

RESEARCH ARTICLE

Two lines of evidence for physiological control of insensible evaporative water loss by a tiny marsupial

Christine Elizabeth Cooper^{1,2,*} and Philip Carew Withers^{1,2}**ABSTRACT**

We present two independent lines of evidence that a tiny dasyurid marsupial, the ningai (*Ningai* spp.), has acute physiological control of its insensible evaporative water loss below and within thermoneutrality. Perturbation of the driving force for evaporation by varying relative humidity, and therefore the water vapour pressure deficit between the animal and the ambient air, does not have the expected physical effect on evaporative water loss. Exposure to a helox atmosphere also does not have the expected physical effect of increasing evaporative water loss for live ningais (despite it having the expected effect of increasing heat loss for live ningais), but increases evaporative water loss for dead ningais. We discuss the relative advantages and disadvantages of both experimental approaches for demonstrating physiological control of insensible evaporative water loss. An appreciation of physiological control is important because insensible evaporative water loss contributes to both water and heat balance, is clearly under environmental selection pressure, and potentially impacts the distribution of endotherms and their response to environmental change.

KEY WORDS: Dasyurid, Evaporation, Helox, Ningai, Relative humidity, Temperature

INTRODUCTION

Evaporative water loss (EWL) is important for terrestrial animals. It is critical for their water budget and impacts heat balance (e.g. Schmidt-Nielsen and Schmidt-Nielsen, 1952; MacMillen, 1990; Withers et al., 2016). For endotherms, the thermoregulatory role and control of increased EWL at ambient temperatures (T_a) above the thermoneutral zone (TNZ; the range of T_a at which the animal can maintain heat balance with basal or resting metabolic rate and minimal EWL; Scholander et al., 1950; IUPS Thermal Commission, 2003; Riek and Geiser, 2013) is well understood (Morrison and Nakamura, 2011; Gerson et al., 2014). However, EWL in and below the TNZ (insensible EWL, IEWL; Monteith, 1973; IUPS Thermal Commission, 2003) has been considered a passive physical process, with IEWL proportional to the water vapour pressure differential (ΔWVP) between the animal and its environment (Cossins and Bowler, 1987; Campbell and Norman, 1998; Withers et al., 2016) as a consequence of the inevitable permeability of the skin and respiratory surfaces to water vapour. Some studies of IEWL for birds and mammals, which modified the

ΔWVP by manipulating the ambient relative humidity (RH), concluded that IEWL conforms to this physical model (Chew and Dammann, 1961; Edwards and Haines, 1978; Webster and King, 1987; Powers, 1992; Klüg-Baerwald and Brigham, 2017). Other studies, however, suggest that IEWL at and below the TNZ is under physiological control (Webster et al., 1985; Webster and Bernstein, 1987; Withers and Cooper, 2014; Cooper and Withers, 2017; Eto et al., 2017; Cooper et al., 2020), identifying a previously unappreciated physiological process.

It is important to understand the dynamics of IEWL at and below the TNZ because it is the primary form of evaporation for many endotherms for the majority of the time (even small desert species are often at $T_a \leq TNZ$; e.g. Schmidt-Nielsen, 1975; Cooper et al., 2019). Correlations between IEWL and ecological and environmental factors (Williams, 1996; Withers et al., 2006; Van Sant et al., 2012; Song and Beissinger, 2020) indicate selective pressure on IEWL, similar to that observed for body temperature (T_b) and metabolic rate (MR, e.g. basal MR, field MR; Nagy, 1987; McNab, 2003; White and Seymour, 2004; Withers et al., 2006; Withers et al., 2016). Consequently, understanding regulation of IEWL is just as important for understanding the distribution of endotherms and their potential response to environmental change as regulation of metabolic heat production (MHP) for proportional thermoregulatory control, especially considering that control of IEWL is likely to have a thermoregulatory role (Eto et al., 2017; Cooper and Withers, 2017; Cooper et al., 2020). However, assessing whether IEWL is under physiological control (i.e. deviates from the physical model) is not straightforward, as there is no expected slope for EWL versus RH (Eto et al., 2017). This can be resolved by determining the slope for EWL/ ΔWVP versus RH, as a slope of 0 indicates conformity to the physical model, and a slope >0 indicates physiological control. However, calculation of ΔWVP requires an estimation of the WVP saturation at the animal's mean evaporative surface temperature (T_{evap}), which is between T_a and body temperature (T_b); overestimating T_{evap} (using T_b) underestimates the slope, and underestimating T_{evap} (using T_a) overestimates the slope (Cooper et al., 2020).

An alternative approach for perturbing the evaporative environment is to compare EWL in helox (21% oxygen in helium) and air (Cooper and Withers, 2014). Helox theoretically increases an endotherm's EWL for two reasons. Water diffuses two to three times faster in helox owing to its lower density (Kingdon, 1963; Paganelli and Kurata, 1977; Parkhurst and Mott, 1990), increasing cutaneous EWL (CEWL). Helox is also four times more thermally conductive, increasing heat loss (Leon and Cook, 1960; Rosenmann and Morrison, 1974), which requires increased MHP (Rosenmann and Morrison, 1974; Thomas et al., 1998) and hence increased respiratory EWL (REWL) to accommodate the higher O_2 demand. Therefore, we expect EWL to be higher in helox than air, owing to enhancement of both CEWL and REWL. Helium mixtures have been used to modify the evaporative environment of plants

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List of symbols and abbreviations

C_{dry}	dry thermal conductance
CEWL	cutaneous evaporative water loss
C_{wet}	wet thermal conductance
EHL	evaporative heat loss
EWL	evaporative water loss
IEWL	insensible evaporative water loss
MHP	metabolic heat production
MR	metabolic rate
RER	respiratory exchange ratio
REWL	respiratory evaporative water loss
RH	relative humidity
T_a	ambient temperature
T_b	body temperature
T_{evap}	evaporative surface temperature
TNZ	thermoneutral zone
\dot{V}_{CO_2}	rate of carbon dioxide production
\dot{V}_{O_2}	rate of oxygen consumption
V_I	respiratory minute volume
WVP	water vapour pressure
ΔWVP	water vapour pressure deficit

(Egorov and Karpushkin, 1988; Parkhurst and Mott, 1990; Mott and Parkhurst, 1991), but only one study has examined this effect of helox for a mammal (Cooper and Withers, 2014).

Here, we examined the dynamics of IEWL at or below the TNZ ($T_a \leq 30^\circ\text{C}$; Geiser and Baudinette, 1988; Riek and Geiser, 2013; C.E.C. and P.C.W., unpublished data) for a tiny arid-habitat dasyurid marsupial, the ningau (*Ningau* spp.), using both altered RH and helox to perturb the evaporative environment. The basic thermoregulatory physiology of the ningau is typical of other small dasyurid marsupials (Geiser and Baudinette, 1988), and because two other species of small dasyurid (little red kaluta, *Dasykaluta rosamondae*; Withers and Cooper, 2014; red-tailed phascogale, *Phascogale calura*; Cooper and Withers, 2017) control their IEWL, the ningau is a useful model for this study. We applied the two methodologies of both altered RH and helox together for the first time to seek strong evidence of IEWL control, compared the relative merits of each approach, and considered the importance of identifying EWL regulation.

MATERIALS AND METHODS

Ten ningaus (*Ningau ridei* and/or *Ningau yvonnae*; these species cannot be reliably distinguished in the field at this site, where they are sympatric) were captured in the Goldfields ($30^\circ 24' \text{S}$, $119^\circ 38' \text{E}$), Western Australia. They were maintained at Curtin University with *ad libitum* food (invertebrates, kangaroo meat, cat food) and water for a period of 3 months, during which time experiments were conducted. Ningaus were fasted overnight, but had access to *ad libitum* water before measurement for 6–8 h the following day, during their inactive period to ensure resting values were achieved (e.g. Cooper and Withers, 2009; Connelly and Cooper, 2014). Each ningau was measured during one experimental treatment per day, with at least 3 days between measurements. Experiments followed the Australian Code of Practise for the care and use of animals for scientific purposes, were approved by the Curtin University Animal Ethics Committee (AEC_2016_01) and were conducted under licence from the West Australian Department of Biodiversity, Conservation and Attractions (FO2500069).

We used open-flow respirometry (Withers, 2001) to measure EWL and rates of oxygen consumption (\dot{V}_{O_2}) and carbon dioxide production (\dot{V}_{CO_2}) of ningaus in air at varying RH, and in helox.

Live ningaus were measured at T_a of 20, 25 and 30°C (below or within thermoneutrality; Geiser and Baudinette, 1988; Riek and Geiser, 2013; C.E.C. and P.C.W., unpublished data), at five RH levels of 7 to 81% in air, and in helox, in random order. Not every ningau was measured under each experimental condition for logistical reasons; $N=7-9$ for the various RH/ T_a combinations and $N=6-7$ for the helox measurements. At the conclusion of the study, ningaus were killed with an intraperitoneal injection of sodium pentobarbitone, and EWL of seven individuals was immediately measured in both dry air and helox at $T_a=33^\circ\text{C}$ (approximate T_b of live ningaus), to quantify the physical effects of helox on EWL for animals that were not physiologically regulating. Four of the ningau carcasses were measured in air first and then switched to helox, and vice versa for the other three; the gas mix was switched once EWL had stabilised (~ 120 min).

The respirometry system consisted of a ~ 400 ml glass metabolic chamber, inside a temperature-controlled room, through which flow of air (dried with Drierite; W. A. Hammond Co., Xenia, OH, USA) was regulated at 118 ml min^{-1} with an Aalborg GFC17 (Orangeburg, NY, USA) mass flow controller. At each T_a , incurrent air RH was controlled by saturating the air with water using an aerator in a water-filled portable refrigerator (Engel, Varsity Lakes, QLD, Australia), which was regulated at a known temperature using a Ritek heater circulator (Boronia, VIC, Australia), then warmed to the experimental T_a with RH calculated using the equations of Parish and Putnam (1977). Excurrent RH and T_a were measured with a Vaisala HMP45A (Helsinki, Finland) probe, then a subsample was dried with Drierite, passed through a carbon dioxide analyser (Sable Systems CA-2A; Las Vegas, NV, USA) and finally a paramagnetic oxygen analyser (Sable Systems PA-10). Analysers were interfaced to a PC via a Sable Systems UI2 A/D converter and excurrent O_2 , CO_2 , RH and T_a were recorded every 20 s throughout the experiment, using custom-written Visual Basic (Microsoft VB v6; Redmond, WA, USA) software. At the end of each experiment, when O_2 , CO_2 and RH data indicated that the ningau was resting and had attained minimal and steady-state MR and EWL, the animal was removed from the chamber and T_b was measured immediately. A lubricated plastic-tipped thermocouple, connected to a RadioSpares 611–234 thermocouple meter (Smithfield, NSW, Australia), was inserted into the cloaca while the animal was restrained by hand by the scruff, a process that was typically achieved within seconds of removing the animal from the chamber. For measurements in helox, incurrent air was replaced by a helox mix (21.1% oxygen in helium; BOC Gases, Perth, WA, Australia). Baselines of background O_2 , CO_2 and RH were established for at least 30 min before and after each experiment.

Mass flow meters were calibrated volumetrically using a Gilibrator 2 (Sensidyne, Clearwater, FL, USA) for both air and helox, corrected to standard temperature and pressure. RH probes were calibrated by comparing the measured and theoretical RH of the initial baselines at each RH. The Vaisala temperature sensor and thermocouple meter were calibrated against a mercury thermometer traceable to a national standard. Gas analysers were two-point calibrated with compressed nitrogen (BOC Gases; 0% O_2 and CO_2) and dry ambient air (20.95% O_2) and a certified gas mix (0.53% CO_2 ; BOC Gases). We used a custom-written (P. C. Withers) VB v6 program to calculate EWL, \dot{V}_{O_2} and \dot{V}_{CO_2} for a single ~ 20 -min period towards the end of each experiment, when all variables were constant and minimal, after Withers (2001). Respiratory exchange ratio (RER) was calculated as $\dot{V}_{\text{CO}_2}/\dot{V}_{\text{O}_2}$, and this was used to convert MR to MHP after Withers et al. (2016). Wet (C_{wet}) and dry (C_{dry})

thermal conductance were calculated as $MHP/(T_b - T_a)$ and $(MHP - EHL)/(T_b - T_a)$, respectively, with evaporative heat loss (EHL) calculated from EWL assuming $2.4 \text{ J mg}^{-1} \text{ H}_2\text{O}$ (Monteith, 1973). Hygrometeorological equations (Parish and Putnam, 1977) were used to calculate saturation WVP at T_b , and ambient WVP was calculated as saturation WVP at $T_a \times RH/100$. The ΔWVP was then calculated as saturation WVP animal minus ambient WVP.

We used linear mixed-effect models, with Helmert and reverse Helmert *a priori* contrasts (Withers and Cooper, 2011), with RH as a fixed factor and individual as a random factor, to determine whether IEWL and other physiological variables were affected by RH, and whether IEWL/ ΔWVP deviated from the physical model, i.e. if the slope of IEWL/ ΔWVP versus RH was >0 . We analysed these data separately at each T_a because of non-equivalence of RH, and of WVP relative to saturation, at different T_a . We used lme4 (<http://CRAN.R-project.org/package=lme4>) and lmerTest (<http://CRAN.R-project.org/package=lmerTest>) in RStudio (<http://www.rstudio.com/>). Values in helox were compared with those in dry air (7% chamber RH), to achieve similar chamber RH for the two treatments, using a linear mixed-effect model, with air/helox and T_a as fixed factors and individual as a random factor. Owing to significant interactions between T_a and air/helox, differences in physiological variables in air and helox were explored separately at each T_a for live animals, and at $T_a = 33^\circ\text{C}$ for dead ningaus, using a paired *t*-test, accomplished with statistiXL (www.statistiXL.com, Perth, WA, Australia). Expected rates of EWL in helox were calculated assuming 50:50 REWL:CEWL partitioning (Chew, 1955; Tracy and Walsberg, 2000) and the measured diffusive ($1.3\times$) and respiratory ($1.5\times$) effects of helox for ningaus. Values are presented as means \pm s.e.m., with N =number of individuals and n =number of measurements.

RESULTS

The mass of ningaus ($N=10$, $n=145$) was $7.8 \pm 0.09 \text{ g}$. Relative humidity had no effect on T_b , MR or C_{wet} at any T_a ($F_{1,24-37} \leq 3.47$, $P \geq 0.072$). There was a significant negative linear effect of RH for EWL at all T_a ($F_{1,29-34} \geq 11.37$, $P \leq 0.002$), although this was driven by lower IEWL at higher RH (reverse Helmert contrasts; $F_{1,27-32} \geq 5.72$, $P \leq 0.023$; Fig. 1A). RH had a positive linear effect on IEWL/ ΔWVP at all T_a ($F_{1,28-30} \geq 5.22$, $P \leq 0.030$), although the effect was attenuated at the two highest RH values (reverse Helmert contrasts; $F_{1,27-30} \leq 1.93$, $P \geq 0.062$; Fig. 1B).

For live ningaus, T_b was maintained constant in air and helox ($F_{1,36} = 1.97$, $P = 0.169$), but there was an overall T_a effect ($F_{1,36} = 6.97$, $P = 0.012$; Fig. 2A). Wet conductance was higher ($\sim 1.68\times$) in helox compared with air below ($t_{5,6} \geq 3.96$, $P \leq 0.007$) but not in the TNZ ($t_6 = 0.05$, $P = 0.958$; Fig. 2B). Consequently, MR was influenced by both T_a ($F_{1,30} = 190$, $P < 0.001$) and helox ($F_{1,30} = 17.8$, $P < 0.001$; Fig. 2C), increasing both at low T_a and in helox compared with air, but with a significant interaction ($F_{1,30} = 11.2$, $P < 0.001$) as the helox/air differential ($\sim 1.5\times$) was only apparent below the TNZ ($< 30^\circ\text{C}$). IEWL was not influenced by helox ($F_{1,30} = 0.561$, $P = 0.460$), although there was a significant T_a effect ($F_{1,30} = 21.8$, $P < 0.001$; Fig. 2D). Predicted rates of IEWL in helox were $1.15\text{--}1.4\times$ those measured in air. The IEWL of dead ningaus was higher in helox ($3.04 \pm 0.366 \text{ mg g}^{-1} \text{ h}^{-1}$) than in air ($2.30 \pm 0.308 \text{ mg g}^{-1} \text{ h}^{-1}$; $t_6 = 9.82$, $P < 0.001$; Fig. 2D).

DISCUSSION

It is important to understand the control of IEWL because it is a contributor to the overall heat and water budgets of endotherms, which in turn relate to their distribution and response to

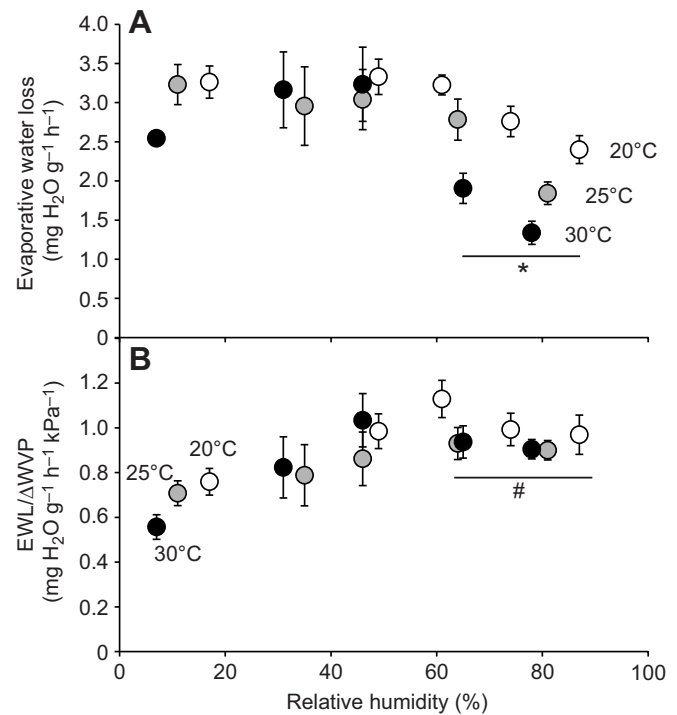


Fig. 1. Effect of relative humidity on insensible evaporative water loss for ningaus at ambient temperatures of 20, 25 and 30°C. (A) Evaporative water loss and (B) evaporative water loss per water vapour pressure deficit (EWL/ ΔWVP). * indicates values significantly lower than the mean of the previous levels, and # indicates values that do not differ from the previous levels (reverse Helmert contrasts). Values are means \pm s.e.m. ($N=7\text{--}9$).

environmental change. We demonstrated, for the first time using two independent methodologies (RH and helox), that IEWL is under physiological control. If IEWL was not controlled, we would expect it to follow physical predictions, i.e. be higher in helox than in air, and decrease in proportion to a decrease in the WVP deficit with increasing RH. As neither of these physical effects were observed, we conclude that IEWL must be under physiological control.

Although EWL decreased overall with increasing RH, this was driven by decreases in EWL at higher RH. Clearly, a small ΔWVP near saturation makes the maintenance of constant EWL challenging; this has been observed for other marsupials (Cooper and Withers, 2008; Withers and Cooper, 2014) and a bird (Eto et al., 2017). At lower RH (higher ΔWVP), EWL remained constant. Consequently, there was an overall linear relationship for EWL/ ΔWVP versus RH, indicating that ΔWVP alone was not determining IEWL, which deviated significantly from the linear relationship with ΔWVP predicted by the physical model. It was only at high RH that the ΔWVP was sufficiently small to inhibit EWL. This is particularly compelling evidence for IEWL control as we used a conservative approach to calculate the ΔWVP gradient using T_b for the 'animal end', which underestimates the EWL/ ΔWVP versus RH slope (Cooper and Withers, 2017; Eto et al., 2017; Cooper et al., 2020). Estimating T_{evap} , which provides a more accurate calculation of ΔWVP and a steeper, more significant slope, requires partitioning of IEWL into REWL and CEWL, and estimation of the animal's surface and expired air temperatures, all of which are difficult to measure non-invasively (e.g. Muñoz-García et al., 2012a; Minnaar et al., 2014), especially for small mammals. Physiological regulation of EWL was sufficient to maintain MR, T_b and C_{wet} independent of RH, by minimising the impact on evaporative heat loss.

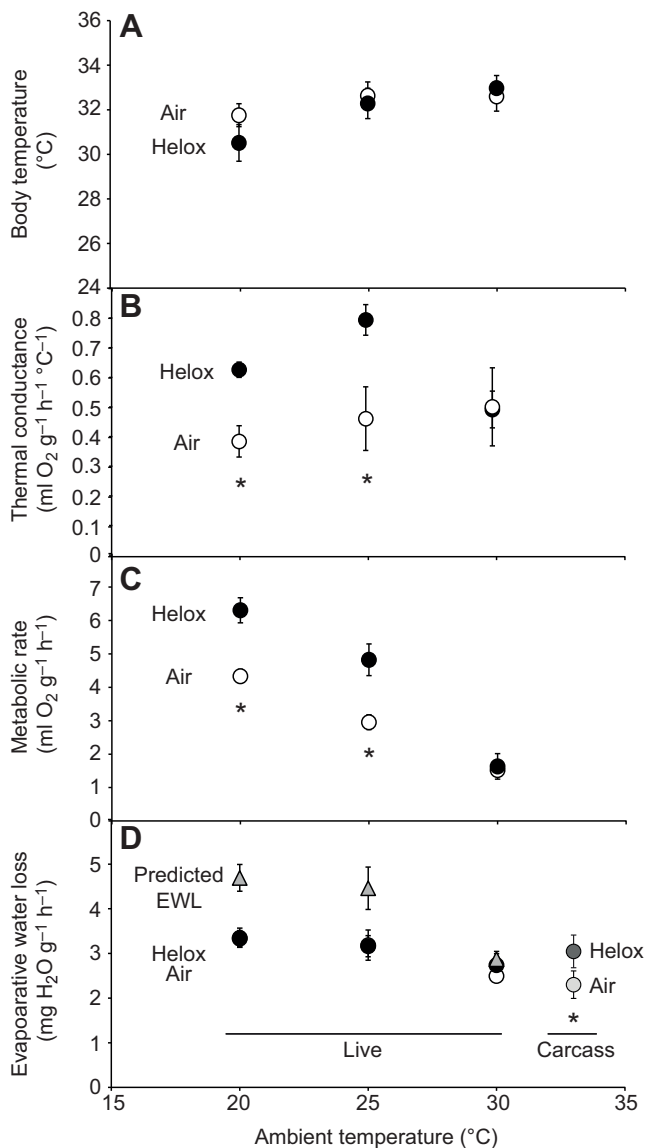


Fig. 2. Effect of air and helox on physiological variables of ningaus. (A) Body temperature, (B) metabolic rate, (C) wet thermal conductance and (D) evaporative water loss (EWL) for live ningaus at ambient temperatures of 20, 25 and 30°C, and ningau carcasses at $T_a=33^\circ\text{C}$ in helox (dark circles) and air (light circles). Expected rates of EWL in helox, calculated assuming 50:50 REWL:CEWL partitioning and the measured diffusive (1.3 \times) and respiratory (1.5 \times) effects of helox for ningaus are shown with grey triangles. Values are means \pm s.e.m. ($N=6-7$). There was a significant effect of ambient temperature for all variables except wet thermal conductance. * indicates a significant effect of helox compared with air at a particular T_a .

The thermal and metabolic responses of ningaus to a helox atmosphere were typically endothermic. Values for MR and C_{wet} were equivalent in both air and helox for ningaus at $T_a=30^\circ\text{C}$, consistent with the results of previous studies (e.g. Leon and Cook, 1960; Rhoades et al., 1967; Holloway and Geiser, 2001; Cooper and Withers, 2014) that there is no helox effect on C_{wet} or MR in thermoneutrality. Below the TNZ, normothermic responses to T_a were typical for this and other very small dasyurids (Dawson and Wolfers, 1978; Geiser and Baudinette, 1988; Warnecke et al., 2010). Helox increased C_{wet} , but MR increased sufficiently (1.5–1.7 \times) to maintain T_b . For small endotherms, this helox/air MR ratio does not exceed 2.6, and a low ratio is expected for a tiny,

poorly insulated mammal (Rosenmann and Morrison, 1974; Cooper and Withers, 2014). The significant increase in EWL of dead ningaus in helox was also expected because there was no possibility for physiological control, and although the increase was lower (1.3 \times) than predicted for a purely diffusional effect (2.3 \times ; Parkhurst and Mott, 1990), it was similar to the increase in EWL for a water-filled plastic vial in helox compared with air (1.7 \times ; Cooper and Withers, 2014). This suggests that there were convective as well as diffusional effects on EWL.

Despite the expected thermal and metabolic effects of helox for live ningaus, and physical effects on evaporation for carcasses, we did not observe the increase in EWL predicted by a physical model for live ningaus; EWL in air was indistinguishable from that in helox. Increased respiratory ventilation that must accompany a higher MR in helox (Cooper and Withers, 2014) should increase REWL, and the increased diffusion observed for dead ningaus should increase CEWL. Thus, exposure to helox also provides additional strong evidence that insensible EWL is controlled, and is not simply a passive consequence of the evaporative environment.

The two approaches for assessing regulation of IEWL, compared here for the first time, were consistent in indicating that IEWL is under physiological control. Of the two approaches, modification of WVP by controlling the inlet RH is a less expensive and more straightforward technique. However, there are logistical issues: it is difficult to generate and maintain low RHs at low T_a values, and condensation at high RH and high T_a values can be problematic. The major challenge with the RH experimental approach is interpreting the results. With no theoretical slope for EWL against RH, it is necessary to calculate the slope of EWL/ Δ WVP versus RH (or WVP). Calculation of Δ WVP is not straightforward owing to challenges associated with estimating T_{evap} ; direct efforts to physically partition EWL into REWL and CEWL and instrument animals to measure expired air and surface temperatures can overestimate IEWL (Muñoz-García et al., 2012a; Minnaar et al., 2014; Cooper et al., 2018). The approach of using T_b for calculating Δ WVP is conservative; it will avoid falsely claiming IEWL control (type I error) but it may fail to identify IEWL control (type II error; Cooper et al., 2020). However, an advantage of using the slope for EWL/ Δ WVP versus RH to assess IEWL control is that the relative abilities of different species for IEWL regulation can be compared (Cooper et al., 2020).

Using helox has the advantage of requiring fewer experiments to assess IEWL control over the same T_a range (most individuals were measured six times for the helox/air experiment and 15 times for the RH experiment), with ethical, logistical and financial benefits. It is also straightforward to interpret results; estimation of T_{evap} is not necessary. However, helox is relatively expensive and it is a finite resource (Nuttall et al., 2012) so its use is problematic for large species. It is difficult to detect helox effects on EWL within the TNZ, where there are no C_{wet} or MR impacts on REWL. However, a sensitivity analysis (Cooper and Withers, 2014) demonstrated that elevated EWL should still be detected in helox if mammals conform to physical predictions.

The role of the acute control of IEWL that we have shown for this small arid-habitat marsupial could be related to either water conservation or heat balance. Although the purpose of this control is not yet understood, current data suggest that a thermoregulatory rather than water balance role is more likely for both birds and mammals (Eto et al., 2017; Cooper and Withers, 2017; Cooper et al., 2020). If IEWL control has a thermoregulatory function, then the well-developed thermoregulatory feedback system of endotherms provides a possible sensory system for IEWL control, although we

do not understand the specific effector mechanism(s) that achieve control. Control could result from changes to REWL and/or CEWL.

Respiratory EWL is determined by the temperature and RH of the expired air and by respiratory minute volume (V_1). For ash-grey mice (Cooper and Withers, 2014), changes in V_1 in helox compared with air accommodated the increased V_{O_2} , and there were no changes in oxygen extraction, suggesting that oxygen extraction is already optimised in air and that there is little scope for modification of V_1 to control IEWL. Cooper et al. (2020) also concluded that V_1 was an unlikely mechanism of IEWL control for parrots at different RH. Changes in the temperature and RH of expired air is a more likely avenue of REWL control. REWL can be cooled below T_b by nasal counter-current water and heat exchange (e.g. Schmidt-Nielsen et al., 1970), and although the RH of expired air is assumed to be 100%, some mammals such as camels (*Camelus dromedarius*; Schmidt-Nielsen et al., 1981) and sheep (*Ovis aries*; Johnson et al., 1988) can expire unsaturated air. Cutaneous blood flow, posture, fur positioning and skin lipids may all affect CEWL via their impact on surface temperature and the resistance of the skin, pelt and boundary layer. Modifying peripheral blood flow can change surface temperature, and therefore CEWL, along with changes in posture and piloerection, and these adjustments can be near-instantaneous and could account for the acute control of IEWL we observed here. Adjustments to skin lipid composition impact CEWL of bats (Muñoz-García et al., 2012b), and for birds, these micro-structural changes in the skin occur within time frames of hours to weeks (Menon et al., 1996; Muñoz-García and Williams, 2008; Muñoz-García et al., 2008), so possibly contribute to the control we observed here.

It is inconceivable that we would try to understand the distribution of endotherms without appreciating the acute thermoregulatory control afforded by proportional MHP; we propose that the consequences of IEWL control that we demonstrate here are also important. Evidence for acute physiological control of IEWL is growing (Webster et al., 1985; Webster and Bernstein, 1987; Cooper and Withers 2008, 2014, 2017; Withers and Cooper, 2014; Eto et al., 2017; Cooper et al., 2020), and here we use two different techniques to unequivocally demonstrate this for a tiny arid-habitat dasyurid marsupial. Further studies are required to elucidate the extent, purpose and, importantly, the mechanism of this control system, by examining this phenomenon in a broad range of phylogenetic, ecological and allometric contexts.

Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: C.E.C., P.C.W.; Methodology: C.E.C., P.C.W.; Software: P.C.W.; Formal analysis: C.E.C., P.C.W.; Investigation: C.E.C., P.C.W.; Resources: C.E.C., P.C.W.; Data curation: C.E.C., P.C.W.; Writing - original draft: C.E.C.; Writing - review & editing: P.C.W.; Visualization: C.E.C.; Project administration: C.E.C., P.C.W.; Funding acquisition: C.E.C., P.C.W.

Funding

This research was supported by the Australian Research Council Discovery Research Projects funding scheme (DP160103627).

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