RESEARCH ARTICLE

Does control of insensible evaporative water loss by two species of mesic parrot have a thermoregulatory role?

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ABSTRACT

Insensible evaporative water loss (EWL) at or below thermoneutrality is generally assumed to be a passive physical process. However, some arid zone mammals and a single arid zone bird can control their insensible water loss, so we tested the hypothesis that the same is the case for two parrot species from a mesic habitat. We investigated red-rumped parrots (*Psephotus haematonotus*) and eastern rosellas (*Platycercus eximius*), measuring their EWL, and other physiological variables, at a range of relative humidities at ambient temperatures of 20 and 30°C (below and at thermoneutrality). We found that, despite a decrease in EWL with increasing relative humidity, rates of EWL were not fully accounted for by the water vapour deficit between the animal and its environment, indicating that the insensible EWL of both parrots was controlled. It is unlikely that this deviation from physical expectations was regulation with a primary role for water conservation because our mesic-habitat parrots had equivalent regulatory ability as the arid habitat budgerigar (*Melopsittacus undulatus*). This, together with our observations of body temperature and metabolic rate, instead support the hypothesis that acute physiological control of insensible water loss serves a thermoregulatory purpose for endotherms. Modification of both cutaneous and respiratory avenues of evaporation may be involved, possibly via modification of expired air temperature and humidity, and surface resistance.

KEY WORDS: Body temperature, Evaporative water loss, Humidity, Metabolic rate, Physiological control, Respirometry, Thermoregulation, Water balance, Water vapour pressure

INTRODUCTION

Evaporative water loss (EWL) is important for terrestrial endothermic animals, affecting both water balance and thermoregulation. As it can account for more than 70% of the total water loss of an animal, it is a critical component of the water budget (Dawson, 1982; MacMillen, 1990; Williams and Tieleman, 2005), and as a consequence EWL is subject to plastic and adaptive responses to environmental conditions (e.g. Williams, 1996; Tieleman and Williams, 2000, 2002; Tracy and Walsberg, 2000, 2001; Withers et al., 2006; Van Sant et al., 2012; Song and Beissinger, 2020). Heat balance is also affected by EWL, as metabolic and environmental heat loads are dissipated by evaporation (latent heat of evaporation is 2.4 J mg^{-1} H$_2$O;

Monteith, 1973). For endotherms, thermoregulatory EWL occurs at ambient temperatures (T_a) above thermoneutrality via increases in respiratory (REWL) and/or cutaneous (CEWL) evaporation, as a consequence of active thermoregulatory mechanisms controlled by the autonomic nervous system, such as panting, salivation and sweating (Gerson et al., 2014; Withers et al., 2016; Fuller et al., 2019). High environmental relative humidity (RH) may have an impact on thermoregulatory EWL, but there are effective physiological mechanisms that can maintain high rates of thermoregulatory EWL even at high environmental water vapour pressures (WVP; e.g. Gerson et al., 2014; van Dyk et al., 2019).

EWL that occurs due to the inevitable permeability of the animal's surface to water vapour (e.g. not sweating or panting) is said to be insensible (Monteith, 1973; IUPS Thermal Commission, 2003). It has long been a central paradigm of animal physiology that the water vapour pressure differential (ΔWVP) between the evaporative surface(s) of the animal and its environment is the driver of insensible EWL at and below thermoneutrality (Campbell and Norman, 1998; Withers et al., 2016), and that this insensible EWL is passive, i.e. not controlled or regulated (IUPS Thermal Commission, 2003). Many studies of insensible EWL of birds and mammals have concluded that EWL at and below the thermoneutral zone (TNZ) is positively related to the ΔWVP (e.g. Chew and Dammann, 1961; Edwards and Haines, 1978; Webster and King, 1987; Powers, 1992; Klüg-Baerwald and Brigham, 2017). However, Webster et al. (1985) and Webster and Bernstein (1987) proposed that there is physiological control of insensible EWL for columbiform birds, which have an unusual ability to augment CEWL, and Ro and Williams (2010) observed different rates of CEWL for live and dead birds, which they interpreted as evidence of physiological control of CEWL. Subsequently, several studies of acute responses of a psittacine bird (budgerigar, *Melopsittacus undulates*; Eto et al., 2017) and placental and marsupial mammals (Cooper and Withers, 2008, 2014, 2017; Withers and Cooper, 2014) to perturbations of the evaporative environment have concluded that rates of insensible EWL are not necessarily passive, as predicted by a physical model (Campbell and Norman, 1998; Withers et al., 2016). Re-analysis of published EWL data further suggested that many species diverge from physical expectations (Withers and Cooper, 2014), suggesting widespread acute physiological control of insensible EWL.

To date, a specific physiological role has not been identified for control of insensible EWL. It is possible that reduction of EWL below that expected at high ΔWVP (e.g. low environmental RH) is important for water conservation, and currently the mammals and birds for which acute regulation of insensible EWL has been described have been from arid or semi-arid habitats (Cooper and Withers, 2008, 2014, 2017; Withers and Cooper, 2014; Eto et al., 2017). If maintaining water balance was the major driver of EWL regulation, then we would expect regulation of insensible EWL to be restricted to, or better developed by, species that have an ecological requirement for water conservation, and we would

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List of abbreviations

BTPS	body temperature and pressure, saturated
C_{dry}	dry thermal conductance
CEWL	cutaneous evaporative water loss
C_{wet}	wet thermal conductance
EHL	evaporative heat loss
E_{O_2}	oxygen extraction
EWL	evaporative water loss
f_R	respiratory frequency
MHP	metabolic heat production
MR	metabolic rate
N	number of individuals
n	number of measurements
R	cutaneous resistance
REWL	respiratory evaporative water loss
RH	relative humidity
STPD	standard temperature and pressure, dry
T_a	ambient temperature
T_b	body temperature
T_{evap}	evaporative surface temperature
T_{exp}	expired air temperature
TNZ	thermoneutral zone
T_{surf}	surface temperature
$\dot{V}_{CO_2}$	rate of carbon dioxide consumption
V_I	minute volume
$\dot{V}_{O_2}$	rate of oxygen consumption
V_T	tidal volume
WVP	water vapour pressure
ΔWVP	water vapour pressure differential

hypothesise that arid habitat species are able to better regulate their EWL against physical drivers compared with mesic species. We know that desert birds and mammals overall have lower rates of insensible EWL (e.g. Williams et al., 1991; Williams, 1996; Withers et al., 2006; Williams and Tieleman, 2005; Van Sant et al., 2012; Song and Beissinger, 2020), and that they can acclimate to chronic conditions of heat and/or aridity by reducing their EWL over periods of weeks to months (e.g. Tieleman and Williams, 2000, 2002; Williams and Tieleman, 2000; Tracy and Walsberg, 2000, 2001). However, we currently do not have quantitative, comparable data for acute (hours rather than days or weeks) regulation of insensible EWL by mesic compared with arid habitat birds or mammals.

An alternate hypothesis is that control of insensible EWL, even at and below thermoneutrality, is a consequence of physiological thermoregulation. If EWL is affected by varying ΔWVP, then evaporative heat loss (EHL) will consequently be modified, requiring adjustment of metabolic heat production (MHP) or dry thermal conductance (C_{dry}) to regulate body temperature (T_b). Controlling EWL despite varying ΔWVP simplifies thermoregulatory responses (Cooper and Withers, 2017; Eto et al., 2017). In support of this thermoregulatory hypothesis, red-tailed phascogales (*Phascogale calura*) control their insensible EWL when thermoregulating, but not when thermoconforming during torpor (Cooper and Withers, 2017), and the EWL of torpid, thermoconforming big brown bats (*Eptesicus fuscus*) is significantly higher at low RH compared with high RH (Klüg-Baerwald and Brigham, 2017). Observations of EWL control by both placental and marsupial mammals, and convergently by endothermic birds, adds further weight to the hypothesis that control of insensible EWL is an important characteristic of the thermoregulatory physiology of endotherms (Eto et al., 2017). If indeed control of insensible EWL serves a thermoregulatory purpose as opposed to a role in water balance, then mesic and arid habitat species might be expected to be equally

competent with respect to control of EWL under perturbing environmental conditions.

Here we examined the capacity for acute control of insensible EWL of two psittacine birds, the red-rumped parrot [*Psephotus haematonotus* (Gould 1838)] and the eastern rosella [*Platycercus eximius* (Shaw 1792)], from a mesic environment, the Northern Tablelands of New South Wales, Australia. We measured EWL, along with metabolic rate (MR), T_b and respiratory variables, over a range of RH at two T_a at and below thermoneutrality (20 and 30°C; Williams et al., 1991), to determine if EWL deviated significantly from physical predictions indicating physiological control. We examined the effect of ΔWVP on other physiological variables, and assessed the degree of EWL control for these mesic-habitat parrots compared with the other bird species for which there are comparable data, the arid-habitat budgerigar (Eto et al., 2017). We tested the hypothesis that the major purpose of physiological control of insensible EWL at and below thermoneutrality is thermoregulatory.

MATERIALS AND METHODS

Experiments followed the Australian Code of Practice for the care and use of animals for scientific purposes, approved by the University of New England animal ethics committee (ARE 2016-3) and with reciprocal approval from Curtin University and the University of Western Australia, and were conducted under licence from the New South Wales National Parks and Wildlife Service.

Ten red-rumped parrots and six eastern rosellas were captured in mist nets on the University of New England campus, Armidale, New South Wales (30°30′S, 151°40′E). Parrots were held in outdoor aviaries and fed seed (small parrot mix) and fresh fruit, with *ad libitum* water; experiments were carried out over a period of 9–10 weeks, after approximately 1 week acclimation to captivity. On the day of experiments, birds were fasted from 11.00 h; water was always available in the aviaries. Birds were measured overnight, at one set of environmental conditions, individually in a 3 litre glass metabolic chamber, for a period of 6–9 h, until MR and EWL had become constant and minimal. Birds were exposed to differing RH and T_a (measured to the nearest 0.1°C) combinations in random order. At the conclusion of the experiment, which was generally between 00.00 and 03.00 h, within the bird's rest phase, the bird was removed from the chamber and its T_b measured immediately with a plastic-tipped thermocouple, connected to a RadioSpares thermocouple meter (Smithfield, NSW, Australia), inserted into the cloaca. Birds were weighed to 0.1 g before and after each experiment on an electronic balance, and the mean used in subsequent calculations. Birds were rested for at least 3 days between measurements, and were released at the site of capture at the conclusion of the study.

Experimental temperature was regulated by placing the metabolic chamber in a custom-built temperature cabinet (G. Körtner) set to 20 or 30°C. Baselines of background levels of O_2, CO_2 and RH were established for at least 30 min before and after each experiment; gas analysers were in a regulated temperature room to eliminate temperature-induced baseline drift. Outside air was dried with Drierite (W.A. Hammond Co., Xenia, OH, USA) and then pushed through the chamber with a diaphragm pump at a rate of 1000–1600 ml min^{-1} (red-rumped parrots) or 1100–2000 ml min^{-1} (eastern rosellas), regulated by a mass-flow controller (Alborg, Orangeburg, NY, USA or Cole-Palmer, Vernon Hills, IL, USA) or a Sable Systems FoxBox (Las Vegas, NV, USA). To achieve the desired chamber RH (approximately 8, 30, 45, 63 and 78%), air was passed through either a Sable Systems DG4 dew point controller or an aerator in a temperature-regulated water bath [Engel, Carole Park, Queensland, Australia; portable refrigerator with a Ratek (Boronia,

Victoria, Australia) heater circulator] that saturated the air at a specified temperature (i.e. the dew point), which was then warmed to the experimental T_a. The RH and temperature of chamber excurrent air was measured with a Vaisala (Helsinki, Finland) HMP45A RH and T_a probe, before it passed through a small Drierite column, and then through a carbon dioxide (Sable Systems CA-2A or FoxBox) and finally an oxygen analyser (Sable Systems PA-10 or FoxBox). The RH and T_a probe and gas analysers were interfaced to a PC via a Sable Systems UI2 A/D converter, or the serial port of the FoxBox, and data were recorded every 30 s throughout the experimental period using custom-written (P. C. Withers) Visual Basic (Microsoft VB version 6; Microsoft, Redmond, WA, USA) software.

Ventilatory parameters [respiratory frequency (f_R) and tidal volume (V_T)] were measured by open-flow whole-body plethysmography (Withers, 1977). A custom-built pressure transducer (constructed with a MPX 2010 Motorola transducer, Denver, CO, USA) detected changes in pressure caused by the warming and humidifying of inspired air, and its voltage output was interfaced to a PC via a Pico AD 11 A/D converter (Pico Technology, St Neots, UK) and measured every 20 ms for approximately 30 s using PicoScope. Ventilatory measurements were made towards the end of each experimental period, just before measurement of T_b and when low and constant physiological variables indicated that the birds were calm and resting.

Flow meters were volumetrically calibrated, corrected to standard temperature and pressure, dry (STPD). The gas analysers were two-point calibrated with compressed nitrogen [BOC Gas (North Ryde, NSW, Australia), 0% O_2 and 0% CO_2] and either dry ambient air (20.95% O_2) or a certified gas mix (1.5% CO_2; BOC Gas). Measured baseline RH values for the five experimental RH treatments were compared with the theoretical baseline RH values calculated from the dew point using hygrometeorological equations of Parish and Putnam (1977) to calibrate the Vaisala RH probes, and the temperature sensors and thermocouple meter were calibrated to the nearest 0.1°C against a mercury thermometer traceable to a national standard. The plethysmograph system was calibrated by injecting 1 ml of air into the chamber several times for every RH and T_a combination, and determining the pressure displacement and wash-out characteristics of the resulting pulse, then mathematically converting the open system to a closed system plethysmograph (Malan, 1973; Szewczak and Powell, 2003).

Metabolic rate [oxygen consumption ($\dot{V}_{O_2}$) and carbon dioxide production ($\dot{V}_{CO_2}$)] and EWL were calculated using a custom-written (P. C. Withers) VB version 6 programme, for an approximately 20 min period from each experiment when all variables were minimal and constant, after Withers (2001). The respiratory exchange ratio (RER) was calculated as $\dot{V}_{CO_2}/\dot{V}_{O_2}$. 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 MR converted to MHP using the measured RER after Withers et al. (2016), and EHL calculated from EWL assuming 2.4 J mg^{-1} H_2O (Monteith, 1973). Calculations for f_R, V_T, minute volume (V_I) and oxygen extraction (E_{O_2}) were also made using the custom-written VB data analysis program, after Malan (1973), Szewczak and Powell (2003) and Cooper and Withers (2004). Three to five ventilatory data sets were analysed and then averaged to provide a single value for each individual at each T_a and RH combination.

Values are presented as means±s.e.m. with N individuals and n measurements. All individuals were measured at each T_a and RH combination, except at T_a=20°C, where eight red-rumped parrots were measured at each RH. Gas volumes are presented at STPD, except for V_T and V_I, which are expressed at body temperature and pressure, saturated (BTPS). Effects of RH on physiological variables

were assessed separately for 20 and 30°C, because of non-equivalence of RH and of WVP relative to saturation at different T_a. Overall effects were assessed using a full-factorial multivariate repeated measures analysis of variance (ANOVA), with a priori polynomial contrasts to examine specific hypotheses for the pattern of the response at varying RH. A priori contrasts can be more powerful and more appropriate than the overall repeated measures ANOVA because they address more specific hypotheses (e.g. if there is a linear relationship for ordered categories; Withers and Cooper, 2011). Repeated measures ANOVA and contrast analyses were achieved with a custom-written macro (Withers and Cooper, 2011).

To compare EWL of parrots at different RH with physical expectations, EWL was expressed relative to ΔWVP between the animal and the ambient air (i.e. EWL/ΔWVP), and the significance of the slope (physical expectation is slope=0) of the relationship between EWL/ΔWVP and RH was assessed with a linear a priori contrast for repeated measures ANOVA, after Eto et al. (2017). Hygrometeorological equations (Parish and Putnam, 1977) were used to calculate saturation WVP at T_a, T_b and the animal's overall evaporative surface temperature (T_{evap}), and ambient WVP was calculated as saturation WVP T_a×RH/100. The ΔWVP was then calculated as saturation WVP animal – ambient WVP. Use of T_a, T_b and T_{evap} to calculate the 'animal end' of the ΔWVP provided the upper bound (T_a), lower bound (T_b) and best estimate (T_{evap}) of the slope for EWL/ΔWVP against RH. We use T_{evap} as the temperature of an imaginary surface that conceptually combines the temperatures of the animal's various evaporative surfaces, in proportion to their contribution to total EWL. We calculated T_{evap} using the parrots' estimated expired air temperature (T_{exp}) and surface temperature (T_{surf}), adjusted by the proportional contribution of each to T_{evap} determined by the percentage partitioning of REWL and CEWL, after Eto et al. (2017). We used the iterative model of Withers et al. (2012) to non-invasively calculate T_{surf}, T_{exp} and cutaneous resistance to evaporation (R, s cm^{-1};), and partition total EWL into CEWL and REWL, to avoid potential measurement issues with physical partitioning approaches that may over-estimate total EWL and presumably have an impact on partitioning (e.g. Muñoz-Garcia et al., 2012; Minnaar et al., 2014; but see Wolf and Walsberg, 1996).

RESULTS
Red-rumped parrots
Mean mass of all red-rumped parrots over all experiments (N=10, n=90) was 57.4±0.37 g. Linear contrasts for mass with RH were insignificant for both T_a=20°C and T_a=30°C ($t_{7–9}$≤2.20, P≥0.064). At T_a=20°C, there were no overall RH effects on EWL ($F_{4,4}$=1.74, P=0.303; N=8, n=40) or any of the other physiological variables ($F_{4,4}$≤6.04, P≥0.055; Fig. 1). The only significant linear contrast was for EWL (P=0.027). A similar absence of overall RH effects on the physiology of red-rumped parrots was observed at T_a=30°C ($F_{4,6}$≤3.79, P≥0.072; Fig. 1), except there was an overall RH effect for EWL ($F_{4,6}$=5.59, P=0.032; N=10, n=50), described by a significant linear contrast (P=0.002). No overall RH effects were apparent for respiratory variables at T_a=20°C ($F_{4,6}$≤3.14, P≥0.146; Fig. 2) but at T_a=30°C the RH influenced all respiratory variables ($F_{4,6}$≥4.90, P≤0.042) except for E_{O_2} ($F_{4,6}$=0.765, P=0.585). However, there were no linear patterns for any respiratory variables at either T_a (P>0.127).

The partitioning model at T_a=20°C calculated a T_{surf} of 29.3±0.64°C and T_{exp} of 29.5±1.20°C (Fig. 3); neither was influenced by RH ($F_{4,4}$≤1.49, P≥0.354). Cutaneous resistance declined linearly with RH (P=0.033; range 333±59 to
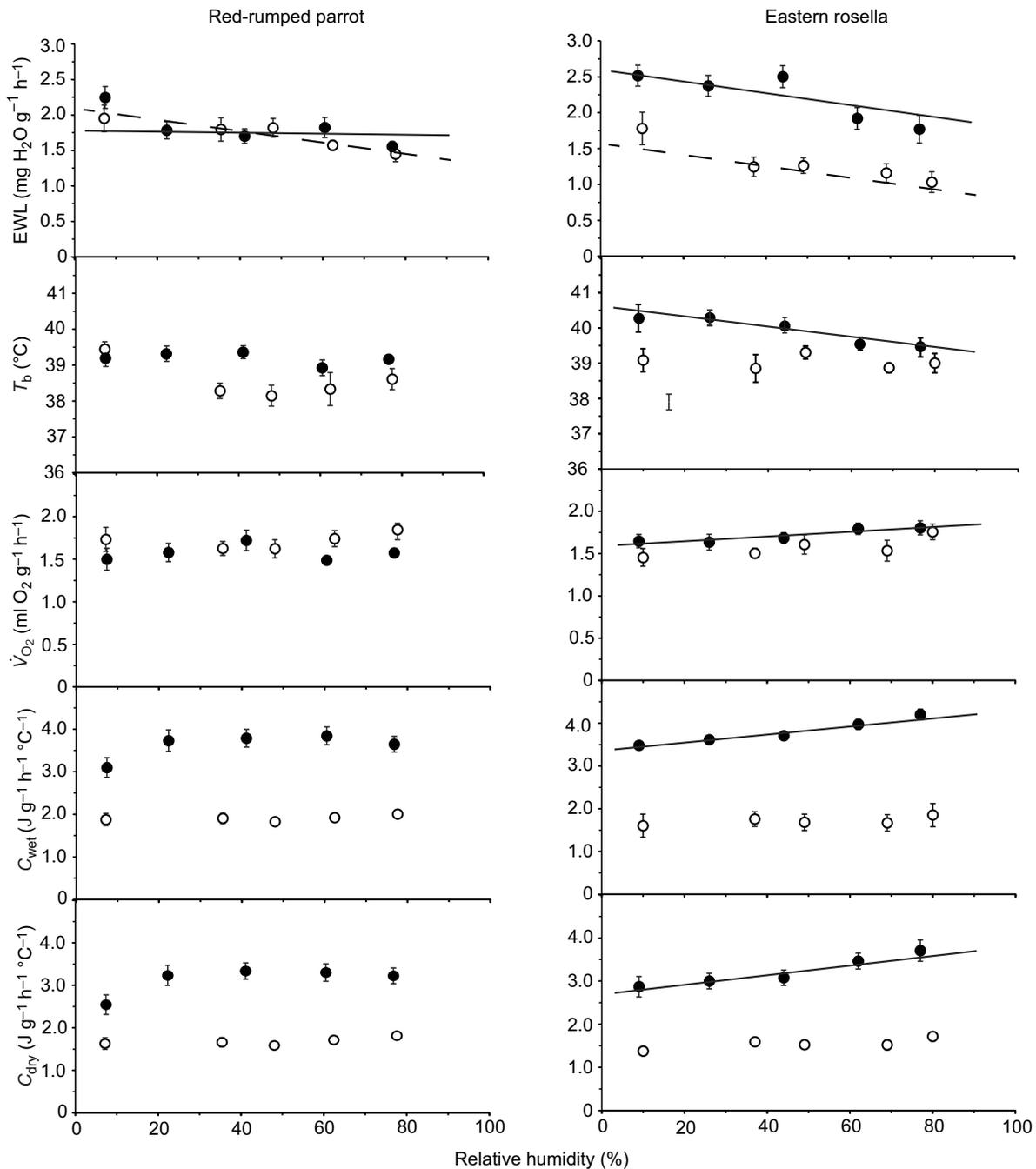
Fig. 1. The effect of ambient relative humidity on evaporative water loss, body temperature, metabolic rate (oxygen consumption) and wet and dry thermal conductance for red-rumped parrots (*Psephotus haematonotus*) and eastern rosellas (*Platycercus eximius*). EWL, evaporative water loss; T_b, body temperature; $\dot{V}_{O_2}$, metabolic rate (oxygen consumption); C_{wet}, wet thermal conductance; C_{dry}, dry thermal conductance. White symbols represent measurements made at an ambient temperature (T_a) of 20°C, and black symbols are measurements made at T_a=30°C. Dashed lines are significant linear contrasts at T_a=20°C, and continuous lines are significant linear contrasts at T_a=30°C. Values are means±s.e.m.; N=10 for red-rumped parrots at T_a=30°C and N=8 at T_a=20°C, and N=6 for eastern rosellas at both T_a.

658±94 s cm^{-1}). Partitioning of EWL was 41±4.5% CEWL and 59 ±4.5% REWL (Fig. 4). From these values, T_{evap} was calculated as 29.4°C, compared with an overall mean T_b of 38.6±0.15°C. Patterns were slightly different at T_a=30°C, with both T_{exp} ($F_{4,6}$=4.67, P=0.047) and R ($F_{4,6}$=7.64, P=0.016) influenced by RH, with linear contrasts that were positive (P=0.001; range 26±2.6 to 36.5±0.55°C) and negative (P=0.008; 356±98 to 172±21 s cm^{-1}), respectively (Fig. 3). Overall mean T_{surf} was 34.3±0.56°C, and EWL partitioning was 55±4.1% CEWL and 45±3.1% REWL

(Fig. 4). T_{evap} at T_a=30°C was calculated as 33.3°C compared with an overall mean T_b of 39.2±0.10°C.

Linear contrasts for the relationship between EWL/ΔWVP and RH (Fig. 5) were significant at T_a=20°C when the 'animal end' of ΔWVP was calculated from T_a [P<0.001; 0.024×(EWL/ΔWVP)+1.57] and T_{evap} [P=0.037; 0.002×(EWL/ΔWVP)+0.584], but not from T_b (P=0.699; mean=0.58±0.021 mg H$_2$O g^{-1} h^{-1} kPa^{-1}). At T_a=30°C, all linear contrasts for EWL/ΔWVP against RH were significant, regardless of whether ΔWVP was calculated from T_a [P<0.001;
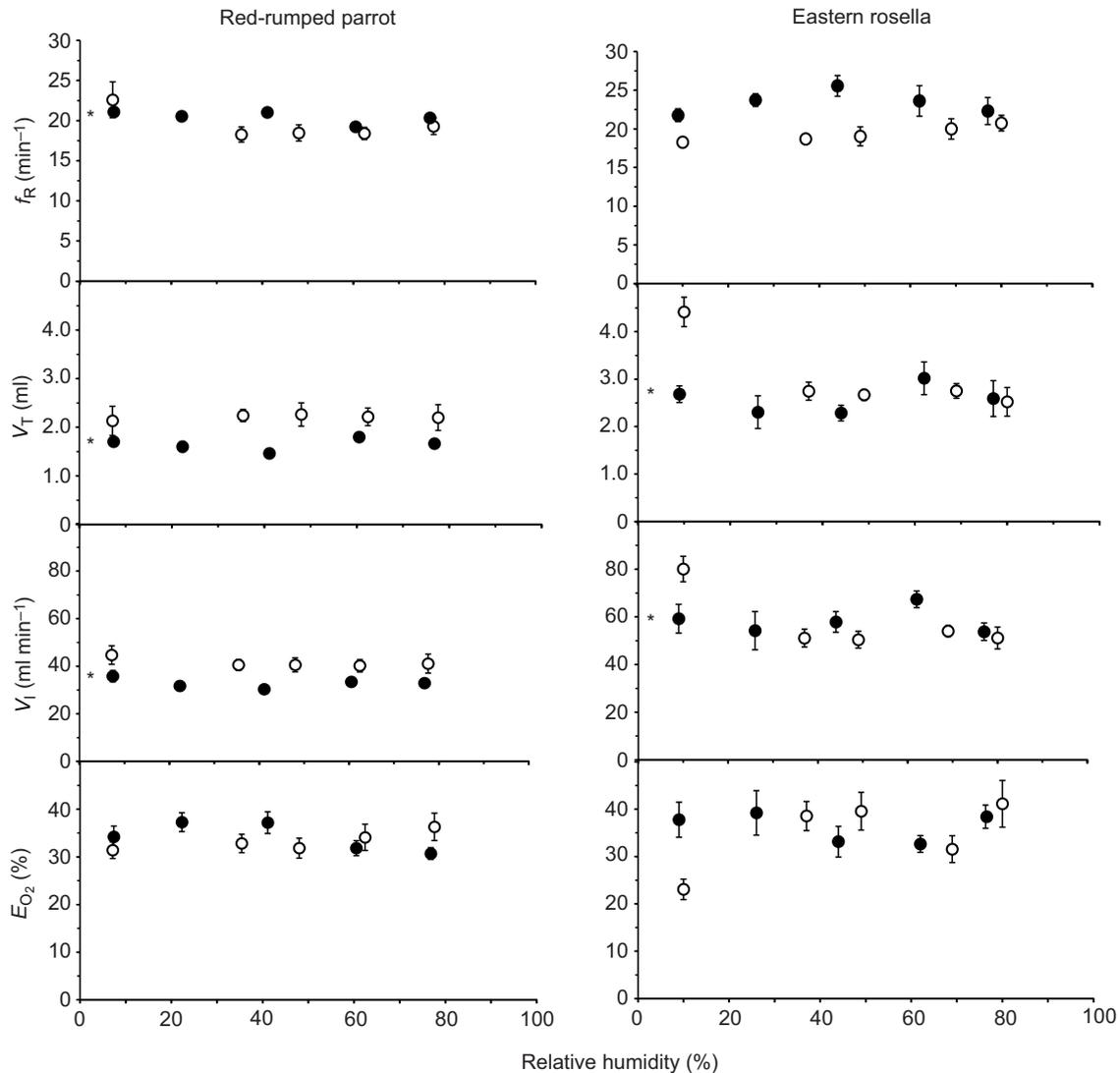
Fig. 2. The effect of ambient relative humidity on ventilatory variables; respiratory frequency, tidal volume, minute volume and oxygen extraction for red-rumped parrots and eastern rosellas. f_R, respiratory frequency; V_T, tidal volume; V_I, minute volume; E_{O_2}, oxygen extraction. White symbols represent measurements at an ambient temperature (T_a) of 20°C, and black symbols are measurements at T_a=30°C. Values are means±s.e.m.; N=10 for red-rumped parrots at T_a=30°C and N=8 at T_a=20°C, and N=6 for eastern rosellas at both T_a.

$0.008 \times (EWL/\Delta WVP) + 0.724]$, T_{evap} [P=0.001; $0.006 \times (EWL/\Delta WVP) + 0.547$] or T_b [P=0.001; $0.002 \times (EWL/\Delta WVP) + 0.334$].

Eastern rosellas
Mean mass of all eastern rosellas over all experiments (N=6, n=60) was 97.4±0.99 g. Linear contrasts for mass with RH were insignificant for both T_a=20°C and T_a=30°C ($t_5 \leq 2.21$, $P \geq 0.089$). At T_a=20°C, the overall repeated measures ANOVA model suggested that thermal, metabolic and hygric variables, including EWL, were independent of RH ($F_{4,2} \leq 12.7$, $P \geq 0.074$), but there was a significant linear contrast for EWL (P=0.004; Fig. 1). Although there were no significant overall effects of RH on EWL ($F_{4,2}$=5.93, P=0.150) or the other physiological variables ($F_{4,2} \leq 12.8$, $P \geq 0.073$) at T_a=30°C, there were significant linear contrasts for all variables ($P \leq 0.027$; Fig. 1). No overall RH effects were apparent for respiratory variables at T_a=20°C ($F_{4,2} \leq 11.3$, $P \geq 0.081$; Fig. 2), but at T_a=30°C RH influenced V_T ($F_{4,2}$=82.4, P=0.012) and V_I ($F_{4,2}$=151, P=0.007), although these effects could not be described by a linear pattern (P>0.713).

None of the variables calculated from the partitioning model varied with RH at either T_a=20°C ($F_{4,2} \leq 12.7$, $P \geq 0.074$) or T_a=30°C ($F_{4,2} \leq 4.16$, $P \geq 0.203$). At T_a=20°C, the overall mean calculated T_{surf} was 29.9±0.97°C, T_{exp} was 28.5±1.29°C and R was 439±48.5 s cm^{-1} (Fig. 3). Overall partitioning was 42±3.9% CEWL and 58±3.9% REWL (Fig. 4). From these values, T_{evap} was calculated as 29.1°C, compared with an overall mean T_b of 39.0±0.12°C (N=6, n=30). At T_a=30°C, calculated overall mean T_{surf} was 35.9±0.68°C, T_{exp} was 32.5±1.32°C and R was 189±18 s cm^{-1} (Fig. 3). Partitioning was 61±3.6% CEWL and 39±3.6% REWL (Fig. 4), with T_{evap} at T_a=30°C calculated as 34.6°C, compared with an overall mean T_b of 39.9±0.12°C.

Linear contrasts for the relationship EWL/ΔWVP versus RH (Fig. 5) were significant at T_a=20°C when the 'animal end' of ΔWVP was calculated from T_a [P=0.006; $0.010 \times (EWL/\Delta WVP) + 0.762$], but not T_b (P=0.367; mean=0.23±0.012 mg H$_2$O g^{-1} h^{-1} kPa^{-1}). The linear contrast for T_{evap} at T_a=20°C was not significant (P=0.234), although the quadratic contrast was (P=0.030); at RH≥37% there was a significant linear contrast (P=0.014, $0.003 \times (EWL/$
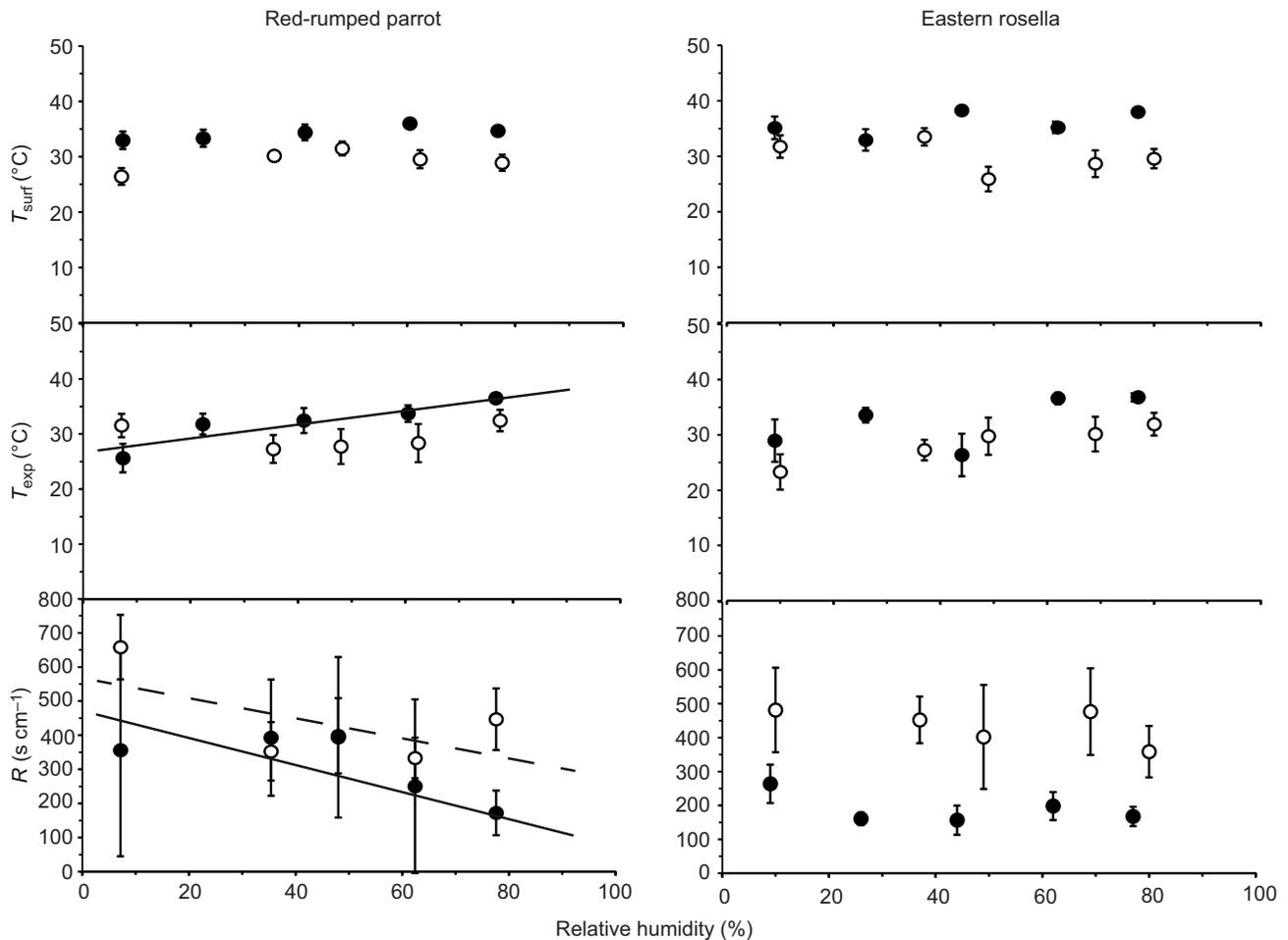
Fig. 3. The effect of ambient relative humidity on surface temperature, expired air temperature and resistance for red-rumped parrots and eastern rosellas, calculated after Withers and Cooper (2011). T_{surf}, surface temperature; T_{exp}, expired air temperature; R, resistance. White symbols represent calculations at an ambient temperature (T_a) of 20°C, and black symbols are calculations at T_a=30°C. Dashed line is significant linear contrast at T_a=20°C and continuous lines are significant linear contrasts at T_a=30°C. Values are means±s.e.m.; N=10 for red-rumped parrots at T_a=30°C and N=8 at T_a=20°C, and N=6 for eastern rosellas at both T_a.

ΔWVP)+0.469; Fig. 5). At T_a=30°C, all linear contrasts for the EWL/ΔWVP *versus* RH relationship were positive and significant, regardless of whether ΔWVP was calculated from T_a [P=0.005; 0.010×(EWL/ΔWVP)+0.947], T_{evap} [P=0.007; 0.005×(EWL/ΔWVP)+0.640] or T_b [P=0.043; 0.002×(EWL/ΔWVP)+0.413].

DISCUSSION

We present here clear evidence that two species of mesic habitat parrot can control their insensible EWL. Our conclusion is supported by the lack of conformity of insensible EWL to the predicted linear physical dependency on the ΔWVP between the animal and its environment. The data show that acute insensible EWL control occurs in multiple species of parrot, and is not restricted to arid habitat species. Our results support the hypothesis of Eto et al. (2017) and Cooper and Withers (2017) that physiological control of insensible EWL serves a thermoregulatory purpose for endotherms.

Although increasing RH resulted in a linear decrease of EWL at both T_a for red-rumped parrots and eastern rosellas, the main question was whether the magnitude of this effect was consistent with physical predictions, as there is no theoretical slope for the effect of RH on EWL (Cooper and Withers, 2017). The widely accepted physical model is that insensible EWL is proportional to the ΔWVP (Campbell and Norman, 1998; Withers et al., 2016). Therefore

EWL/ΔWVP should theoretically be independent of RH (i.e. slope=0), whereas a slope≠0 implies EWL control (Cooper and Withers, 2017; Eto et al., 2017). However, it is not straightforward to calculate the ΔWVP. When we calculated the 'animal end' of the ΔWVP using WVP saturation at T_a (e.g. Withers and Cooper, 2014), the slope for EWL/ΔWVP against RH was >>0 for both parrot species at both T_a. However, using T_a under-estimates ΔWVP (as T_{evap} is >T_a), over-estimates EWL/ΔWVP, and exaggerates the effect of RH. While this is not robust evidence for control of insensible EWL, it does give us an upper bound for the actual EWL/ΔWVP against RH slope. Using T_b to calculate ΔWVP is a much more conservative approach (Cooper and Withers, 2017; Eto et al., 2017) for assessing EWL control. As T_{evap} is <T_b, using T_b to calculate the ΔWVP will overestimate ΔWVP and consequently under-estimate the EWL/ΔWVP change with ambient RH. At T_a=30°C, this highly conservative approach provides clear evidence for EWL control by both species, but not at 20°C. The most realistic determination of ΔWVP is calculated from the animal's T_{evap}, which is intermediate between T_a and T_b, and calculated here from the partitioning of REWL and CEWL along with estimates of T_{exp} and T_{surf}. For red-rumped parrots, slopes for the relationship of EWL/ΔWVP *versus* RH were significant at T_a=20°C and T_a=30°C, and for eastern rosellas at T_a=30°C and at T_a=20°C for RH ≥37%, providing evidence of
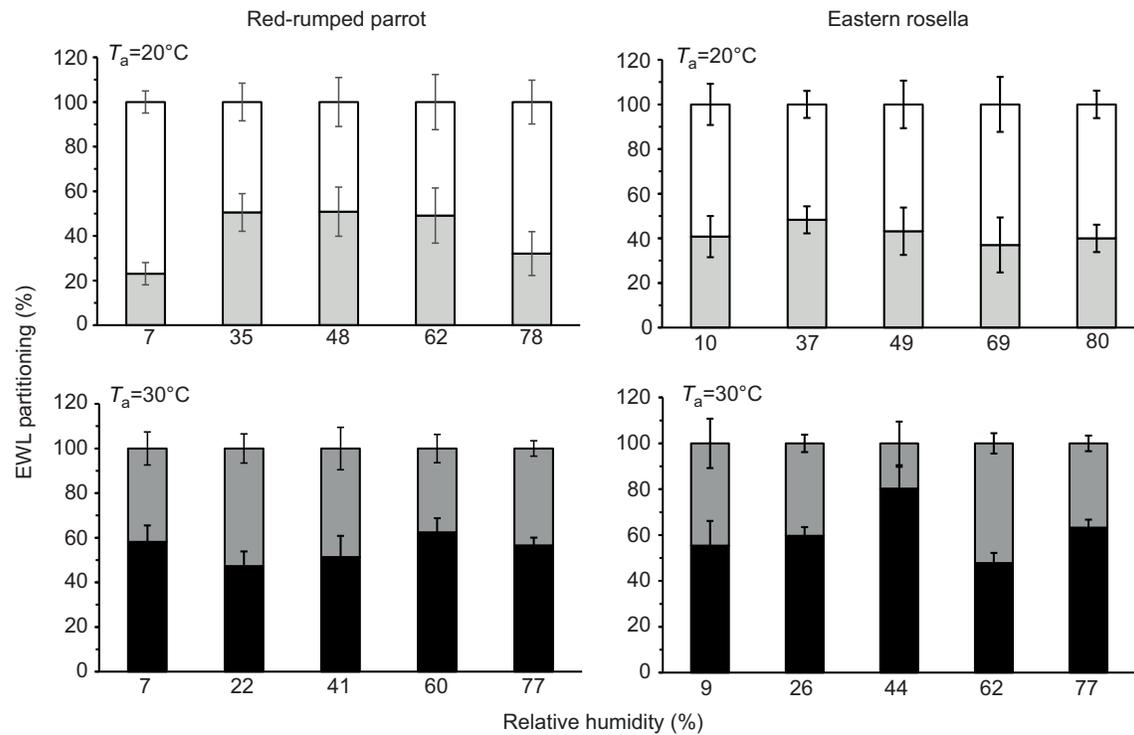
Fig. 4. The effect of ambient relative humidity on the partitioning of evaporative water loss into respiratory and cutaneous evaporative water loss for red-rumped parrots and eastern rosellas, calculated after Withers and Cooper (2011) at ambient temperatures of 20 and 30°C. EWL, evaporative water loss; T_a, ambient temperature. Darker, bottom bars denote respiratory EWL and lighter, top bars denote cutaneous EWL. Values are means±s.e.m.; N=10 for red-rumped parrots at T_a=30°C and N=8 at T_a=20°C, and N=6 for eastern rosellas at both T_a.

EWL control by both species of mesic habitat parrots. Therefore, although EWL did decline with increasing RH for both species at T_a=20°C and T_a=30°C, this was not as substantial as predicted by the ΔWVP (Campbell and Norman, 1998; Withers et al., 2016).

Diminishing the RH effect on EWL at any given T_a means that EHL remains relatively stable, and therefore the requirement for endotherms to adjust other aspects of heat balance (MHP or thermal conductance) to regulate T_b is reduced. For red-rumped parrots, EWL control achieved this, with no RH effects on T_b, MR or C_{wet} and C_{dry} at either T_a=20°C or T_a=30°C, despite the theoretical inhibition of EHL that would be expected at high RH if EWL was proportional to the ΔWVP. We calculate (using the equation for C_{dry}, and assuming C_{dry}, MR and EWL/ΔWVP remain constant at the values for the lowest RH) that this theoretical inhibition would increase T_b up to 0.4°C in the absence of control of EWL. For eastern rosellas, this was also the case at T_a=20°C. However, at T_a=30°C, despite clear evidence of EWL control, all other physiological variables were also affected by RH (which prevented the calculation of the magnitude of the thermal effect of EWL control for this species). However, the effect on MR and T_b was the opposite of what would be expected if EHL was hampered by high RH; T_b decreased and MR increased with increasing RH. This was presumably a consequence of increased non-evaporative heat loss (C_{dry}) at high RH, which may have resulted from changes in cutaneous blood flow, posture or plumage that are likely mechanisms for regulating the cutaneous component of total EWL (see below). A T_a of 30°C is presumably closer to the upper critical temperature of the TNZ for the larger eastern rosella compared with red-rumped parrots, and therefore we might expect eastern rosellas to initiate increased non-evaporative heat loss.

One hypothesis for the regulation of EWL is that it conserves water at low RH and therefore aids in maintaining water balance.

Considering that EWL can comprise up to 70% of an endotherm's water loss (Dawson, 1982; MacMillen, 1990; Williams and Tieleman, 2005), minimising EWL is potentially an important part of achieving water balance, especially for arid habitat species (e.g. Williams, 1996; Tieleman and Williams, 2000, 2002; Tracy and Walsberg, 2000, 2001; Withers et al., 2006; Van Sant et al., 2012; Song and Beissinger, 2020).

If EWL is reduced at low RH to conserve water, why then is EWL/ΔWVP higher at high RH? If water conservation was the primary role of EWL regulation, then the parrots could exploit high RH to limit EWL by allowing it to decline in proportion to the ΔWVP. In addition, there is currently no obvious mechanism for hygrosensory control of EWL by birds (Eto et al., 2017). To further explore the potential role of insensible EWL control, we compared the slope for the relationship between EWL/ΔWVP and RH for our two mesic parrots with that of the arid habitat budgerigar (data from Eto et al., 2017). The higher the slope, the more EWL deviates from the physical model of ΔWVP proportionally driving EWL. When EWL data were standardised for body mass using a scaling exponent of 0.635 for EWL (Douglas et al., 2017), there was no significant difference in the EWL/ΔWVP versus RH slope for the three species at T_a=20°C (ANCOVA $F_{2,92}$=2.73, P=0.071) or T_a=30°C ($F_{2,99}$=2.16, P=0.121; Fig. 6). We consequently conclude that there is no evidence that the arid habitat budgerigar has 'better' regulation of EWL than the mesic habitat parrots (i.e. it does not have a higher slope of EWL/ΔWVP versus RH), at least under the conditions we examined. We therefore find no support for the hypothesis that regulation of insensible EWL has a major water balance function. Another avenue for future investigation of potential contribution to water balance would be to examine the degree of regulation of insensible EWL for water-restricted birds compared with those maintained with *ad libitum* water.
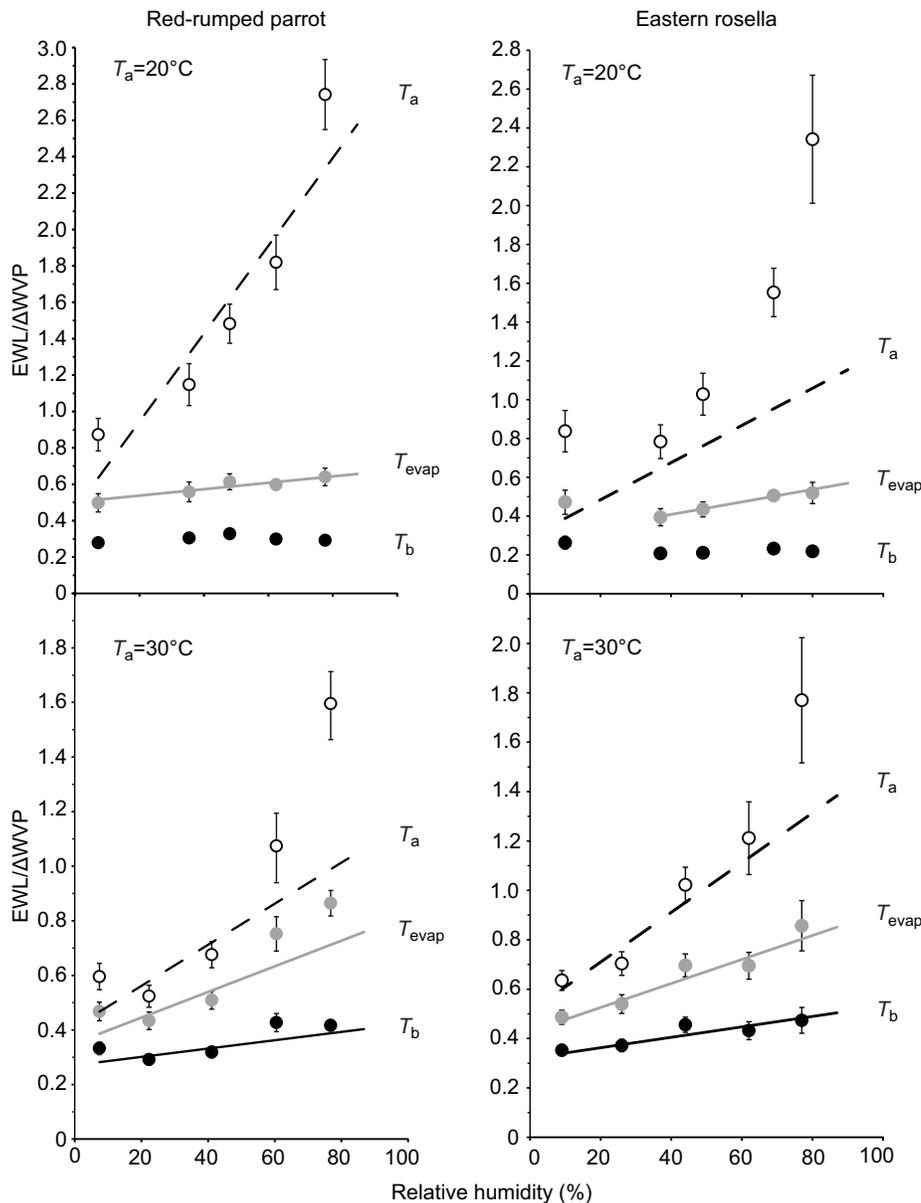
Fig. 5. Comparison of evaporative water loss divided by the water vapour deficit ($mg^{-1} g^{-1} h^{-1} kPa^{-1}$) between the bird and the ambient air, where the 'animal end' of the deficit is calculated from ambient temperature, calculated evaporative surface temperature and body temperature for red-rumped parrots) and eastern rosellas at ambient temperatures of 20 and 30°C. EWL, evaporative water loss; ΔWVP, water vapour deficit; T_a, ambient temperature (white symbols); T_{evap}, evaporative surface temperature (grey symbols); T_b, body temperature (black symbols). Lines are the significant linear contrast equations. Values are means±s.e.m.; N=10 for red-rumped parrots at T_a=30°C and N=8 at T_a=20°C, and N=6 for eastern rosellas at both T_a.

We do not currently understand the specific mechanism(s) by which (non-columbiform) birds control their EWL at and below thermoneutrality, but our iterative partitioning model provides some insights. This control could result from changes to REWL or CEWL, or both, but we did not observe any consistent pattern in EWL partitioning changing with increasing RH, suggesting that modification of both avenues may be involved. EWL partitioning for these two parrot species was remarkably consistent, approximately 40%:60% CEWL:REWL at T_a=20°C and approximately 55–60%:45–40% CEWL:REWL at T_a=30°C, reflecting greater evaporation from the cutaneous surface at higher T_a. These values are consistent with the 45–80% CEWL measured for other birds by invasive partitioning methodologies (Lasiewski et al., 1971; Wolf and Walsberg, 1996; Tieleman and Williams, 2002; Muñoz-Garcia and Williams, 2005; Ro and Williams, 2010). They are, however, considerably higher than the CEWL proportions of 30 and 38% estimated by Eto et al. (2017) for small budgerigars at T_a=20°C and T_a=25°C, but consistent with the 53% calculated at T_a=30°C, using the same iterative model of Withers et al. (2012). It is possible there are allometric effects on the partitioning of EWL into cutaneous and

respiratory avenues that account for these differences, and these allometric effects may also interact with T_a.

Cutaneous EWL is determined by T_{surf} and the skin, plumage and boundary layer resistances, which in turn may be determined by cutaneous blood flow, posture, plumage position and skin lipids. Skin temperature is expected to approximate core T_b (Körtner et al., 2001; McKechnie et al., 2007; Nord et al., 2013), but the insulation of the plumage means that the effective T_{surf} is considerably lower than skin temperature, especially at lower T_a. Modifying peripheral blood flow can change T_{surf}, but there was no evidence that T_{surf} was changing with RH for either species. We know that birds adjust their skin lipid composition over periods of days to weeks to modify CEWL (Muñoz-Garcia and Williams, 2008; Muñoz-Garcia et al., 2008), and for zebra finches (*Taeniopygia guttata*), CEWL changed even within 16 h, related to changes in the microstructure of the skin (Menon et al., 1996), so their ability to do so over our experimental period of 6–9 h is possible. It is also possible that changes in skin and/or feather hydration with RH contribute to changes in R, cf. mammalian skin (Grice et al., 1969). Posture and ptiloerection can, however, be adjusted almost instantaneously. They are more likely
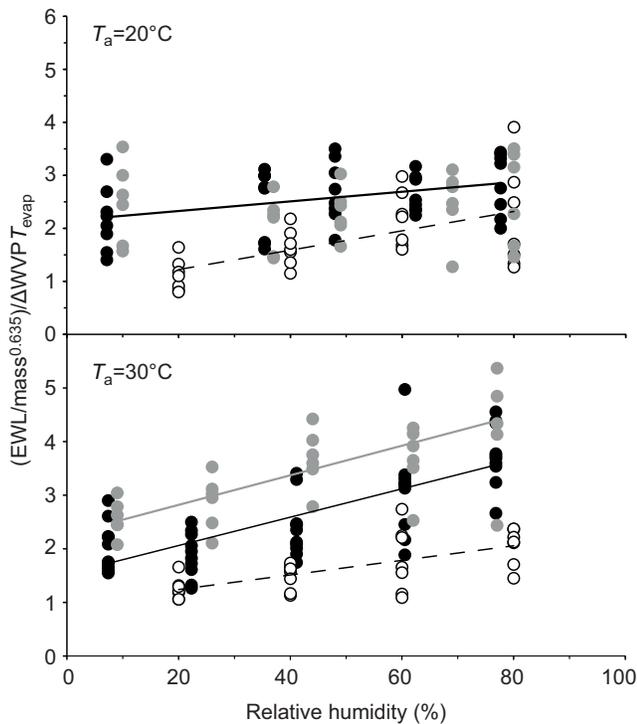
Fig. 6. Comparison of mass-independent evaporative water loss divided by the water vapour deficit (mg^{-1} g^{-1} h^{-1} kPa^{-1}) between the bird (calculated from surface temperature) and the ambient air for red-rumped parrots, eastern rosellas and budgerigars at ambient temperatures of 20 and 30°C. EWL, mass-independent evaporative water loss (EWL mg g$^{-0.635}$ h^{-1}); ΔWVP, water vapour deficit; T_a, ambient temperature; T_{evap}, evaporative surface temperature. Black symbols, red-rumped parrots (*Psephotus haematonotus*); grey symbols, eastern rosellas (*Platycercus eximius*); white symbols, budgerigars (*Melopsittacus undulatus*; data from Eto et al., 2017). Lines are the least-square regression equations for each species. Values are means±s.e.m.; N=10 for red-rumped parrots at T_a=30°C and N=8 at T_a=20°C, and N=6 for eastern rosellas at both T_a.

to contribute to acute changes in R with RH, such as observed for red-rumped parrots at T_a=30°C, and may be reflected in changes in C_{dry} with RH, as observed for eastern rosellas, also at T_a=30°C.

Respiratory EWL is the other avenue by which EWL can be modified, and it is determined by T_{exp}, V_I and the RH of the expired air. The T_{exp} of many birds is cooled below T_b by nasal counter-current water and heat exchange, as we calculated for these two species, and is often considerably lower than T_b, especially at low T_a (e.g. Schmidt-Nielsen et al., 1970; Brent et al., 1984; Kaiser and Bucher, 1985; Engel et al., 2006; Eto et al., 2017). Increasing T_{exp} at higher RH, as seen for red-rumped parrots at T_a=30°C, is one mechanism by which EWL may be increased under conditions of lower ΔWVP. However, we found no evidence that V_I was modified in any consistent fashion with increasing RH, so it is likely that V_I, driven by a combination of f_R and V_T, is predominantly adjusted to accommodate metabolic demand, maintaining E_{O_2} high and constant. We assumed that expired air was saturated; this is a common assumption, as the RH of expired air is very difficult to measure for small species, especially non-invasively (Welch, 1984). However, the ostrich (*Struthio camelus*) can expire unsaturated air (Withers et al., 1981), as can some mammals (camel, *Camelus dromedarius*, Schmidt-Nielsen et al., 1981; sheep, *Ovis aries*, Johnson et al., 1988) so it is not inconceivable that modification of expired air humidity contributes to control of REWL.

In conclusion, our measurements of EWL over a range of environmental RH for these two mesic parrot species provide further support for the conclusion of Eto et al. (2017), based on their study of EWL for the budgerigar and earlier suggestions for birds (Webster et al., 1985; Webster and Bernstein, 1987; Ro and Williams, 2010), that birds as well as mammals can control their insensible EWL. This control appears to be a feature of the two major groups of convergently endothermic vertebrates, and we demonstrate here that it is not limited to arid habitat species. Considering the remarkable physiological convergence between birds and mammals (Lasiewski and Calder, 1971; Ruben, 1995), it is not unexpected that they share this additional element of physiological control, but it does imply that physiological control of insensible EWL is of fundamental importance for endotherms, and we suggest that it is more likely to have a primarily thermoregulatory, rather than a water balance, role. As such, we conclude that EWL at and below the TNZ should not be termed passive EWL (IUPS Thermal Commission, 2003), as there is now clear evidence that this insensible EWL is not passive but is a controlled physiological variable that presumably contributes to the sophisticated thermoregulatory system of endothermic vertebrates.

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