

1 **Can birds do it too? Evidence for convergence in evaporative water loss regulation for**
2 **birds and mammals**

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15 Running head: Bird regulates insensible evaporation

16 **ABSTRACT**

17 Birds have many physiological characteristics that are convergent with mammals. In light of
18 recent evidence that mammals can maintain a constant insensible evaporative water loss over
19 a range of perturbing environmental conditions, we hypothesised that birds might also regulate
20 insensible evaporative water loss, reflecting this convergence. We found that budgerigars
21 (*Melopsittacus undulatus*) maintain evaporative water loss constant over a range of relative
22 humidities at three ambient temperatures. Evaporative water loss, expressed as a function of
23 water vapour pressure deficit, differed from a physical model where the water vapour pressure
24 deficit between the animal and the ambient air is the driver of evaporation, indicating
25 physiological control of evaporative water loss. Regulating evaporative water loss avoids
26 thermoregulatory impacts of varied evaporative heat loss; changes in relative humidity had no
27 effect on body temperature, metabolic rate or thermal conductance. Our findings that a small
28 bird can regulate evaporative water loss is evidence that this is a common feature of
29 convergently-endothermic birds and mammals, and may therefore be a fundamental
30 characteristic of endothermy.

31 Key words: evaporative water loss, water vapour pressure deficit, budgerigar, endotherm, air
32 temperature, relative humidity, body temperature

33 1. INTRODUCTION

34 Evaporative water loss (EWL) is an important physiological variable, as it may account for the
35 majority of an animal's water loss and has implications for heat balance [1]. Total EWL may
36 occur as the consequence of insensible and thermoregulatory EWL. Insensible EWL occurs via
37 both respiratory and cutaneous avenues, and is an inevitable consequence of the permeability
38 of the skin and respiratory surfaces to water vapour [2]. Thermoregulatory EWL refers to
39 augmented water loss at high ambient temperatures (T_a), arising from responses such as panting
40 and sweating to dissipate heat [2].

41

42 It is well known that endothermic mammals and birds are able to regulate their
43 thermoregulatory EWL [1,3]. However, insensible EWL has been traditionally thought to be
44 passive, determined physically by the water vapour pressure deficit (Δwvp) between an animal
45 and its environment [4-6]. A high Δwvp e.g. low environmental relative humidity (RH) should
46 lead to high rates of EWL and *vice versa*. Consequently, we would expect $EWL/\Delta wvp$ to be
47 independent of environmental wvp, and RH, if insensible EWL was simply a physical process
48 [6].

49

50 Recently it has been demonstrated that some mammals have a constant insensible EWL under
51 perturbing evaporative conditions [6-8], suggesting some form of acute physiological control.
52 This apparent regulation of insensible EWL has been observed for both marsupial [6,7] and
53 placental [8] mammals, which diverged some 148 MYBP [1], suggesting that it may be a
54 fundamental characteristic of mammalian physiology. Birds are the other major group of
55 endothermic vertebrates; they evolved endothermy independently to mammals from different
56 reptilian groups (divergence ~ 300 MYBP) [9, 10], but are remarkably convergent in many
57 aspects of their physiology [11]. A convergent capacity for birds to also regulate insensible
58 EWL would suggest that maintaining EWL independent of perturbing environmental
59 conditions is of fundamental importance for endotherms.

60

61 Birds have the capacity to modify their insensible EWL over an acclimatory period of weeks
62 to months [12,13], presumably as a consequence of changes in skin lipids [14,15] and/or
63 modification of expired air temperature (T_{exp}) [16,17]. However, there is as yet no unequivocal
64 evidence of an acute response over a time frame of hours, as has been observed for mammals,
65 although our re-analysis of published EWL data for a suite of birds and mammals suggests that

66 birds may also possess this ability [6], and [18] noted that live and dead passerine birds had
67 different rates of cutaneous EWL (CEWL) suggesting physiological regulation. [5] and [19]
68 suggested the possibility of physiological control of EWL measured at different RH for
69 pigeons, although the unusual capacity of columbiform birds to augment CEWL [20-22] makes
70 this observation difficult to unequivocally interpret as regulation of insensible EWL.

71

72 We investigate here whether a psittaciform bird (the budgerigar *Melopsittacus undulatus*) is
73 able to control its insensible evaporative water loss independent of ambient relative humidity.
74 We measure EWL for budgerigars at varying T_a and RH, along with metabolic rate and
75 ventilatory parameters, and partition total EWL into CEWL and respiratory (REWL) [23]
76 components and estimate their respective evaporative surface temperatures (T_{surface} and T_{exp}) to
77 evaluate evidence for EWL control for this small granivorous desert bird.

78

79 **2. METHODS**

80 Seven wild-type male budgerigars (*Melopsittacus undulatus*) were obtained from a parrot
81 fancier in Perth, Western Australia, and housed in outdoor aviaries. Wild-type budgerigars can
82 be morphologically and physiologically quite different to aviary strains (e.g. “English”
83 budgies), particularly with respect to obesity and obesity-related physiology [24]. Open-flow
84 respirometry [25] was used to measure oxygen consumption ($\dot{V}O_2$), carbon dioxide production
85 ($\dot{V}CO_2$) and EWL of budgerigars at each RH (approximately 15%, 40%, 60% and 80%) and
86 T_a (20°C, 25°C and 30°C) combination. Post-absorptive birds were measured for a minimum
87 of 8 h at night. Chamber RH was controlled by pre-conditioning inlet air to <2% RH (using
88 Drierite) for measuring flow rate (Sierra Instruments 901C-PE, Monterey, CA, USA; 400 ml
89 min^{-1}), and then humidifying it to the required RH by saturating the inlet air at the appropriate
90 water temperature for the required chamber RH at that particular T_a after [6,7,26]. Temperature
91 and RH of excurrent chamber air was measured with a Vaisala HMP 45A RH and T_a probe.
92 The air was then dried with drierite and the O_2 and CO_2 content measured with a Foxbox (Sable
93 Systems, Las Vegas, NV, USA) or Combo (David Bishop, Cambridgeshire, UK) analyser;
94 analog outputs were interfaced to a PC, running custom-written (VB6) data acquisition
95 software. Whole body plethysmography [27, 28] was used to measure ventilatory variables of
96 respiratory frequency (f_R) and tidal volume (V_T), using a Motorola MPX2010DP pressure
97 sensor (Denver, CO, USA) and a Pico ADC11/12bit A/D converter (Pico Technology, St

98 Neots, Cambs, UK) and a custom-manufactured analog amplifier/offset signal conditioner,
99 sampling at 50 Hz. At the conclusion of each experiment, T_b was measured using a RS 611-
100 234 thermocouple meter (Radiospares, Smithfield, NSW, Australia) with a plastic-tipped
101 thermocouple inserted into the cloaca, typically within 30-60 sec after the end of the
102 experiment. A baseline of background O_2 , CO_2 and RH values was established for at least 30
103 min before and after each experiment.

104 Calculations [25] of metabolic rate (MR; VO_2 and VCO_2) and EWL were after for a period of
105 at least 20 min during which values were low and constant. Wet (C_{wet}) and dry (C_{dry}) thermal
106 conductance were calculated as $MR/(T_b-T_a)$ and $(MR-EHL)/(T_a-T_b)$ respectively, where MR
107 was converted to joules $g^{-1} h^{-1}$ using the measured respiratory exchange ratio (VCO_2/VO_2) for
108 that experiment, and evaporative heat loss (EHL) calculated as $EWL \times 2.4 J g^{-1} h^{-1}$, after [1,3].
109 We used whole-body plethysmography [27, 28, 29] to determine f_R and V_T , and minute volume
110 (V_I) was calculated as $f_R \times V_T$; oxygen extraction efficiency (EO_2) was calculated after [30],
111 using VO_2 measured at the time of ventilatory measurements. Calibration of the mass flow
112 controller was achieved using a volumetric flow meter (Gilibrator 2, Sensidyne, St. Petersburg,
113 FL, USA). The gas analysers were calibrated using nitrogen (0% O_2 and CO_2), room air (21%
114 O_2) and a precision gas mix (BOC gasses, Perth, WA, Australia) of 0.53% CO_2 . The RH probe
115 was calibrated against the known baseline incurrent humidities, and temperature with a
116 mercury thermometer traceable to a national standard.

117

118 EWL can be expressed relative to the water vapour pressure deficit (i.e. $EWL/\Delta wvp$) that drives
119 evaporation from the animal to the ambient air in three ways. First, we calculated Δwvp as the
120 difference between the wvp at ambient RH and saturation wvp at T_a . Second, we calculated
121 Δwvp as the difference between the wvp at ambient RH, and saturation wvp at T_b . Third, we
122 calculated an average Δwvp using the effective evaporative surface temperature (hence
123 saturation wvp) for the respiratory and cutaneous components of total EWL, and their %
124 partitioning. The effective evaporative surface temperature is T_{exp} for REWL, and $T_{surface}$ for
125 CEWL. We estimated $T_{surface}$, T_{exp} and cutaneous resistance (R), and partitioned total EWL into
126 CEWL and REWL, using the iterative model of [23] to solve for the most likely $T_{surface}$, T_{exp}
127 and % CEWL and REWL. The iterative model calculates REWL from V_I and T_{exp} , and CEWL
128 from $T_{surface}$ and overall resistance to cutaneous evaporative water loss (R_v), and finds the best
129 fit of CEWL+REWL to measured total EWL. $T_{surface}$ is expected to be slightly lower than T_{skin} ,

130 because of the additional resistance of the plumage and air boundary layer to cutaneous
131 resistance to evaporation. The iterative model parameters varies T_{exp} and T_{surface} within
132 biological limits (T_a and T_b) and $R_v > 0$ to estimate the best fit for T_{exp} , T_{surface} and R_v .

133

134 Hygrometeorological equations were used to calculate saturation wvp at the appropriate
135 temperature [26] and wvp was calculated as saturation wvp $T_a \times \text{RH}/100$. The water vapour
136 pressure deficit (Δwvp) was then calculated as saturation wvp animal – ambient wvp, and was
137 used to correct EWL for water vapour pressure deficit (i.e. $\text{EWL}/\Delta\text{wvp}$).

138

139 Values are presented as mean \pm standard error of the mean (s.e.m) with N = number of
140 individuals and n = number of measurements. Multivariate repeated measures ANOVA was
141 used to examine an overall effect of temperature and RH treatments for body mass. Linear and
142 reverse Helmert *a priori* repeated measures contrasts [31] were used to statistically describe
143 the pattern of the RH treatments (SPSS v23) at each T_a .

144

145 **3. RESULTS**

146 The body mass of budgerigars was 28.6 ± 0.39 g over all experiments ($N = 7$, $n = 84$) and did
147 not vary with T_a ($F_{2,5} = 2.30$, $P = 0.196$) or RH ($F_{3,4} = 0.19$, $P = 0.897$). There was however, a
148 significant difference in the body mass of individuals ($F_{1,6} = 860$, $P < 0.001$).

149

150 **a. Evaporative water loss**

151 For EWL (Figure 1), the negative linear pattern in EWL with RH, expected from physical
152 principles, was not apparent at $T_a = 20^\circ\text{C}$ (linear contrast $F_{1,6} = 1.66$, $P = 0.246$) or 30°C ($F_{1,6}$
153 $= 1.27$, $P = 0.302$), where the mean EWL for all individuals at all RH ($n = 28$, $N = 7$) was 1.28
154 ± 0.067 and 2.25 ± 0.086 $\text{mg g}^{-1} \text{h}^{-1}$ respectively. There was a significant effect of RH on EWL
155 at $T_a = 25^\circ\text{C}$ ($F_{1,6} = 11.4$, $P = 0.015$), but this reflected a significant decline in EWL (reverse
156 Helmert contrast $F_{1,6} = 20.6$, $P = 0.004$) to 1.02 $\text{mg g}^{-1} \text{h}^{-1}$ at 80% RH compared to 1.64 mg g^{-1}
157 h^{-1} (20%), 1.48 $\text{mg g}^{-1} \text{h}^{-1}$ (40%) and 1.55 $\text{mg g}^{-1} \text{h}^{-1}$ (60%). There was no significant effect
158 of RH on EWL ($F_{1,6} = 0.19$, $P = 0.682$) without the 80% data.

159

160 There were some T_a and RH effects on the respiratory variables used to model CEWL and
161 REWL. There were significant positive (V_T , $F_{1,6} = 72.9$, $P < 0.001$; V_I , $F_{1,6} = 19.29$, $P = 0.005$)
162 and negative (EO_2 ; $F_{1,6} = 22.8$, $P = 0.003$) linear contrasts. The partitioning of total EWL was
163 30% CEWL at $T_a = 20^\circ\text{C}$, 38% at 25°C , and 53% at 30°C respectively. The effective T_{surface}
164 and T_{exp} at each T_a were calculated to be 28 and 26°C respectively at $T_a = 20^\circ\text{C}$, 31 and 29°C
165 at $T_a = 25^\circ\text{C}$, and 36 and 32°C at $T_a = 30^\circ\text{C}$. For R, there were significant linear contrasts at T_a
166 of 20°C and 30°C ($F_{1,6} \geq 9.42$, $P \leq 0.022$) but not at 25°C ($F_{1,6} = 1.39$, $P = 0.284$). R decreased
167 at 20°C from about 1143 at 80% RH to 600 sec cm^{-1} at 20% RH, R was $664 \pm 87 \text{ sec cm}^{-1}$ at
168 25°C , and decreased at 30°C from 751 (20% RH) to 280 at 80% RH. There were no effects of
169 RH on %CEWL at any T_a ($F_{1,6} \leq 2.12$, $P \geq 0.195$), T_{surface} ($F_{1,6} \leq 1.67$, $P \geq 0.244$) or T_{exp} ($F_{1,6}$
170 ≤ 2.57 , $P \geq 0.160$).

171

172 For EWL expressed as a function of Δwvp , calculated using saturation wvp at T_a – ambient
173 wvp (Figure 2), contrasts indicated a linear and positive RH effect at each T_a ($F_{1,6} \geq 10.0$, $P \leq$
174 0.019). When Δwvp was calculated using saturation at T_b – ambient wvp, RH effects were
175 positive and linear at T_a of 20°C and 30°C ($F_{1,6} \geq 9.16$, $P \leq 0.023$) but not 25°C ($F_{1,6} = 0.08$, P
176 $= 0.787$), even with the 80% RH data excluded ($F_{1,6} = 2.12$, $P = 0.195$). Using our best estimate
177 of the driving force for evaporation, saturation wvp using skin and expired air temperatures
178 weighted by % EWL_{skin} and % EWL_{resp} from the partitioned model, – ambient wvp, indicated
179 significant RH effects at T_a of 20°C ($F_{1,6} = 14.3$, $P = 0.009$) and 30°C ($F_{1,6} = 22.7$, $P = 0.003$).
180 At 25°C , there was no effect of RH ($F_{1,6} = 4.99$, $P = 0.067$) but there was when the 80% RH
181 data were excluded ($F_{1,6} = 7.94$, $P = 0.031$).

182

183 **b. Body temperature, metabolism and thermal conductance**

184 There was no evidence of a linear pattern for body temperature with RH (Figure 3B) at any
185 individual T_a ($F_{1,6} \leq 3.3$, $P \geq 0.244$). Mean T_b was $39.0 \pm 0.1^\circ\text{C}$ at $T_a = 30^\circ\text{C}$, $38.2 \pm 0.2^\circ\text{C}$ at
186 25°C and $37.4 \pm 0.2^\circ\text{C}$ at 20°C (over all RH; $N = 7$, $n = 28$). Linear contrasts for MR with RH
187 were insignificant at all T_a ($F_{1,6} \leq 0.03$, $P \geq 0.866$). MR (averaged over all RH) was lowest at
188 25°C ($1.95 \pm 0.028 \text{ ml O}_2 \text{ g}^{-1} \text{ h}^{-1}$), significantly higher at 30°C (2.29 ± 0.013 ; $P = 0.013$) and
189 highest at 20°C (2.58 ± 0.050 ; $P < 0.001$).

190

191 There was no RH effect for either C_{wet} or C_{dry} (Figure 3C) at any of the T_a ($F_{1,6} \leq 0.593$, $P \geq$
192 0.470) but there were effects of T_a on both C_{wet} and C_{dry} ($F_{2,5} \geq 41.1$, $P \leq 0.001$). Over all RH
193 ($N = 7$, $n = 28$), both C_{wet} and C_{dry} were significantly higher at 30°C ($5.02 \pm 0.21 \text{ J g}^{-1} \text{ h}^{-1} \text{ }^\circ\text{C}^{-1}$;
194 $4.59 \pm 0.21 \text{ J g}^{-1} \text{ h}^{-1} \text{ }^\circ\text{C}^{-1}$ respectively) than 25°C (3.03 ± 0.04 ; 2.77 ± 0.05) and 20°C ($3.00 \pm$
195 0.06 ; 2.82 ± 0.05); there was no difference between 25°C and 20°C ($P \geq 0.775$).

196 4. DISCUSSION

197 Wild-type budgerigars did not conform to a physical model of insensible EWL decreasing
198 linearly with increasing wvp. There was no significant effect of RH on EWL, and we found
199 that $\text{EWL}/\Delta\text{wvp}$ vs RH had a significant positive slope, so Δwvp is not the only driver of
200 insensible EWL for budgerigars. We conclude that this small desert bird shares the ability of
201 some marsupial and placental mammals [6-8,] to acutely control insensible EWL. That birds
202 and convergently endothermic mammals can maintain insensible EWL independent of
203 perturbing environmental conditions suggests that this may be a regulatory ability of
204 fundamental importance to endothermic vertebrates.

205

206 Our results for budgerigars clearly show there is a general constancy of EWL as a function of
207 RH. Only at the highest RH (80%) was there any indication, particularly at a thermoneutral T_a
208 of 25°C , that EWL was lower at high RH. Presumably, high RH pushes the limits of the
209 budgerigar's EWL regulatory mechanism. In contrast to the lack of RH effects, there were
210 highly significant effects of T_a on EWL, in accord with similar studies of other birds and
211 mammals. Other published data indicated EWL of budgerigars increased curvilinearly with T_a ,
212 being about $2.32 \text{ mg g}^{-1} \text{ h}^{-1}$ (compared to our value of 1.42) at $T_a = 25^\circ\text{C}$ [32]. Presumably our
213 lower value is a consequence of longer measurement duration; it was consistent with the 1.4
214 $\text{mg g}^{-1} \text{ h}^{-1}$ reported by [33]. These temperature effects presumably result from changes in T_{surface}
215 and T_{exp} at higher T_a vs T_b gradients, and possibly some minor thermoregulatory EWL at $T_a =$
216 30°C .

217

218 The generally-accepted physical model for insensible EWL is that Δwvp is the driving force
219 [e.g. 5,6,7,8,19,40], predicting that $\text{EWL}/\Delta\text{wvp}$ is independent of RH (slope = 0) and so a slope
220 $\neq 0$ implies some form of EWL regulation. To examine the hypothesis that $\text{EWL}/\Delta\text{wvp}$ is

221 independent of RH, it is straightforward to statistically determine if the slope of the function
222 of $EWL/\Delta wvp$ vs $RH = 0$, but it is not so straightforward to calculate $EWL/\Delta wvp$. The wvp at
223 the animal's evaporative surfaces is often considered to be saturation wvp at the ambient T_a [6
224 and references therein] but this will underestimate Δwvp , over-estimate $EWL/\Delta wvp$ and
225 exaggerate the effect of RH (Figure 2), as the temperatures of the evaporative surfaces are
226 generally $>T_a$. Calculating saturation wvp at T_b provides a more realistic estimate of the Δwvp ,
227 albeit an overly conservative approach, as using T_b will over-estimate Δwvp and underestimate
228 its change with ambient RH (Figure 2).

229

230 The most realistic determination of the Δwvp is calculated from the partitioning of REWL and
231 CEWL and their respective evaporative temperatures. However, accurate measurement of these
232 variables is logistically challenging, particularly if care is taken to ensure the animal is resting
233 and able to adopt a natural posture [5,23]. Consequently, we partitioned EWL and calculated
234 evaporative temperatures using an iterative model that incorporated metabolic, thermal, hygric
235 and ventilatory variables [23] for our unrestrained budgerigars to avoid potential experimental
236 artefacts from stress or restraint (e.g. hyperventilation). This partitioning resulted in CEWL
237 estimates of 30, 38 and 53% of total EWL, compared to CEWL partitioning of 45-80% for
238 direct but invasive measurements of birds at and below thermoneutrality [18,34-37].
239 Corresponding evaporative temperatures for CEWL ($T_{surface}$) and REWL (T_{exp}) were
240 substantially lower than T_b at low T_a , but more closely approximated T_b at higher T_a , as
241 expected from thermoregulatory responses to heat loss. The expired air of most birds and
242 mammals is cooled by nasal counter-current water and heat exchange so T_{exp} is expected to be
243 lower than T_b [16]. Skin temperature is usually close to or slightly less than core T_b [38,39],
244 but the additional resistance of the pelage to EWL means that the effective body surface
245 temperature we calculated for evaporation ($T_{surface}$) is less than skin temperature. Using Δwvp
246 calculated from these estimates of T_{exp} and $T_{surface}$, and %CEWL and %REWL, resulted in an
247 estimate of $EWL/\Delta wvp$ that was (as expected) intermediate between that calculated from T_a
248 and T_b , but more closely approximated T_b (Figure 2).

249

250 Overall, we conclude that $EWL/\Delta wvp$ increased linearly with RH, deviating from the physical
251 model of no linear relationship, for all three potential calculations for Δwvp . Even with the
252 most conservative approach, significant linear RH effects on $EWL/\Delta wvp$ were confirmed for
253 two of the three T_a . For our best estimate of Δwvp we statistically showed clear divergence
254 from a predicted slope of 0 for $EWL/\Delta wvp$ at T_a of 20, 25 and 30°C, with slopes of $0.00414 \pm$

255 0.00137, 0.00246 ± 0.00094 and 0.00390 ± 0.00113 $\text{mg g}^{-1} \text{h}^{-1} \text{kPa}^{-1}$ respectively. Our findings
256 support earlier suggestions [6,18,40] that the insensible EWL of birds may be under
257 physiological regulation and, together with recent data for marsupial and placental mammals
258 [6-8] provides clear evidence that this phenomenon occurs in representatives of the two major
259 groups of convergently-endothermic vertebrates. The implication of this is that regulation of
260 insensible EWL has fundamental importance for the function for endotherms, so how and why
261 these endothermic birds and mammals might regulate their insensible EWL needs to be
262 considered further.

263

264 Regulation of insensible EWL has ramifications for water status, as an unregulated increase of
265 EWL at low ambient RH could compromise water balance; reducing EWL at low RH has
266 intuitive adaptive significance. However, if EWL can be reduced at low RH to conserve water,
267 then why is EWL not also minimised at higher RH (where $\text{EWL}/\Delta w_{vp}$ increases)? If water
268 conservation was the primary role, then budgerigars could minimise EWL at high and low RH
269 by keeping $\text{EWL}/\Delta w_{vp}$ minimal. Regulation of EWL for water conservation also requires a
270 control system, including a hygrosensory receptor and feedback control loop; although this has
271 not been described for birds or mammals, it is not unrealistic. Nematodes and insects can sense
272 RH, and hygrosensitive neurones have been identified in these animals [41-43]. For nematodes,
273 both thermosensory and mechanosensory pathways contribute to hygrosensation, and similar
274 dual hygrosensory systems have been hypothesised for insects and humans [43].

275

276 It is more likely that regulation of EWL has a thermoregulatory, rather than water balance, role
277 because EWL directly impacts evaporative heat loss (EHL) and therefore has implications for
278 control of T_b and other physiological variables. If increased RH (decreased Δw_{vp}) reduced
279 EWL, then EHL would also be reduced, resulting in either an increased T_b , or a reduced MR
280 and/or C. There was a consistent lack of an effect of RH on T_b , MR, C_{wet} or C_{dry} (Figure 3),
281 suggesting that the observed change in $\text{EWL}/\Delta w_{vp}$ with RH obviated any detectable changes
282 in these thermal and metabolic variables, which were also in close agreement with other
283 comparable measurements for budgerigars at low RH [31,32]. A thermoregulatory role means
284 that the sensory feedback system for maintenance of constant EWL may be the well-established
285 thermoregulatory system.

286

287 There is little comparative information on the effects of RH on thermal and metabolic variables
288 for other birds, and mammals. There is a suggestion of a slightly decreased T_b at low RH for
289 banner-tailed kangaroo rat (*Dipodomys spectabilis*) [44] but no effect of RH on MR or T_b for
290 California ground squirrels (*Citellus beecheyi*) [45]. For brushtail possums (*Trichosurus*
291 *vulpecula*) at $T_a = 25$ °C, where EWL was regulated, there was no RH effect on metabolic rate
292 or C_{wet} , but MR was higher and C_{wet} lower at lower RH at $T_a = 30$ °C, corresponding to a
293 significantly lower EWL at higher RH [7]. For the little red kaluta (*Dasykaluta rosomondae*),
294 there was no effect of RH on T_b , MR, or C at RH where EWL was regulated, but at high RH
295 (78%) where EWL regulation appeared compromised, T_b increased reflecting a decrease in
296 EHL [6]. Together with our budgerigar data, these findings support the hypothesis that
297 regulation of EWL plays a thermoregulatory role, and may explain why mammals and birds,
298 which convergently regulate T_b using similar endothermic mechanisms, may show similar
299 EWL regulation as well.

300

301 Potential mechanisms for regulation of insensible EWL involve modification of cutaneous
302 and/or respiratory EWL. REWL is determined primarily by T_{exp} and RH and reflects V_I , which
303 is determined by VO_2 and EO_2 . Our estimates of T_{exp} for budgerigars (28, 31 and 36°C at T_a s
304 of 20, 25 and 30°C respectively) are similar to but slightly higher than previously reported for
305 budgerigars (25, 28 and 32 °C respectively) [16], and we assumed 100% RH for expired air.
306 We found no effect of RH on T_{exp} , but slight RH effects for V_I and EO_2 that would reduce
307 REWL at low RH. CEWL is affected by T_{skin} , cutaneous resistance to evaporation, and
308 diffusive resistance of feathers and the air boundary layer [5]. Our partitioning results, which
309 combine effects of skin, feather and boundary layer resistance as a single effect ($T_{surface}$),
310 indicated an increase in effective evaporative surface temperature with T_a , but not RH. RH
311 regulation of CEWL would therefore seem to occur via changes in cutaneous resistance to
312 evaporation rather than $T_{surface}$ or a change in plumage resistance (consistent with the lack of
313 an RH effect on C_{dry}). The partitioning model suggested that R increased with lower RH at T_a
314 = 20°C, and decreased with increasing RH at 30°C, from similar R values as at 25°C. Chronic
315 decreases in cutaneous R at low RH have been reported for birds, being linked to changes in
316 skin lipid composition [14,15], but it is not clear whether similar changes could occur acutely,
317 as in our experiments. Alternatively, changes in R might reflect changes in skin hydration

318 (water content) at varying RH; [46] reported lower cutaneous EWL at low RH (and presumably
319 low water content) for human skin, although a substantial linear decrease in CEWL with
320 increasing RH has been reported for newborn infants [47] and pig skin *in vitro* [48].

321 **5. CONCLUSIONS**

322 We conclude that there is now unequivocal evidence for regulation of insensible water loss for
323 the two major groups of endothermic vertebrates; birds (budgerigars) and mammals [6-8]. The
324 EWL/ Δwvp of budgerigars increases with ambient RH such that EWL remains independent of
325 RH; consequently, there is no effect of RH on other thermoregulatory and metabolic variables.
326 This suggests that the role of regulation of insensible EWL may be to maintain
327 thermoregulatory constancy, and that the sensory mechanism might be thermoregulatory
328 feedback control. Changes in both REWL, via changes in T_{exp} and CEWL, via changes in skin
329 R may provide mechanisms for this observed phenomenon. The convergence of EWL
330 regulation in these phylogenetically different endothermic lineages is strong evidence that
331 insensible EWL regulation is a fundamental aspect of the physiology of endothermic
332 vertebrates.

333

334 **ETHICS**

335 All experiments were performed according to the Australian Code of Practice for the care and
336 use of animals for scientific purposes, with approval by the University of Western Australia
337 Animal Ethics Committee (RA/3/100/1354).

338 **DATA, CODE and MATERIALS**

339 Supporting data can be found in the Supplementary Material

340 **COMPETING INTERESTS**

341 The authors have no competing interests.

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346 **AUTHOR'S CONTRIBUTIONS**

347 ECE contributed to experimental design, was responsible for data acquisition, contributed to
348 data analysis and interpretation, drafted the original manuscript and approved the final
349 manuscript. PCW contributed to experimental design, data acquisition, analysis and
350 interpretation, drafting and revising the manuscript and approved the final manuscript. CEC
351 contributed to experimental design, statistical analysis and data interpretation, drafting and
352 revising the manuscript and approved the final manuscript.

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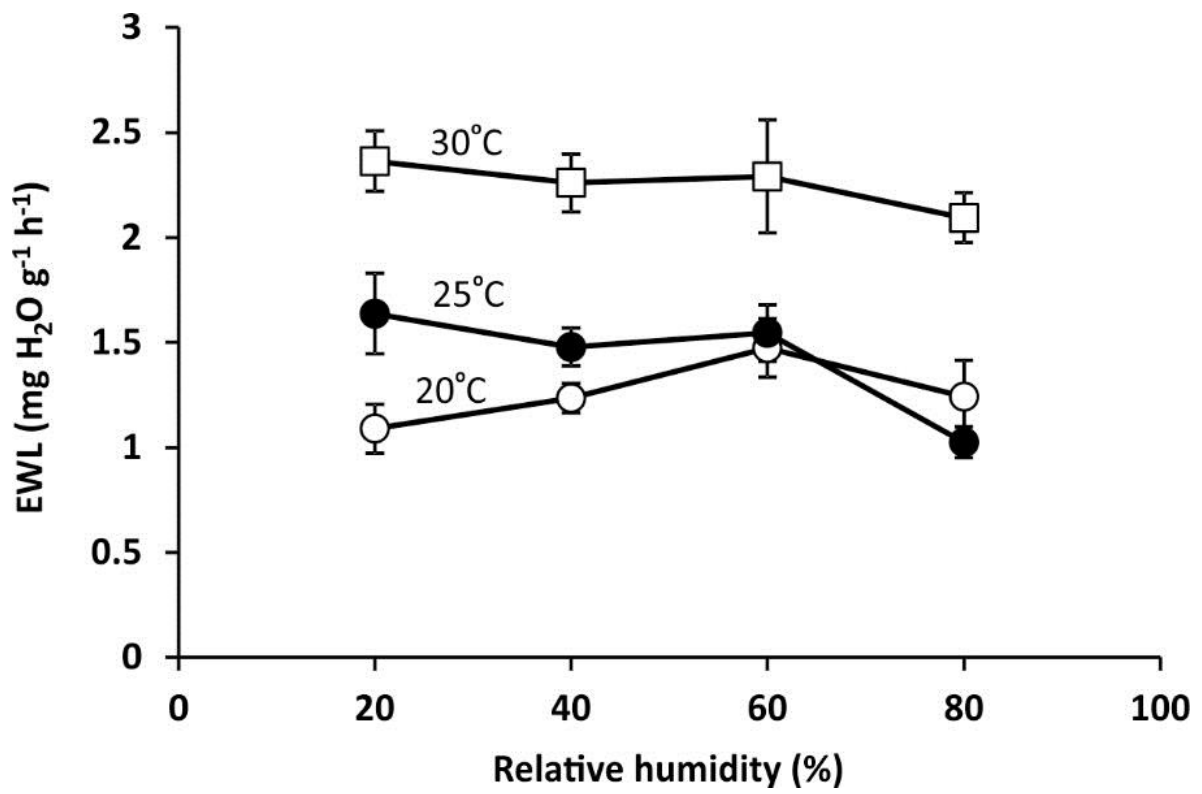
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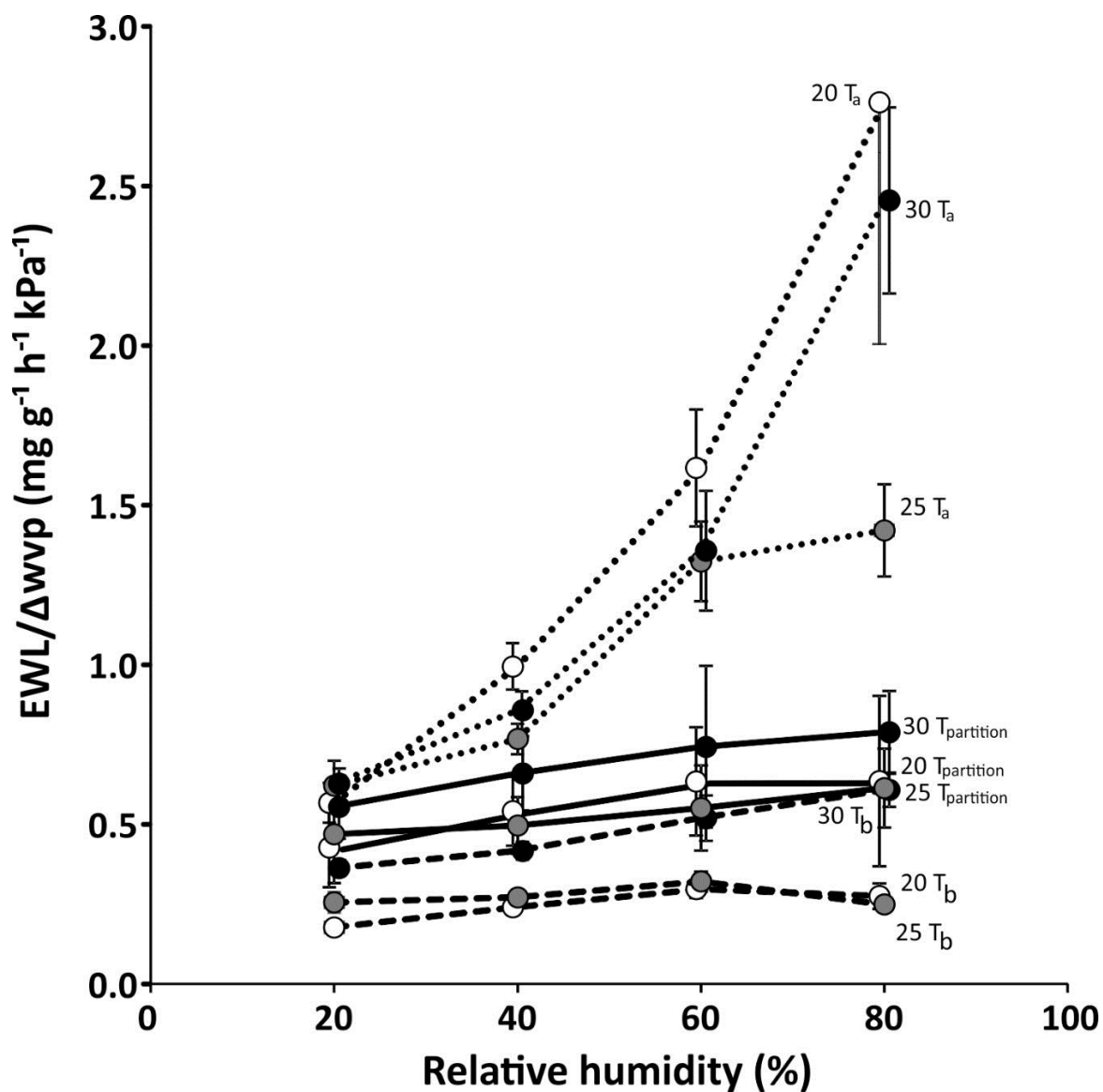
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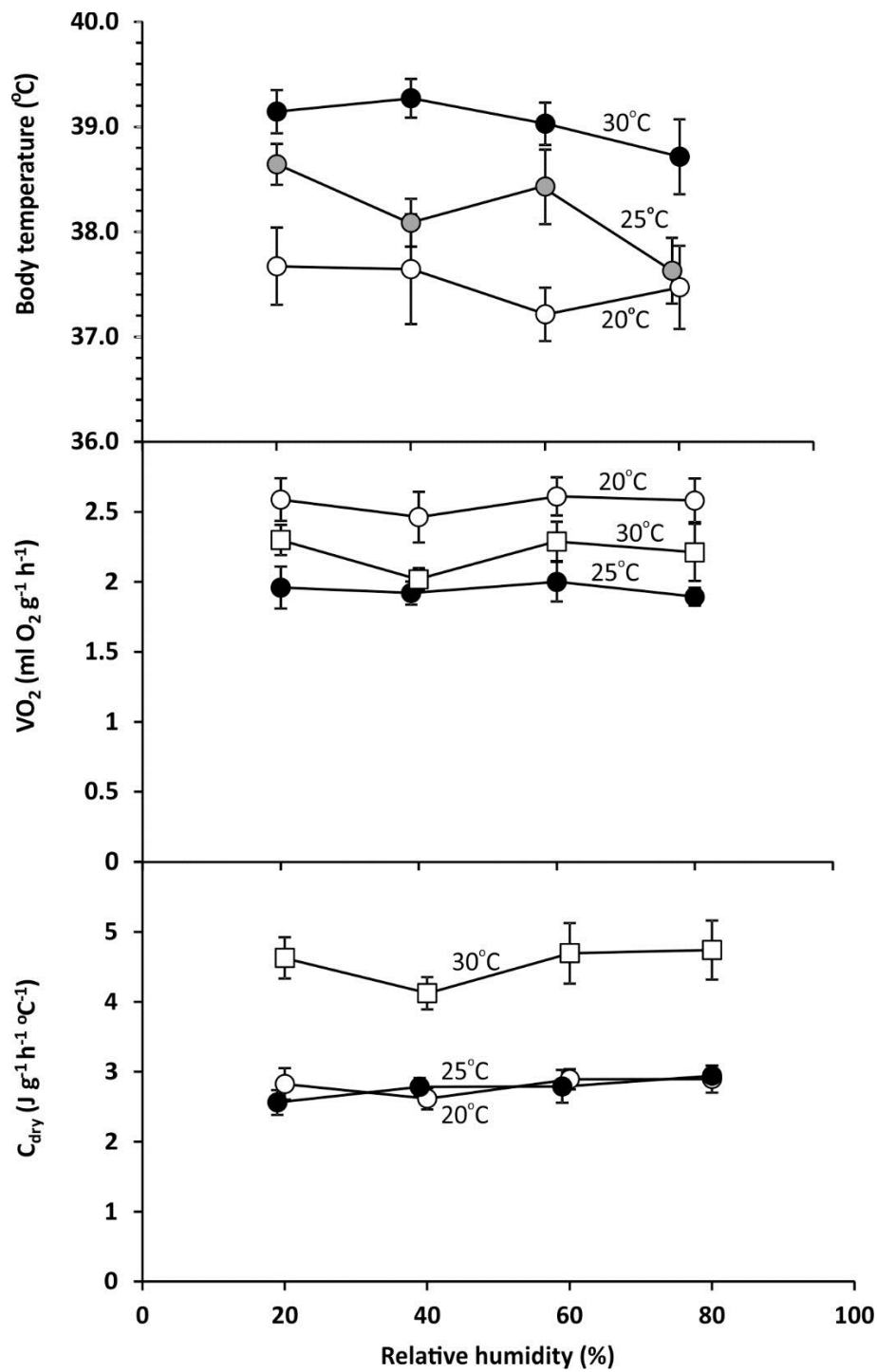
472

473 Fig. 1. Evaporative water loss (EWL) of budgerigars over a range of ambient relative humidities at
474 ambient temperatures of 20°C, 25°C and 30°C. Values are mean ± s.e.m; N = 7.



476

477 Fig. 2. Evaporative water loss per water vapour pressure deficit ($EWL/\Delta wvp$) over a range of ambient
 478 relative humidities at ambient temperatures of 20°C (white), 25°C (grey) and 30°C (black), using Δwvp
 479 calculated from ambient temperature (T_a ; dotted lines), body temperature (T_b ; dashed lines) and from
 480 the CEWL-REWL partitioning model ($T_{partition}$; solid lines). Values are mean \pm s.e.m; $N = 7$.



482

483 Fig. 3. Relationships between relative humidity and body temperature (T_b), oxygen consumption (VO_2),
 484 and dry thermal conductance (C_{dry}) at ambient temperatures of 20°C (white circles), 25°C (grey circles)
 485 and 30°C (black circles). Values are mean \pm s.e.m., N = 7.