Two independent approaches to assessing the constancy of evaporative water loss for birds under varying evaporative conditions

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Running Head: Control of evaporative water loss by a mesic parrot

Author Contributions

CEC and PCW designed the study, obtained funding, and provided logistical support; LNG and CEC carried out experimental procedures; LNG and CEC analysed the data and drafted the manuscript; PCW wrote data acquisition and analysis software; CEC, PCW, and MMG supervised the project; PCW and MMG edited the manuscript.

Abstract:

We examine here the effects on evaporative water loss, at and below thermoneutrality, of perturbing the evaporative environment for the red-capped parrot (*Purpureicephalus spurius*) by modifying the ambient relative humidity or the diffusive properties of the ambient environment using a helium-oxygen mix (helox). We found that evaporative water loss did not change with relative humidity at an ambient temperature of 30°C, but there was a negative relationship for evaporative water loss with relative humidity at 20 and 25°C. The evaporative water loss per water vapour pressure deficit between the bird and its ambient environment was not constant with relative humidity, as would be expected for a physical effect (slope = 0); rather there was a significant positive relationship with relative humidity at ambient temperatures of 25 and 30°C. Consequently, we conclude that the red-capped parrot can physiologically control its EWL over a range of relative humidities. For the first time for a bird species, we also confirmed EWL control using a second methodology to perturb the evaporative environment, and demonstrated that a more diffusive helox atmosphere has no effect on evaporative water loss of live birds, but evaporative water loss was higher for dead birds in helox compared to air. Our results for evaporative water loss and other physiological variables for red-capped parrots are consistent with the hypothesis that evaporative water loss is under physiological control.

Keywords: evaporative water loss, helox, water vapour pressure, humidity, temperature, metabolism, thermoregulation, physiological control

List of abbreviations

C_{dry}	dry thermal conductance
C_{wet}	wet thermal conductance
CEWL	cutaneous evaporative water loss
D	diffusion coefficient
EHL	evaporative heat loss
EWL	evaporative water loss
F_E	fractional excurrent
F_I	fractional incurrent
MHP	metabolic heat production
PIT	Passive integrated transponders
RER	respiratory exchange ratio
RH	relative humidity
REWL	respiratory evaporative water loss
STPD	standard temperature and pressure, dry
T_a	ambient temperature
T_b	body temperature
T_{evap}	temperature of the animal’s evaporative surface
TNZ	thermoneutral zone
V_I	incurrent air flow
V_E	excurrent air flow
VO_2	oxygen consumption
VCO_2	carbon dioxide production
wvp	water vapour pressure
Δwvp	water vapour pressure deficit
$\Delta wvpT_a$	water vapour pressure deficit with the “animal end” calculated from ambient temperature

$\Delta wvpT_b$	water vapour pressure deficit with the “animal end” calculated from body temperature
wvp_{sat}	saturation water vapour pressure
$wvpT_a$	water vapour pressure at ambient temperature
$wvpT_b$	water vapour pressure at body temperature

INTRODUCTION

The evaporative water loss (EWL) of endothermic birds and mammals has been traditionally classified as either thermoregulatory EWL, occurring at high ambient temperatures (T_a), or “insensible” evaporative water loss occurring passively at T_a within or below the thermoneutral zone (TNZ; Monteith, 1973; Commission for Thermal Physiology, 2003). Thermoregulatory EWL is augmented through avenues such as panting and sweating, and is vital for dissipating metabolic heat at high T_a (Morimoto, 1998). Physiological control of EWL at high T_a via the autonomic nervous system is well-understood (Dawson, 1982; Morrison and Nakamura, 2011), and EWL can be maintained at high relative humidity (RH) to sustain evaporative heat loss (EHL; Gerson et al., 2014; van Dyk et al., 2019). “Insensible” EWL, by contrast, was historically thought to be a physical process resulting from the inevitable permeability of the skin and respiratory surfaces to water vapour, driven by the water vapour pressure deficit (Δwvp) between an animal and its environment (Cossins and Bowler, 1987; Campbell and Norman, 1998; Withers et al., 2016). This concept was supported by several studies of EWL for both birds and mammals (e.g. Chew and Dammann, 1961; Baudinette, 1972; Christian, 1978; Edwards and Haines, 1978; Webster and King, 1987; Powers, 1992; Webb et al., 1995; Klüg-Baerwald and Brigham, 2017). However, there is growing evidence 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; Cooper and Withers, 2014; Eto et al., 2017; Cooper et al., 2020) that EWL at low to moderate T_a does not necessarily follow the expectations of this physical model; EWL may remain constant under environmental conditions expected to enhance EWL.

This study focuses on “insensible” EWL at and below the TNZ. Physiological control of EWL can be assessed by quantifying EWL under conditions expected to perturb it (Lasiewski et al., 1966; Procter and Studier, 1970), and then assessing if EWL responds according to physical predictions or deviates from the expected physical model. The evaporative environment can be manipulated by altering one of two components of Fick’s (1855) law of diffusion (Monteith and Campbell, 1980; Withers, 1992), the Δwvp or the diffusion coefficient of water vapour in the environment. The concentration difference for water vapour may be readily manipulated by modifying the ambient water vapour pressure (wvp), achieved by changing the ambient RH at a particular T_a (e.g. Cooper and Withers, 2008, 2017; Kurta, 2014; Withers and Cooper, 2014; Eto et al., 2017; van Dyk et al., 2019; Cooper et al., 2020). If EWL conforms to physical expectations, then there will be a negative linear relationship between the ambient wvp and EWL (or a positive relationship between EWL and the Δwvp; Webster and King, 1987; Powers, 1992; Webb et al., 1995; Finkler, 2001). This is the most common conceptual approach to examining an animal's EWL under varying evaporative conditions.

There are some challenges associated with assessing the effect of the ambient wvp on EWL and therefore identifying whether there is physiological control of EWL. There is no theoretical expectation for the magnitude of the slope describing the negative linear relationship between EWL and RH, so it is problematic to test if an observed relationship differs from physical expectations (Eto et al., 2017; Cooper and Withers, 2020). This issue can be overcome by calculating EWL/Δwvp and assessing this relative to the RH (Withers and Cooper, 2014; Eto et al., 2017; Cooper and Withers, 2020; Cooper et al., 2020). It is statistically straightforward to determine if the slope ≠ 0 (Zar, 2010; Withers and Cooper, 2011), indicating deviation from physical expectations of a slope = 0. However, calculation of the Δwvp is not straightforward. To calculate the saturation wvp at the "animal end" of the Δwvp, it is necessary to determine the average temperature of the animal's evaporative surface (T_{evap}). One approach is to assume T_{evap} is equal to body temperature (T_b), but T_b will probably overestimate T_{evap}, and therefore underestimate the EWL/Δwvp vs RH slope (e.g. Cooper and Withers, 2017, 2020). Alternatively the animal's wvp can be estimated from T_a (e.g. Coulombe, 1970; Withers and Cooper, 2014) which will likely underestimate T_{evap} and overestimate the EWL/Δwvp vs RH slope. Together these provide upper and lower bounds for the actual EWL/Δwvp vs RH slope.

An alternative approach to determining if EWL conforms to biophysical expectations is to modify the diffusion coefficient of the ambient air (Cooper and Withers, 2014, 2020). Water vapour diffuses 2.3 times faster in helox (21% O_2 in He) than in air due to the lower density of helium compared to nitrogen (Paganelli et al., 1975; Paganelli and Kurata, 1977; Egorov and Karpushkin, 1988; Parkhurst and Mott, 1990; Mott and Parkhurst, 1991). Consequently, an animal's cutaneous evaporative water loss (CEWL) should be higher in helox than in air. Helox is also 4 times more conductive than air and will therefore increase the animal's dry heat flux. An endotherm will require a proportional increase in metabolic heat production (MHP) to remain homeothermic (Cook et al., 1951; Leon and Cook, 1960; Rosenmann and Morrison, 1974; Maddocks and Geiser, 1999), which in turn must be accommodated by increased respiratory minute volume (Hallam and Dawson, 1993; Chappell and Dawson, 1994; Cooper and Withers, 2014) that should increase respiratory EWL (REWL). Consequently, because helox should augment both CEWL and REWL, the physical model predicts that EWL will be higher in helox than in air (Paganelli et al., 1975; Cooper and Withers, 2014, 2020).

Helox has been used to modify the evaporative environment of plants (Egorov and Karpushkin, 1988; Parkhurst and Mott, 1990; Mott and Parkhurst, 1991) and bird eggs (Paganelli et al., 1975), but until recently it was assumed that helox did not influence EWL of small endotherms (Leon and Cook, 1960; Rosenmann and Morrison, 1974; Holloway and Geiser, 2001) although Paganelli et al. (1975) predicted that helox would increase human insensible EWL. Cooper and Withers (2014, 2020) used helox to modify the evaporative environment of a placental (ash-grey mouse; *Pseudomys albocinereus*) and marsupial (ningaui; *Ningaui sp.*) mammal to assess their potential for physiological control of EWL. They found that the EWL of these small mammals did not increase in helox for live animals but did increase for carcasses, indicating physiological control of EWL by live animals. To our knowledge helox has not been used to modify the evaporative environment for a bird, although it is routinely used to modify their thermal environment (e.g. Rosenmann and Morrison, 1974; Maddocks and Geiser, 1999; Oswald et al., 2021). Here we manipulate two components of Fick's law, the Δwvp and the diffusion coefficient to examine avian physiological control of EWL, using the mesic-habitat red-capped parrot (*Purpureicephalus spurius*) as a model.

METHODS

Capture and care of animals

Seven red-capped parrots (3 females, 4 males) were captured with mist nets in Carmel (32° 01′ S, 116° 04′ E), 25 km east of Perth, Western Australia. Parrots were housed in outdoor aviaries at Curtin University's Bentley campus (Perth, Western Australia). Passive integrated transponders (PITs; e-Mitter G2, Starr Life Sciences; 15.5 x 6.5 mm, 1.09 g, <1% of body mass) were surgically implanted into the abdominal cavity using sterile techniques under isoflurane anaesthesia (~ 4% induction, 2% maintenance) to record core T_b throughout experiments. Post-operative analgesia was provided by sub-cutaneous injection of meloxicam (Metacam, Boehringer Ingelheim Vetmedica, 0.2 mg kg^{-1} body mass); birds were allowed at least 10 days for recovery prior to commencement of measurements. Birds had access to *ad libitum* water and food (commercial "wild bird" seed mix supplemented with fresh fruit and greens) during daylight hours on days that they were not measured. Birds were fasted (but had access to drinking water) for at least 6 hours during the day prior to overnight measurements, and were rested for at least three days between successive measurements.

We used open flow-through respirometry (Withers, 2001) to measure EWL, oxygen consumption (VO_2) and carbon dioxide production (VCO_2) at relative humidities of ~12, 36, 51, 66, and 81% RH at T_a of 20, 25 and 30°C. Additional measurements at ~12% RH were made in air at T_a = 15°C, and in helox at T_a = 15, 20, 25 and 30°C. Birds were measured individually in a 5 L Perspex metabolic chamber placed in a controlled-temperature cabinet (Arcus 400E) or room. Compressed air flow into the chamber was dried using columns of drierite (W.A. Hammond Co.) and regulated at 1000 mL min^{-1} standard temperature and pressure, dry (STPD) using a mass-flow controller (Aalborg GFC171 or Cole-Parmer 32708-28). To achieve experimental RHs, incurrent air was humidified after the mass flow controller by saturating it at the water temperature appropriate to provide the required RH when warmed to the experimental T_a (calculated from the psychrometric equations of Parish and Putnam, 1997). The water vapour produced by the bird added to the incurrent RH to generate the experimental chamber RH, which was assumed to be the same as that of the excurrent air.

The RH and T_a of excurrent chamber air were measured using a Vaisala RH/T_a probe (MNP45A or MNP35B). Excurrent air then passed through a drierite column, a CO_2 analyser (Qubit Systems S153 or Sable Systems CA-2A), and finally a paramagnetic O_2 analyser (Servomex 574 or Sable Systems PA-10). Outputs from these instruments were interfaced to a PC via a Sable Systems UI2 A/D converter, and data were recorded every 30 sec using a custom-written data acquisition program (Visual Basic v.6, P.C. Withers). The O_2 analyser output was recorded as the differential between the experimental and a reference cell, to eliminate background drift from changes in room temperature and pressure during measurements. Baselines for background levels of O_2, CO_2, and RH were established for at least 30 min at the start and end of each measurement. Post-absorptive birds were measured overnight for about 10 h until EWL was minimal and stable for at least 20 min (Page et al., 2011). Body temperature was recorded every 10 sec throughout experiments using telemetry receiver pads (Starr Life Sciences) positioned below the respirometry chamber, in conjunction with VitalView monitoring software (Starr Life Sciences). At the conclusion of the study the birds were euthanised with an intraperitoneal injection of sodium pentobarbitone (Lethabarb, Virback) and EWL from the carcasses was measured immediately in either air or helox (N = 3 for each treatment) at T_a = 24˚C and RH of 8-9%.

Emitter PITs were calibrated at T_a from 30 to 45°C against a mercury thermometer traceable to a national standard (AMA®, -5 to 50˚C), before implantation and again after removal. Body temperature data were corrected using the individual calibration curve generated for each PIT. Mass flow controllers were calibrated using a volumetric flow meter (Sensidyne Gilibrator-2), separately for both air and helox. Oxygen and CO_2 analysers were two-point calibrated with compressed N_2 and a precision gas mix (0.538% CO_2; BOC Gas) or dry compressed air (20.95% O_2), and RH and T_a probes were calibrated against the known baseline incurrent humidities.

Standard mass balance equations were used to calculate VO_2, VCO_2, and EWL from the measured dry incurrent air flow (V_I) and the calculated STPD excurrent air flow (V_E), using the fractional incurrent (F_I) O_2 and CO_2 concentrations and excurrent (F_E) O_2, CO_2,and H_2O concentrations (Withers 2001). For oxygen consumption $VO_2 = [V_I.F_IO_2 - V_E.F_EO_2]$ and carbon dioxide production $VCO_2 = [V_E.F_ECO_2 - V_I.F_ICO_2]$, where V_E is calculated for dry excurrent air as $V_I (1 - F_IO_2)/(1 - F_EO_2 - F_ECO_2)$. Evaporative water loss was calculated as $EWL = [V_E.F_EH_2O - V_I.F_IH_2O]$ where V_E is calculated for excurrent air (with water vapour present) as $V_I (1 - F_IO_2)/(1 - F_EO_2 - F_ECO_2 - F_EH_2O)$. Calculations were made using a custom-written data acquisition program (Visual Basic v.6, P.C. Withers). Minimal and stable values were averaged over an approximately 20 min period. Mean T_b was calculated from calibrated PIT data for the same period. Wet (C_{wet}) and dry (C_{dry}) thermal conductance (C; J g^{-1} h^{-1} $°C^{-1}$) were calculated as $C_{wet} = MHP/(T_b\text{-}T_a)$ and $C_{dry} = (MHP\text{-}EHL)/(T_a\text{-}T_b)$ respectively. The oxy-calorific coefficient for the measured respiratory exchange ratio ($RER = VCO_2/VO_2$) was used to convert VO_2 to J, and the latent heat of vaporisation (2.4 J mg^{-1}; Monteith 1973) used to convert EWL to EHL, after Withers et al. (2016). Hygrometeorological equations (Parish and Putnam, 1977) were used to calculate saturation wvp (wvp_{sat}) at T_a ($wvpT_a$) and T_b ($wvpT_b$), and ambient wvp was calculated as wvp_{sat} at $T_a \cdot RH/100$. The Δwvp was then calculated as wvp_{sat} animal – ambient wvp, using either $wvpT_a$ or $wvpT_b$ as the “animal end” of the equation, as $\Delta wvpT_a$ or $\Delta wvpT_b$ respectively.

Values are presented as mean ± standard error (SE), with gas volumes at STPD, with number of individuals = N and number of measurements = n. Responses for VCO_2 mirrored those for VO_2, and so VCO_2 data are not presented separately. Effects of RH on physiological variables were analysed with multivariate repeated measures analyses of variance (RMANOVA) separately for each T_a because of the non-equivalence of RH and of wvp relative to saturation at different T_a. We used linear and

Helmert *a priori* contrasts to examine specific hypotheses about the pattern of physiological response to increasing RH (Withers and Cooper, 2011). Effects of helox on physiological variables were analysed by RMANOVA with two levels of repeat; T_a and ambient gas (air or helox). We used a two-sample t-test (with a test, and correction if necessary, for unequal variance) for comparison of EWL from carcasses in dry air and helox. Statistical analyses were accomplished with SPSS (V. 25; IBM) and Statisti*XL* (2.1; statisti*XL*.com).

RESULTS

Body mass was 116 ± 0.1 g for all birds over all experiments (N = 7, n = 136). There was no overall RH effect for T_b, VO_2, C_{wet} or C_{dry} ($F_{4,3} \leq 3.80$; $P \geq 0.157$; Fig. 1) by RMANOVA, but there was a positive linear relationship with RH for VO_2 and C_{dry} at T_a = 25 and 30˚C by linear contrasts, which accounts for the order of RH treatments ($F_{1,6} \geq 7.18$, $P \leq 0.037$; Fig. 1). There was no overall ($F_{4,3} = 1.55$, $P = 0.374$) or linear ($F_{1,6} = 1.01$, $P = 0.368$) RH effect on EWL at T_a = 30°C (Fig 2A). However, there was a significant overall effect of RH on EWL at T_a = 20˚C (RMANOVA; $F_{4,3} = 31.6$, $P = 0.009$) and 25°C ($F_{4,3} = 13.1$, $P = 0.030$), that was explained by significant negative linear contrasts ($F_{1,6} \geq 6.83$, $P \leq 0.040$). The positive linear contrasts relating RH and EWL/$\Delta wvpT_a$ were significant for all T_a ($F_{1,6} \geq 22.9$, $P \leq 0.003$), while for EWL/$\Delta wvpT_b$ they were significant at T_a = 25 and 30°C ($F_{1,6} \geq 13.12$, $P \leq 0.011$) but not at 20°C ($F_{1,6} = 2.39$, $P = 0.173$; Fig. 2B).

Body temperature was not influenced by T_a ($F_{3,3} = 0.58$, $P = 0.599$) or helox ($F_{1,5} = 0.81$, $P = 0.331$; Fig 3A), but both T_a ($F_{3,3} \geq 15.0$, $P \leq 0.026$) and helox ($F_{3,3} \geq 29.4$, $P \leq 0.003$) had significant effects (Fig 3B & C) on VO_2, C_{wet}, and C_{dry}. Oxygen consumption increased with decreasing T_a, with a more pronounced increase in helox compared to air; VO_2 was 1 (T_a = 30°C) to 1.62 (T_a = 15°C) times higher in helox than in air. Wet thermal conductance in helox was 0.98 times that in air at T_a = 30°C, increasing to 1.71 times at T_a = 15°C, with a similar pattern for C_{dry} (0.98 - 1.81 times than in air from T_a = 30 to 15°C). Neither T_a ($F_{3,3} = 0.586$, $P = 0.608$) nor helox ($F_{1,5} = 0.595$, $P = 0.479$) significantly affected EWL of live red-capped parrots, but EWL of red-capped parrot carcasses was significantly higher (t_4 = 6.90, P = 0.002) in helox (1.05 ± 0.02 mg H_2O g^{-1} h^{-1}) than in air (0.76 ± 0.04 mg H_2O g^{-1} h^{-1}; Fig. 3D).

DISCUSSION

We present strong evidence of control of EWL by a bird at and below the TNZ, by using two different approaches to modify the evaporative environment. Previous studies (Webster et al., 1985; Webster and Bernstein, 1987; Ro and Williams, 2010) suggested a capacity for EWL control at varying ambient RH. More recently Eto et al. (2017) and Cooper et al. (2020) used the slope of the EWL/Δwvp vs RH relationship as more definitive evidence for control of EWL by birds. We also found that the EWL of the mesic-habitat red-capped parrot deviated from expectations of a physical model, suggesting physiological control of EWL. For the first time for a bird, we also verified constancy of EWL in a helox atmosphere, despite the increased diffusion coefficient for evaporation. Consequently we have provided two independent lines of evidence for physiological control of EWL by a bird. This is important because understanding how EWL is impacted by environmental conditions provides insight into how animals manage their water balance and/or thermoregulation, which in turn can influence their current and future distribution (Williams et al., 2012).

At T_a = 30°C, EWL was unequivocally independent of the ambient RH, and thus clearly deviated from a physical diffusion model. At T_a = 20 and 25°C however, EWL decreased with increasing RH (decreasing Δwvp) , suggesting conformity to a linear model at lower T_a as has been proposed for a number of birds and mammals (Lasiewski et al., 1966; Richards, 1976; Webster and King, 1987; Edwards and Haines, 1978). For normothermic endotherms within and below their TNZ T_b is however typically higher than T_a and therefore the EWL vs RH relationship will not extrapolate to 0 at 100% RH, because the higher wvp at T_{evap} still allows for EWL. Consequently the expected slope of EWL vs RH is unknown, so that a negative EWL vs RH relationship is not necessarily evidence that EWL is passively driven by the Δwvp. When we assessed EWL relative to the Δwvp, positive slopes for the EWL/Δwvp vs RH relationship provided evidence of deviation from physical expectations (i.e. slope ≠ 0) at T_a = 25 and 30 but not 20°C.

Establishing that EWL at moderate T_a is controlled and not passive raises the question of why EWL is physiologically regulated. Physiological regulation of EWL could assist in water conservation at low RH, or alternatively could minimise the impact of varying RH on EWL, minimising impacts of varying RH on EHL and consequently thermoregulation. Similar patterns of EWL control by both mesic- and arid-habitat birds (Cooper et al., 2020) and by a thermoregulating but not thermoconforming torpid marsupial (Cooper and Withers, 2017) suggest that a thermoregulatory role is most likely. Consistent with this hypothesis, the red-capped parrots maintained a constant T_b with increasing RH and C_{wet} also remained constant; C_{wet} would be expected to decrease if EWL was inhibited at high RH. We did observe an increase in C_{dry} (and a corresponding increase in VO_2) at increasing RH, which may be a consequence of the mechanisms involved in the maintenance of EWL constancy. Modifying posture and the extent of ptilo-erection are potential mechanisms for enhancing CEWL at high RH to maintain total EWL and C_{wet}, but could consequently increase C_{dry} and the requirement for MHP.

It is possible that our observations of the non-conformity of EWL to physical predictions is not due to any physiological response by the bird, but is a physical effect of a change in ambient RH. Humidification of the skin's stratum corneum at high RH may increase EWL (Grice et al., 1972). Consequently, we applied a second methodology to modify the evaporative environment without modifying the ambient RH. The constancy of EWL in a helox environment provides a second line of evidence for physiological EWL control.

We observed increases in C_{dry} and consequently C_{wet} and VO_2 in helox compared to air below the TNZ, occurring as a consequence of maintaining T_b constant under conditions of greater conductive heat loss in helium, as is typically observed for endotherms (e.g. Leon and Cook, 1960; Rosenmann and

Morrison, 1974; Maddocks and Geiser, 1999; Oswald et al., 2021). Helox:air ratios for C_{wet} and VO_2 were ~1 at T_a = 30°C and increased with decreasing T_a, confirming that T_a = 30°C was within the TNZ. Below the TNZ the expected helox:air ratio for C_{wet} and VO_2 is 4 for conductive heat loss, but it is substantially lower (2.1) for convective heat loss, and may be even lower due to radiative and evaporative heat losses. The helox:air ratios of 1.6 - 1.7 that we observed are typical of mammals and birds (helox:air ratio ≤ 2.6; Rosenmann and Morrison, 1974; Thomas et al., 1998; Maddocks and Geiser, 1999; Cooper and Withers, 2014, 2020), reflecting heat loss from a combination of these avenues.

In striking contrast, there was no effect of helox on EWL. The observed increase in VO_2 in helox below the TNZ should be accompanied by increased REWL, as an increase in respiratory ventilation typically accommodates the VO_2 demand of birds (Maloney and Dawson, 1994). In addition, the diffusive effect of helox on EWL should increase CEWL, even in thermoneutrality. Increased CEWL was observed for parrot carcasses; the CEWL increased 1.3 times in helox compared to air, as for carcases of a small marsupial (1.3 times; Cooper and Withers, 2020) but lower than for rodent carcasses (2.9 times; Cooper and Withers, 2014). If we assume 50:50 partitioning of EWL into cutaneous and respiratory components (e.g. Wolf and Walsberg, 1996; Cooper et al., 2020), then total EWL should be 1.45 times higher in helox than in air, but the ratio we observed was 1.04, i.e. EWL was the same in helox and air, providing unequivocal evidence of physiological control of EWL by red-capped parrots.

That the mesic-zone red-capped parrot can control EWL over a range of RH is consistent with previous studies demonstrating constancy of EWL over a range of RH for the arid-zone budgerigar (Eto et al., 2017) and two other mesic-zone parrots (eastern rosella and red-rumped parrots; Cooper et al., 2020). We confirmed this constancy of EWL using a helox atmosphere as a second methodology to perturb the evaporative environment, as has been established previously for mammals; a rodent (Cooper and Withers, 2014) and a marsupial (Cooper and Withers, 2020). Our results for this mesic-habitat parrot are consistent with the hypothesis that EWL control functions in the context of thermoregulatory control rather than as a water-conserving mechanism at low RH (Eto et al., 2017; Cooper et al., 2020).

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Figure Legends

Figure One: Body temperature (A), metabolic rate (oxygen consumption; B), and wet (C) and dry (D) thermal conductance of red-capped parrots (*Purpureicephalus spurius*) measured at five relative humidities and ambient temperatures of 20°C (white symbols), 25°C (grey symbols) and 30°C (black symbols). Significant linear contrasts are indicated with grey (ambient temperature = 25°C) and black (30°C) lines. Values are mean ± SE, N = 7.

Figure Two: Evaporative water loss (A) and evaporative water loss/water vapour pressure deficit between the animal and the environment (EWL/Δwvp; B) calculated from T_a (upper bound) or T_b (lower bound) for red-capped parrots (*Purpureicephalus spurius*)measured at five relative humidities and ambient temperatures of 20°C (white symbols), 25°C (grey symbols) and 30°C (black symbols). Significant linear contrasts are indicated with dashed (ambient temperature = 20°C), grey (25°C) and black (30°C) lines. Values are mean ± SE, N = 7.

Figure Three: Body temperature (A), wet thermal conductance (B), metabolic rate (oxygen consumption; C) and evaporative water loss (D) of red-capped parrots (*Purpureicephalus spurius*) in air (black symbols) and helox (white symbols), measured at ambient temperatures of 15-30°C. Evaporative water loss of parrot carcasses measured at 24°C is indicated with grey symbols. There were significant ambient temperature and helox effects on metabolic rate and wet thermal conductance, and helox effects on carcass EWL. Values are mean ± SE, N = 7.