Small Alpine Marsupials Regulate Evaporative Water Loss, Suggesting a Thermoregulatory Role Rather than a Water Conservation Role

Philip Carew Withers[1,2,3,*]
Christine Elizabeth Cooper[2,3]
Gerhard Körtner[3]
Fritz Geiser[3]
[1]School of Biological Sciences, University of Western Australia, Perth, Western Australia, Australia; [2]School of Molecular and Life Sciences, Curtin University, Perth, Western Australia, Australia; [3]School of Environmental and Rural Science, Zoology, University of New England, Armidale, New South Wales, Australia

Accepted 3/3/2022; Electronically Published 4/18/2022

ABSTRACT

We show here that evaporative water loss (EWL) is constant over a wide range of ambient relative humidity for two species of small, mesic habitat dasyurid marsupials (*Antechinus agilis* and *Antechinus swainsonii*) below thermoneutrality (20°C) and within thermoneutrality (30°C). This independence of EWL from the water vapor pressure deficit between the animal and its environment indicates that EWL is physiologically controlled by both species. The magnitude of this control of EWL was similar to that of two other small marsupials from more arid habitats, which combined with the observation that there were no effects of relative humidity on body temperature or metabolic rate, suggests that control of EWL is a consequence of precise thermoregulation to maintain heat balance rather than a water-conserving strategy at low relative humidities. The antechinus appear to manipulate cutaneous EWL rather than respiratory EWL to control their total EWL by modifying their cutaneous resistance and/or skin temperature. We propose that there is a continuum between enhanced thermoregulatory EWL at high ambient temperature and so-called insensible EWL at and below thermoneutrality.

Keywords: Antechinus, body temperature, evaporative water loss (EWL), humidity, metabolic rate, regulation, thermoregulation, water balance, water vapor pressure deficit.

Introduction

Water is the primary constituent of animal body tissues, being about 61% of total mass (Calder and Braun 1983) or 74% of fat-free lean mass (Wang et al. 1999) for mammals. The evaporative water loss (EWL) of terrestrial mammals is an important component (up to 70%) of their water budget and also has consequences for heat balance (Chew 1951; Schmidt-Nielsen and Schmidt-Nielsen 1952; Hinds and MacMillen 1985; Ostrowski et al. 2006; Withers et al. 2016). For endothermic mammals and birds, EWL increases at ambient temperatures (T_a's) above the upper critical temperature of the thermoneutral zone (TNZ; Scholander et al. 1950; Commission for Thermal Physiology IUPS 2003) to increase heat loss, and evaporative heat loss (EHL) is the only mechanism by which metabolic heat can be dissipated when environmental temperature exceeds body temperature (T_b; Wolf 2000; Withers et al. 2016). Physiological regulation of thermoregulatory EWL at high T_a is well appreciated (e.g., Kanosue et al. 1998; Simon 1999; Morrison and Nakamura 2011), and it can be enhanced under environmental conditions, such as high relative humidity (RH), that physically inhibit EWL (Gerson et al. 2014; van Dyk et al. 2019).

In contrast, EWL in and below the TNZ, which has historically been termed "insensible" EWL (Benedict and Root 1926; Commission for Thermal Physiology IUPS 2003), is considered a cost that can compromise water balance but is not usually considered to be of thermoregulatory importance. It is well established that there are environmental correlates of EWL between and within species (e.g., Williams et al. 1991; Williams 1996; Williams and Tieleman 2005; Withers et al. 2006; Van Sant et al. 2012; Song and Beissinger 2020). EWL also varies with chronic acclimation and acclimatization to varied temperature and water availability (Tieleman and Williams 2000, 2002; Tracy and Walsberg 2000, 2001; Williams and Tieleman 2000), driven predominantly by changes in cutaneous EWL (CEWL; Tieleman et al. 1999; Tracy and Walsberg 2000; Tieleman and Williams 2002) as a consequence of changes in skin lipid composition (Cox et al. 2008).

Despite these adaptive and plastic responses, insensible EWL has traditionally been considered to be passive, that is, not acutely regulated, reflecting the permeability of the animal's skin and lung surfaces to water vapor (Monteith 1973; Edwards and Haines 1978; Campbell and Norman 1998). The driver of insensible EWL is the water vapor pressure differential (ΔWVP) between the animal and its environment (Chew and Dammann 1961; Edwards and Haines 1978; Webster and King 1987; Powers 1992; Klüg-Baerwald and Brigham 2017). There is growing evidence, however,

---

*Corresponding author; email: philip.withers@uwa.edu.au.

Physiological and Biochemical Zoology, volume 95, number 3, May/June 2022. © 2022 The University of Chicago. All rights reserved. Published by The University of Chicago Press. https://doi.org/10.1086/719735

that insensible EWL is not simply a passive consequence of the evaporative environment but is under acute physiological control (like thermoregulatory EWL at high T_a) for both mammals (Cooper and Withers 2008, 2014, 2017, 2020; Withers and Cooper 2014) and birds (Webster et al. 1985; Webster and Bernstein 1987; Ro and Williams 2010; Eto et al. 2017; Cooper et al. 2020; Gilson et al. 2021). Manipulation of the evaporative environment by modifying two components of Fick's Law (Gilson et al. 2021)—the ΔWVP by modifying the ambient RH (and thus the driving force for evaporation; Webster et al. 1985; Webster and Bernstein 1987; Ro and Williams 2010; Cooper and Withers 2008, 2017; Withers and Cooper 2014; Eto et al. 2017; Cooper et al. 2020; Gilson et al. 2021) and/or the diffusion coefficient by placing the animal in a helox atmosphere (21% oxygen in helium), which has a greater diffusivity than air (Cooper and Withers 2014; 2020; Gilson et al. 2021)—does not necessarily change EWL as expected if EWL was physically determined, suggesting a physiological control response.

The physiological role of EWL control is not clear, but there are two likely hypotheses: water conservation and thermoregulation. Control of EWL could contribute to water balance by reducing EWL under conditions expected to exacerbate water loss (e.g., low environmental RH, high ΔWVP). If so, we would expect control of EWL to be better developed by desert species compared with mesic species. However, this comparison is problematic because the majority of species for which acute control of EWL has been comprehensively described are from arid or semiarid habitats (Cooper and Withers 2008, 2014, 2017, 2020; Withers and Cooper 2014; Eto et al. 2017). Gilson et al. (2021) recently demonstrated EWL control for a mesic habitat parrot using two independent methodologies, and Cooper et al. (2020) showed that two mesic habitat parrots have a similar capacity for control of EWL as the arid habitat budgerigar (*Melopsittacus undulatus*; Eto et al. 2017), but for mammals no direct comparable data for acute EWL control for mesic habitat species are available. Support for the water conservation hypothesis also requires identification of a hygrosensory receptor and feedback control system. Hygroreceptors have been described for insects, and nematodes achieve hygrosensation via thermosensory and mechanosensory pathways (e.g., Dethier and Schoonhoven 1968; Yokohari and Tateda 1976; Sayeed and Benzer 1996; Liu et al. 2007). Hygroreceptors have been hypothesized for mice and humans because of the presence of orthologous mechanosensitive proteins (Russell et al. 2014), but there is currently no description of a complete receptor and feedback control system for mammals.

An alternative hypothesis for control of EWL is that it is a consequence of the precise thermoregulatory control by mammals and birds. If EWL varies with RH, then EHL will be affected, requiring adjustment of metabolic heat production (MHP) or thermal conductance to maintain T_b constant. Keeping EWL constant despite varying RH simplifies thermoregulation (Cooper and Withers 2017; Eto et al. 2017; Cooper et al. 2020). In support of this thermoregulatory hypothesis, convergently endothermic birds and mammals regulate their EWL at and below thermoneutrality (Eto et al. 2017). In addition, heterothermic mammals that thermoconform during torpor or hibernation do not appear to regulate their EWL (Cooper and Withers 2017; Klüg-Baerwald and Brigham 2017) but do regulate their EWL when thermoregulating during normothermia (Cooper and Withers 2017). The sensory feedback system for a thermoregulatory role of acute EWL control at and below the TNZ is most likely the already well-described thermoregulatory system (e.g., Kanosue et al. 1998; Simon 1999; Morrison and Nakamura 2011).

We examine here the capacity of two alpine (mesic) species of dasyurid marsupial, agile antechinus (*Antechinus agilis*) and dusky antechinus (*Antechinus swainsonii*), for acute control of EWL. We determine whether EWL at $T_a = 20°C$ and 30°C (below thermoneutrality and in thermoneutrality, respectively; Cooper et al. 2016) deviates significantly from physical predictions of the effect of RH, indicating physiological control, and we examine the effect of ΔWVP on other metabolic, thermoregulatory, and ventilatory variables to investigate possible mechanisms of control. This study of alpine antechinus enables us to question whether the control of EWL is likely an environmental adaptation for water conservation by mammals or a general consequence of thermoregulation.

Methods

We captured 10 agile and 10 dusky antechinus in Kosciuszko National Park, New South Wales (36.1°S, 148.3°E), using Elliot traps with universal peanut butter/oats bait; traps were filled with Dacron insulation and fitted with plastic rain covers. Animals were transported to the University of New England, where they were held individually in large plastic crates and fed a mixture of kangaroo mince, wet cat food, and mealworms with ad lib. water. Individuals were allowed approximately 1 wk for their food intake and activity to stabilize under captive conditions before experiments commenced. They were fasted overnight preceding measurement the following day. Each individual had at least 3 d of rest with ad lib. food and water between successive measurements.

Four separate open-flow respirometry systems were used to measure EWL and metabolic rate (MR) as oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$). Each respirometry system consisted of a 500-mL glass metabolic chamber, a Vaisala HMP45A RH/T_a probe, a carbon dioxide analyzer (Sable Systems CA-2A or FoxBox), and an oxygen analyzer (Sable Systems PA-10 or FoxBox). The analog voltage outputs of the gas analyzers and RH/T_a probe were interfaced to a PC (Sable Systems UI2 A/D converter or FoxBox serial port) running custom-written data acquisition software (Microsoft Visual Basic ver. 6; P. C. Withers).

Animals were measured during the day for 6–9 h, until MR and EWL were constant and minimal. Each animal was measured at only one T_a and RH combination per day. Positive pressure flow of dried ambient air (using Drierite; W.A. Hammond) was maintained through the system at 200–1,000 mL min^{-1} (agile antechinus) or 300–1,400 mL min^{-1} (dusky antechinus), regulated by a mass-flow controller (Aalborg, Cole-Palmer, or FoxBox internal pump). Desired chamber RH (approximately 8%, 30%, 45%, 65%, and 78%) was achieved by passing incurrent air through either a dew point controller (Sable Systems DG4) or an aerator to saturate air at the appropriate temperature (using an Engel portable refrigerator fitted with a Ratek heater circulator) to give the required RH when warmed to the experimental T_a (calculated using hygrometerological equations of Parish and Putnam [1977]), in concert with the water vapor added by the animal. Excurrent RH was assumed to represent chamber RH. A custom-built controlled-temperature cabinet (G. Körtner) regulated experimental temperature at 20°C or 30°C (below thermoneutrality and at thermoneutrality, respectively; Cooper et al. 2016), and the whole metabolic system was located in a controlled-temperature room to eliminate O_2 analyzer baseline drift from varying room temperature. Experimental combinations of RH and T_a were measured in random order.

Body mass was measured to ±0.1 g, using an A&D HL-200i electronic balance before and after each experiment and the mean used for calculations. Baseline values for background O_2, CO_2, and RH levels were recorded for at least 30 min before and after each experiment, and values for O_2, CO_2, and RH were recorded every 30 s. At the end of the experiment, which was before the end of the rest phase while physiological variables were still low and stable, the antechinus were removed from the chamber and their T_b's were measured immediately using a RadioSpares thermocouple meter with a plastic-tipped thermocouple inserted into the cloaca.

EWL (mg g^{-1} h^{-1}), $\dot{V}O_2$ (mL g^{-1} h^{-1}), and $\dot{V}CO_2$ (mL g^{-1} h^{-1}) were calculated after Withers (2001) using a custom-written VB6 program (P. C. Withers), over an approximately 20-min period when all variables were minimal and constant. The respiratory exchange ratio (RER) was calculated as $\dot{V}CO_2/\dot{V}O_2$. Wet thermal conductance (C_{wet}) and dry thermal conductance (C_{dry}) were calculated as $MHP/(T_b - T_a)$ and $(MHP - EHL)/(T_b - T_a)$, respectively, with MR converted to MHP using the appropriate oxycalorific equivalent for the measured RER after Withers et al. (2016) and EHL calculated from EWL assuming 2.4 J mg H_2O^{-1} (Monteith 1973).

Ventilatory variables (respiratory frequency [f_R]; STPD tidal volume [V_T]; minute volume [$V_I = f_R \cdot V_T$]) were measured using the metabolic chamber as an open-flow whole-body plethysmograph (Malan 1973; Withers 1977). Two to six sets of ventilatory measurements were made toward the end of each experiment, before the animal was removed from the chamber. A custom-built pressure transducer (with a Motorola MPX 2010 differential transducer) measured pressure deflections in the metabolic chamber caused by the warming and humidifying of inspired air, and its voltage output was interfaced to a PC via an A/D converter (Pico AD11, Pico Technology). Breathing data were recorded as approximately 30-s traces (sampled at 50 Hz) using PicoScope software (Pico Technology). Room temperature and barometric pressure were recorded at the time of ventilatory measurements using an electronic weather station. Oxygen extraction (EO_2; %) was calculated from $\dot{V}O_2$, V_I, and excurrent fractional O_2 content (FEO_2) as $(100 \cdot \dot{V}O_2)/(FEO_2 \cdot V_I)$. Calculations were made using a custom-written VB6 data analysis program after Malan (1973) and Szewczak and Powell (2003).

Flow meters were volumetrically calibrated to standard temperature and pressure. O_2 and CO_2 analyzers were two-point calibrated with compressed nitrogen (0% O_2 and CO_2; BOC Gas) and either dry ambient air (20.95% O_2) or a certified gas mix (1.5% CO_2; BOC Gas). The Vaisala probes were RH calibrated using the theoretical and measured baseline RH for experimental trials. These probes and the thermocouple meter were calibrated to 0.1°C against a mercury thermometer traceable to a national standard. The plethysmograph system was calibrated after Szewczak and Powell (2003) by injecting 0.2 mL of air into the chamber at the end of experiments.

Total EWL was partitioned noninvasively into CEWL and respiratory EWL (REWL) using the iterative model of Withers et al. (2012) to determine the combination of expired air temperature (T_{exp}), skin temperature (T_{skin}), and cutaneous (skin and pelage) resistance ($R_{cutaneous}$; s cm^{-1}; Monteith and Campbell 1980) that best estimated total EWL from calculated CEWL and REWL. $R_{cutaneous}$ was calculated from the water vapor density difference (ΔWVD) between saturation at T_{skin} to ambient air (mg cm^{-3}) divided by CEWL (mg cm^{-2} s^{-1}) expressed per body surface area (cm^2) calculated as 10 $M^{0.667}$ (Dawson and Hulbert 1970; Walsberg and King 1978), that is, $R_{cutaneous} = \Delta WVD/CEWL$. Note that resistance essentially consolidates the diffusion coefficient (D), the diffusion path length (L), and skin surface area terms from Fick's law of diffusion into a resistance term. These estimates of T_{skin}, T_{exp}, and the partitioning of total EWL into percentage of cutaneous and pulmonary EWL were used to calculate an average surface temperature for evaporation (T_{evap}) as $T_{evap} = ((CEWL \cdot T_{skin}) + (REWL \cdot T_{exp}))/EWL$. The respiratory surface temperature is considered to be T_{exp} rather than T_b because the nasal countercurrent heat exchange typically reduces the T_{exp} to less than T_b (Schmidt-Nielsen et al. 1970), so T_{exp} is the functional respiratory evaporative surface temperature.

Total EWL was expressed relative to ΔWVP between the animal and the ambient air to compare EWL/ΔWVP at different RH with the physical expectation that its slope is 0 (Eto et al. 2017). The animal's WVP was calculated as WVP saturation ($WVP_{saturation}$) at either T_b or T_{evap} using hygrometeorological equations of Parish and Putnam (1977). Ambient WVP was calculated as $(RH/100)WVP_{saturation}$ at T_a.

Values are presented as mean ± SE with N as the number of animals and n as the number of measurements. It was not possible to measure all individuals at all T_a and RH combinations, precluding analysis by multivariate repeated-measures ANOVA, so effects of RH on the various physiological variables were assessed with linear mixed models (package lme4; Bates et al. 2015) in the R environment (ver. R-4.0.0; R Core Team 2020) using RStudio (ver. 1.2.5042; RStudio Team 2020). Repeated measures for individual animals were included as a random factor. Deviation of values at the highest RH from the pattern at lower RH was evaluated using Helmert contrasts. Data were analyzed separately for each T_a because of the nonequivalence of saturation WVP to RH at different T_a.

Results

Evaporative, Thermal, and Metabolic Variables

Body mass of agile antechinus was 21.7 ± 0.45 g over all experiments ($N = 10$, $n = 93$; see table A1 for summary data).

The EWL of agile antechinus did not change with RH ($F_{1,36-40} \leq 2.50$, $P \geq 0.124$) at $T_a = 20$°C (1.81 ± 0.05, $N = 9$, $n = 43$) and RH < 70% at 30°C (2.30 ± 0.06, $N = 10$, $n = 34$), but at $T_a = 30$°C EWL was 23% lower at the highest RH than at lower RH (Helmert, $P < 0.001$; fig. 1). There was no effect of RH on T_b at either $T_a = 20$°C (34.8°C ± 0.21°C, $N = 9$, $n = 44$) or $T_a = 30$°C (34.9°C ± 0.12°C, $N = 10$, $n = 46$; $F_{1,37-44} \leq 1.53$, $P \geq 0.223$), $\dot{V}O_2$ at 20°C ($F_{1,36-37} \leq 2.94$, $P \geq 0.095$; 2.47 ± 0.06 mL O_2 g^{-1} h^{-1}; $N = 9$, $n = 44$) and 30°C (1.34 ± 0.03 mL O_2 g^{-1} h^{-1}; $N = 10$, $n = 46$), or C_{wet} and C_{dry} at $T_a = 20$°C ($F_{1,36-37} \leq 0.620$, $P \geq 0.436$). However, at $T_a = 30$°C, both C_{wet} and C_{dry} were 44% and 56% higher at >50% RH (Helmert, $P \leq 0.004$) than at lower RH.

Body mass was 59.8 ± 1.61 g ($N = 10$, $n = 77$) for dusky antechinus (see table A2 for summary data). EWL was independent of RH < 70% ($F_{1,21-33} \leq 1.59$, $P \geq 0.221$) at both $T_a = 20$°C (1.34 ± 0.05 mg g^{-1} h^{-1}; $N = 8$, $n = 23$) and 30°C (1.76 ± 0.05 mg g^{-1} h^{-1}; $N = 10$, $n = 43$) but decreased by 24%–37% at >70% RH (Helmert, $P < 0.001$). There were no RH effects at either T_a for T_b, $\dot{V}O_2$, C_{wet}, or C_{dry} ($F_{1,26-34} \leq 1.96$, $P \geq 0.174$) for dusky antechinus. T_b was 35.8°C ± 0.19°C ($N = 8$, $n = 34$) at $T_a = 20$°C and 36.0°C ± 0.16°C ($N = 10$,

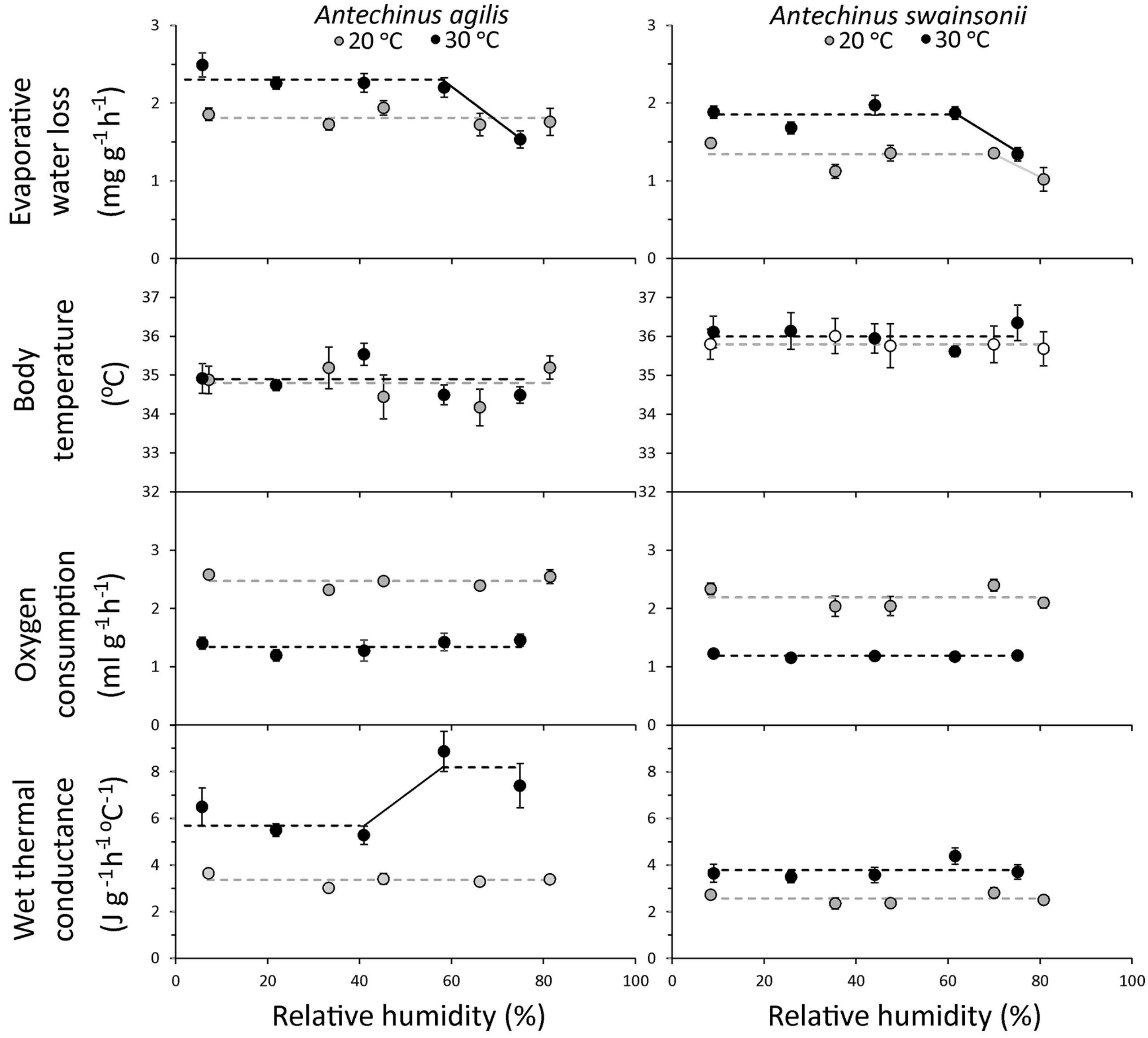

Figure 1. Patterns of thermoregulatory, metabolic, and hygric values for agile antechinus (*Antechinus agilis*; $N = 10$) and dusky antechinus (*Antechinus swainsonii*; $N = 10$) at varying relative humidities at ambient temperatures of 20°C (gray symbols and lines) and 30°C (black symbols and lines). Dashed lines indicate nonsignificant relative humidity effects, and solid lines indicate significant linear or a priori contrast effects. Values are mean ± SE.

$n = 43$) at $T_a = 30°C$; $\dot{V}O_2$ was 2.19 ± 0.06 ($N = 8$, $n = 34$) and 1.19 ± 0.02 ($N = 10$, $n = 43$), respectively.

Respiratory Variables

For agile antechinus, RH did not affect any respiratory variables at $T_a = 20°C$ ($F_{1,28–32} \leq 0.590$, $P \geq 0.448$; fig. 2). At $T_a = 30°C$, f_R increased 35% at the highest RH (Helmert, $P = 0.047$), and V_I increased with RH ($F_{1,25} = 4.30$, $P = 0.048$), while V_T was independent of RH ($F_{1,25} = 0.275$, $P = 0.604$) and EO_2 decreased with RH ($F_{1,25} = 6.67$, $P = 0.016$). For dusky antechinus there was no effect of RH on f_R, V_T, or V_I at $T_a = 20°C$ ($F_{1,31} = 0.441$, $P = 0.511$) or V_T at 30°C ($F_{1,24–30} = 0.441$, $P > 0.511$), but at $T_a = 30°C$, f_R and V_I increased 33% and 66%, respectively, at RH > 70% compared with lower RH (Helmert, $P < 0.001$). EO_2 decreased linearly with RH at $T_a = 20°C$ ($F_{1,29} = 7.41$, $P = 0.011$) but was constant at 30°C for RH < 70% ($F_{1,25} = 0.668$, $P = 0.421$) and decreased for RH > 70% (Helmert, $P = 0.032$).

Pulmocutaneous Partitioning

RH had no effect for agile antechinus at $T_a = 20°C$ on CEWL or $R_{cutaneous}$ ($F_{1,32–35} < 2.10$, $P > 0.156$), but REWL declined

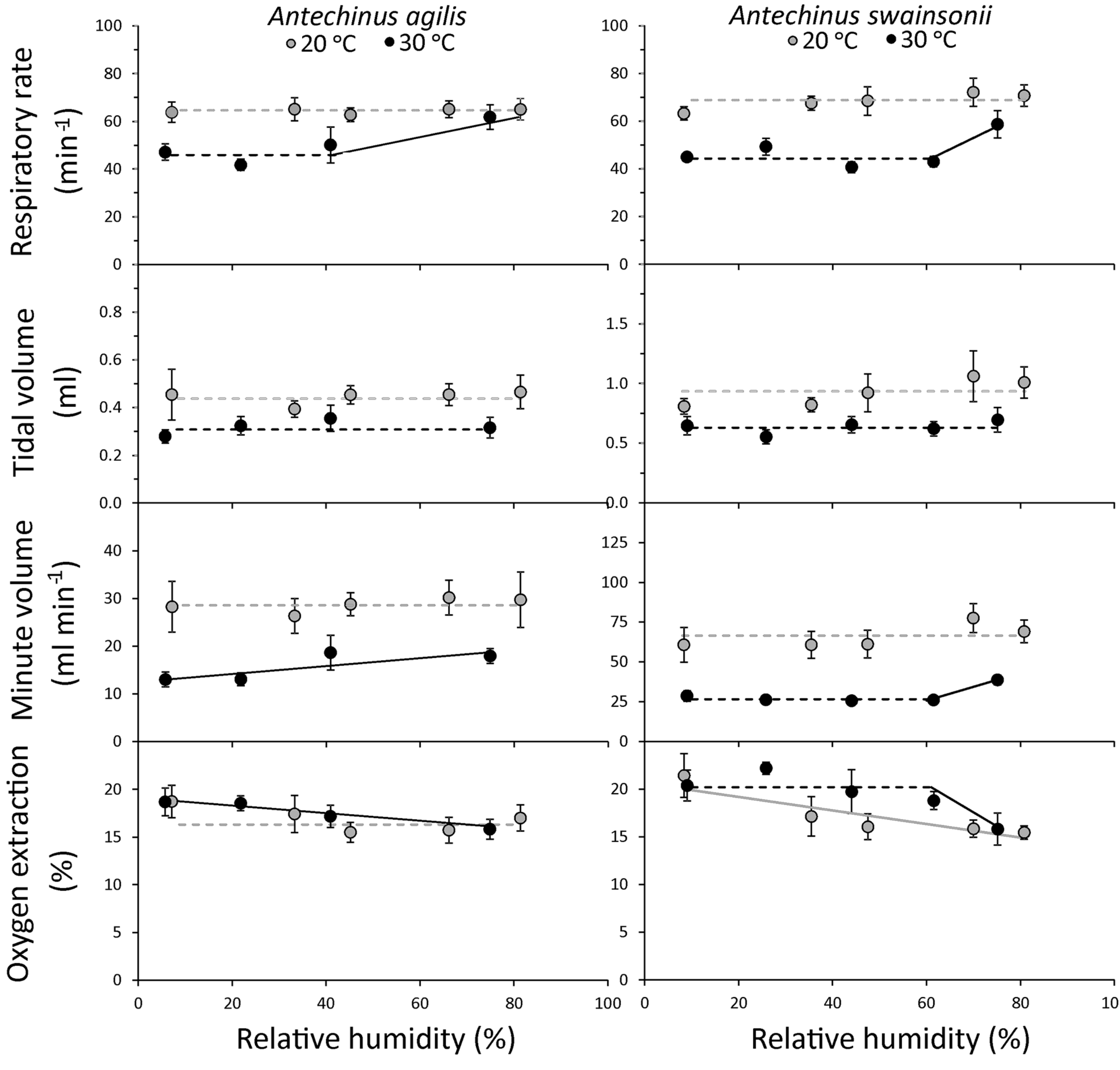

Figure 2. Patterns of respiratory variables for agile antechinus (*Antechinus agilis*; $N = 10$) and dusky antechinus (*Antechinus swainsonii*; $N = 10$) at varying relative humidities at ambient temperatures of 20°C (gray symbols and lines) and 30°C (black symbols and lines). Dashed lines indicate nonsignificant relative humidity effects, and solid lines indicate significant linear or a priori contrast effects for relative humidity. Values are mean ± SE.

from 1.79 at RH < 20% (Helmert, $P = 0.022$) to 1.23 at RH > 20% ($F_{1,31} = 0.15$, $P = 0.702$). There was no effect of RH at $T_a = 30$°C on CEWL ($F_{1,23} = 0.799$, $P = 0.308$; fig. 3), but there was a significant negative effect on REWL and $R_{\text{cutaneous}}$ ($F_{1,23\text{–}30} \geq 3.96$, $P < 0.001$), which declined from 440 to 190 s cm^{-1} with increasing RH. For dusky antechinus at $T_a = 20$°C, CEWL was constant at 0.41 ± 0.06 mg g^{-1} h^{-1} ($F_{1,24} = 0.006$, $P = 0.938$), but there was a significant negative linear RH effect for REWL ($F_{1,25} = 4.37$, $P = 0.047$) at low RH, and $R_{\text{cutaneous}}$ decreased logarithmically with RH ($F_{1,28} = 6.13$, $P = 0.020$) from about 530 to 335 s cm^{-1}. However at $T_a = 30$°C, CEWL increased with RH ($F_{1,31} = 15.7$, $P < 0.001$), then declined at RH > 70% (Helmert, $P = 0.006$). $R_{\text{cutaneous}}$ decreased logarithmically with RH at 30°C ($F_{1,36} = 61.1$, $P < 0.001$) from 450 to 130 s cm^{-1}, and REWL decreased linearly ($F_{1,30} = 16.3$, $P < 0.001$).

CEWL as a percentage of total EWL was independent of RH at both $T_a = 20$°C and 30°C for agile antechinus ($F \leq 2.79$, $P \geq 0.105$; fig. 4), averaging 32.6% ± 2.7% ($N = 9$, $n = 37$) and 48.3% ± 2.9% ($N = 10$, $n = 32$) of total EWL, respectively. T_{skin}, T_{exp}, and T_{evap} increased linearly with RH at $T_a = 20$°C ($F_{1,30\text{–}35} < 5.22$, $P > 0.029$) but not at 30°C ($F_{1,20\text{–}30} < 4.20$, $P > 0.053$). At $T_a = 20$°C, T_{evap} increased with RH from 23.1°C to 29.5°C and was 11.8°C to 5.7°C less than T_b; the random residual variance was 6.80 (cf. the random residual variance of 1.6 for T_b with RH). At $T_a = 30$°C, T_{evap} averaged 31.2°C ± 0.46°C ($N = 9$; $n = 29$), 3.7°C < T_b, and the random residual variance was 3.22 (cf. 1.04 for T_b).

For dusky antechinus, CEWL as a percentage of total EWL was independent of RH at 20°C (31.6% ± 3.7%; $F_{1,25} = 0.263$, $P = 0.613$), but at $T_a = 30$°C it increased for RH < 70% to 69.1% ($F_{1,26} = 16.7$, $P < 0.001$), then decreased at RH > 70% (Helmert, $P < 0.001$) to 60.8%. Calculated T_{skin} was independent of RH at 20°C ($F_{1,24} = 3.24$, $P = 0.084$), but T_{exp} and T_{evap} increased with RH ($F_{1,23\text{–}29} > 2.16$, $P < 0.041$), whereas T_{skin}, T_{exp}, and T_{evap} were all independent of RH at 30°C ($F_{1,36} < 3.47$, $P > 0.071$). At $T_a = 20$°C, T_{evap} increased with RH from 21.1°C to 27.8°C, which was 14.7°C to 7.9°C less than T_b; the random

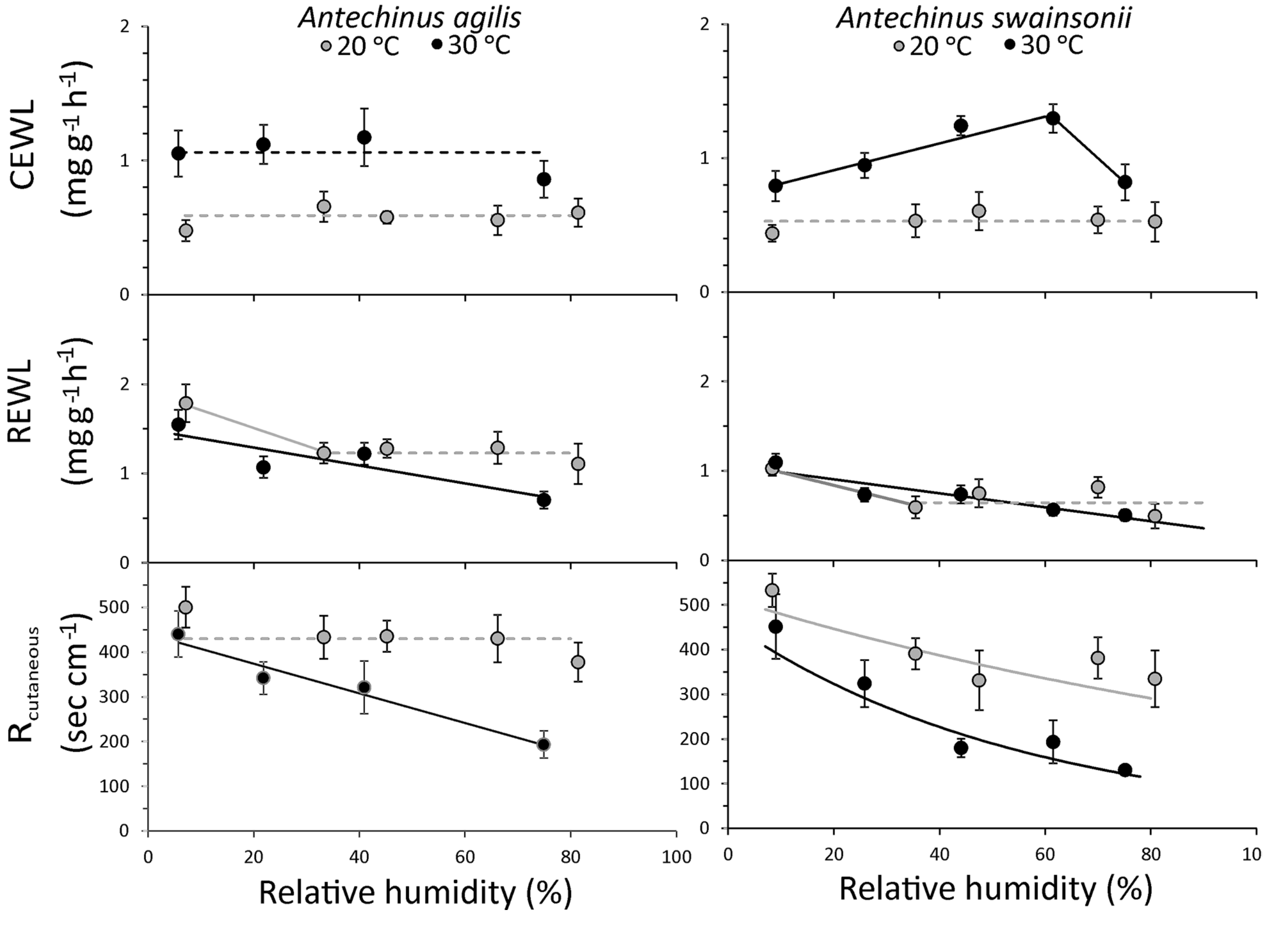

Figure 3. Cutaneous evaporative water loss (CEWL), respiratory evaporative water loss (REWL), and cutaneous resistance ($R_{\text{cutaneous}}$) for agile antechinus (*Antechinus agilis*; $N = 10$) and dusky antechinus (*Antechinus swainsonii*; $N = 10$) at varying relative humidities at ambient temperatures of 20°C (gray symbols and lines) and 30°C (black symbols and lines). Dashed lines indicate nonsignificant relative humidity effects, and solid lines indicate significant linear or a priori contrast effects for relative humidity. Values are mean ± SE.

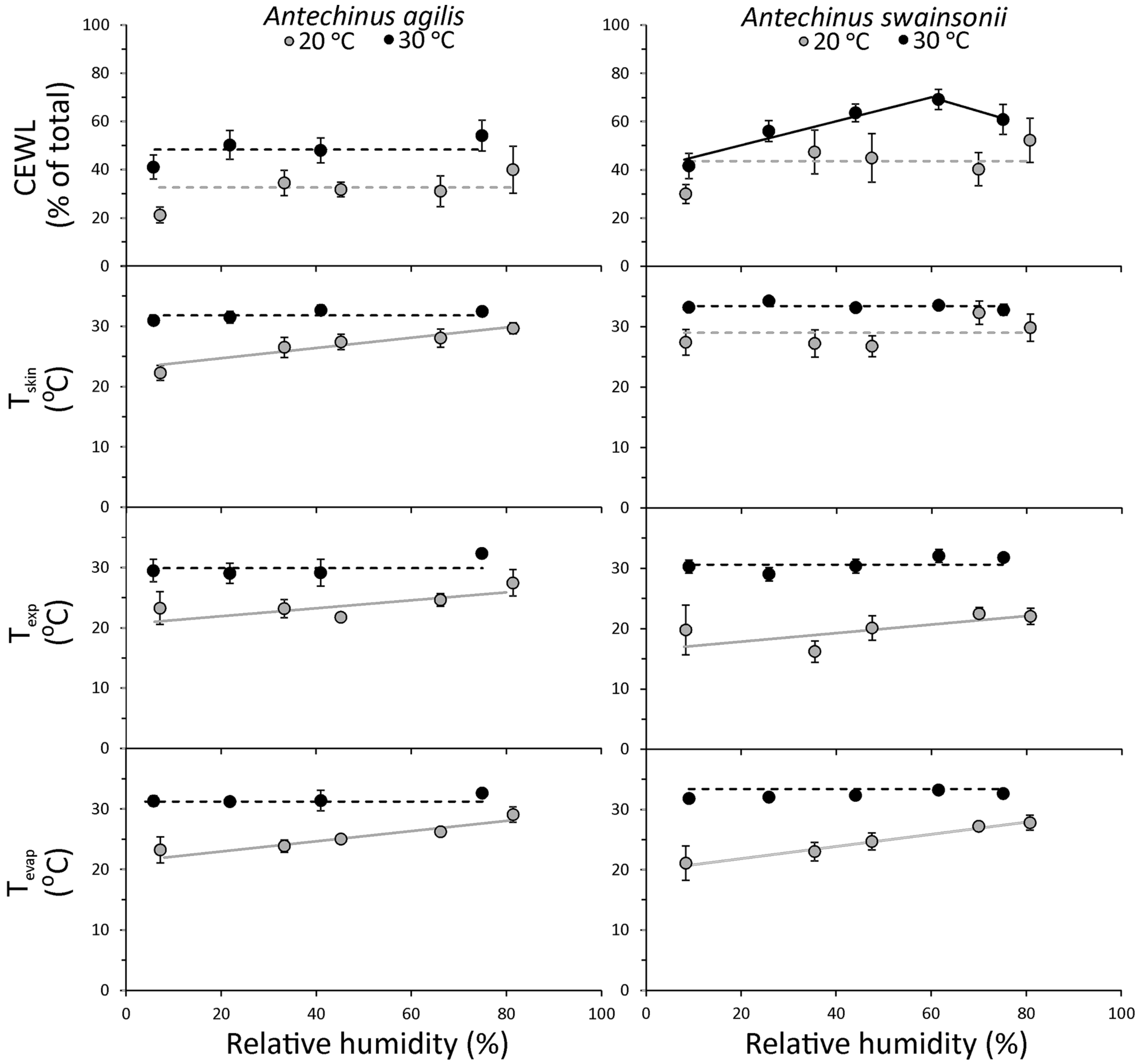

Figure 4. Pulmonary-cutaneous partitioning of evaporative water loss for agile antechinus (*Antechinus agilis*; $N = 10$) and dusky antechinus (*Antechinus swainsonii*; $N = 10$), with calculated surface temperature (T_{skin}), expired air temperature (T_{exp}), and integrated pulmonary-cutaneous surface temperature (T_{evap}) at varying relative humidities at ambient temperatures of 20°C (gray symbols and lines) and 30°C (black symbols and lines). Dashed lines indicate nonsignificant relative humidity effects, and solid lines indicate significant linear or a priori contrast effects for relative humidity. Values are mean ± SE. CEWL = cutaneous evaporative water loss.

residual variance was 24.3 (cf. the random residual variance for T_b with RH was 0.71). At $T_a = 30$°C, it was 31.2°C ± 0.5°C ($N = 10$, $n = 29$), 3.6°C less than T_b; the random residual variance was 3.22 (cf. 1.04 for T_b).

Water Loss per ΔWVP

The relationship for EWL/ΔWVP (mg g^{-1} h^{-1} kPa^{-1}; fig. 5) with RH when the "animal surface" WVP was calculated from T_b had a slope > 0 for agile antechinus at both $T_a = 20$°C ($F_{1,41} = 16.3$, $P < 0.001$) and 30°C for RH < 70% ($F_{1,31} = 50.3$, $P < 0.001$) and for dusky antechinus at $T_a = 20$°C and 30°C for RH < 70% ($F_{1,14\text{–}33} > 4.84$, $P < 0.045$). For the relationship between EWL/ΔWVP (mg g^{-1} h^{-1} kPa^{-1}) and RH when the animal surface WVP was calculated from T_{evap}, the slope was >0 for agile and dusky antechinus at $T_a = 30$°C ($F_{1,28\text{–}30} \geq 19.7$, $P \leq 0.001$) but not at $T_a = 20$°C ($F_{1,28\text{–}35} < 0.670$, $P > 0.3418$).

Discussion

For both species of alpine antechinus, our measurements of physiological variables were consistent with those measured previously for these species at low RH by Cooper et al. (2016) with similar effects of T_a. Our current data show that the EWL of agile and dusky antechinus within and below thermoneutrality does not conform to biophysical predictions of independence of RH and EWL/ΔWVP between the animal and the environment (fig. 5). This is evidence that these small mesic habitat mammals can physiologically control their insensible EWL and that this control is not restricted to mammals from more arid environments. We then examine the efficacy of EWL control by these alpine antechinus to that of arid and semiarid habitat dasyurid marsupials (fig. 6), along with the RH effect on other physiological variables, and find support for the hypothesis that EWL regulation serves a thermoregulatory purpose rather than facilitating water balance.

Effect of RH on EWL and EWL/ΔWVP

If EWL is independent of RH, then it is unequivocal that the effect of RH on EWL is inconsistent with physical predictions. We observed this independence for antechinus at RH $< 70\%$ at all T_a's and for agile antechinus over all RH at $T_a = 20°C$ (fig. 1). This is clear evidence of EWL control over a range of RH by these mesic habitat marsupials. Similar independence of RH with EWL has been reported for other mammals (Cooper and Withers 2008, 2017, 2020; Withers and Cooper 2014). It was only at the highest RH, where a small ΔWVP near saturation makes the maintenance of constant EWL challenging, that any inhibition of EWL by increasing RH was observed, as has been observed for a bird and some other small mammals (Cooper and Withers 2008, 2020; Withers and Cooper, 2014; Eto et al. 2017). It is typical for physiologically regulated variables to deviate from constancy under more extreme conditions, for example, T_b at low and high T_a (Lovegrove et al. 1991; Withers and Cooper 2009; Tattersall et al. 2012).

Linear declines in EWL with RH reported for some other mammals (Chew and Dammann 1961; Baudinette 1972; Kay 1977; Christian 1978; Edwards and Haines 1978; Welch 1980) are difficult to evaluate whether there is EWL control or if the decrease in EWL with increasing RH is consistent with physical predictions, because there is no theoretical slope for the effect of RH on EWL (Welch 1980; Eto et al 2017; Cooper and Withers 2017, 2020). However, EWL/ΔWVP should theoretically be independent of RH (slope $= 0$) for the physical model, since ΔWVP is the biophysical driving force. So a slope $\neq 0$ for EWL/ΔWVP implies EWL control (Eto et al. 2017), with a steeper slope suggesting greater deviation from the physical model (Cooper et al. 2020). Consequently, we evaluated the independence of EWL/ΔWVP from RH to provide more definitive evidence for nonconformity with the biophysical model and to enable comparison of the degree of EWL control with other species (fig. 6).

To calculate EWL/ΔWVP, we need to calculate the ΔWVP. It is straightforward to calculate the "ambient end" of the deficit from the T_a and ambient RH, but it is more complex to calculate the "animal end" of the ΔWVP. Using WVP saturation at T_a as the animal end underestimates ΔWVP because the animal's T_{evap} is typically greater than T_a. Using T_a overestimates EWL/ΔWVP and exaggerates the effect of RH (Cooper and Withers 2017; Eto et al. 2017; Cooper et al. 2020), so we do not use T_a for calculating ΔWVP here. Using T_b to calculate the animal end for ΔWVP is a

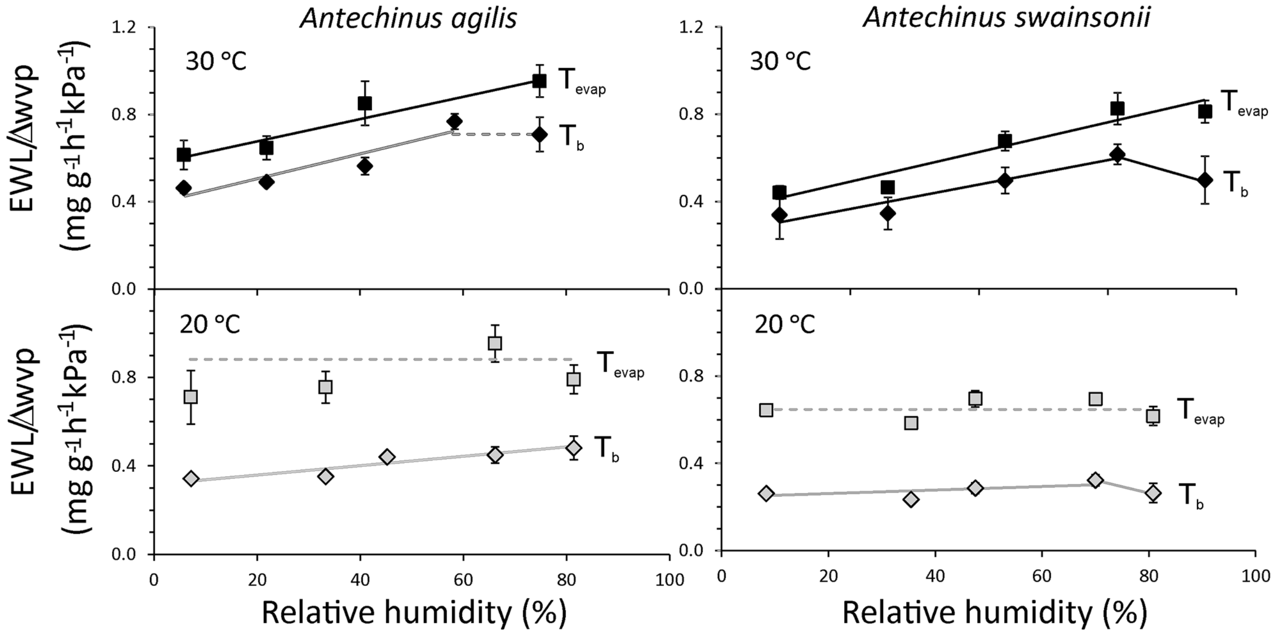

Figure 5. Evaporative water loss (EWL) per water vapor pressure deficit (ΔWVP) for agile antechinus (*Antechinus agilis*; $N = 10$) and dusky antechinus (*Antechinus swainsonii*; $N = 10$) at varying relative humidities at ambient temperatures of 20°C (gray symbols and lines) and 30°C (black symbols and lines). Diamonds indicate EWL/ΔWVP calculated from body temperature (T_b), and squares indicate EWL/ΔWVP calculated from average surface temperature for evaporation (T_{evap}). Dashed lines indicate nonsignificant relative humidity effects, and solid lines indicate significant linear or a priori contrast effects for relative humidity. Values are mean ± SE.

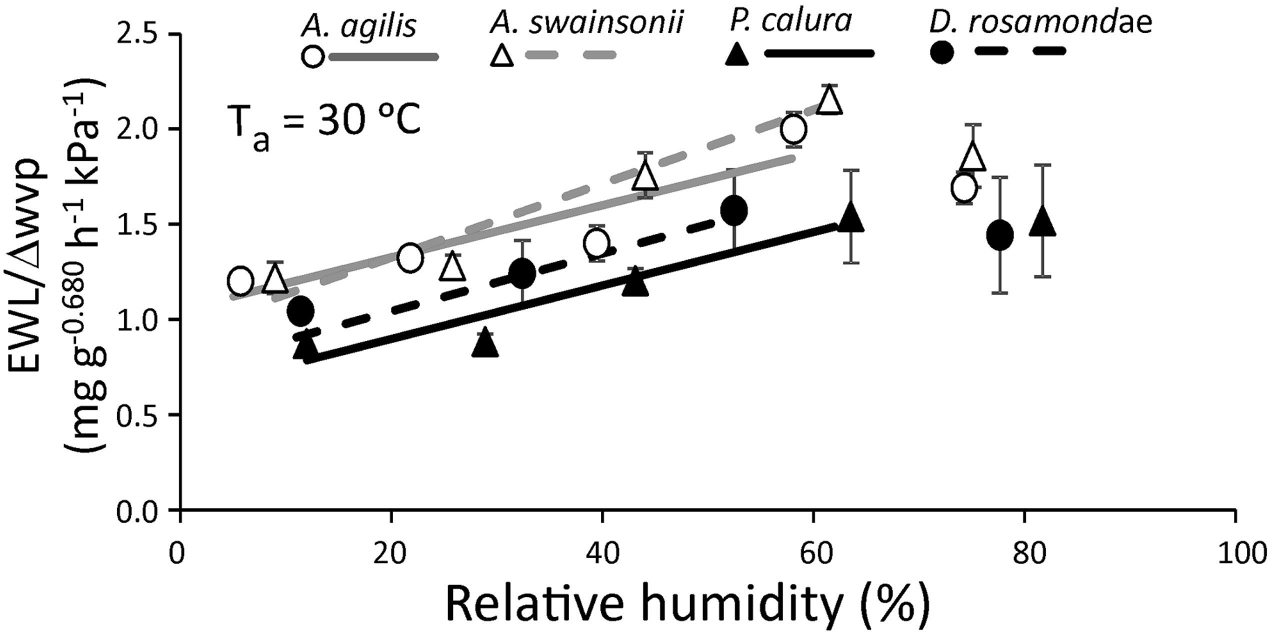

Figure 6. Evaporative water loss (EWL) per water vapor pressure deficit (ΔWVP) for mesic habitat antechinus (*Antechinus agilis* and *Antechinus swainsonii*; open symbols and gray lines) compared with two dasyurid marsupials from arid and semiarid habitats (red-tailed phascogale, *Phascogale calura*, and little red kaluta, *Dasykaluta rosamondae*; black symbols and lines), corrected for allometric scaling, at varying relative humidities and an ambient temperature of 30°C. Lines indicate significant linear effects ($P < 0.05$); highest relative humidity values are excluded from linear regression analysis. Values are mean ± SE.

conservative approach to assess EWL control, as $T_b > T_{evap}$. This approach will overestimate ΔWVP and underestimate the EWL/ΔWVP change with ambient RH (fig. 5). The most realistic calculation of ΔWVP uses the animal's T_{evap}, which is intermediate between T_a and T_b, but calculating T_{evap} requires the partitioning of EWL into CEWL and REWL and the estimation of T_{skin} and T_{exp}.

Our results for the most conservative approach of evaluating EWL control using T_b to calculate ΔWVP are consistent with the constancy of EWL with RH we observed and provide further robust evidence for EWL control by these mesic habitat antechinus. Despite underestimating the slopes of EWL/ΔWVP versus RH, these slopes were still significantly >0 for both species at $T_a = 20$°C and 30°C. As expected, our estimate of T_{evap} was lower than T_b, so EWL/ΔWVP values were higher than for T_b calculations (fig. 5).

Environment and Effect of RH on EWL/ΔWVP

To test our hypotheses that RH control of EWL has either a water balance or a thermoregulatory function for mammals, we compared the effect of RH on EWL/ΔWVP for the two alpine antechinuses with that for the semiarid habitat red-tail phascogale (*Phascogale calura*; Cooper and Withers 2017) and arid habitat kaluta (*Dasykalua rosamondae*; Withers and Cooper 2014), for which the EWL response to RH was examined with the same methodology within thermoneutrality (fig. 6). To account for species differences in body mass we mass standardized absolute EWL (mg $g^{-0.68}$ g^{-1} h^{-1}) using a scaling exponent of 0.68 (Withers et al. 2006; Van Sant et al. 2012). We found that the slopes for the effect of RH on EWL/ΔWVP were not different for the alpine species compared with arid/semiarid species (contrast $t_{118} = 1.41$, $P = 0.160$). Cooper et al. (2020) found similar comparable EWL control for mesic and arid habitat parrots and argued that this indicates that the control of EWL does not have a water conservation role for birds. Consequently, we conclude that mesic habitat mammals can control their EWL, and there is no evidence that arid/semiarid habitat species have a more pronounced RH response for EWL/ΔWVP than mesic species from an alpine environment.

Partitioning of CEWL and REWL

Partitioning total EWL into its cutaneous and pulmonary components is important for evaluating their relative contributions to, and their potential roles in, control of EWL. The experimental procedure for partitioning typically involves physically separating the head (REWL) and body (CEWL) with a membrane or mask, but here we used the noninvasive modeling approach of Withers et al. (2012) to avoid potential impacts of more direct approaches (e.g., Muñoz-Garcia et al. 2012; Minnaar et al. 2014).

Total EWL was predominately REWL (~70%) for both species at $T_a = 20$°C (fig. 4), while in the TNZ, cutaneous and respiratory pathways contributed equally (although there was a RH effect for dusky antechinus, with the proportion of CEWL increasing with increasing RH). These are similar to partitioning of EWL for the monito del monte (*Dromiciops gliroides*) using the same modeling methodology (Withers et al. 2012), which had CEWL with REWL ratios of 23 : 77 (at $T_a = 14$°C) to 37 : 63 (30°C). Partitioning for other small mammals using more invasive methods results in a similar to higher cutaneous component of ~50% to 80% at and below thermoneutrality (Chew 1955; Tracy and Walsberg 2000; Muñoz-Garcia et al. 2012; Minnaar et al. 2014).

REWL typically decreased with increasing RH (fig. 3) as predicted by modeling of nasal countercurrent exchange (Welch and Tracy 1977), suggesting that REWL played a limited role in maintaining EWL constancy at varying RH. In contrast, CEWL remained constant or increased with increasing RH, presumably driven by decreasing $R_{\text{cutaneous}}$ (as observed at $T_a = 30°C$) and/or increasing T_{skin} at higher RH (fig.3). There are few $R_{\text{cutaneous}}$ values for other mammals to compare with values for antechinus; values of about 100–400 s cm^{-1} have been reported for humans, spiny mice, and laboratory mice (Campbell and Norman 1998; Lillywhite 2006). Our calculated values for resting, undisturbed animals encompassed but exceeded this range, from ~200 to 500 s cm^{-1}, depending on the T_a and RH combination. Edwards and Haines (1978) reported significant decreases of both REWL and CEWL with RH for deer mice (*Peromyscus maniculatus*) and house mice (*Mus musculus*) at $T_a = 20°C$ and 30°C, but it is possible that their short measurement durations, physical partitioning of CEWL and REWL, and previous exposure to ether may have affected the animal's propensity to control EWL independent of EWL.

The mechanisms by which $R_{\text{cutaneous}}$ might respond to varying RH include changes in skin blood flow and temperature, changes in piloerection, low RH decreasing the water content and permeability of the stratum corneum layer (see Grice et al. 1972), and changes to skin lipids. Chronic changes to the CEWL of bats can be achieved by changes in their skin lipid composition (Muñoz-Garcia et al. 2012), and for birds these microstructural changes in the skin occur within time frames of hours to weeks (Menon et al. 1996; Muñoz-Garcia and Williams 2008; Muñoz-Garcia et al. 2008), so it is not unreasonable to suggest that changes to skin structure that impact CEWL contribute to the acute EWL control we observed here for small mammals. For kangaroo rats (*Dipodomys merriami*; Tracy and Walsberg 2000, 2001), developmental, acclimatory, and adaptive changes in total EWL are most likely driven by CEWL. A CEWL-mediated mechanism for EWL control would explain why EWL control is typically more pronounced at thermoneutrality than at lower T_a where REWL becomes a greater component of total EWL; there is more scope for moderation of total EWL when CEWL is a larger component.

Effects of RH on Other Physiological Variables

For both species of antechinus, RH had little effect on most metabolic and respiratory variables (figs. 1, 2). Both T_b and $\dot{V}O_2$ were independent of RH, and conductance was affected at only the highest RHs for agile antechinus at $T_a = 30°C$, where EWL and consequently EHL declined significantly. A similar lack of effect of RH on T_b, $\dot{V}O_2$, and $C_{\text{wet}}/C_{\text{dry}}$ has been reported for various other small mammals (Baudinette 1972; Ewing and Studier 1973; Cooper and Withers 2008, 2017; Withers and Cooper 2014), although Kay (1975) noted some inconsistent effects of RH on T_b for kangaroo rats, and $\dot{V}O_2$ of white mice is aberrant at high RH (Ewing and Studier 1973). As respiratory variables typically accommodate $\dot{V}O_2$ demand for small mammals at and below the TNZ (Hallam and Dawson 1993), these were generally unaffected by RH in keeping with constant $\dot{V}O_2$, with the exception of EO_2, which typically decreased with RH. The increase in f_R and V_I at the highest RH at $T_a = 30°C$ presumably reflected some inhibition of EHL and some initial heat challenge. This supports our early hypothesis that the highest RH posed a regulatory challenge for EWL control.

The general constancy of the thermal physiology over a range of RH for both antechinus species supports our alternative hypothesis for the role of EWL control for small mammals; we suggest that it is important for maintaining thermoregulation by minimizing the impact of environmental RH on EHL and therefore heat balance. This hypothesis is consistent with observations of heterothermic endotherms controlling their EWL when thermoregulating but not when thermoconforming (Cooper and Withers 2017; Klüg-Baerwald and Brigham 2017) and with conclusions based on equivalent EWL control by both mesic and arid habitat birds (Cooper et al. 2020). We conclude that control of EWL is a convergent feature of thermoregulation by endothermic mammals and birds. Furthermore, we suggest that RH control of EWL at and below the TNZ is an extension of the same physiological regulatory processes for thermoregulatory EWL at high T_a and is probably under the same autonomic control (Jessen 2001; Gerson et al. 2014; Smith and Johnson 2016; Fuller et al. 2019). Consequently, we suggest that EWL in and below the TNZ should not be considered a passive, insensible physical process but rather is part of a continuum of processes that become sensible thermoregulatory processes at high T_a.

Acknowledgments

This study was supported by an Australian Research Council Discovery Project grant (DP160103627) to P.C.W. and C.E.C. Experiments followed the Australian Code of Practice for the care and use of animals for scientific purposes and were approved by the University of New England Animal Ethics Committee (ARE 2016-3), with reciprocal approval from Curtin University and the University of Western Australia. Collection of antechinuses was conducted under license from the New South Wales National Parks and Wildlife Service; we thank Ken Green and Mel Schroder for advice on trapping antechinuses and logistical support.

APPENDIX

Table A1: Summary data for *Antechinus agilis* at ambient temperatures of 20°C and 30°C and varying relative humidity (RH)

	Ambient temperature = 20°C				
	RH = 7%	RH = 33%	RH = 45%	RH = 66%	RH = 81%
Body mass (g)	23.5 ± 1.53 (9)	22.1 ± 1.57 (8)	21.8 ± 1.33 (10)	22.5 ± 1.64 (8)	22.5 ± 1.40 (9)
Body temperature (°C)	34.9 ± .35 (9)	35.2 ± .53 (8)	34.4 ± .57 (10)	34.2 ± .47 (8)	35.26 ± .30 (9)
Oxygen consumption (mL O_2 g^{-1} h^{-1})	2.58 ± .106 (9)	2.32 ± .099 (8)	2.47 ± .182 (10)	2.39 ± .150 (8)	2.55 ± .102 (9)
Carbon dioxide production (mL CO_2 g^{-1} h^{-1})	1.96 ± .082 (9)	1.59 ± .068 (8)	1.67 ± .113 (10)	1.50 ± .106 (8)	1.67 ± .064 (9)
Respiratory exchange ratio (mL CO_2 mL O_2^{-1})	.762 ± .025 (9)	.690 ± .027 (8)	.680 ± .019 (10)	.630 ± .028 (8)	.665 ± .035 (9)
Evaporative water loss (EWL; mg g^{-1} h^{-1})	1.86 ± .081 (9)	1.73 ± .073 (8)	1.94 ± .095 (10)	1.73 ± .145 (8)	1.76 ± .174 (8)
Wet thermal conductance (J g^{-1} h^{-1} °C^{-1})	3.65 ± .157 (9)	3.02 ± .153 (8)	3.40 ± .234 (10)	3.29 ± .187 (8)	3.38 ± .196 (9)
Dry thermal conductance (J g^{-1} h^{-1} °C^{-1})	3.33 ± .154 (9)	2.72 ± .145 (8)	3.08 ± .223 (10)	3.00 ± .179 (8)	3.17 ± .200 (8)
Respiratory frequency (min^{-1})	63.8 ± 4.22 (4)	65.1 ± 4.81 (8)	62.8 ± 2.87 (10)	65.1 ± 3.47 (8)	65.1 ± 4.54 (8)
Tidal volume (mL STPD)	.454 ± .106 (4)	.394 ± .035 (8)	.454 ± .038 (10)	.454 ± .045 (8)	.466 ± .070 (8)
Respiratory minute volume (mL min^{-1} STPD)	28.2 ± 5.35 (4)	26.3 ± 3.68 (8)	28.8 ± 2.42 (10)	30.2 ± 3.64 (8)	29.7 ± 5.85 (8)
Oxygen extraction (%)	18.7 ± 1.70 (4)	17.4 ± 1.93 (8)	15.5 ± 1.03 (10)	15.7 ± 1.35 (8)	17.0 ± 1.36 (8)
Cutaneous EWL (mg g^{-1} h^{-1})	.476 ± .080 (4)	.657 ± .113 (8)	.576 ± .046 (10)	.555 ± .109 (8)	.611 ± .104 (7)
Cutaneous EWL (%)	21.2 ± 3.29 (4)	34.5 ± 5.22 (8)	31.7 ± 3.03 (10)	31.1 ± 6.43 (8)	40.0 ± 9.75 (7)
Skin temperature (°C)	22.3 ± 1.24 (4)	26.5 ± .165 (8)	27.4 ± 1.27 (10)	28.1 ± 1.49 (8)	29.7 ± .931 (7)
Cutaneous resistance (s cm^{-1})	501 ± 45 (4)	433 ± 48 (8)	436 ± 35 (10)	431 ± 53 (8)	378 ± 44 (7)
Respiratory EWL (mg g^{-1} h^{-1})	1.79 ± .212 (4)	1.23 ± .115 (8)	1.28 ± .105 (10)	1.29 ± .139 (8)	1.11 ± .224 (7)
Expired air temperature (°C)	23.3 ± 2.70 (4)	23.2 ± 1.52 (8)	21.8 ± 1.02 (10)	24.6 ± 1.06 (8)	27.5 ± 2.20 (7)
Average evaporative temperature (°C)	23.2 ± 2.15 (4)	23.9 ± 1.02 (8)	25.0 ± .76 (10)	26.2 ± .80 (8)	29.1 ± 1.29 (7)
EWL ΔWVP T_b^{-1} (mg g^{-1} h^{-1} kPa^{-1})	.343 ± .016 (9)	.353 ± .017 (8)	.441 ± .021 (10)	.450 ± .037 (8)	.482 ± .054 (8)
EWL ΔWVP T_{evap}^{-1} (mg g^{-1} h^{-1} kPa^{-1})	.710 ± .121 (4)	.755 ± .072 (8)	1.054 ± .077 (10)	.953 ± .083 (8)	.791 ± .065 (7)

	Ambient temperature = 30°C				
	RH = 6%	RH = 22%	RH = 41%	RH = 58%	RH = 75%
Body mass (g)	20.3 ± 1.18 (8)	22.8 ± 1.24 (11)	18.9 ± 1.17 (10)	18.7 ± .81 (9)	23.1 ± 1.75 (8)
Body temperature (°C)	34.9 ± .38 (8)	34.7 ± .14 (11)	35.5 ± .28 (10)	34.5 ± .26 (9)	34.5 ± .21 (8)
Oxygen consumption (mL O_2 g^{-1} h^{-1})	1.41 ± .072 (8)	1.20 ± .046 (11)	1.28 ± .058 (10)	1.42 ± .053 (9)	1.46 ± .117 (8)
Carbon dioxide production (mL CO_2 g^{-1} h^{-1})	.911 ± .056 (8)	.862 ± .033 (11)	.937 ± .044 (10)	1.07 ± .047 (9)	.960 ± .102 (8)
Respiratory exchange ratio (mL CO_2 mL O_2^{-1})	.652 ± .031 (8)	.720 ± .012 (11)	.746 ± .047 (10)	.755 ± .030 (9)	.662 ± .041 (8)
EWL (mg g^{-1} h^{-1})	2.49 ± .157 (8)	2.26 ± .079 (11)	2.26 ± .120 (7)	2.20 ± .126 (8)	1.53 ± .110 (8)
Wet thermal conductance (J g^{-1} h^{-1} $°C^{-1}$)	6.49 ± .816 (8)	5.50 ± .272 (11)	5.28 ± .395 (10)	8.87 ± .863 (9)	7.40 ± .948 (8)
Dry thermal conductance (J g^{-1} h^{-1} $°C^{-1}$)	5.13 ± .716 (8)	4.23 ± .229 (11)	4.26 ± .469 (7)	7.60 ± .665 (8)	6.46 ± .841 (8)
Respiratory frequency (min^{-1})	47.1 ± 3.53 (8)	41.7 ± 2.39 (11)	50.1 ± 7.56 (8)	...	61.8 ± 5.13 (7)
Tidal volume (mL STPD)	.280 ± .028 (8)	.324 ± .038 (11)	.355 ± .054 (8)	...	.316 ± .044 (7)
Respiratory minute volume (mL min^{-1} STPD)	13.0 ± 1.56 (8)	13.1 ± 1.34 (11)	18.7 ± 3.64 (8)	...	18.0 ± 1.55 (7)
Oxygen extraction (%)	18.7 ± 1.45 (8)	18.6 ± .78 (11)	17.2 ± 1.17 (8)	...	15.8 ± 1.05 (7)
Cutaneous EWL (mg g^{-1} h^{-1})	1.05 ± .172 (8)	1.12 ± .146 (10)	1.17 ± .214 (7)	...	.860 ± .137 (7)
Cutaneous EWL (%)	41.1 ± 4.96 (8)	50.2 ± 5.95 (10)	48.0 ± 5.12 (7)	...	54.1 ± 6.36 (7)
Skin temperature (°C)	31.0 ± .70 (8)	31.5 ± .99 (10)	32.7 ± .833 (7)	...	32.5 ± .408 (7)
Cutaneous resistance (s cm^{-1})	441 ± 52 (8)	342 ± 37 (10)	321 ± 59 (7)	...	193 ± 30 (7)
Respiratory EWL (mg g^{-1} h^{-1})	1.55 ± .164 (8)	1.07 ± .120 (10)	1.22 ± .122 (7)	...	.71 ± .095 (7)
Expired air temperature (°C)	29.5 ± 1.87 (8)	29.0 ± 1.64 (10)	29.1 ± 2.20 (7)	...	32.3 ± .68 (7)
Average evaporative temperature (°C)	31.3 ± .84 (8)	31.2 ± .60 (8)	31.4 ± 1.70 (6)	...	32.6 ± .44 (7)
EWL ΔWVP T_b^{-1} (mg g^{-1} h^{-1} kPa^{-1})	.463 ± .023 (8)	.490 ± .017 (11)	.564 ± .039 (7)	.768 ± .035 (7)	.709 ± .079 (8)
EWL ΔWVP T_{evap}^{-1} (mg g^{-1} h^{-1} kPa^{-1})	.615 ± .067 (8)	.648 ± .053 (8)	.851 ± .101 (6)	...	.953 ± .073 (7)

Note. Values are mean ± SE with N (number of animals) in parentheses. Average surface temperature (T_{evap}) was calculated from skin temperature and expired air temperature, weighted for the partitioning of total EWL into cutaneous and pulmonary components. EWL was also calculated per water vapor pressure differential (ΔWVP), determined as the difference between saturation water vapor pressure at body temperature (ΔWVP T_b^{-1}) and at average evaporative temperature (ΔWVP T_{evap}^{-1}).

Table A2: Summary data for *Antechinus swainsonii* at ambient temperatures of 20°C and 30°C and varying relative humidity (RH)

	Ambient temperature = 20°C				
	RH = 8%	RH = 35%	RH = 47%	RH = 70%	RH = 81%
Body mass (g)	64.1 ± 4.8 (8)	63.4 ± 6.9 (6)	63.9 ± 6.8 (6)	61.9 ± 5.7 (7)	63.7 ± 6.9 (7)
Body temperature (°C)	35.8 ± .39 (8)	36.0 ± .45 (6)	35.8 ± .56 (6)	35.8 ± .47 (7)	35.7 ± .44 (7)
Oxygen consumption (mL O_2 g^{-1} h^{-1})	2.34 ± .10 (8)	2.04 ± .17 (6)	2.04 ± .16 (6)	2.40 ± .16 (7)	1.10 ± .09 (7)
Carbon dioxide production (mL CO_2 g^{-1} h^{-1})	1.60 ± .066 (8)	1.41 ± .094 (6)	1.43 ± .110 (6)	1.70 ± .096 (7)	1.42 ± .055 (7)
Respiratory exchange ratio (mL CO_2 mL $O_2{}^{-1}$)	.725 ± .014 (8)	.697 ± .022 (6)	.703 ± .023 (6)	.705 ± .013 (7)	.679 ± .015 (7)
Evaporative water loss (EWL; mg g^{-1} h^{-1})	1.48 ± .05 (8)	1.12 ± .09 (6)	1.35 ± .10 (6)	1.35 ± .06 (7)	1.02 ± .15 (7)
Wet thermal conductance (J g^{-1} h^{-1} $°C^{-1}$)	2.97 ± .101 (8)	2.53 ± .247 (6)	2.59 ± .201 (6)	3.03 ± .245 (7)	2.68 ± .106 (7)
Dry thermal conductance (J g^{-1} h^{-1} $°C^{-1}$)	2.73 ± .095 (8)	2.36 ± .241 (6)	2.38 ± .187 (6)	2.82 ± .232 (7)	2.52 ± .096 (7)
Respiratory frequency (min^{-1})	63.2 ± 2.81 (5)	67.5 ± 2.94 (6)	68.5 ± 6.02 (6)	72.1 ± 5.91 (7)	70.7 ± 4.57 (7)
Tidal volume (mL STPD)	.808 ± .066 (5)	.821 ± .059 (6)	.921 ± .158 (6)	1.060 ± .214 (7)	1.008 ± .131 (7)
Respiratory minute volume (mL min^{-1} STPD)	60.7 ± 10.94 (5)	60.7 ± 8.38 (6)	61.1 ± 8.77 (6)	77.5 ± 9.17 (7)	69.0 ± 7.12 (7)
Oxygen extraction (%)	21.4 ± 2.29 (5)	17.1 ± 2.05 (6)	16.1 ± 1.36 (6)	15.8 ± .90 (7)	15.5 ± .70 (7)
Cutaneous EWL (mg g^{-1} h^{-1})	.488 ± .061 (5)	.582 ± .123 (6)	.605 ± .143 (6)	.538 ± .102 (7)	.526 ± .148 (7)
Cutaneous EWL (%)	30.0 ± 3.9 (5)	47.4 ± 9.1 (6)	44.9 ± 10.1 (6)	40.2 ± 6.9 (7)	52.2 ± 9.2 (7)
Skin temperature (°C)	27.4 ± 2.14 (4)	27.2 ± 2.25 (6)	26.8 ± 1.72 (6)	32.3 ± 1.93 (7)	29.8 ± 2.39 (7)
Cutaneous resistance (s cm^{-1})	533 ± 38 (5)	391 ± 35 (6)	331 ± 67 (6)	382 ± 46 (6)	334 ± 63 (7)
Respiratory evaporative water loss (mg g^{-1} h^{-1})	1.025 ± .079 (5)	.590 ± .123 (6)	.748 ± .158 (6)	.815 ± .114 (7)	.492 ± .137 (7)
Expired air temperature (°C)	19.8 ± 4.15 (4)	16.2 ± 1.78 (6)	20.1 ± 2.04 (6)	22.5 ± 1.05 (7)	22.0 ± 1.35 (7)
Average evaporative temperature (°C)	21.1 ± 2.83 (5)	23.0 ± 1.55 (6)	24.7 ± 1.40 (6)	27.2 ± .51 (7)	27.8 ± 1.24 (7)
EWL ΔWVP $T_b{}^{-1}$ (mg g^{-1} h^{-1} kPa^{-1})	.262 ± .011 (8)	.235 ± .012 (5)	.287 ± .025 (6)	.323 ± .026 (7)	.264 ± .044 (7)
EWL ΔWVP $T_{evap}{}^{-1}$ (mg g^{-1} h^{-1} kPa^{-1})	.645 ± .111 (4)	.585 ± .074 (6)	.696 ± .059 (6)	.696 ± .046 (7)	.617 ± .110 (7)

	Ambient temperature = 30°C				
	RH = 9%	RH = 26%	RH = 44%	RH = 62%	RH = 75%
Body mass (g)	56.3 ± 4.1 (8)	62.6 ± 4.5 (8)	50.9 ± 3.9 (9)	52.7 ± 3.6 (10)	63.8 ± 4.9 (8)
Body temperature (°C)	36.1 ± .41 (8)	36.1 ± .47 (8)	36.0 ± .38 (9)	35.6 ± .13 (10)	36.4 ± .46 (8)
Oxygen consumption (mL O_2 g^{-1} h^{-1})	1.23 ± .56 (8)	1.15 ± .053 (8)	1.19 ± .059 (9)	1.18 ± .053 (10)	1.19 ± .053 (8)
Carbon dioxide production (mL CO_2 g^{-1} h^{-1})	.859 ± .026 (8)	.766 ± .026 (8)	.875 ± .032 (9)	.871 ± .031 (10)	.864 ± .046 (8)
Respiratory exchange ratio (mL CO_2 mL O_2^{-1})	.705 ± .020 (8)	.669 ± .029 (8)	.746 ± .026 (9)	.748 ± .026 (10)	.723 ± .021 (8)
EWL (mg g^{-1} h^{-1})	1.88 ± .08 (8)	1.68 ± .08 (8)	1.97 ± .13 (9)	1.87 ± .08 (10)	1.34 ± .09 (8)
Wet thermal conductance (J g^{-1} h^{-1} °C^{-1})	4.49 ± .460 (8)	4.26 ± .298 (8)	4.49 ± .370 (9)	5.46 ± .426 (10)	4.30 ± .336 (8)
Dry thermal conductance (J g^{-1} h^{-1} °C^{-1})	3.65 ± .387 (8)	3.50 ± .249 (8)	3.58 ± .323 (9)	4.39 ± .355 (10)	3.71 ± .306 (8)
Respiratory frequency (min^{-1})	45.0 ± 1.90 (8)	49.3 ± 3.47 (8)	40.68 ± 2.35 (9)	43.0 ± 2.17 (9)	58.7 ± 5.66 (5)
Tidal volume (mL STPD)	.645 ± .077 (8)	.552 ± .164 (8)	.654 ± .070 (9)	.620 ± .062 (9)	.695 ± .103 (5)
Respiratory minute volume (mL min^{-1} STPD)	28.6 ± 3.30 (8)	26.2 ± 1.72 (8)	25.5 ± 1.74 (9)	26.0 ± 2.25 (9)	38.6 ± 3.01 (5)
Oxygen extraction (%)	20.4 ± 1.63 (8)	22.2 ± .64 (8)	19.7 ± 2.29 (9)	18.8 ± .94 (9)	15.8 ± 1.69 (5)
Cutaneous EWL (mg g^{-1} h^{-1})	.792 ± .113 (7)	.946 ± .094 (8)	1.241 ± .072 (9)	1.296 ± .107 (9)	.820 ± .134 (5)
Cutaneous EWL (%)	41.6 ± 5.2 (7)	56.0 ± 4.3 (8)	63.6 ± 3.7 (9)	69.1 ± 4.2 (9)	60.8 ± 6.2 (5)
Skin temperature (°C)	33.2 ± .73 (7)	34.3 ± .67 (8)	33.1 ± .58 (9)	33.5 ± .53 (9)	32.8 ± .91 (5)
Cutaneous resistance (s cm^{-1})	451 ± 73 (7)	324 ± 53 (8)	180 ± 21 (9)	193 ± 48 (9)	130 ± 9 (5)
Respiratory EWL (mg g^{-1} h^{-1})	1.094 ± .096 (7)	.733 ± .075 (8)	.736 ± .099 (9)	.563 ± .063 (9)	.502 ± .063 (5)
Expired air temperature (°C)	30.3 ± 1.08 (7)	29.0 ± 1.13 (8)	30.4 ± 1.14 (9)	32.0 ± 1.09 (9)	31.8 ± .60 (5)
Average evaporative temperature (°C)	31.8 ± .60 (7)	32.1 ± .73 (8)	32.3 ± .62 (9)	33.2 ± .57 (9)	32.7 ± .60 (5)
EWL ΔWVP T_b^{-1} (mg g^{-1} h^{-1} kPa^{-1})	.339 ± .021 (8)	.346 ± .016 (8)	.496 ± .037 (9)	.616 ± .024 (10)	.499 ± .043 (8)
EWL ΔWVP T_{evap}^{-1} (mg g^{-1} h^{-1} kPa^{-1})	.441 ± .030 (7)	.465 ± .028 (8)	.677 ± .044 (9)	.827 ± .073 (9)	.813 ± .050 (5)

Note. Values are mean ± SE with N (number of animals) in parentheses. Average surface temperature (T_{evap}) was calculated from skin temperature and expired air temperature, weighted for the partitioning of total EWL into cutaneous and pulmonary components. EWL was also calculated per water vapor pressure differential (ΔWVP), determined as the difference between saturation water vapor pressure at body temperature (ΔWVP T_b^{-1}) and at average evaporative temperature (ΔWVP T_{evap}^{-1}).

Literature Cited

Bates D., M. Mächler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using lme4. J Stat Softw 67:1–48.

Baudinette R.V. 1972. Energy metabolism and evaporative water loss in the California ground squirrel. J Comp Physiol 81:57–72.

Benedict F.G. and H.F. Root. 1926. Insensible perspiration: its relation to human physiology and pathology. Archiv Int Med 38:1–35.

Calder W.A. and E.J. Braun. 1983. Scaling of osmotic regulation in mammals and birds. Am J Physiol 244:R601–R606.

Campbell G.S. and J.M. Norman. 1998. An introduction to environmental biophysics. Springer, New York.

Chew R.M. 1951. The water exchanges of some small mammals. Ecol Monogr 21:215–225.

———. 1955. The skin and respiratory water losses of *Peromyscus maniculatus sonoriensis*. Ecology 36:463–467.

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

Christian D.P. 1978. Effects of humidity and body size on evaporative water loss in three desert rodents. Comp Biochem Physiol A 60:425–430.

Commission for Thermal Physiology of the International Union of Physiological Sciences (IUPS). 2003. Glossary of terms for thermal physiology. 3rd ed. J Therm Biol 28:75–106.

Cooper C.E. and P.C. Withers. 2008. Allometry of evaporative water loss in marsupials: implications of the effect of ambient relative humidity on the physiology of brushtail possums (*Trichosurus vulpecula*). J Exp Biol 211:2759–2766.

———. 2014. Physiological responses of a rodent to heliox reveal constancy of evaporative water loss under perturbing environmental conditions. Am J Physiol 307:R1042–R1048.

———. 2017. Thermoregulatory role of insensible evaporative water loss constancy in a heterothermic marsupial. Biol Lett 13:20170537.

———. 2020. Two lines of evidence for physiological control of insensible evaporative water loss by a tiny marsupial. J Exp Biol 223:jeb234450.

Cooper C.E., P.C. Withers, A. Hardie, and F. Geiser. 2016. Marsupials don't adjust their thermal energetics for life in an alpine environment. Temperature 3:484–498.

Cooper C.E., P.C. Withers, G. Körtner, and F. Geiser. 2020. Does control of insensible evaporative water loss by two species of mesic parrot have a thermoregulatory role? J Exp Biol 223:jeb229930.

Cox R.M., A. Muñoz-Garcia, M.S. Jurkowitz, and J.B. Williams. 2008. *β*-glucocerebrosidase activity in the stratum corneum of house sparrows following acclimation to high and low humidity. Physiol Biochem Zool 81:97–105.

Dawson T.J. and A.J. Hulbert. 1970. Standard metabolism, body temperature, and surface areas of Australian marsupials. Am J Physiol 218:1233–1238.

Dethier V.G. and L.M. Schoonhoven. 1968. Evaluation of evaporation by cold and humidity receptors in caterpillars. J Insect Physiol 14:1049–1054.

Edwards R.M. and H. Haines. 1978. Effects of ambient water vapor pressure and temperature on evaporative water loss in *Peromyscus maniculatus* and *Mus musculus*. J Comp Physiol 128:177–184.

Eto E., P.C. Withers, and C.E. Cooper. 2017. Can birds do it too? evidence for convergence in evaporative water loss regulation for birds and mammals. Proc R Soc B 284:20171478.

Ewing W.G. and E.H. Studier. 1973. A method for control of water vapor pressure and its effect on metabolism and body temperature in *Mus musculus*. Comp Biochem Physiol A 45:121–125.

Fuller A., S.K. Maloney, D. Blache, and C.E. Cooper. 2019. Endocrine and metabolic consequences of climate change for terrestrial mammals. Curr Opin Endocr Metab Res 11:9–14.

Gerson A.R., E.K. Smith, B. Smit, A.E. McKechnie, and B.O. Wolf. 2014. The impact of humidity on evaporative cooling in small desert birds exposed to high air temperatures. Physiol Biochem Zool 87:782–795.

Gilson L.N., C.E. Cooper, P.C. Withers, and M.M. Gagnon. 2021. Two independent approaches to assessing the constancy of evaporative water loss for birds under varying evaporative conditions. Comp Biochem Physiol A 261: 111041.

Grice K., H. Sattar, and H. Baker. 1972. The effect of ambient humidity on transepidermal water loss. J Invest Derm 58: 343–346.

Hallam J.F. and T.J. Dawson. 1993. The pattern of respiration with increasing metabolism in a small dasyurid marsupial. Respir Physiol 93:305–314.

Hinds D.S. and R.E. MacMillen. 1985. Scaling of energy metabolism and evaporative water loss in heteromyid rodents. Physiol Zool 58:282–298.

Jessen C. 2001. Temperature regulation in humans and other mammals. Springer, Berlin.

Kanosue K., T. Hosono, Y.-H. Zhang, and X.-M. Chen. 1998. Neuronal networks controlling thermoregulatory effectors. Prog Brain Res 115:49–62.

Kay F.R. 1975. Environmental physiology of the banner-tailed kangaroo rat. I. Influences of ambient temperature, humidity and carbon dioxide on body temperature. Comp Biochem Physiol A 50:483–488.

———. 1977. Environmental physiology of the banner-tailed kangaroo rat. II. Influences of the burrow environment on metabolism and water loss. Comp Biochem Physiol A 57: 471–477.

Klüg-Baerwald B.J. and R.M. Brigham. 2017. Hung out to dry? intraspecific variation in water loss in a hibernating bat. Oecologia 183:977–985.

Lillywhite H.B. 2006. Water relations of tetrapod integument. J Exp Biol 209:202–226.

Liu L., Y. Li, R. Wang, C. Yin, Q. Dong, H. Hing, and M.J. Welsh. 2007. *Drosophila* hygrosensation requires the TRP channels water witch and nanchung. Nature 450:294–298.

Lovegrove B.G., G. Heldmaier, and T. Ruf. 1991. Perspectives of endothermy revisited: the endothermic temperature range. J Therm Biol 16:184–197.

Malan A. 1973. Ventilation measured by body plethysmography in hibernating mammals and in poikilotherms. Respir Physiol 17:32–44.

Menon G.H., P.F.A. Maderson, R.C. Drewes, L.F. Baptista, L.F. Price, and P.M. Elias. 1996. Ultrastructural organization of avian stratum corneum lipids as the basis for facultative cutaneous waterproofing. J Morph 227:1–13.

Minnaar I.A., N.C. Bennett, C.T. Chimimba, and A.E. McKechnie. 2014. Partitioning of evaporative water loss into respiratory and cutaneous pathways in Wahlberg's epauletted fruit bats (*Epomophorus wahlbergi*). Physiol Biochem Zool 87:475–485.

Monteith J.L. 1973. Principles of environmental physics. Edward Arnold, London.

Monteith J.L. and G.S. Campbell. 1980. Diffusion of water vapour through integuments: potential confusion. J Therm Biol 5:7–9.

Morrison S.F. and K. Nakamura. 2011. Central neural pathways for thermoregulation. Front Biosci 16:74–104.

Muñoz-Garcia A., M. Ben-Hamo, B. Pinshow, J.B. Williams, and C. Korine. 2012. The relationship between cutaneous water loss and thermoregulatory state in Kuhl's pipistrelle *Pipistrellus kuhlii*, a vespertilionid bat. Physiol Biochem Zool 85:516–525.

Muñoz-Garcia A., R.M. Cox, and J.B. Williams. 2008. Phenotypic flexibility in cutaneous water loss and lipids of the stratum corneum in house sparrows (*Passer domesticus*) following acclimation to high and low humidity. Physiol Biochem Zool 81:87–96.

Muñoz-Garcia A. and J.B. Williams. 2008. Developmental plasticity of cutaneous water loss and lipid composition in stratum corneum of desert and mesic nestling house sparrows. Proc Natl Acad Sci USA 105:15611–15616.

Ostrowski S., J.B. Williams, P. Mésochina, and H. Sauerwein. 2006. Physiological acclimation of a desert antelope, Arabian oryx (*Oryx leucoryx*), to long-term food and water restriction. J Comp Physiol B 176:191–201.

Parish O.O. and T.W. Putnam. 1977. Equations for the determination of humidity from dewpoint and psychrometric data. NASA Technical Note D-8401. Dryden Flight Research Center, Edwards, CA.

Powers D.R. 1992. Effect of temperature and humidity on evaporative water loss in Anna's hummingbird (*Calypte anna*). J Comp Physiol B 162:4–84.

R Core Team. 2020. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna.

Ro J. and J.B. Williams. 2010. Respiratory and cutaneous water loss of temperate-zone passerine birds. Comp Biochem Physiol A 156:237–246.

RStudio Team. 2020. RStudio: integrated development for R. RStudio, Boston.

Russell J., A.G. Vidal-Gadea, A. Makay, C. Lanam, and J.T. Pierce-Shimomura. 2014. Humidity sensation requires both mechanosensory and thermosensory pathways in *Caenorhabditis elegans*. Proc Natl Acad Sci USA 111:8269–8274.

Sayeed O. and S. Benzer. 1996. Behavioral genetics of thermosensation and hygrosensation in *Drosophila*. Proc Natl Acad Sci USA 93:6079–6084.

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

Schmidt-Nielsen K. and B. Schmidt-Nielsen. 1952. Water metabolism of desert mammals. Physiol Rev 32:135–166.

Scholander P.F., R. Hock, V. Walters, F. Johnson, and L. Irving. 1950. Heat regulation in some arctic and tropical mammals and birds. Biol Bull 99:237–258.

Simon E. 1999. Thermoregulation as a switchboard of autonomic nervous and endocrine control. Jap J Physiol 49:297–323.

Smith C.J. and J.M. Johnson. 2016. Responses to hyperthermia—optimizing heat dissipation by convection and evaporation: neural control of skin blood flow and sweating in humans. Auto Neurosci 196:5–36.

Song S. and S.R. Beissinger. 2020. Environmental determinants of total evaporative water loss in birds at multiple temperatures. Auk 137:ukz069.

Szewczak J.M. and F.L. Powell. 2003. Open-flow plethysmography with pressure decay compensation. Respir Physiol Neurobiol 134:57–67.

Tattersall G.J., B.J. Sinclair, P.C. Withers, P.A. Fields, F. Seebacher, C.E. Cooper, and S.K. Maloney. 2012. Coping with thermal challenges: physiological adaptations to environmental temperatures. Compr Physiol 2:2151–2202.

Tieleman B.I. and J.B. Williams. 2000. The adjustment of avian metabolic rates and water fluxes to desert environments. Physiol Biochem Zool 73:461–479.

———. 2002. Cutaneous and respiratory water loss in larks from arid and mesic environments. Physiol Biochem Zool 75:590–599.

Tieleman B.I., J.B. Williams, G. Michaeli, and B. Pinshow. 1999. The role of nasal passages in the water economy of crested larks and desert larks. Physiol Biochem Zool 72: 219–226.

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

———. 2001. Developmental and acclimatory contributions to water loss in a desert rodent: investigating the time course of adaptive change. J Comp Physiol B 171:669–679.

van Dyk M., M.J. Noakes, and A.E. McKechnie. 2019. Interactions between humidity and evaporative heat dissipation in a passerine bird. J Comp Physiol B 189:299–308.

Van Sant M.J., C.E. Oufiero, A. Muñoz-Garcia, K.A. Hammond, and J.B. Williams. 2012. A phylogenetic approach to total evaporative water loss in mammals. Physiol Biochem Zool 85: 526–532.

Walsberg G.E. and J.R. King. 1978. The relationship of the external surface area of birds to skin surface area and body mass. J Exp Biol 76:185–189.

Wang Z., P. Deurenberg, W. Wang, A. Pietrobelli, R.N. Baumgartner, and S.B. Heymsfield. 1999. Hydration of fat-free body mass: review and critique of a classic body-composition constant. Am J Clin Nutr 69:833–841.

Webster M.D. and M.H. Bernstein. 1987. Ventilated capsule measurements of cutaneous evaporation in mourning doves. Condor 89:863–868.

Webster M.D., G.C. Campbell, and J.R. King. 1985. Cutaneous resistance to water-vapor diffusion in pigeons and the role of the plumage. Physiol Zool 58:58–70.

Webster M.D. and J.R. King. 1987. Temperature and humidity dynamics of cutaneous and respiratory evaporation in pigeons, *Columba livia*. J Comp Physiol B 157:253–260.

Welch W.R. 1980. Evaporative water loss from endotherms in thermally and hygrically complex environments: an empirical approach for interspecific comparisons. J Comp Physiol 139: 135–143.

Welch WR and C.R. Tracy. 1977. Respiratory water loss: a predictive model. J Theor Biol 65:253–265.

Williams J.B. 1996. A phylogenetic perspective of evaporative water loss in birds. Auk 113:457–472.

Williams J.B. and B.I. Tieleman. 2000. Flexibility in basal metabolism and evaporative water loss in hoopoe larks from the Arabian Desert. J Exp Biol 203:153–159.

———. 2005. Physiological adaptation in desert birds. Bioscience 55:416–425.

Williams J.B., P.C. Withers, S.D. Bradshaw, and K.A. Nagy. 1991. Metabolism and water flux of captive and free-living Australian parrots. Aust J Zool 39:131–142.

Withers P.C. 1977. Metabolic, respiratory and haematological adjustments of the little pocket mouse to circadian torpor cycles. Respir Physiol 31:295–307.

———. 2001. Design, calibration and calculation for flow-through respirometry systems. Aust J Zool 49:445–461.

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

———. 2014. Physiological regulation of evaporative water loss in endotherms: is the little red kaluta (*Dasykaluta rosamondae*) an exception or the rule? Proc R Soc B 281: 20140149.

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

Withers P.C., C.E. Cooper, S.K. Maloney, F. Bozinovic, and A.P. Cruz-Neto. 2016. Ecological and environmental physiology of mammals. Oxford University Press, Oxford.

Withers P.C., C.E. Cooper, and R.F. Nespolo. 2012. Evaporative water loss, relative water economy and evaporative partitioning of a heterothermic marsupial, the monito del monte (*Dromiciops gliroides*). J Exp Biol 215:2806–2813.

Wolf B. 2000. Global warming and avian occupancy of hot deserts; a physiological and behavioral perspective. Rev Chil de Hist Nat 73:395–400.

Yokohari F. and H. Tateda. 1976. Moist and dry hygroreceptors for relative humidity of the cockroach, *Periplaneta americana* L. J Comp Physiol 106:137–152.