5	26.34
<i>Perameles bougainville</i> ⁶	255	342	3	11.85
<i>Isoodon auratus</i> ⁷	428	402	7.0	16.22
<i>Myrmecobius fasciatus</i> ⁸	500	300	2.6	10.27
<i>Dasyurus hallucatus</i> ¹	593.8	814	16	51.36
<i>Perameles gunnii</i> ⁹	681	892	10.4	41.59
<i>Dasyurus viverrinus</i> ¹	900.5	1072	22.5	114.72
<i>Potorous tridactylus</i> ⁹	1000	1410	10	113.85
<i>Isoodon obesulus</i> ⁶	1197	862	3.6	30.51
<i>Isoodon macrourus</i> ⁶	1609	1094	5.0	40.81
<i>Trichosurus vulpecula</i> ¹⁰	1980	1445	2.4	67.54
<i>Lagorchestes conspicillatus</i> ¹¹	2660	1835	7.5	75.40
<i>Phalanger maculatus</i> ¹²	4250	2423	4.2	110.27
<i>Macropus eugenii</i> ¹³	4960	2728	3.1	132.01
<i>Macropus rufus</i> ¹⁴	25,000	11,200	1.5	610.97
<i>Macropus robustus</i> ¹⁴	30,000	9300	1.3	441.09

Source of data: ¹(Hinds and MacMillen, 1986), ²(Withers et al., 1990), ³(Cooper et al., 2005), ⁴(Cooper et al., in press), ⁵(Bartholomew and Hudson, 1962), ⁶(Larcombe, 2004), ⁷(Withers, 1992a), ⁸(Cooper and Withers, 2002), ⁹(Hudson and Dawson, 1975), ¹⁰(Dawson, 1969), ¹¹(Dawson and Bennett, 1978), ¹²(Dawson and Degabrielle, 1973), ¹³(Dawson et al., 1969) and ¹⁴(Dawson, 1973).

mean square=0.053; Fig. 1). An *F*-test of the residual mean squares for the regressions indicates that the regression with EWL data corrected for ΔWVP was significantly more variable than that for uncorrected data ($F_{25,25}=2.32$, $P=0.020$).

Brushtail possums

The mean body mass of the brushtail possums over all experiments ($N=6$ individuals, measured 10 times each, so total $N=60$), was 1847 ± 22.3 g (range 1450–2186 g). Observations of possums in the metabolic chamber indicated that they settled after about 30 min, and then remained resting for the duration of the

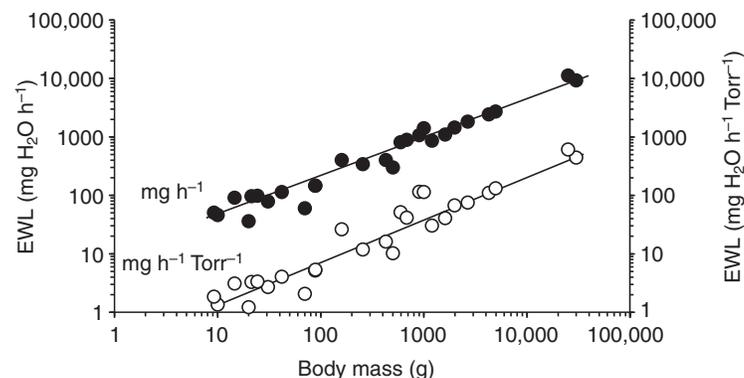


Fig. 1. Allometric relationship for evaporative water loss (EWL) of marsupials before (filled symbols) and after (open symbols) correction for chamber water vapour pressure deficit (Torr).

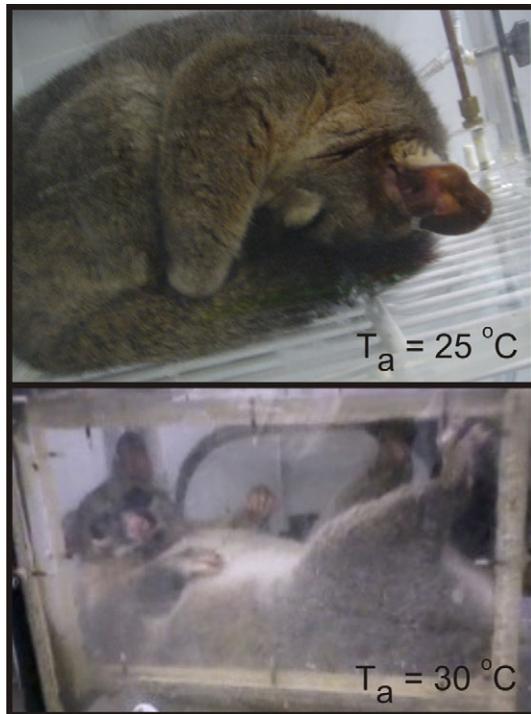


Fig. 2. Posture of brushtail possums curled with nose tucked under body fur at ambient temperature (T_a)=25°C (top) and stretched out with limbs extended at T_a =30°C (bottom).

experiment, curled up in a sphere at 25°C and stretched out on their backs at 30°C (Fig. 2). There was no evidence of the possums spreading saliva on their fur or paws to enhance evaporative cooling in any of the experiments. Excurrent chamber RH during experiments was $26 \pm 1.3\%$, $43 \pm 0.9\%$, $63 \pm 0.9\%$, $82 \pm 1.0\%$ and $93 \pm 1.2\%$ at T_a =25°C, and $26 \pm 1.0\%$, $44 \pm 3.0\%$, $60 \pm 1.2\%$, $79 \pm 0.5\%$ and $93 \pm 0.8\%$ at 30°C.

A two-way (T_a and RH) multivariate (T_b , \dot{V}_{O_2} , EWL, C_{wet} and C_{dry}) ANOVA revealed a highly significant effect of both T_a ($F_{6,45}=108.5$, $P<0.001$) and RH ($F_{24,158}=6.6$, $P<0.001$) on these physiological variables for brushtail possums. In the light of a significant T_a -RH interaction ($F_{24,158}=2.0$, $P=0.006$), we further examined the physiological variables separately with univariate one- and two-way ANOVA and linear regression to determine specifically the effects of T_a and RH on EWL, T_b , MR and C .

There was a highly significant effect of both T_a ($F_{1,50}=100.4$, $P<0.001$) and RH ($F_{4,50}=59.2$, $P<0.001$; Fig. 3A) on EWL by two-way ANOVA, with EWL significantly lower at 25°C than at 30°C, and lower at high RH. There was also a highly significant interaction between T_a and RH ($F_{1,50}=6.2$, $P<0.001$). At T_a =25°C, the effect of RH on EWL by one-way ANOVA was highly significant ($F_{4,25}=20.24$, $P<0.001$); there was no significant difference in EWL at RH values of 25%, 43% and 63% (SNK $P \geq 0.426$), but these values were different to those at RH of 82% and 92% (SNK $P<0.001$). There was a significant linear regression ($F_{1,28}=34.3$, $P<0.001$, $R^2=0.55$, residual sum of squares=0.071) for RH and EWL at 25°C. A regression of EWL with RH from 63% to 92% was also highly significant (EWL= $-0.0040RH+0.518$; $F_{1,16}=26.7$, $P<0.001$, $R^2=0.63$; residual sum of squares=0.029), but regression of RH from 26% to 63% was not significant (EWL= $0.000RH+0.287$; $F_{1,16}=0.0$, $P=0.983$, $R^2<0.001$, residual sum of squares=0.03). The sum of the

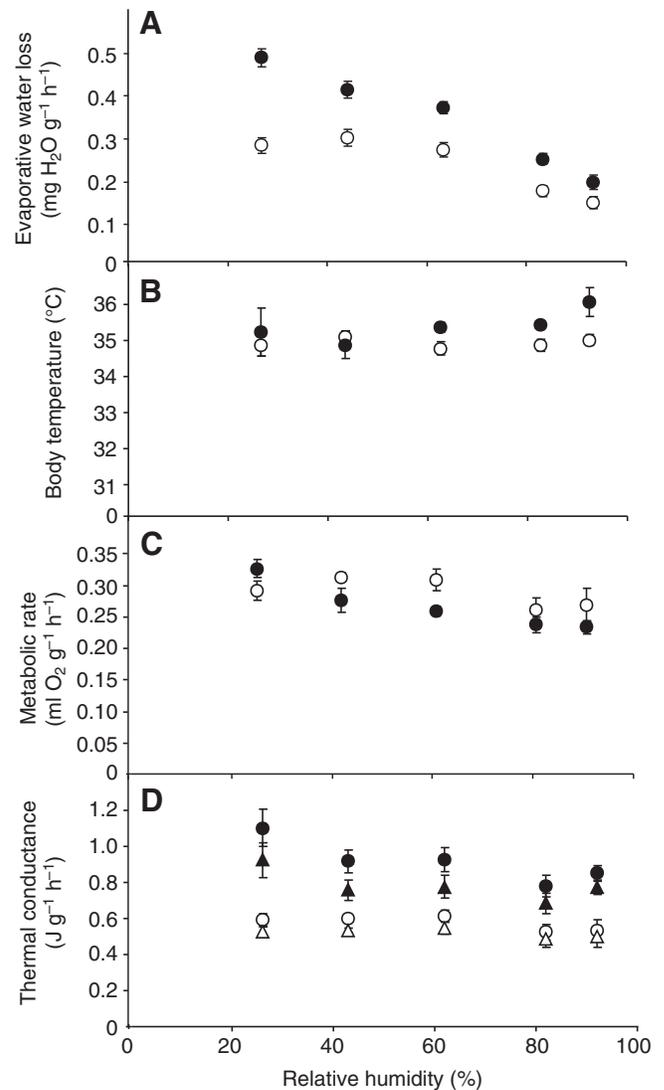


Fig. 3. Evaporative water loss (A), body temperature (B), metabolic rate (C), and wet (circles) and dry (triangles) thermal conductance (D) of brushtail possums over a range of ambient relative humidities at ambient temperatures of 25°C (open symbols) and 30°C (filled symbols). Values are means \pm s.e.m. ($N=6$).

residual sum of squares for two separate regressions, 26–43% and 63–92%, of 0.062 was less than that of the overall regression (0.071), indicating a break point at RH=63%.

At T_a =30°C, the effect of RH on EWL by one-way ANOVA was highly significant ($F_{4,25}=44.3$, $P<0.001$). There was a significant difference in EWL at each RH (SNK $P \leq 0.038$) except 43% and 62%. There was a highly significant relationship between RH and EWL for all 30°C experiments; EWL= $-0.0042RH+0.602$ ($F_{1,28}=34.3$, $P<0.001$, $R^2=0.80$). A regression between EWL and RH from 63% to 92% was also highly significant ($F_{1,16}=52.5$, $P<0.001$, $R^2=0.77$), as was the regression for EWL and RH from 26% to 63% ($F_{1,16}=11.7$, $P<0.001$, $R^2=0.42$).

The slopes of the EWL regression relationships at 25°C and 30°C at RH 63–92% were statistically indistinguishable ($F_{1,40}=0.02$, $P=0.887$; common slope= -0.0042), but the relationship at 30°C had a significantly higher elevation ($F_{1,33}=19.8$, $P=0.001$). However, at low RH (26–63%) there was a significant difference between slopes

at $T_a=25$ and 30°C ($F_{1,41}=19.1$, $P<0.001$). The relationship between EWL and RH over the range 26–92% at $T_a=30^\circ\text{C}$ had an x -axis intercept of 144% RH, while at $T_a=25^\circ\text{C}$ (over the RH range 63–92%) the x -axis intercept was 129% RH.

A univariate two-way ANOVA indicated that T_b was influenced by T_a ($F_{1,50}=5.6$, $P=0.021$), being higher at 30°C than at 25°C , but was not influenced by RH ($F_{4,50}=0.98$, $P=0.425$; Fig. 3B). Further analysis (one-way ANOVA) of the effect of RH separately at $T_a=25$ and 30°C confirmed that T_b was independent of RH at both T_a values ($F_{4,25}=0.40$, $P=0.809$; $F_{4,25}=1.14$, $P=0.359$).

\dot{V}_{O_2} was influenced by both T_a (univariate two-way ANOVA; $F_{1,50}=4.7$, $P=0.001$) and RH ($F_{4,50}=4.7$, $P=0.035$; Fig. 3C). \dot{V}_{O_2} was generally higher at $T_a=25$ than 30°C . One-way ANOVAs examining the effect of RH on \dot{V}_{O_2} at each T_a indicated that at $T_a=30^\circ\text{C}$ the \dot{V}_{O_2} at 25% RH was significantly higher than at all other RH values ($F_{4,25}=7.58$, $P<0.001$, with SNK $P\leq 0.017$) but at $T_a=25^\circ\text{C}$ the \dot{V}_{O_2} was independent of RH ($F_{4,25}=1.63$, $P=0.199$). The linear regression between RH and \dot{V}_{O_2} at 30°C was highly significant; $\dot{V}_{O_2}=-0.001(\pm 0.0003)\text{RH}+0.35(\pm 0.017)$ ($F_{1,28}=30.8$, $P<0.001$, $R^2=0.52$). The regression at 25°C was not significant ($F_{1,28}=2.77$, $P=0.107$, $R^2=0.09$).

There was a highly significant effect of both T_a (univariate two-way ANOVA; $F_{1,50}=101.3$, $P<0.001$) and RH ($F_{4,50}=22.1$, $P<0.001$) on the ratio of EWL to \dot{V}_{O_2} (evaporative quotient, EQ). For RH, one-way ANOVA indicated that it significantly influenced EQ at both $T_a=25^\circ\text{C}$ ($F_{4,25}=7.5$, $P<0.001$) and 30°C ($F_{4,25}=14.8$, $P<0.001$). EQ was constant at low humidities at both T_a , but decreased at higher RH (SNK $P<0.05$; Fig. 4). At 25°C , EQ was 0.90–0.98 at RH of 26–62%, and declined to 0.71 at 82% and 0.58 at 92%. At 30°C , EQ was higher than at 25°C , being 1.44–1.53 at RH of 26–62%, and declining to 1.09 at 82% and 0.85 at 92%.

Both T_a ($F_{1,50}=90.8$, $P<0.001$) and RH ($F_{4,50}=3.4$, $P=0.015$) had a significant influence (by univariate two-way ANOVA) on C_{wet} . At $T_a=30^\circ\text{C}$, C_{wet} was higher than at 25°C and decreased with increasing RH (Fig. 3D). However, for C_{dry} only the effect of T_a ($F_{1,50}=66.6$, $P<0.001$) not RH ($F_{4,50}=1.7$, $P=0.162$) was significant (Fig. 3D). Analysis of the influence of RH on C_{wet} by one-way ANOVA separately at each T_a indicated that at 25°C there was no effect ($F_{4,25}=0.97$, $P=0.441$), but that there was a significant influence at $T_a=30^\circ\text{C}$ ($F_{4,25}=2.94$, $P=0.040$), as described by the linear relationship $C_{\text{wet}}=-0.004(\pm 0.001)\text{RH}+1.152(\pm 0.085)$ ($F_{1,28}=8.94$, $P=0.006$). C_{dry} was independent of RH at $T_a=25$ and 30°C ($F_{4,25}=0.42$, $P=0.792$; $F_{4,25}=1.84$, $P=0.155$).

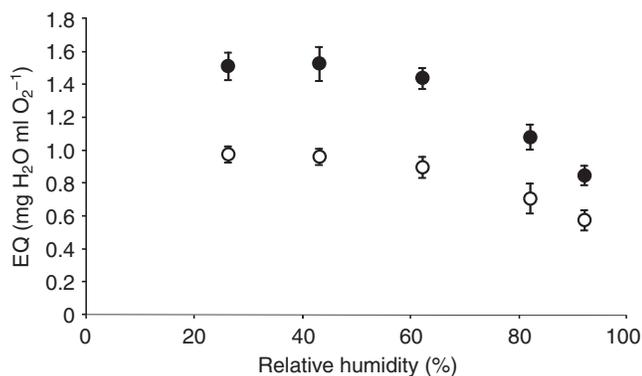


Fig. 4. Relationship between evaporative quotient (EQ) and ambient relative humidity for brushtail possums, at ambient temperatures of 25°C (open symbols) and 30°C (filled symbols). Values are means \pm s.e.m. ($N=6$).

DISCUSSION

We have found that correcting EWL data of marsupials to account for variation in chamber WVP increases rather than decreases the residual variability of allometric analysis. To account for this unexpected result, we present here the first data for the effect of RH on the physiology of a marsupial, the brushtail possum, which is the largest mammal for which RH effects have been examined. We found that the expected theoretical relationship between ambient RH and EWL for an endotherm does not always apply to brushtail possums; behavioural modification of the possum's immediate microclimate by curling into a sphere may be the mechanism by which possums deviate from the expected EWL vs RH relationship. Deviation from the theoretical relationship between EWL and RH for brushtail possums may account for the failure of ambient WVP correction to improve the allometric EWL relationship for marsupials.

Allometry of EWL in marsupials

The allometric relationship for marsupial EWL presented here is derived from 14 separate studies, that used a range of different measurement techniques, and WVP within the metabolic chamber during these measurements varied considerably (Table 1). We expected that variation in chamber WVP would contribute substantial variability to the marsupial EWL data set, due to the influence of chamber RH on EWL demonstrated for other endotherms (Lasiewski et al., 1966; Proctor and Studier, 1970; Baudinette, 1972; Christian, 1978; Edwards and Haines, 1978; Webb et al., 1995). Consequently, it was surprising that correcting EWL for WVP within the metabolic chamber significantly degraded the allometric relationship for EWL, by accounting for less of the variability in EWL. The influence of ambient RH on EWL (and other physiological variables) that we measured for a medium-sized marsupial, the brushtail possum, suggests why Δ WVP correction of marsupial EWL did not reduce variability in the marsupial EWL dataset.

Brushtail possum EWL and RH

Our EWL values for brushtail possums were lower than previously published values (Dawson, 1969) at $\text{RH}\leq 25\%$, at both 25°C (0.28 compared with $0.45\text{ mg H}_2\text{O h}^{-1}$) and 30°C (0.49 compared with $1.5\text{ mg H}_2\text{O h}^{-1}$). These lower values presumably result from a combination of longer experiment durations (Cooper and Withers, in press), measurement of minimum EWL by continuous hygrometric vs time-averaged gravimetric measurement, and allowing the possums to adopt their preferred posture in the metabolic chamber.

At 30°C , the relationship between ambient RH and EWL for the brushtail possum was the expected inverse relationship, consistent with the general relationship found for other small endotherms [rodents, bats and birds (Lasiewski et al., 1966; Proctor and Studier, 1970; Baudinette, 1972; Christian, 1978; Edwards and Haines, 1978; Webb et al., 1995)]. When EWL was expressed as a function of Δ WVP, which in theory is the driving force for evaporation, the expected positive linear relationship was observed at $T_a=30^\circ\text{C}$ (Fig. 5). As RH in the metabolic chamber increased, Δ WVP between the possum and the ambient air decreased, reducing evaporation from the skin and/or respiratory surface.

At $T_a=25^\circ\text{C}$, EWL was lower than that at 30°C , reflecting the reduced capacity for air to hold water vapour. The expected inverse relationship between EWL and ambient RH was observed at $\text{RH}\geq 63\%$; the slope of the relationship ($-0.0040\text{ mg H}_2\text{O}\%$ RH^{-1}) was the same as that observed at $T_a=30^\circ\text{C}$ (-0.0042).

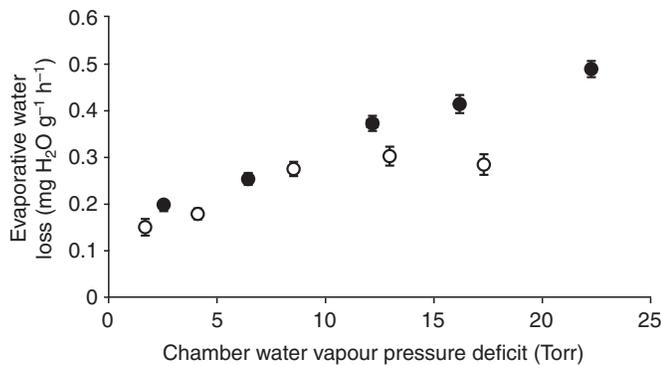


Fig. 5. Relationship between evaporative water loss and ambient water vapour pressure deficit for brushtail possums, at ambient temperatures of 25°C (open symbols) and 30°C (filled symbols). Values are means \pm s.e.m. ($N=6$).

Expressing EWL as a function of ΔWVP to remove the T_a effect resulted in the expected positive linear relationship below 10 Torr WVP, as at 30°C (Fig. 5). Unexpectedly, EWL was independent of ΔWVP above 10 Torr at $T_a=25^\circ\text{C}$. At 25°C the possums were tightly curled into a sphere (Fig. 2). In this posture, they would humidify the airspace within the fur around their nose and mouth with expired air. Re-breathing this humid air therefore reduces the ΔWVP between the respiratory surfaces and the inspired air, reducing respiratory water loss below that expected from the overall chamber RH. As the majority of EWL for possums at the T_a values examined is respiratory (Dawson, 1969), a reduction in respiratory water loss in this manner would reduce total water loss. Cutaneous water loss will also be somewhat reduced in a spherical posture as the animal's ventral surface and limbs are tucked up, reducing the evaporative surface area and allowing the build-up of a boundary layer of moist air, which will be further humidified by respiratory water from the nose and mouth. So, we suggest that the possum's posture at $T_a=25^\circ\text{C}$ modifies its immediate microclimate and eliminates the RH effect at low chamber RH. At 30°C the resting possums stretched out on their backs, presumably to maximise heat loss, and in this posture would not 're-breathe' humidified air from within their fur and the majority of the body surface along with the respiratory passages would be exposed to chamber RH. This is presumably why EWL was directly related to RH at $T_a=30^\circ\text{C}$ but not at 25°C.

Brushtail possum EQ

The EQ at 30°C and low RH values for brushtail possums ($1.49\text{ mg H}_2\text{O ml O}_2^{-1}$) is less than EQ values for other marsupial species [mean $2.7\text{ mg H}_2\text{O ml O}_2^{-1}$, calculated from previously published data (Withers et al., 2006)] at BMR and similarly low RHs. This reflects our lower EWL for the brushtail possums rather than a higher \dot{V}_{O_2} . EQ values for brushtail possums at 25°C were constant at $\text{RH}\leq 65\%$ because EWL and \dot{V}_{O_2} were constant. The decline in EQ at high RH reflected the decrease in EWL while \dot{V}_{O_2} remained constant. In effect, rebreathing air within the sphere they created by curling added a diffusive resistance [cf. figures 16–22 of Withers (Withers, 1992b)]. At $T_a=30^\circ\text{C}$, EQ was higher, but showed a similar pattern with RH, but for a different reason. Both EWL and \dot{V}_{O_2} changed with RH, but the increase in \dot{V}_{O_2} was less at lower RH than that of EWL, so EQ at $T_a=30^\circ\text{C}$ also flattened out at $\text{RH}\leq 65\%$.

EWL and chamber RH

Most studies have found a highly significant effect of chamber RH (or ΔWVP) on EWL (Lasiewski et al., 1966; Proctor and Studier, 1970; Coulombe, 1970; Baudinette, 1972; Bernstein et al., 1977; Christian, 1978; Edwards and Haines, 1978; Webb et al., 1995), consistent with the expected negative relationship with RH or positive relationship with ΔWVP . However, there are exceptions. Non-reproductive little brown bats *Myotis lucifugus* (Proctor and Studier, 1970) at $T_a=28$ and 33°C have a reduced EWL at low RH, and a highly positive relationship at $T_a=37^\circ\text{C}$ (presumably reflecting compromised thermoregulatory capacity at high T_a). Bernstein et al. (Bernstein et al., 1977) measured the expected inverse relationship of EWL over a range of ambient WVPs for pigeons (*Columba livia*) at $T_a=40^\circ\text{C}$, as was observed for possums at 30°C , but EWL of pigeons was independent of WVP at 20°C , as for possums at 25°C and $<62\%$ RH. They did not discuss these findings in detail, but suggested that 'adjustments of respiratory ventilation or in temperature of exhaled air may account for the independence of EWL at 20°C '. No observations of posture, such as the head being tucked under a wing, were reported.

The unexpected independence of EWL from low ambient RH at 25°C for brushtail possums presumably explains why our WVP correction for marsupial EWL increased, rather than decreased, the residual variability of the allometric relationship. Clearly there is not necessarily a direct relationship between ambient RH (or WVP) and EWL. Correcting EWL for RH is spurious if there is no effect, or if the effect is not understood (i.e. not the assumed linear relationship). Postural adjustments may have a significant impact on EWL, by altering the animal's immediate microclimate. Thus, although the impact of chamber RH on EWL needs to be considered in comparative studies, a 'correction factor' for this problem is not straightforward. A simplistic approach based on the theoretical relationship between EWL and WVP will not account for behavioural modification of the animal's immediate surroundings and thus the potential independence of EWL from chamber RH. We suggest that enabling the animal to adopt its preferred posture during experiments, and maintaining a low chamber RH will probably result in the most meaningful and realistic measure of EWL for comparative studies.

EWL at 100% RH

Christian (Christian, 1978) suggested that EWL should theoretically be 0 at 100% RH, but a ΔWVP remains for both cutaneous and respiratory water loss if skin temperature (T_{skin}) and/or T_b is $>T_a$, even if the ambient RH is 100% at T_a , due to the greater capacity of air to hold water vapour at higher T_a . Thus when $T_b>T_a$ and $T_{\text{skin}}>T_a$, the warmer air in the respiratory passages and near the body surface will continue to drive EWL. However, this water loss would presumably condense in the metabolic system because the air at T_a would be saturated, and would not actually be measured as EWL (measured EWL would plateau at the value calculated for saturation water vapour density of ambient air). The magnitude of EWL at 100% RH will depend on the body core to skin thermal gradient and the extent of nasal counter-current heat and water exchange (Schmidt-Nielsen et al., 1970). We calculate from the regression relationship for EWL and RH at $T_a=30^\circ\text{C}$ that a theoretical RH of 144% would be required for zero EWL. A RH of 144% at $T_a=30^\circ\text{C}$ ($WVP=45$ Torr) is equivalent to a RH of 100% at 36.6°C , calculated using hygrometric equations (Parish and Putnam, 1977). We consider 36.6°C as the effective evaporative temperature (T_{ce}) of the possums at $T_a=30^\circ\text{C}$, and it closely approximates the possums' actual T_b at $T_a=30^\circ\text{C}$ and $\text{RH}=92\%$ of

36.1°C. This close approximation of T_{ce} and T_b suggests that the possums were not recovering any water *via* nasal counter-current heat and water exchange, and did not have a large body core to skin thermal gradient at $T_a=30^\circ\text{C}$. However, it is likely that nasal counter-current heat and water exchange mechanisms were operating at $T_a=25^\circ\text{C}$. We calculate from the regression relationship for EWL and RH (from 62% to 92% RH) at $T_a=25^\circ\text{C}$ that a theoretical RH of 129% would result in zero EWL at $T_a=25^\circ\text{C}$. A RH of 129% at $T_a=25^\circ\text{C}$ (WVP=29.6 Torr) is equivalent to a RH of 100% at only 29°C calculated from hygrometric equations (Parish and Putnam, 1977); this T_{ce} is substantially lower than the actual T_b of 35.0°C at 25°C and 93% RH. Thus nasal counter-current heat and water exchange and/or a large body core to skin thermal gradient were reducing EWL below that expected if the T_{ce} approximated T_b at $T_a=25^\circ\text{C}$. This may have contributed to the independence of EWL from RH at RH \leq 62%, together with postural adjustments.

Effect of RH on T_b , MR and C

Body temperatures of the brushtail possums measured in this study were somewhat lower than those previously measured (Dawson, 1969) at both $T_a=25^\circ\text{C}$ and $T_a=30^\circ\text{C}$ (34.9 \pm 0.08°C compared with 35.9°C, and 35.4 \pm 0.18°C compared with 36.5°C, respectively). \dot{V}_{O_2} at both $T_a=25^\circ\text{C}$ and $T_a=30^\circ\text{C}$ (means over all RH values at each T_a were 0.29 \pm 0.01 and 0.27 \pm 0.01 ml O₂ g⁻¹ h⁻¹, respectively; $N=30$) was slightly lower than previous measurements (Dawson and Hulbert, 1970; 0.31 ml O₂ g⁻¹ h⁻¹ at $T_a=27^\circ\text{C}$). Long experiment durations (Cooper and Withers, in press), allowing possums to adopt their preferred posture within the metabolic chamber, and possible intraspecific differences between possum populations presumably account for these differences.

The effect of ambient RH has rarely been examined for physiological variables other than EWL. RH has not previously been shown to affect MR or T_b (Baudinette, 1972; Ewing and Studier, 1973; Edwards and Haines, 1978), although Kay (Kay, 1975) suggested that RH may have an effect on T_b at high T_a ($>T_{uc}$). C_{wet} must be affected by RH if EWL is, as evaporative heat loss is a substantial component of C_{wet} , especially at higher T_a . Thus the significant relationship between C_{wet} and RH at 30°C but not at 25°C is consistent with the relationship between EWL and RH that we observed at these T_a values. \dot{V}_{O_2} was significantly related to RH at $T_a=30^\circ\text{C}$ (but not at $T_a=25^\circ\text{C}$), increasing with decreasing RH. As T_b of possums was independent of RH, they had to adjust their MR and/or C_{dry} to account for the effect of RH on EWL and thus evaporative heat loss (and C_{wet}) at $T_a=30^\circ\text{C}$. C_{dry} was independent of RH at both T_a values, and therefore \dot{V}_{O_2} (and thus metabolic heat production) had to vary with RH to counteract the effect of RH on evaporative heat loss to maintain a constant T_b . We observed that the pattern of \dot{V}_{O_2} change with RH mirrored that of EWL (Fig. 3A,C), as expected. However, at $T_a=25^\circ\text{C}$, where possums can maintain EWL independent of RH, the effect of RH on C_{wet} and therefore \dot{V}_{O_2} was not significant.

Our finding that the MR of brushtail possums within the thermoneutral zone can be influenced by RH has important implications for the measurement of standard physiological variables such as BMR, T_b and C_{wet} . If valid intraspecific and interspecific comparisons are to be made, then it is essential that comparative standards (e.g. BMR) are measured under comparable environmental conditions. Although the effect of chamber RH has been considered for the comparison of standard EWL data, little attention has been paid to the possible confounding effect of chamber RH on commonly measured variables such as BMR. Here we demonstrated that under

conditions where RH has a significant effect on EWL, other interrelated variables such as BMR, T_b and C_{wet} may also be influenced. Thus chamber RH should be considered when defining standard conditions for measurement of physiological variables such as EWL and BMR.

LIST OF ABBREVIATIONS

BMR	basal metabolic rate
C_{dry}	dry thermal conductance
C_{wet}	wet thermal conductance
EHL	evaporative heat loss
EQ	evaporative quotient
EWL	rate of evaporative water loss
M	body mass
MHP	metabolic heat production
MR	metabolic rate
RH	relative humidity
SNK	Student–Newman–Keuls <i>post hoc</i> test
T_a	ambient temperature
T_b	body temperature
T_{ce}	effective evaporative temperature
T_{skin}	skin temperature
T_{uc}	upper critical temperature
\dot{V}_{CO_2}	rate of carbon dioxide production
\dot{V}_{O_2}	rate of oxygen consumption
WVP	water vapour pressure
Δ WVP	water vapour pressure deficit

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