Interactions between humidity and evaporative heat dissipation in a passerine bird

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Abstract

Environmental humidity is thought to be a major determinant of evaporative cooling capacity at high air temperatures (T_a), but the technical challenges of experimentally manipulating humidity in respirometry chambers have resulted in far less being known about the effects of humidity compared to those of T_a. We tested the prediction that at T_a approaching and exceeding normothermic body temperature (T_b), high humidity would result in higher T_b, lower evaporative water loss (EWL) and/or higher resting metabolic rate (RMR) in a passerine bird, the white-browed sparrow-weaver (*Plocepasser mahali*). We used open-system flow-through respirometry to measure EWL, RMR and T_b in sparrow-weavers experiencing T_a = 36–44 °C and chamber humidities of 6, 13, 19 or 25 g m^{-3}. Increasing humidity was associated with significantly higher T_b. The strongest effect of humidity, however, involved significant increases in RMR; at T_a = 40 °C, RMR at a humidity of 25 g m^{-3} was ~ 40% higher compared to a humidity of 6 g m^{-3}. Moreover, the interaction between T_a and humidity exerted a significant effect on the ratio of evaporative heat loss (EHL) to metabolic heat production (MHP), evident as an increasing effect of humidity with increasing T_a. Our results, when compared with those of the limited number of previous studies that involved similar ranges of T_a and humidity, reveal that the relative effects of humidity on EWL and RMR vary among avian taxa, and support the notion that the overall effect of high humidity is a reduction in maximum EHL/MHP.

Introduction

Evaporative heat dissipation provides the physiological basis for defending body temperature (T_b) below lethal limits during heat exposure and is the only avenue of heat flux that permits animals to maintain T_b below environmental temperature (Cowles and Dawson 1951; Dawson 1954; Dawson and Schmidt-Nielsen 1964). Evaporative cooling is particularly important for taxa inhabiting hot environments, with some arid-zone birds capable of maintaining T_b at 10–20 °C below air temperature (T_a) by evaporatively dissipating heat at rates equivalent to ~ 5 × metabolic heat production (McKechnie et al. 2016; O'Connor et al. 2017). Heat dissipation behaviors such as panting are associated with consequential trade-offs between thermoregulation and foraging, constraining the ability of birds in hot environments to maintain body condition and/or successfully breed (du Plessis et al. 2012; van de Ven 2017).

Maximum rates of evaporative heat dissipation are determined by the water vapor gradients between organisms and the surrounding air (Campbell and Norman 1998), and

increasing humidity is associated with decreasing rates of evaporative water loss in birds and other animals (Edwards and Haines 1978; Webster and King 1987; Powers 1992; Gerson et al. 2014). Environmental humidity is therefore a critical variable for understanding thermoregulatory constraints in birds (e.g., Smit et al. 2013) and other taxa (Welch 1980; Walters et al. 2004; Sherwood and Huber 2010) in hot environments.

Most studies of the interactions between environmental humidity and avian thermo-regulation have involved birds experiencing T_a below normothermic T_b (Webster and King 1987; Eto et al. 2017) or approaching normothermic T_b (Powers 1992). Far less is known about how humidity affects avian thermoregulation at T_a approaching and exceeding T_b (Lasiewski et al. 1966). In one of the few such studies of which we are aware, Gerson et al. (2014) evaluated the influence of humidity on interactions between evaporative water loss (EWL), resting metabolic rate (RMR) and T_b in two arid-zone species at $T_a > 40$ °C. Thermoregulation was less affected by humidity in a 24-g passerine (predominantly respiratory EWL via panting) than in a 39-g columbid in which cutaneous EWL predomi-nates (Gerson et al. 2014).

The paucity of studies quantifying interactions between avian evaporative heat dissipation and humidity at high T_a reflects, to a large extent, methodological challenges inherent in experimentally manipulating humidity during measurements of gas exchange by flow-through respirometry. In broad terms, the water vapor gradient experienced by an animal in a metabolic chamber is determined by three variables: T_a, the humidity of incurrent air, and the rate of EWL by the animal itself (Lighton 2008). Hence, it becomes very challenging for investigators to regulate chamber humidity at constant levels, one consequence of which is that previous workers had little choice but to plot relationships between EWL and humidity separately for different T_a values and/or use three-dimensional plots to illustrate the interactive effects of between T_a and humidity on EWL (Powers 1992; Gerson et al. 2014).

In this study, we quantified interactions between humidity, EWL, RMR and T_b over T_a values approaching and exceeding normothermic T_b in a passerine bird, using a respirometry system that allowed us to control chamber humidity much more precisely than previous studies. We predicted that the efficiency of evaporative heat dissipation decreases with increasing humidity, such that higher environmental humidity is associated with higher T_b, higher RMR and/or reduced EWL.

Materials and methods

Study species

White-browed Sparrow-weavers (*Plocepasser mahali*, Smith 1836) are ploceid passerines that occur in woodland and savanna habitats across southern Africa (du Plessis 2005). The ten sparrow-weavers [mean body mass (M_b) = 43.64 ± 2.95 g] used for this study had been caught 4 months previously at Polokwane in the Limpopo province of South Africa (23°56′ S, 29°28′E), at the same site and using the same methods as described by Noakes et al. (2016). The average maximum and dewpoint daily temperatures for the hottest summer month (January) at this site are 27.7 ± 1.1 °C and 14.2 ± 1.4 °C, respectively (data for 2012–2018 provided by the South African Weather Service). After being transported to Pretoria by road in modified pet transport crates, birds were housed in a climate-controlled room

at the Small Animal Physiological Research Facility of the University of Pretoria. The T_a and relative humidity in each room was 20.4 ± 0.5 °C and 43.3 ± 2.1% respectively, and the photoperiod 12:12 LL:DD. Sparrow-weavers were housed in pairs (male and female) in cages 600 mm long × 400 mm wide × 400 mm high, with each cage equipped with perches and food and water dispensers. Each bird was provided with wild bird seed and water ad libitum, and received four superworms and four mealworms daily, a diet that allowed the maintenance of body condition. All experiments took place during the day, the active phase of this species. Food was removed 90 min before each set of measurements to ensure that birds were postabsorptive (predicted mean digesta retention time for a 47-g bird is ~ 67.5 min; Karasov 1990). Individuals were weighed before each set of measurements, and were allowed a minimum of 3 days to rest between measurements.

Measurements of air and body temperature

Air temperature within the metabolic chamber was measured using a thermistor probe (model TC-100, Sable systems, Las Vegas, USA) inserted through a hole in the chamber sealed with a rubber grommet. For T_b measurements, a temperature-sensitive passive integrated transponder (PIT) tag (Biomark, Boise, ID, USA) was injected into the abdominal cavity of each sparrow-weaver. The T_b of each individual was continually recorded every ~ 15 s during measurements using a portable transceiver system (model HPR+, BioMark, Biose ID, USA). PIT tags were calibrated in a water bath at temperatures ranging from 39 to 46 °C against a digital thermocouple reader (model RDXL12SD, Omega, Stamford, CT, USA) with Cu–Cn thermocouples (Physitemp, Clifton, NJ, USA). The thermocouple reader had previously been calibrated against a mercury-in-glass thermometer with NIST-traceable accuracy.

Measurements of gas exchange and regulation of chamber humidity

Oxygen consumption ($V_{O_2}$$V_{O_2}$), carbon dioxide production ($V_{CO_2}$$V_{CO_2}$) and EWL were measured at T_a between 36 and 44 °C using an open-flow through respirometry system. Each bird was placed in a 4-L plastic chamber (Lock & Lock, Seoul, South Korea), known not to absorb measurable quantities of water vapor (Whitfield et al. 2015). To prevent evaporation from urine and feces affecting EWL measurements, a 1-cm layer of mineral oil was placed at the bottom of each chamber, with a plastic mesh platform positioned approximately 10 cm above the oil layer. The chamber was placed either in a modified ice chest (~ 75 L) in which T_a was regulated by a Peltier device (model AC-162, TE Technology Inc., Traverse City, MI, USA) and a custom-built controller (Whitfield et al. 2015), or in a temperature-controlled cabinet (Binder, Tuttlingen, Germany). Dry air supplied by a compressor was split into an experimental and a baseline channel using Bev-A-Line IV tubing (Thermoplastic Processes Inc., Warren, NJ, USA). The flow rate to the baseline channel ("dry baseline") was kept constant at ~ 1 L min^{-1} using a needle valve (Swagelok, Solon, OH, USA), and the flow rates of 1–3 L min^{-1} to the experimental channel were maintained using a mass flow controller (Alicat Scientific Inc., Tuscon AZ, USA) calibrated using a soap-bubble flow meter (Gilibrator 2, Sensidyne, St Petersburg, FL, USA). Downstream of the mass flow controller, experimental channel air was passed through a bubbler constructed from a 1.5-L screw-top bottle with fittings installed in the lid and incurrent air passing through an aquarium stone. The bubbler was placed in a temperature-

controlled chamber (PELT-5, Sable Systems, Las Vegas NV, USA) set to regulate T_a = 17 °C. Humidified air generated in this way then passed through a dew point generator (DG-4, Sable Systems, Las Vegas NV, USA) to precisely regulate incurrent humidity. Downstream of the dew point generator, the humid air stream was again split into a secondary baseline channel ("humid baseline") with flow rate regulated using a needle valve and the supply to the chamber. Before entering the chamber, the latter passed through a flow meter of a subsampler (SS-4, Sable Systems, Las Vegas NV, USA), also calibrated using a Gilibrator 2 flow meter. Flow rate through the SS-4 was included in data acquisition by connecting the voltage output to a UI-2 analog–digital convertor (see below). Incurrent flow rates to the chamber varied between 230 and 1900 mL min^{-1}. All adjustments of flow rates and/or incurrent humidity took place at the start of transition periods to maximize the likelihood that equilibrium conditions (Lasiewski et al. 1966) were reached before data were collected from stable O_2, CO_2 and H_2O traces.

By periodically adjusting the humidity and flow rate of incurrent air, we were able to regulate the absolute humidity of excurrent air (i.e., the humidity experienced by a bird in the chamber) at one of four setpoint values (mean ± SD): 6.41 ± 0.63 g H_2O m^{-3} (n = 35), 12.55 ± 0.53 g H_2O m^{-3} (n = 35), 18.61 ± 0.50 g H_2O m^{-3} (n = 28) and 25.04 ± 0.64 g H_2O m^{-3} (n = 26). These values, which we hereafter refer to as 6, 13, 19 and 25 g m^{-3}, respectively, are similar to the values used by previous authors (Powers 1992; Gerson et al. 2014), and are equivalent to relative humidities at T_a = 40 °C of 12.6 ± 1.2%, 24.6 ± 1.03%, 36.5 ± 1.0% and 49.1 ± 1.2%, respectively, and vapor pressure deficits (VPD) at T_a = 40 °C of 6.46 ± 0.09 kPa, 5.57 ± 0.08 kPa, 4.69 ± 0.07 kPa and 3.78 ± 0.09 kPa, respectively. Because 25 g m^{-3} is equivalent to a dewpoint of ~ 27 °C, for most runs we set up the equipment in a controlled climate room set to regulate T_a = 35 °C to avoid condensation in the tubing and analysers.

Excurrent air or air from the two baseline channels (dry and humid) was sequentially subsampled using a respirometry multiplexer (model MUX3-1101-18M, Sable Systems) in manual mode, at a flow rate ~ 200 mL min^{-1} regulated by a custom-built subsampling pump, and pulled through a CO_2/H_2O analyser (LI-840A, LI-COR, Lincoln NE, USA) followed by an O_2 analyser (FC-10A, Sable Systems, Las Vegas NV, USA). The CO_2/H_2O analyser was regularly zeroed using pure nitrogen (AFROX, Johannesburg, South Africa) and spanned using a 2000 ppm CO_2 in N_2 gas mix (AFROX) or humidified air with a dewpoint 3–4 °C below ambient generated using the dew point generator. The O_2 analyser was periodically spanned to 20.95% using dry, CO_2-free air scrubbed of CO_2 using soda lime and water vapor using sequential columns of silica gel, drierite and magnesium perchlorate.

Data were acquired every 5 s from the analysers and SS-4 using an analog–digital converter (model UI-3, Sable Systems, Las Vegas NV, USA) to convert voltage inputs into digital values, which we then recorded using a personal computer with Expedata software (Sable Systems, Las Vegas NV, USA).

Experimental protocol

We measured T_b, EWL and RMR at each of the four humidity setpoints at the following T_a values (mean ± SD): 36.18 ± 0.43 °C, 38.66 ± 0.27 °C, 40.46 ± 0.29 °C, and 42.25

± 0.30 °C (hereafter 36, 38, 40, and 42 °C, respectively). The sample size for each T_a/humidity combination was generally 5–12, but we were only able to obtain data for a single individual at 42 °C and 25 g H_2O m^{-3}. We also included data at T_a = 43.91 ± 0.32 °C for a single individual at each of 6, 13 and 19 g H_2O m^{-3}; at these combinations, most birds became agitated in the chamber and had to be excluded from analyses.

Each set of measurements typically lasted 2–3 h and began with a bird placed in a chamber at T_a = 35 °C, after which it was given at least 15 min to habituate. All the birds involved in this study had previously been used for a separate study involving similar measurements of gas exchange and T_b; we are thus confident that the birds were well-habituated to the metabolic chambers, and any stress response to being placed in the chambers was likely much less pronounced than would have been the case had we used freshly caught birds (Jacobs and McKechnie 2014). After this initial period, T_a and humidity values were set to one of the experimental combinations, with transitions between treatments taking a maximum of 10–15 min to achieve. Once T_a and humidity had stabilized at the new values, birds were exposed to these conditions for a minimum of 15 min until traces of O_2, CO_2 and H_2O were stable. Thereafter, T_a and humidity were set to new setpoints, and measurements repeated under the new conditions. The sequence of T_a and humidity combinations within each set of measurements was randomized to the extent possible, with each set of measurements involving 4–8 such combinations.

Data analyses

All traces were corrected for analyser drift and lag using the relevant Expedata algorithms. Excurrent flow rates were calculated using equation [9.3] of Lighton (2008) and equations [9.4]—[9.6] used to calculate V_{O_2}, V_{CO_2} and EWL. RMR and EWL were calculated from the lowest 5-min period of V_{O_2} per trace. Respiratory exchange ratio (RER) was calculated as V_{CO_2}/V_{O_2}, and thermal equivalence data (Withers 1992, Table 4-2) were used to convert rates from respiratory gas exchange to metabolic rates (W). RER averaged 0.863 ± 0.081 and in the small number of instances where estimates of RER fell outside the range of 0.71–1.00 we assumed either of the latter values. Rates of EWL were converted to EHL (W) assuming 2.406 J mg H_2O^{-1} at 40 °C (Tracy et al. 2010). We excluded all data from individuals that were active in the chambers (e.g., sustained escape behavior) from our analysis. All conversions from water vapor pressure (kPa) to absolute humidity (g H_2O m^{-3}) and calculated saturation water vapor pressures were based on equations provided by Campbell and Norman (1998).

All statistical analyses were computed in the R 3.5.1 (R Core Team) environment, using R Studio 1.1.463 (RStudio, Inc.). We used Levene's tests implanted in the R package *lawstat* (Gastwirth et al. 2017) to confirm that no significant heteroscedasticity existed in T_b, EWL or RMR data among humidity or temperature categories. We used general linear models to model the responses of T_b, EWL, RMR and EHL/MHP to the continuous predictor variables T_a, absolute humidity and the T_a X absolute humidity interaction term. To select the models with highest explanatory power, we used the *model.sel* function of the R package *MuMIn* (Bartoń 2013) to identify the best model for each response variable on the basis of Akaike information criterion values corrected for small sizes (AIC$_c$) (Sakamoto et al. 1986). For T_b, RMR and EHL/MHP, the best models included T_a, absolute humidity, T_a X absolute humidity, M_b, sex and identity as predictors.

For EWL, the best model included just T_a, absolute humidity, T_a X absolute humidity. However, as the difference in AIC$_c$ values between the latter EWL model and one including M_b, sex and identity was only 1.37, we fitted models with T_a, absolute humidity, T_a X absolute humidity, M_b, sex and identity as predictors to all the response variables. Individual identity was included as a random fixed effect in all models. After confirming that no obvious deviations from normality or homoscedasticity were evident in residual plots, we fitted linear mixed effect models using the *nlme* package (Pinheiro et al. 2009).

Results

The T_b of the sparrow-weavers varied from ~ 42 °C at T_a = 36–38 °C to ~ 43–44 °C at the highest T_a values at which we obtained data (Table 1; Fig. 1). There was a significant influence of absolute humidity on T_b ($F_{1,111}$ = 4.05, p = 0.047), with T_b typically 0.2–0.5 °C higher in the high humidity treatments compared to the low humidity treatments (Table 1; Fig. 1). T_b was also strongly related to T_a ($F_{1,111}$ = 84.43, p < 0.001) but there was no significant T_a X absolute humidity interaction ($F_{1,111}$ = 0.42, p = 0.519).
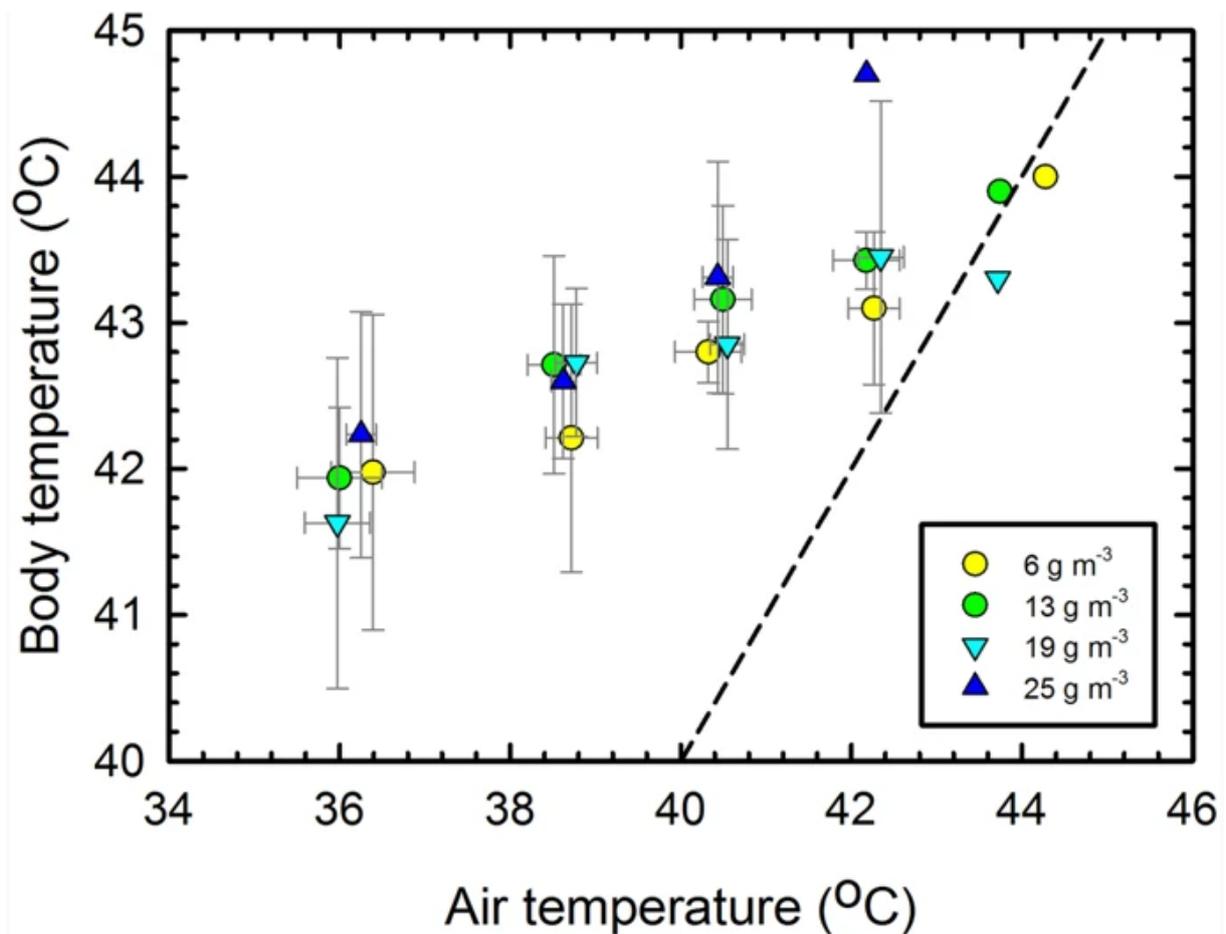

Fig. 1. Relationship between body temperature (T_b) and air temperature (T_a) over absolute humidities of 6–25 g H_2O m^{-3} in white-browed sparrow-weavers (*Plocepasser mahali*). Values are means with standard deviations shown by error bars whenever sample sizes were large enough (see Table 1). The dashed line shows $T_b = T_a$. A scatter plot of the data is available in ESM Figure S1

Table 1 Mean $\pm$ SD body temperature, evaporative water loss, resting metabolic rate and ratio of evaporative heat loss/metabolic heat production in white-browed sparrow-weavers (*Plocepasser mahali*) exposed to five air temperature (T_a) and four humidity treatments

T_a (°C)	Body temperature (°C)				Evaporative water loss (g h^{-1})			
	6 g m^{-3}	13 g m^{-3}	19 g m^{-3}	25 g m^{-3}	6 g m^{-3}	13 g m^{-3}	19 g m^{-3}	25 g m^{-3}
36	41.98 $\pm$ 1.08 (11)	41.94 $\pm$ 0.48 (8)	41.63 $\pm$ 1.13 (7)	42.23 $\pm$ 0.84 (9)	0.175 $\pm$ 0.057 (11)	0.174 $\pm$ 0.075 (8)	0.166 $\pm$ 0.081 (7)	0.253 $\pm$ 0.122 (9)
38	42.21 $\pm$ 0.92 (10)	42.71 $\pm$ 0.75 (8)	42.73 $\pm$ 0.51 (8)	42.60 $\pm$ 0.53 (8)	0.312 $\pm$ 0.099 (10)	0.362 $\pm$ 0.087 (8)	0.406 $\pm$ 0.227 (8)	0.413 $\pm$ 0.141 (8)
40	42.80 $\pm$ 0.21 (6)	43.16 $\pm$ 0.64 (12)	42.85 $\pm$ 0.72 (8)	43.31 $\pm$ 0.79 (8)	0.467 $\pm$ 0.076 (6)	0.514 $\pm$ 0.078 (12)	0.478 $\pm$ 0.117 (8)	0.527 $\pm$ 0.254 (8)
42	43.10 $\pm$ 0.52 (7)	43.43 $\pm$ 0.20 (6)	43.45 $\pm$ 1.07 (4)	44.70 (1)	0.549 $\pm$ 0.078 (7)	0.539 $\pm$ 0.109 (6)	0.403 $\pm$ 0.141 (4)	0.819 (1)
44	44.00 (1)	43.90 (1)	44.30 (1)		0.663 (1)	0.627 (1)	0.788 (1)	

T_a (°C)	Resting metabolic rate (W)				Evaporative heat loss/metabolic heat production			
	6 g m^{-3}	13 g m^{-3}	19 g m^{-3}	25 g m^{-3}	6 g m^{-3}	13 g m^{-3}	19 g m^{-3}	25 g m^{-3}
36	0.329 $\pm$ 0.052 (11)	0.330 $\pm$ 0.058 (8)	0.352 $\pm$ 0.045 (7)	0.399 $\pm$ 0.104 (9)	0.351 $\pm$ 0.085 (11)	0.345 $\pm$ 0.113 (8)	0.310 $\pm$ 0.153 (7)	0.415 $\pm$ 0.095 (9)
38	0.348 $\pm$ 0.079 (10)	0.414 $\pm$ 0.094 (8)	0.416 $\pm$ 0.103 (8)	0.413 $\pm$ 0.103 (8)	0.593 $\pm$ 0.136 (10)	0.602 $\pm$ 0.169 (8)	0.629 $\pm$ 0.211 (8)	0.673 $\pm$ 0.104 (8)
40	0.332 $\pm$ 0.065 (6)	0.433 $\pm$ 0.102 (12)	0.380 $\pm$ 0.065 (8)	0.471 $\pm$ 0.119 (8)	0.965 $\pm$ 0.204 (6)	0.815 $\pm$ 0.129 (12)	0.848 $\pm$ 0.193 (8)	0.726 $\pm$ 0.236 (8)
42	0.323 $\pm$ 0.081 (7)	0.387 $\pm$ 0.056 (6)	0.421 $\pm$ 0.031 (4)	0.626 (1)	1.170 $\pm$ 0.215 (7)	0.933 $\pm$ 0.168 (6)	0.650 $\pm$ 0.262 (4)	0.875 (1)
44	0.306 (1)	0.389 (1)	0.517 (1)		1.449 (1)	1.078 (1)	1.019 (1)	

Rates of EWL varied approximately from minima of ~ 0.17 g h^{-1} at lower T_a up to maxima of ~ 0.55 g h^{-1} at $T_a = \sim 42$ °C and ~ 0.7 g h^{-1} in the small number of birds for which we obtained data at $T_a = \sim 44$ °C (Table 1; Fig. 2). There was a marginally non-significant influence of absolute humidity on EWL ($F_{1,111} = 3.71$, $p = 0.057$), and EWL was significantly related to T_a ($F_{1,111} = 136.041$, $p < 0.001$) but not to the T_a X absolute humidity interaction ($F_{1,111} = 0.037$, $p = 0.848$).
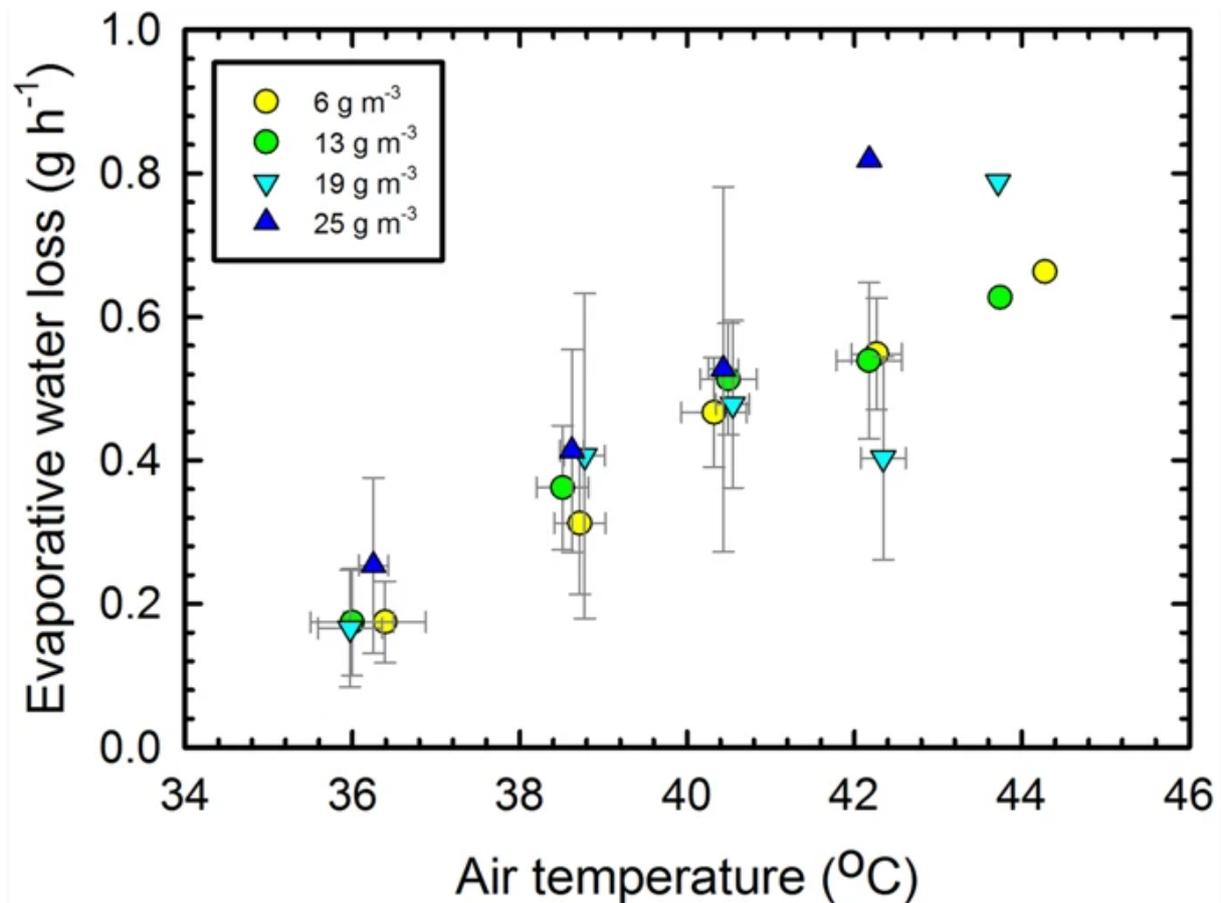
Fig. 2. Relationship between evaporative water loss and air temperature over absolute humidities of 6–25 g H$_2$O m^{-3} in white-browed sparrow-weavers (*Plocepasser mahali*). Values are means with standard deviations shown by error bars whenever sample sizes were large enough (see Table 1). A scatter plot of the data is available in ESM Figure S2

Resting metabolic rate was significantly influenced by absolute humidity ($F_{1,111} = 19.30$, $p < 0.001$) and T_a ($F_{1,111} = 4.782$, $p = 0.031$), with increasing humidity resulting in higher rates of increasing RMR with increasing T_a (Fig. 3). The T_a X absolute humidity interaction term emerged as a significant ($F_{1,111} = 5.494$, $p = 0.021$), confirming that the effect of humidity on RMR became more pronounced at higher T_a (Fig. 3). At $T_a \approx 40$ °C, mean RMR at a humidity of 25 g m^{-3} was equivalent to 142% of the corresponding value at a humidity of 6 g m^{-3} (Table 1; Fig. 3). In the single sparrow-weaver for which we obtained data at a humidity of 25 g m^{-3} at $T_a = 42$ °C, RMR was equivalent to 194% of the mean value for 7 birds at a humidity of 6 g m^{-3}.
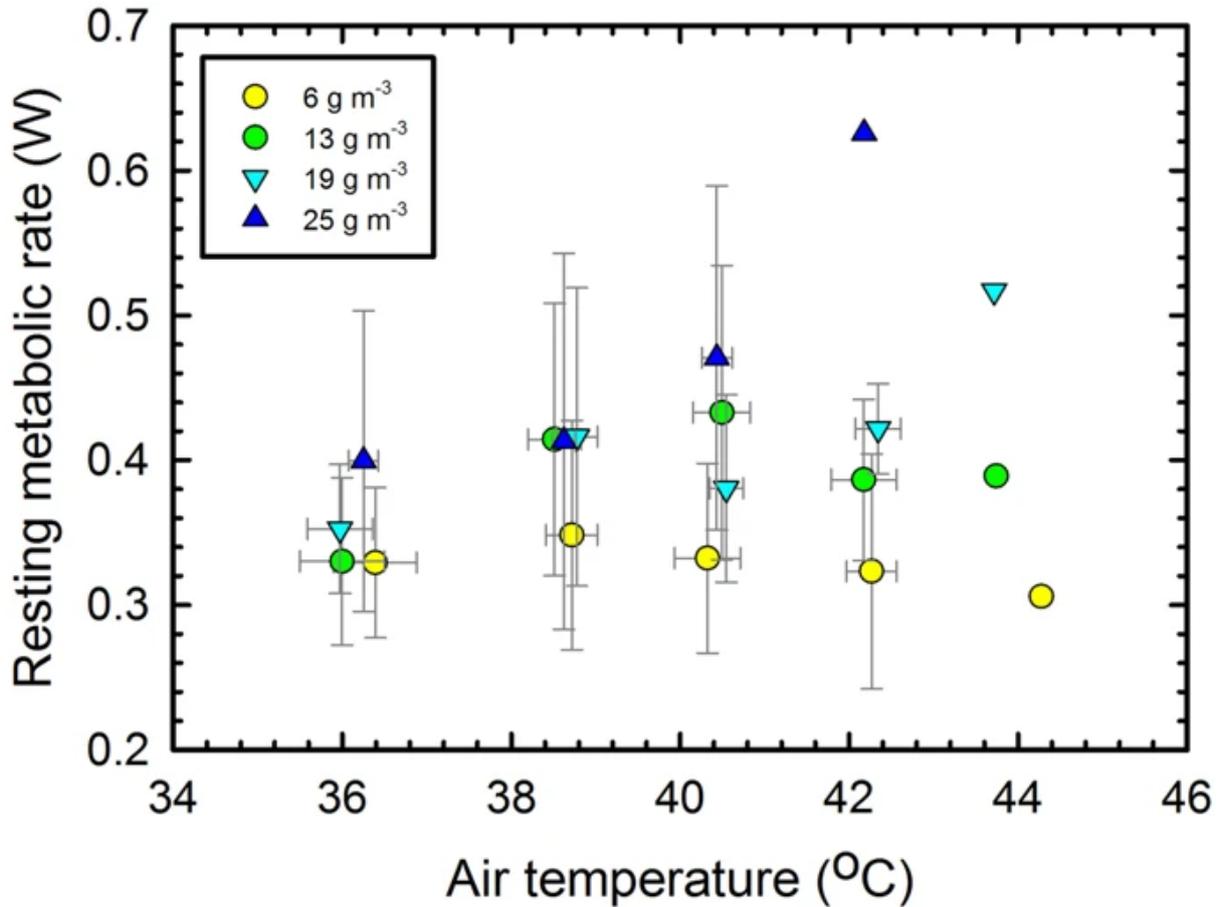
Fig. 3. Resting metabolic rate increased with increasing absolute humidity over air temperatures between 36 and 44 °C in white-browed sparrow-weavers (*Plocepasser mahali*). Values are means with standard deviations shown by error bars whenever sample sizes were large enough (see Table 1). A scatter plot of the data is available in ESM Figure S3

Overall, evaporative cooling efficiency (i.e., EHL/MHP) was significantly predicted by T_a ($F_{1,111} = 96.161$, $p < 0.001$) and T_a X absolute humidity ($F_{1,111} = 6.839$, $p = 0.010$), but not absolute humidity alone ($F_{1,111} = 0.707$, $p = 0.402$). The significant interaction between T_a and absolute humidity was evident as a divergence in the relationship between EHL/MHP and increasing T_a among humidity treatments at $T_a > 38$ °C (Fig. 4).
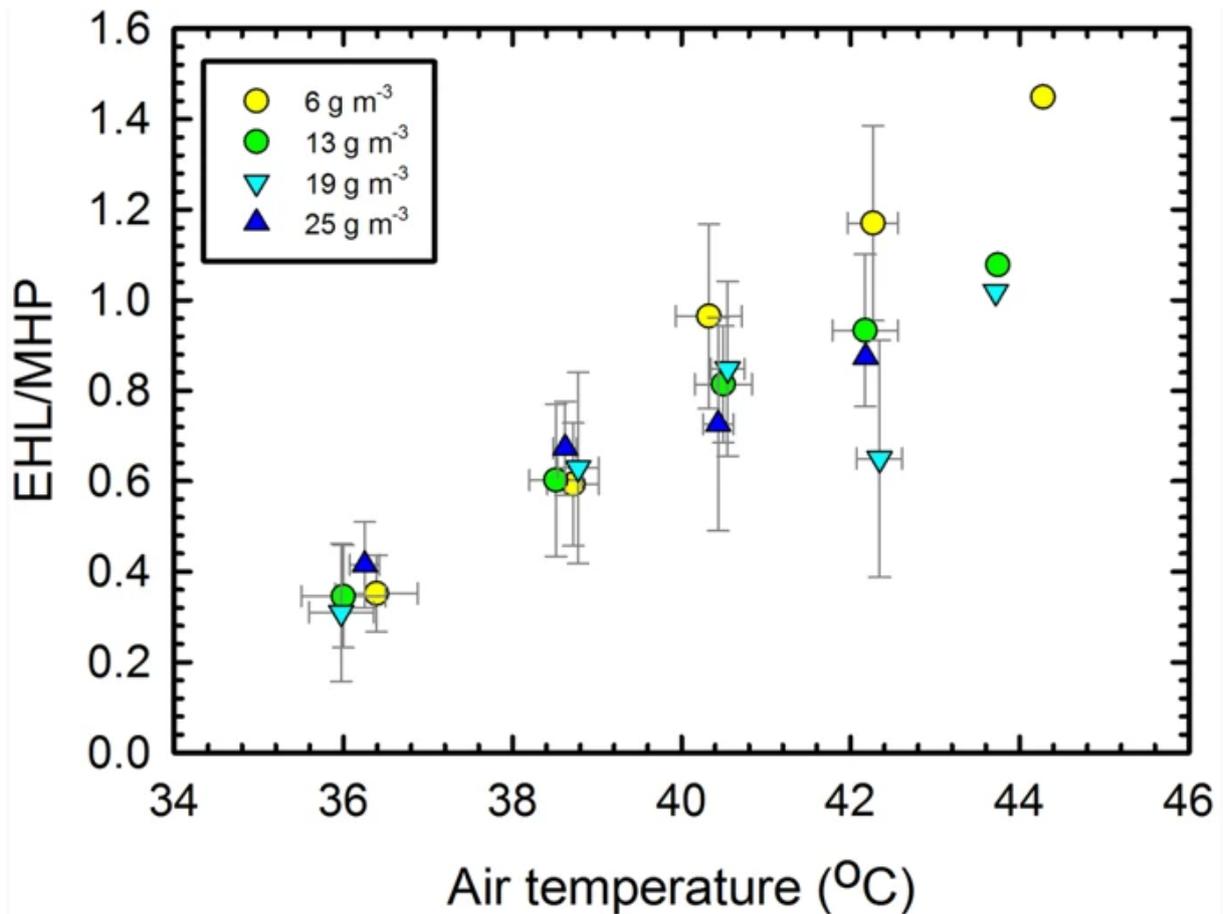
Fig. 4. Relationship between ratio of evaporative heat loss (EHL) and metabolic heat production (MHP) and air temperature over absolute humidities of 6–25 g H_2O m^{-3} in white-browed sparrow-weavers (*Plocepasser mahali*). Values are means with standard deviations shown by error bars whenever sample sizes were large enough (see Table 1). A scatter plot of the data is available in ESM Figure S4

Discussion

Thermoregulation by white-browed sparrow-weavers was significantly influenced by humidity, with increasing absolute humidity correlated with slight increases in T_b and much more substantial increases in RMR. At T_a = 40 °C and above, the large increases in RMR resulted in significant decreases in EHL/MHP, confirming that humidity constrains evaporative cooling efficiency at high T_a. The magnitude of the increases in T_b with increasing humidity were quantitatively similar to those reported for mean and maximum T_b during the hottest part of the day in free-ranging sparrow-weavers (Smit et al. 2013), confirming that humidity is a significant predictor of the T_b of this species under both natural and laboratory conditions. Our findings add to the limited literature on the effects of humidity on avian evaporative cooling and have implications for the methods used to quantify metabolic rates and evaporative water loss under laboratory conditions, as well as for our understanding of avian thermoregulation in natural habitats characterized by high humidity.

Our experimental setup allowed us to regulate chamber excurrent humidity more precisely than was the case in previous studies (Powers 1992; Gerson et al. 2014), primarily as a

result of our inclusion of a dew point generator upstream of the chamber. However, this approach also limited the range of T_a over which we were able to obtain data. The dew point generator we used (Sable Systems DG-4) is limited to flow rates < 5 L min^{-1}, and this constrained our capacity to achieve the range of humidity treatments at higher T_a while maintaining adequate flow rates for baselining purposes.

To the best of our knowledge, the only two previous studies that involved experimental manipulation of humidity at T_a ranges overlapping with that used in the present study involved a hummingbird (20–40 °C, Powers 1992) and a passerine and a columbid ($T_a >$ 40 °C, Gerson et al. 2014). In Anna's hummingbirds (*Calypte anna*), the major influence of humidity at T_a between 33 and 37 °C was a decrease in total rates of evaporation; EWL was typically reduced by 2/3 as humidity increased from ~ 6 to ~ 25 g m^{-3} (Powers 1992). The RMR of the hummingbirds, in contrast with our results for *P. mahali*, was not significantly influenced by humidity, and the overall effect in *C. anna* involved decreases in EHL/MHP fractionally similar to those for EWL (Powers 1992). Comparisons between *P. mahali* and *C. anna* are confounded by the small overlap in experimental T_a range between these studies, but the data for these two phylogenetically unrelated species nevertheless suggest that the effects of humidity may vary considerably among avian taxa, with effects on RMR and EWL contributing differentially to decreasing evaporative cooling efficiency.

More recently, Gerson et al. (2014) evaluated the impact of humidity on evaporative cooling in two species in the Kalahari Desert, the 24-g sociable weaver (*Philetairus socius*) and the 39-g Namaqua dove (*Oena capensis*). In *Ph. socius*, humidity had no significant effect on RMR or on EWL at $T_a = 40$ °C or $T_a = 44$ °C; at $T_a = 48$ °C, however, EWL decreased by ~ 50% as humidity increased from 2 to 26 g m^{-3} (Gerson et al. 2014). The effect of humidity on EWL only at $T_a = 48$ °C was mirrored by EHL/MHP, which was negatively related to humidity only at $T_a = 48$ °C. Direct comparisons between Gerson et al.'s data for sociable weavers and our present results for sparrow-weavers are complicated by the difference in M_b between these species, but also the limited overlap in T_a ranges between the two studies and the fact that the birds in the former study were summer-acclimatized, whereas our study population was acclimated to much milder T_a. Nevertheless, it is striking that the major influence of humidity on evaporative cooling in *P. mahali* involved increasing RMR, whereas in *Ph. socius* no such effect was evident. A similarity between these two passerines is that humidity constrains EHL/MHP at higher T_a, whereas little or no effect is evident at lower values.

Implications for comparative analyses

Our findings reiterate the influence of chamber humidity on measurements of metabolic rate, EWL and T_b, and the need to carefully consider these variables in the design of open-flow respirometry systems (Lasiewski et al. 1966). As noted by these authors, the chamber volume and flow rate used can be an important determinant of physiological responses during measurements of gas exchange at high T_a. For example, Dawson's (1954) seminal work on thermoregulation in the heat in Abert's and brown towhees [*Pipilo aberti* and *P. fuscus* (= *Melozone crissalis*), respectively] revealed unexpectedly modest heat tolerance and evaporative cooling capacity, leading the author to conclude that "*Panting thus appears to produce too much heat to be an efficient process for heat dissipation at high air*

temperatures". In Dawson's (1954) study, towhees experienced relative humidity of 38–47% at T_a = 40 °C, and 46–51% at T_a = 42 °C, equivalent to absolute humidities of 26 g m^{-3} and above. There were very steep increases in EWL and RMR at T_a > 35 °C, and none of the birds were able to tolerate T_a > 43 °C (Dawson 1954).

The EWL, RMR and T_b values measured at T_a = 40 °C and humidity = 25–26 g m^{-3} for *P. mahali* (present study) and *Ph. socius* (Gerson et al. 2014) suggest that the towhees studied by Dawson under similar conditions may, in fact, have been comparatively intolerant of high humidity. In the latter study, several individuals of both species became lethally hyperthermic (Dawson 1954). Moreover, fractional increases in EWL and RMR were much larger than the case in the two more recent studies, with RMR at T_a = 40 °C equivalent to 200–300% of values at T_a = 35 °C in both towhee species (Dawson 1954). The notion that the towhees studied by Dawson may have been unusually intolerant of high humidity is supported by the similarities in M_b between *P. mahali* and the towhees (47 g and 44 g for *P. aberti* and *M. crissalis*, respectively).

The significant influence of humidity on avian thermoregulation emphasizes the need to standardize conditions across studies for comparative analyses. In the present study, this effect was most obvious for RMR, mean values of which varied by > 40% among humidity treatments at T_a ≈ 40 °C. Our results reiterate the need to measure EWL, RMR and T_b under conditions of very low humidity when seeking to quantify heat tolerance limits and maximum evaporative cooling capacity and compare these variables among or within taxa (e.g., Whitfield et al. 2015; McKechnie et al. 2017; McWhorter et al. 2018; Smit et al. 2018).

Humidity may, however, also be an important variable to account for in studies investigating thermoregulation at lower T_a, for instance for measuring basal metabolic rates. In rock doves (*Columba livia*), both respiratory and cutaneous EWL was negatively related to humidity at T_a = 20 °C and 30 °C (Webster and King 1987). More recently, Eto et al. (2017) demonstrated that budgerigars (*Melopsittacus undulatus*) maintain constant EWL despite short-term fluctuations in humidity at T_a between 30 and 40 °C and argued that acute regulation of EWL is functionally linked to thermoregulation and has adaptive value in this context. The contrast between the findings of these latter two studies in terms of the effect of humidity on evaporative heat loss at moderate T_a suggests that humidity may indeed be an important variable to consider when measuring basal metabolic rate (BMR) and/or RMR at low T_a. In many studies focusing on BMR measurements, EWL was not measured and water vapor scrubbed from incurrent and/or excurrent air, with only VO_2 being measured (e.g., McNab 2001, 2005). In such instances, it may often be possible to estimate the likely range of chamber humidities from the equations provided by Lasiewski et al. (1966), particularly if EWL for the species involved has been measured in other studies. Evaporative cooling in natural habitats

The absolute humidities (6–25 g m^{-3}) used in the present study and previous ones (Gerson et al. 2014; Powers 1992) represent the majority of the range birds are likely to encounter in natural habitats. The highest humidity on record is a dewpoint of 35 °C, equivalent to ~ 38 g m^{-3}, in Dhahran, Saudi Arabia in July 2003 (Burt and Stroud 2007) and the 33-year average dewpoint at a site in Panama typical of lowland tropical habitats was 22.6 °C (Weathers 1997), equivalent to 18.9 g m^{-3}. The data currently available on the effects of

humidity on avian thermoregulation thus provide the basis for broadly evaluating how avian thermoregulation may be constrained by humidity in habitats ranging from deserts to wet tropical lowlands.

The evolution of avian thermoregulation in response to continuous high humidity has been investigated by Weathers (1977), who examined relationships between T_b, RMR and EWL at high T_a in Neotropical *Sporophila* seed-eaters. Variable seed-eaters (*S. corvina*; previously *S. aurita*) exposed to much lower humidities than they experience naturally exhibited unusually high T_b of up to 47 °C without any adverse effects at T_a = 45 °C together with a modest maximal EHL/MHP of 1.27 (Weathers 1997). These results led Weathers to hypothesize that selection associated with humid environments has resulted in reduced evaporative cooling capacity and increased tolerance of very high T_b during facultative hyperthermia to maintain positive T_b–T_a gradients even when experiencing high operative temperature while foraging in the sun.

Weathers' (1997) hypothesis is certainly supported by the remarkably high T_b values tolerated by *S. corvina*: T_b = 47 °C is well above the range exhibited by phylogenetically diverse taxa inhabiting hot deserts (e.g., Whitfield et al. 2015; McKechnie et al. 2017; Smith et al. 2017; Smit et al. 2018). Similarly, maximum T_b values for nine species spanning three orders were all < 46 °C in individuals held in large outdoor aviaries in the midsummer in the Kalahari Desert (Thompson et al. 2018). However, maximum EHL/MHP in arid-zone passerines are sometimes similar to the value for *S. corvina*: 1.20 in yellow-plumed honeyeater (*Lichenostomus ornatus*) and 1.32 in spiny-cheeked honeyeater *Acanthagenys rufogularis* (McKechnie et al. 2017) and 1.41 in scaly-feathered weavers *Sporopipes squamifrons* (Whitfield et al. 2015). These values were all measured at very low chamber humidities and likely represent the upper limit to these species' physiological capacity for evaporative heat dissipation, thus confirming that modest EHL/MHP is not restricted to passerines inhabiting humid habitats. Too few data on thermoregulation at high T_a currently exist for birds from humid tropical habitats to rigorously evaluate Weathers' (1997) hypothesis.

One variable that may have an important influence on the capacity of birds to thermo-regulate in very humid environments is the primary avenue for evaporative heat dissipation during heat exposure. Whereas passerines rely primarily on elevations in respiratory evaporative water loss (REWL) via panting, cutaneous evaporative water loss (CEWL) predominates in columbids. Gerson et al.'s (2014) data for one passerine and one columbid provided an opportunity to evaluate how the effects of high humidity may differ between taxa that vary in their reliance on these two avenues of heat dissipation. In Namaqua doves, overall rates of EWL were lower than in sociable weavers, but fractional decreases in EWL with increasing humidity were larger. These results suggest that whereas CEWL is generally a more effective way of dissipating heat evaporatively than panting, high humidity may impede CEWL to a greater extent than REWL, a difference that may have important implications for avian thermoregulation in humid environments (Gerson et al. 2014).

Another avenue of avian heat loss that may have considerable adaptive value in humid environments is heat radiation via the bill (Greenberg et al. 2012), particularly in large-billed tropical taxa such as toucans and hornbills (Tattersall et al. 2009; van de Ven et al. 2016). In the former taxon, heat loss via the bill may represent more than 60% of total heat production (Tattersall et al. 2009). However, the capacity of non-evaporative heat dissipation via the bill may also be strongly selected for in arid habitats to minimize water losses via evaporative cooling.

Conclusion

Resting white-browed sparrow-weavers were able to regulate T_b below lethal limits at T_a approaching and exceeding normothermic T_b across a range of humidities that exceeds what they experience naturally. The major effect of high humidity was to increase the metabolic cost of thermoregulation, with a consequent reduction in the efficiency of evaporative cooling at $T_a \geq 40$ °C. The effect of humidity on evaporative cooling differed qualitatively from that observed in species investigated previously, with the major effect in a passerine, a dove and a hummingbird being decreases in EWL rather than RMR (Powers 1992; Gerson et al. 2014). This variation among species suggests that although humidity has a general effect on avian thermoregulation in the heat of reducing evaporative cooling efficiency (i.e., EHL/MHP ratios), the mechanism whereby this occurs may vary among and within taxa.

The data currently available provide some insight into how evaporative cooling is constrained in species inhabiting habitats in which high T_a is combined with high humidity. Too few data currently exist, however, to test hypotheses about how selection arising from humid environments affects the evolution of avian thermal physiology. Although the number of species inhabiting humid, tropical habitats for which thermal physiological data exist has increased dramatically in recent years (e.g., McNab 2005; Londoño et al. 2015; Bushuev et al. 2017), most such studies have focused on variables such as BMR and normothermic T_b. Obtaining enough species data on interactions between humidity and thermoregulation at high T_b to meaningfully test hypotheses such as that formulated by Weathers (1997), that species in hot, humid environments have evolved greater tolerance of very high T_b in response to constraints on evaporative heat dissipation, will be logistically challenging. Such data are also a prerequisite for modeling the effects of increasing T_a associated with anthropogenic climate change on species in habitats such as lowland tropical forests, where a large fraction of global avian biodiversity occurs.

Abbreviations

EWL: Evaporative water loss
RMR: Resting metabolic rate
EHL: Evaporative heat loss
MHP: Metabolic heat production
M_b *:* Body mass
T_a *:* Air temperature
T_b *:* Body temperature
RER: Respiratory exchange ratio
VCO2VCO2 *:* Carbon dioxide production
VO2VO2 *:* Oxygen consumption

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