1 **Physiological regulation of evaporative water loss in endotherms: is the little red**

2 **kaluta (*Dasykaluta rosamondae*) an exception or the rule?**

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15   Summary

16   It is a central paradigm of comparative physiology that the effect of humidity on

17   evaporative water loss (EWL) is determined for most mammals and birds, in and below

18   thermoneutrality, essentially by physics and is not under physiological regulation. Fick's

19   Law predicts that EWL should be inversely proportional to ambient relative humidity

20   (RH) and linearly proportional to the water vapour pressure deficit (Δwvp) between

21   animal and air. However, we show here for a small dasyurid marsupial, the little kaluta

22   (*Dasykaluta rosamondae*), that EWL is essentially independent of RH (and Δwvp) at low

23   RH (as are metabolic rate and thermal conductance). These results suggest regulation of a

24   constant EWL independent of RH, a hitherto unappreciated capacity of endothermic

25   vertebrates. Independence of EWL from RH conserves water and heat at low RH, and

26   avoids physiological adjustments to changes in evaporative heat loss such as

27   thermoregulation. Re-evaluation of previously published data for mammals and birds

28   suggests that a lesser dependence of EWL on RH is observed more commonly than

29   previously thought, suggesting that physiological independence of EWL of RH is not just

30   an unusual capacity of a few species, such as the little kaluta, but a more general

31   capability of many mammals and birds.

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34   Key index words: evaporative water loss, relative humidity, water vapour pressure

35   deficit, ambient temperature, mammal

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37   Running Head: Regulation of water loss in endotherms

38

Introduction

40   Understanding the effects of relative humidity (RH) on physiological parameters,

41   particularly evaporative water loss (EWL), is important because of the theoretical effect

42   of RH on EWL and consequent effects on other physiological variables (e.g. body

43   temperature, T_b; metabolic rate, MR; thermal conductance, C), especially for mammals

44   and birds. It is a central paradigm of comparative physiology, for mammals and birds at

45   T_a in and below thermoneutrality and over the short-term (i.e. hours), that EWL is

46   determined essentially by physics [1-3], and not physiological regulation, although EWL

47   is often actively enhanced for thermoregulation at high ambient temperature (T_a) and can

48   be modified in the longer term by development and acclimationry.

49   Based on Fick's law of diffusion [1-3], EWL depends on the difference in water

50   vapour concentration (χ) between the body surface (χ_{sat}; assumed to be saturated, RH =

51   100%) and ambient air (χ_o) i.e. EWL α (χ_{sat} - χ_o). Ambient water vapour concentration

52   depends on RH and χ_{sat}; $\chi_o = (^{RH}/_{100}) \chi_{sat}$. °K) EWL and χ_{sat} are expected to increase

53   exponentially with T_a (°K) e.g. $\chi_{sat} = 9.16 \ 10^8 \ e^{-(5218/T_a)}$ [4]. Water vapour pressure (wvp)

54   is an equivalent measure of water potential to χ [1] so EWL should be inversely and

55   linearly related to ambient wvp and positively linearly related to water vapour pressure

56   deficit ($\Delta wvp = wvp_{sat}$ - wvp_o). Various studies have reported that EWL changes

57   inversely with RH and wvp, or linearly with Δwvp, for various small endotherms at and

58   below thermoneutrality [e.g. 5-13], as expected from physical principles. However, a few

59   studies have reported a lower or even opposite relationship between EWL and RH, wvp

60   or Δwvp [12-14]. This raises the important question of whether these endotherms have

61   physiological regulation of EWL at low and moderate T_a, which could confer substantial

62   advantages for balancing their water budget. However, complexities with these studies,

63   such as use of RH, wvp or Δwvp, heterothermy [13], postural adjustments [14],

64   measurement method and protocols [13, 15], and use of skin or ambient temperature to

65   calculate Δwvp [16] make it difficult to interpret these patterns.

66   Here, we measure the effect of varying ambient T_a and RH on hygric, metabolic

67   and thermal physiology for the little red kaluta (*Dasykaluta rosamondae*). This small

68   (35g) dasyurid marsupial has several physiological characteristics of arid habitat

69   mammals, including a low and thermolabile T_b, low basal metabolic rate (BMR) and

70   EWL, high point of relative water economy, and ready use of torpor [17]. We might

71   expect the EWL of little red kaluta to deviate from a theoretical linear relationship with

72   Δwvp, if such deviation has adaptive value such as water conservation. We explore the

73   effects of RH and Δwvp on EWL for the kaluta and other small endotherms in

74   comparison with biophysically predicted expectations, then we consider the implications

75   of effects of RH on EWL for thermal and metabolic physiology.

76

77   **2. Materials and Methods**

78   **(a) Study Animals**

79   Eight little red kalutas (seven males, one female) were collected in northern

80   Western Australia [see 17]. They were housed individually at T_a ~20°C, with a 12:12

81   light:dark cycle, and were fed mince, tinned cat food and mealworms, with *ad lib.* water.

82

83   **(b) Respirometry**

84   Metabolic rate (oxygen consumption, VO_2; carbon dioxide production, VCO_2)

85   and EWL were measured by flow-through respirometry [18]. An Aalborg GFC17 mass

86   flow controller regulated compressed air flow through a 265 ml chamber at 350 ml min^{-1},

87   inside a temperature-controlled room at a T_a of 25, 30 and 35°C. RH was controlled at

88   each T_a (approximately 17, 36, 56 and 78 % RH) by saturating inlet air at known

89   temperatures, using a Lauda K-2/R refrigerated water bath, then warming to T_a; RH was

90   calculated from saturation wvp at the water bath temperature and T_a, using standard

91   equations [19]. Excurrent RH and T_a were measured with a Vaisala HMP45A probe, and

92   a subsample was drawn through a column of drierite then a Servomex OA174 or A184

93   O_2 analyser and a Hereus-Leybold Binos or Hartmann and Braun Uras 10E CO_2 analyser.

94   Analysers were interfaced to a PC via digital multimeters and serial ports. Excurrent O_2,

95   CO_2, RH and T_a were recorded every 20 seconds throughout the experiment, using

96   custom-written software (Visual Basic v6). T_b was measured immediately at the end of

97   each experiment using a RadioSpares 611-234 thermocouple meter, with a plastic-tipped

98   thermocouple inserted ~2 cm into the cloaca.

99   The O_2 analysers were two-point calibrated with compressed nitrogen (BOC

100   gases) and dry ambient air (20.95%). The CO_2 analysers were calibrated with compressed

101   N_2 and a certified gas mix (0.53% CO_2; BOC gases). The RH probes were calibrated over

102   a wide range (using air saturated at a known temperature then warmed to ambient T_a as

103   described above), and calibration was routinely confirmed using 1% RH air (dried with

104   Drierite) and 100% RH air (saturated; by breathing on the RH probe). Flow meters were

105   calibrated using a Sensidyne Gilibrator 2.

106   Kalutas were fasted for 24 hours before commencement of experiments, then

107   measured for no less than 6 hours at each T_a and RH combination (measured in random

108   order) until O_2, CO_2 and RH were stable and minimal for at least 20 minutes [see 15].

109   VO_2, VCO_2 and EWL were calculated after [18] using a custom written analysis program

110   (Visual Basic v6). Respiratory exchange ratio (RER) was calculated as VCO_2/VO_2.

111   Metabolic heat production (MHP) was calculated from MR using the measured RER after

112   [2] and evaporative heat loss (EHL) was calculated from EWL using 2.4 J mg^{-1} H_2O [3].

113   Wet thermal conductance (C_{wet}; J g^{-1} h^{-1} $°C^{-1}$) was calculated as MR/$(T_b - T_a)$ and dry

114   thermal conductance (C_{dry}; J g^{-1} h^{-1} $°C^{-1}$) as (MHP– EHL)/(T_b-T_a), for $(T_b - T_a) > 1$ $°C$.

115   EWL was also corrected for Δwvp, which was calculated as the difference

116   between saturation wvp and ambient wvp at the measured RH and T_a. It is customary to

117   calculate Δwvp as the difference between wvp at 100% RH and ambient T_a and wvp at

118   ambient RH and T_a (e.g. 7, 14, 20). Skin temperature is more appropriate than T_a to

119   calculate Δwvp [16], but its measurement is more difficult so ambient temperature is

120   generally used as a proxy. The Δwvp will be similar, particularly at low ambient wvp,

121   unless there is a very large difference between T_a and T_{skin}.

122

123   **(c) Statistics**

124   Values are presented as mean $\pm$ S.E., with N = number of individuals and n =

125   number of measurements. Multivariate repeated measures ANOVA (mRMANOVA) with

126   two levels of within-individual repeat (T_a and RH) and *a priori* polynomial contrasts

127   were used to analyse the repeated measurements of individuals. Statistical analyses were

128   conducted using a custom-written Excel spread sheet [21] and SPSS (v21 for Windows).

129

130   **3. Results**

131   Mean body mass of kalutas was 33 ± 0.6 g over all experiments (N = 8, n = 84).

132   Kalutas rested quietly in the chamber, at all T_a and RH combinations. There was no

133   significant difference in body mass with either T_a or RH treatments.

134

135   **(a) Effects of T_a and RH on EWL**

136   There was a significant effect of T_a on EWL ($F_{2,5} = 20.5$, P = 0.004), but no

137   significant overall effect of RH ($F_{3,4} = 6.03$; P = 0.058) by mRMANOVA (figure 1).

138   However, a significant quadratic contrast for EWL (P = 0.045) over all RH treatments,

139   combined with a very insignificant mRMANOVA effect ($F_{2,3} = 0.059$; P = 0.943) and no

140   significant polynomial contrasts (P > 0.678) for the three lowest RH treatments (RH =

141   78% removed from the model) indicate that EWL was significantly lower at the highest

142   RH but did not differ between the three lower RHs. The insignificant interaction between

143   T_a and RH ($F_{6,1} = 5.23$; P = 0.317) suggests the patterns in EWL with RH were consistent

144   for each T_a.

145   When EWL was expressed per Δwvp (EWL/Δwvp; figure 2), there was a

146   significant effect of T_a ($F_{2,5} = 15.5$; P = 0.007) and RH ($F_{3,4} = 10.5$; P = 0.023) by

147 mRMANOVA. A significant contrast (P = 0.012) indicated that EWL/Δwvp was

148 significantly higher at elevated RH. A significant T_a-RH interaction term (P = 0.011)

149 suggests that the EWL/Δwvp pattern with RH was different for the three T_as. EWL/Δwvp

150 values for kalutas at low RH, from T_a = 11 to 38 °C [17], are included in figure 2.

151

152 **(b) Effects of T_a and RH on Other Physiological Variables**

153   There were significant T_a effects for T_b ($F_{2,5}$ = 93.3, P < 0.001), VO_2 and VCO_2

154 ($F_{2,5} \geq 33.4$, P ≤ 0.007) and C_{wet} and C_{dry} ($F_{1,6} \geq 13.3$, P ≤ 0.011). We did not determine

155 C_{wet} or C_{dry} at T_a = 35 °C because (T_b - T_a) was < 1. There were no overall RH effects for

156 T_b ($F_{3,4}$ = 2.50, P = 0.198), VO_2 and VCO_2 ($F_{3,4} \leq 3.39$, P ≥ 0.134), or C_{wet} and C_{dry} ($F_{3,4} \leq$

157 2.34; P ≥ 0.215). For all RH data, there was a weak linear effect (P = 0.020) of RH on T_b,

158 with higher T_b at the higher RH. There was no significant effect of RH on T_b (P = 0.173)

159 when the highest RH data were excluded, indicating that there was no effect of RH on T_b

160 except at the highest RH where T_b increased slightly.

161

162 **4. Discussion**

163   Previous studies have shown that EWL changes with T_a, RH and Δwvp in a

164 complex manner [5-14]. Our findings for kalutas of considerable independence of EWL

165 from RH suggest physiological control. T_a influences on EWL also differ from

166 biophysical predictions. Furthermore, re-examination of effects of RH and Δwvp on

167 EWL data from previously studies suggests that our results for kalutas are not unusual

168 amongst small endotherms. Maintaining constancy of EWL at low RH not only conserves

169 water but also affects thermal and metabolic physiology.

171 **(a) Effect of T_a on EWL**

172   Above the thermoneutral zone (TNZ), EWL of endotherms typically increases

173 more rapidly than predicted from T_a alone, reflecting augmented EHL for

174 thermoregulation when $T_b > T_a$ [2, 3, 22, 23]. This physiological response is also apparent

175 for kalutas; at T_a above thermoneutrality, EWL is enhanced, with a successively higher

176 EWL/Δwvp between 31 and 38ºC [17; figure 2] and a higher EWL/Δwvp at 35 ºC

177 compared to 25 and 30 ºC (this study; figure 2).

178   At T_a below TNZ, EWL of endotherms is often constant or even decreases with

179 increasing T_a [e.g. 24, 25], rather than increasing exponentially with T_a as would be

180 predicted (by the physical effect). Kaluta are no exception. EWL is relatively constant at

181 T_a in and below thermoneutrality [30 ºC; 17] so EWL/Δwvp decreases as T_a increases

182 from 11 to 31ºC (figure 2); this pattern differs from the physical model that EWL is

183 proportional to Δwvp. This non-exponential effect of T_a on EWL below thermoneutrality

184 is generally attributed to the counteracting effects of T_a on respiratory and cutaneous

185 EWL. Thermoregulatory adjustments in MR, increase respiratory ventilation and EWL at

186 low T_a, which counterbalances the expected decrease in cutaneous EWL [e.g. 24, 25]. In

187 light of our findings for EWL constancy at low RH, an alternative interpretation of this

188 EWL constancy at low T_a is that EWL is acutely regulated over this T_a range. Whatever

189 the explanation for this EWL constancy below the TNZ, marked deviations in EWL from

190 the simple physical effects of T_a indicate some form of physiological regulation, for

191 thermoregulation via proportionally increased metabolic heat production or water

192 conservation by direct regulation of EWL.

8

193

(b) Effect of RH on EWL

195   Just as deviations of EWL from the physical effects of T_a reflect regulatory

196 processes, comparison of EWL responses to variation in RH at a constant T_a with a

197 biophysical model also provides evidence of physiological regulation of EWL. Various

198 studies have reported that EWL changes inversely with RH and wvp (or linearly with

199 Δwvp) for small endotherms [5-13], consistent with a simple physical model. However,

200 some studies have reported a lower or no relationship between EWL and RH or Δwvp,

201 generally at low or moderate T_a [12-14]. EWL is independent of wvp at 20 ºC for rock

202 pigeons; adjustments in respiratory ventilation or expired air temperature might account

203 for this [12]. For heterothermic male and post-lactating female little brown bats, there

204 was no wvp effect on EWL at T_a of 28 or 33 °C [13], suggesting that EWL is a controlled

205 rather than a physical process, and there was an unexpected linear relationship between

206 EWL and wvp at $T_a = 37$ °C. We [14] previously reported that EWL of brushtail possums

207 was constant at low RH, at a thermoneutral T_a (25 °C); we attributed this EWL constancy

208 at low RH to postural changes and a body core to extremity thermal gradient. Our data

209 for kalutas, showing an unexpected constancy of EWL under environmental conditions

210 that would be expected to perturb water balance, suggests that EWL was under active

211 physiological regulation, reducing their EWL at low RH (high Δwvp).

212   Only these few species have been reported to deviate from the expected physical

213 EWL-RH-Δwvp model, whereas most others apparently conform to it. However, we have

214 re-analysed data from other studies examining the effect of RH, wvp or Δwvp, on EWL

215 to evaluate how well those data fit the expectation of a linear physical effect of Δwvp on

216 EWL. We found that the expected linear relationship between Δwvp and EWL is less

217 universal than first thought.

218

219 **(c) Biophysical model for EWL and RH**

220 We use here a simple conceptual framework for the biophysical relationship between

221 EWL and Δwvp (at T_as at or below 35 °C) to assess how EWL data from published

222 studies conform to this model. According to the simple Fickian model for diffusion, we

223 would expect a positive linear relationship between EWL and Δwvp that passes through

224 the origin (no EWL when there is no Δwvp). To compare data from these disparate

225 studies, we normalised the data so that EWL at the lowest Δwvp would fit on an arbitrary

226 "relative EWL" line; data from higher Δwvp should then also fit on the line if the

227 relationship was Fickian. We found that results from some studies conform to the Fickian

228 model, but many results do not (figure 3). In particular, for many species the relative

229 EWL decreases substantially below the expected linear relationship at high Δwvp. The

230 potential significance of these deviations from the simple Fickian model has not

231 previously been evaluated in a conceptual framework, and was often not even recognised

232 in the original studies. Our re-evaluation of previous data for mammals and birds shows

233 that a lesser dependence of EWL on Δwvp is more common than previously thought. Our

234 interpretation is that physiological independence of EWL of Δwvp is not just an unusual

235 capacity of a few species, such as the little kaluta, but is a more general capability of

236 many mammals and birds.

237 Decreasing curvilinear relationships for EWL-Δwvp are of particular interest

238 because they suggest physiological regulation of EWL at low RH. That EWL is plastic

239 and under physiological control over periods of weeks to months in response to

240 developmental and/or acclimatory changes in water requirements has been demonstrated

241 for birds and mammals (26-29). However, active regulation of EWL over a period of only

242 six hours has not been previously considered. Although mechanisms that acutely enhance

243 EWL for thermoregulatory purposes at high T_a or high RH are well documented e.g.

244 salivation, sweating, panting [2-3], mechanisms that could acutely modify EWL in and

245 below thermoneutrality are not. Potential mechanisms for decreasing EWL at high Δwvp

246 will depend on the relative partitioning of cutaneous and respiratory avenues of EWL,

247 and may include decreased cutaneous EWL e.g. by modification of skin lipids [30-32],

248 and/or reduced respiratory EWL e.g. by lowered expired air temperature by nasal

249 counter-current heat and water exchange [25, 33-36].

250   We propose that our description of acute deviation of EWL from the expected

251 physical model for EWL at moderate and low T_a is evidence for acute physiological

252 regulation e.g. over a few hours. This EWL regulation at low RH is presumably an

253 adaptation to conserve body water when EHL is not required for thermoregulation. This

254 likely has considerable adaptive significance, particularly for species with limited access

255 to free water such as the arid-habitat kaluta. A conservative estimate of the water savings

256 for kalutas (calculated by assuming a linear relationship between EWL values at the

257 highest RH, and EWL = 0 at RH = 100%, and extrapolating this relationship to the lowest

258 measured RH then subtracting the observed EWL) indicates that the EWL of kalutas at

259 about 20% RH is only about 40-50% of that predicted if EWL increased as expected

260 biophysically, saving the kaluta at least 1.0 to 4.6 mg H_2O g^{-1} h^{-1}, depending on T_a.

261

(d) Other physiological implications of EWL-RH effects

There is a paucity of data for endotherms that examine the effects of RH on other physiological variables that impact homeostatic thermoregulation, such as T_b, MR and C . If RH had the expected biophysical effect on EWL, then we would expect compensatory thermoregulatory changes in MR, or impacts of changes in EHL on T_b. For example, wvp may affect T_b for kangaroo-rats, particularly at high T_a [37]. For brushtail possums [14] there was a significant RH effect on MR at T_a = 30 ℃, sufficient to maintain a constant T_b when EWL (and thus EHL) was reduced at higher RH. However, RH does not affect T_b or MR for some rodents [6, 7, 38]. For kalutas, T_b increased significantly only at the highest RH, where EWL (and thus EHL) were significantly reduced; at lower RH, EWL as well as T_b, MR and C were independent of RH. Kalutas did not adjust MR or C to maintain constant T_b at the high RH, reflecting their general thermolability [17].

This observation highlights another important consequence of EWL constancy with varying RH/wvp/Δwvp. Constancy of EWL avoids thermoregulatory impacts that would occur as a result of changing EHL. Constant EWL (and thus EHL) at differing RH keeps T_b constant without the need for compensatory changes in MR and/or C.

284 and animals were held under licence from the West Australian Department of

285 Environment and Conservation.

286

287 **Data Accessibility**

288 Raw data are provided online as Electronic Supplementary Material.

289

290 **References**

291 1.   Monteith JL, Campbell GS. 1980 Diffusion of water vapour through integuments –

292   potential confusion. *J. Thermal Biol.* **5**, 7-9.

293 2.   Withers, P.C. 1992 Comparative Animal Physiology, Philadelphia, PA: Saunders

294   College Publishing.

295 3.   McNab BK. 2002 The Physiological Ecology of Vertebrates. Ithaca, NY: Cornell

296   University Press.

297 4.   Daniels F, Alberty RA. 1975 Physical Chemistry. New York, NY: Wiley.

298 5.   Chew RM, Dammann AE. 1961 Evaporative water loss of small vertebrates, as

299   measured with an infrared analyser. *Science* **133**, 384-385.

300 6.   Baudinette, RV. 1972 Energy metabolism and evaporative water loss in the

301   California ground squirrel. Effects of burrow temperature and water vapour

302   pressure. *J. Comp. Physiol*. **81**, 57-72.

303 7.   Edwards RM, Haines H. 1978 Effects of ambient water vapour pressure and

304   temperature on evaporative water loss in *Peromyscus maniculatus* and *Mus*

305   *musculus*. *J. Comp. Physiol*. **128**, 177-184.

13

306   8.   Lasiewski RC, Acosta AL, Bernstein MH. 1966 Evaporative water loss in birds – I.

307   Characteristics of the open flow method of determination, and their relation to

308   estimates of thermoregulatory ability. *Comp. Biochem. Physiol.* **19**, 445-457.

309   9.   Lasiewski RC, Weathers WW, Bernstein MH. 1967 Physiological responses of the

310   giant hummingbird, *Patagonia gigas*. *Comp. Biochem. Physiol.* **23**, 797-813.

311   10. Christian DP. 1978 Effects of humidity and body size on evaporative water loss in

312   three desert rodents. *Comp. Biochem. Physiol.* **60A**, 425-430.

313   11. Webb PI, Speakman JR, Racey PA. 1995 Evaporative water loss in two sympatric

314   species of vespertilionid bat, *Plecotus auritus* and *Myotis daubentoni*: relation to

315   foraging mode and implications for roost site selection. *J. Zool.* **235**, 269-278.

316   12. Bernstein MH, Hudson DM, Stearns JM, Hoyt RW. 1977 Measurement of

317   evaporative water loss in small animals by dew-point hygrometry. *J. Appl. Physiol.*

318   **43**, 382-385.

319   13. Procter JW, Studier, EH. 1970 Effects of ambient temperature and water vapor

320   pressure on evaporative water loss in *Myotis lucifugus*. *J. Mammal.* **51**, 799-804.

321   14. Cooper CE, Withers PC. 2008 Allometry of evaporative water loss in marsupials:

322   implications of the effect of ambient relative humidity on the physiology of

323   brushtail possums (*Trichosurus vulpecula*). *J. Exp. Biol.* **211**, 2759-2766.

324   15. Cooper CE, Withers PC. 2009 Effects of measurement duration on the

325   determination of basal metabolic rate and evaporative water loss of small

326   marsupials: How long is long enough? *Physiol. Biochem. Zool.* **82**, 438–446.

327 16. Lillywhite HB, Menon JG, Menon GK, Sheehy CM, Tu MC. 2007 Water exchange

328   and permeability properties of the skin in three species of amphibious sea snakes

329   (*Laticauda* spp.). *J. Exp. Biol.* **212**, 1921-1929.

330 17. Withers PC, Cooper CE. 2009 Thermal, metabolic, and hygric physiology of the

331   little red kaluta, *Dasykaluta rosamondae* (Dasyuromorphia: Dasyuridae). *J.*

332   *Mammal.* **90**, 752-760.

333 18.   Withers PC. 2000 Design, calibration and calculation for flow-through respirometry

334   systems. *Aust. J. Zool.* **49**, 445-461.

335 19. Parish OO, Putnam TW. 1977 Equations for the determination of humidity from

336   dewpoint and psychrometric data. NASA Technical Note D-8401. Dryden Flight

337   Research Centre, CA: NASA.

338 20.   Coulombe HN. 1970 Physiological and physical aspects of temperature regulation

339   in the Burrowing Owl *Speotyto cunicularia. Comp. Biochem. Physiol.* **35**, 307-337.

340 21. Withers PC, Cooper CE. 2011 Using a priori contrasts for multivariate repeated-

341   measures ANOVA to analyse thermoregulatory responses of the dibbler

342   (*Parantechinus apicalis*; Marsupialia, Dasyuridae). *Physiol. Biochem. Zool.* **84**,

343   514-521.

344 22.   Moritmoto T. 1998 Heat loss mechanisms. In, Physiology and Pathophysiology of

345   Temperature Regulation (ed. CM Blatteis). World Scientific, Singapore. Pp 80-90.

346 23.   Tattersall GJ, Sinclair BJ, Withers PC, Fields PA, Seebacher F, Cooper CE,

347   Maloney SK. 2012 Coping with thermal challenges: Physiological adaptations to

348   environmental temperatures. *Compr. Physiol.* **2**, 2151-2202.

349   24.   Withers PC, Cooper CE. 2009 Thermal, metabolic, hygric and ventilatory

350   physiology of the sandhill dunnart (*Sminthopsis psammophila*; Marsupialia,

351   Dasyuridae). *Comp. Biochem. Physiol.* **153**, 317–323.

352   25.   Withers PC, Cooper CE, Nespolo RF. 2012 Evaporative water loss, relative water

353   economy and evaporative partitioning of a heterothermic marsupial, the monito del

354   monte (*Dromiciops gliroides*). *J. Exp. Biol.* **215**, 2806-2813.

355   26.   Tracey RL, Walsberg GE. 2001 Developmental and acclimatory contributions to

356   water loss in a desert rodent: investigating the time course of adaptive change. *J.*

357   *Comp. Physiol. B* **171**, 669-679.

358   27.   McKechnie AE, Wolf BO. 2004 Partitioning of evaporative water loss in white-

359   winged doves: plasticity in response to short-term thermal acclimation. *J. Exp. Biol.*

360   **207**, 203-210.

361   28.   Muñoz-Garcia A, Williams JB. 2008 Developmental plasticity of cutaneous water

362   loss and lipid composition in stratum corneum of desert and mesic nestling house

363   sparrows. *Proc. Nat. Acad. Sci.* **105**, 15611–15616.

364   29.   Muñoz-Garcia A, Cox RL, Williams JB. 2008 Phenotypic flexibility in cutaneous

365   water loss and lipids of the stratum corneum in house sparrows (*Passer domesticus*)

366   following acclimation to high and low humidity. *Physiol. Biochem. Zool.* **81**, 87–

367   96.

368   30.   Elias PM. 2004 Epidermal permeability barrier: from the early days at Harvard to

369   emerging concepts. *J. Invest. Dermatol.* **122**, xxxvi–xxxix.

370 31. Muñoz-Garcia A, Williams JB. 2005 Cutaneous water loss and lipids of the stratum

371   corneum in house sparrows *Passer domesticus* from arid and mesic environments.

372   *J. Exp. Biol.* **208**, 3689-3700.

373 32. Ro J, Williams JB. 2010 Respiratory and cutaneous water loss of temperate-zone

374   passerine birds. *Comp. Biochem. Physiol. A* **156**, 237–246.

375 33. Bartholomew GA. 1972 The water economy of seed-eating birds that survive

376   without drinking. Proceedings of the XV$_{TH}$ International Ornithological Congress

377   (ed. KH Voous). Brill, Leiden. Pp 237-254.

378 34. Schmidt-Nielsen K, Hainsworth FR, Murrish DE. 1970 Counter-current heat

379   exchange in the respiratory passages: effect on water and heat balance. *Respir.*

380   *Physiol.* **9**, 263-276.

381 35. Collins JC, Pilkington TC, Schmidt-Nielsen K. 1971 A model of respiratory heat

382   transfer in a small mammal. *Biophys. J.* **11**, 886-914.

383 36. Geist NR. 2000 Nasal respiratory turbinate function in birds. *Physiol. Biochem.*

384   *Zool.* **73**, 581-589.

385 37. Kay FR. 1975 Environmental physiology of the banner-tailed kangaroo rat – I

386   Influence of ambient temperature, humidity and carbon dioxide on body

387   temperature. *Comp. Biochem. Physiol. A* **50**, 483-488.

388 38. Ewing WG, Studier EH. 1973 A method for control of water vapour pressure and

389   its effects on metabolism and body temperature in *Mus musculus*. *Comp. Biochem.*
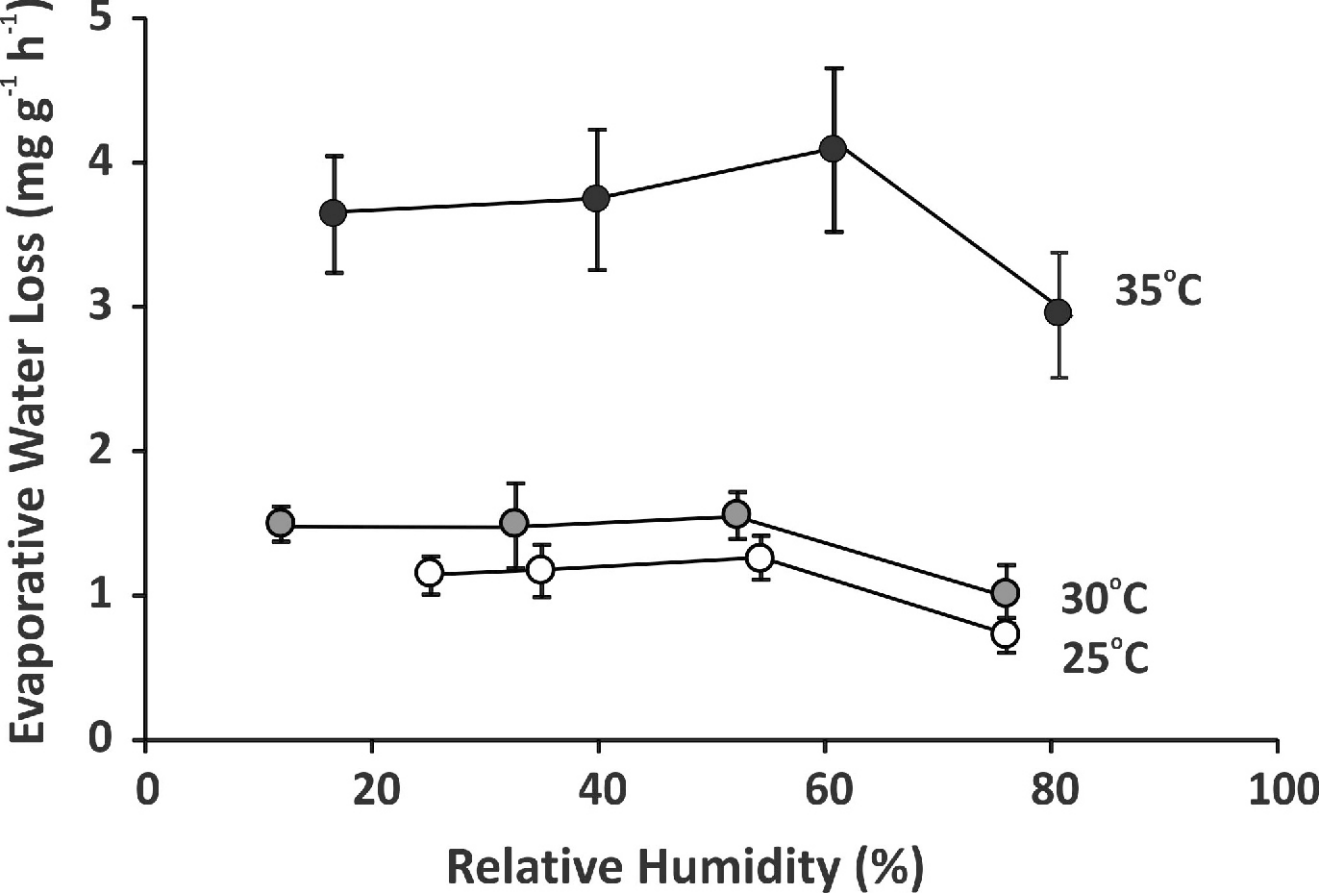
390   *Physiol. A* **45**, 121-125.

391

FIGURE CAPTIONS

393   **Figure 1.** Evaporative water loss of little red kalutas (*Dasykaluta rosamondae*), over a

394   range of ambient relative humidities, measured at ambient temperatures of 25, 30 and 35

395   °C. Values are mean ± SE, N = 8.

396   **Figure 2**. Evaporative water loss relative to water vapour pressure deficit, of little red

397   kalutas (*Dasykaluta rosamondae*), over a range of ambient humidities (RH) and different

398   ambient temperatures (T_a; values in figure). Values from this study (circles) are for four

399   RH at three T_a; values from [17] (diamonds) are for T_a from 11 to 38 ^{o}C, at low RH. A

400   simple physical model of evaporation predicts no effect of T_a or RH on EWL/Δwvp (see

401   text).

402   **Figure 3.** Conceptual model (thick black line) for the expected physical relationship

403   between evaporative water loss (EWL) and water vapour pressure deficit (Δwvp), based

404   on diffusion (Fick's Law), compared with the relative EWL for various endotherms from

405   the literature (numbers indicate source). The Δwvp is calculated as ((100 – ^{ambient}

406   ^{RH})/$_{100}$)*saturation water vapour pressure at the ambient temperature. Evaporative water

407   loss is scaled for each species so that the EWL for the lowest measured Δwvp fits on the

408   expected physical model line. Data that fall below the expected physical line are

409   consistent with a regulated decrease in EWL (i.e. water conservation) whereas data that

410   fall above the expected physical line are consistent with an increased EWL at higher

411   Δwvp (i.e. enhanced water loss).