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ARTICLE

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Evolution of avian heat tolerance: The role of atmospheric humidity

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Abstract

The role of atmospheric humidity in the evolution of endotherms' thermoregulatory performance remains largely unexplored, despite the fact that elevated humidity is known to impede evaporative cooling capacity. Using a phylogenetically informed comparative framework, we tested the hypothesis that pronounced hyperthermia tolerance among birds occupying humid lowlands evolved to reduce the impact of humidity-impeded scope for evaporative heat dissipation by comparing heat tolerance limits (HTLs; maximum tolerable air temperature), maximum body temperatures ($T_b$max), and associated thermoregulatory variables in humid (19.2 g H_2O m^{-3}) versus dry (1.1 g H_2O m^{-3}) air among 30 species from three climatically distinct sites (arid, mesic montane, and humid lowland). Humidity-associated decreases in evaporative water loss and resting metabolic rate were 27%–38% and 21%–27%, respectively, and did not differ significantly between sites. Decreases in HTLs were significantly larger among arid-zone (mean $\pm$ SD $= 3.13 \pm 1.12$°C) and montane species (2.44 ± 1.0°C) compared to lowland species (1.23 ± 1.34°C), with more pronounced hyperthermia among lowland ($T_b\text{max} = 46.26 \pm 0.48$°C) and montane birds ($T_b\text{max} = 46.19 \pm 0.92$°C) compared to arid-zone species (45.23 ± 0.24°C). Our findings reveal a functional link between facultative hyperthermia and humidity-related constraints on evaporative cooling, providing novel insights into how hygric and thermal environments interact to constrain avian performance during hot weather. Moreover, the macrophysiological patterns we report provide further support for the concept of a continuum from thermal specialization to thermal generalization among endotherms, with adaptive variation in body temperature correlated with prevailing climatic conditions.

KEYWORDS
birds, body temperature, endotherm, evaporative water loss, heat tolerance limit, humidity, hyperthermia, thermoregulation

INTRODUCTION

The role of humidity in constraining evaporative heat dissipation has been extensively studied in humans (Coffel et al., 2018; Li et al., 2020; Raymond et al., 2020; Sherwood, 2018; Sherwood & Huber, 2010) but much less so in other endotherms. Impedance of evaporative heat dissipation by high humidity in laboratory settings has long been recognized (e.g., Lasiewski et al., 1966), and elevated humidity has been implicated in heat-related mortalities of birds and bats (e.g., McKechnie et al., 2021; Ratnayake et al., 2019; Welbergen et al., 2008). However, the ways in which humidity constrains evaporative cooling capacity and heat tolerance remain poorly understood, primarily because of the technical challenges associated with experimentally manipulating humidity in laboratory settings. Even less attention has been paid to the notion that environmental humidity acts as a source of selection on the thermal physiology of animals (Angilletta et al., 2010); most analyses of environmental correlates of inter- or intraspecific variation have focused on variables such as temperature, aridity, or primary productivity (Freeman et al., 2022; Lovegrove, 2003; Smit et al., 2013; White et al., 2007).

Birds are an ideal taxon for investigating the possibility of adaptive variation in thermal physiology (Angilletta et al., 2010; Boyles et al., 2011) correlated with humidity, on account of many species' small size combined with diurnal habits and activity often coinciding with high daytime environmental temperatures. In his seminal paper on thermoregulation in birds occupying hot, humid environments, Weathers (1997) hypothesized that small birds occupying open, lowland habitats in the tropics have evolved pronounced hyperthermia tolerance to compensate for humidity-imposed constraints on evaporative heat dissipation. By allowing body temperature (T_b) to increase to values as high as ~47°C, variable seedeaters (*Sporophila aurita*) maintain a positive gradient between T_b and air temperature (T_{air}), facilitating nonevaporative heat dissipation even at the high operative temperatures they experience in sunlit microsites (Weathers, 1997).

Experimental manipulations of humidity within metabolic chambers confirm that elevated humidity impedes evaporative heat dissipation and increases the costs of thermoregulation (Gerson et al., 2014; Powers, 1992; van Dyk et al., 2019). However, these studies focused on just one or two arid-zone species (Gerson et al., 2014; van Dyk et al., 2019) or were restricted to $T_{air} <$ normothermic body temperature ($T_b$norm; e.g., Powers, 1992). In a recent comparative analysis of heat tolerance and evaporative cooling capacity quantified under standardized conditions of very low humidity, Freeman et al. (2022) found that southern African birds occupying mesic climates, particularly humid coastal habitats, tolerated significantly higher maximum body temperature ($T_b$max; maximum T_b before rapid declines in performance, broadly analogous to critical thermal maximum in ectotherms [Lutterschmidt & Hutchison, 1997]) compared to arid-zone species. These findings support the notion of hyperthermia tolerance as a mechanism to mitigate the impeding effects of humidity on evaporative cooling.

Here, we test the hypothesis that pronounced hyperthermia tolerance among birds occupying humid lowlands evolved to reduce the impact of humidity-impeded scope for evaporative heat dissipation. We predicted that (1) compared to birds from less humid environments, reductions in heat tolerance limits (HTLs—highest T_{air} tolerated before the onset of severe hyperthermia) associated with high humidity are more modest among birds from humid lowlands and (2) variation in the effects of humidity on thermoregulation in heat arise primarily from larger increases in T_b above normothermic levels among birds from humid lowlands (Freeman et al., 2022; Weathers, 1997). In addition to body temperature and HTLs, we also quantified resting metabolic rate (RMR; hereafter, metabolic rate), evaporative heat loss, and differences in the T_{air} dependence of panting between humid and dry conditions. Our experimental approach involved comparing relationships between T_{air}, T_b, metabolic heat production, and evaporative heat dissipation at a humidity of ~19 g H_2O m^{-3} (equivalent to a dewpoint of 22.6°C and relative humidity of 37.2% at $T_{air} = 40$°C) to corresponding patterns at ~1 g H_2O m^{-3} (−4.6°C and 5.9%).

MATERIALS AND METHODS

Study areas

We collected data at three climatically distinct areas (hot arid, mesic montane and humid lowland) between latitudes of 27.90° and 30.04° S in South Africa (see Table 1 for general information and climatic data [Fick & Hijmans, 2017; Smit et al., 2011]).

Study species

We collected physiological data under either dry (~1 g H_2O m^{-3}) or humid conditions (~19 g H_2O m^{-3}). Overall, our analysis includes data from 627 individuals (humid, $n = 307$; dry, $n = 320$) representing 30 species, 15 families, and three orders—Passeriformes, Piciformes, and Coraciiformes. Humidity and dry protocol data were collected during the austral summer between

TABLE 1 Location and climate information of our three climatically distinct study areas (hot arid, mesic montane, and humid lowland) within South Africa.

	Hot arid	Mesic montane	Humid lowland
Location and coordinates	Kamiesberg Mountains, western South Africa 30°2′41.58″ S, 17°57′12.88″ E	Mooihoek farm, Harrismith, Free State province, South Africa 28°11′48.74″ S, 29°9′54.70″ E	Hluhluwe, southeastern South Africa 27°53′19.09″ S, 32°21′34.87″ E
Climate	Hot dry summers and cool wet winters	Cool wet summers and cold dry winters	Hot wet humid summers and moderate winters
Mean summer maximum T_{air}	28.4 ± 1.36°C	26.4 ± 1.50°C	30.0 ± 1.85°C
Mean summer minimum T_{air}	14.63 ± 2.60°C	10.3 ± 1.0°C	18.82 ± 1.03°C
Mean summer absolute humidity	$7.1 + 0.7$ g H_2O m^{-3}	10.0 ± 1.4 g H_2O m^{-3}	16.9 ± 1.4 g H_2O m^{-3}
Annual precipitation	~170–220 mm	~713 mm	~895 mm

January and February 2021 (arid-zone species [Appendix S1: Tables S3, S4 and S5a and S5b]) and September 2021 to February 2022 (montane species [Appendix S1: Tables S9 and S10] and lowland species [Appendix S1: Tables S6–S8]). Where available, data for species responses under dry air conditions at our montane and lowland sites were obtained from Freeman et al. (2022) (Appendix S1: Tables S1 and S2).

Air and body temperature measurements

A temperature-sensitive passive integrated transponder (PIT) tag (Biotherm 13, Biomark, Boise, ID, USA) was injected into the peritoneal cavity of each bird prior to the commencement of experimentation to measure T_b. Data from the PIT tags were acquired using a reader and transceiver system (HPR+, Biomark, Boise ID, USA). During experimentation, T_{air} within the metabolic chamber was measured using a thermistor probe (TC-100, Sable Systems, Las Vegas, NV, USA) inserted through a small hole in the side of the chamber and sealed by a rubber grommet.

Experimental protocol

We measured T_b, evaporative water loss, and resting metabolic rate using both the dry and humid protocols. Measurements typically lasted 2–4 h and began with a bird placed in a chamber at $T_{air} = 28$°C and given at least 1 h to habituate. For the dry protocol, T_{air} setpoints beginning from $T_{air} = 28$°C were initially increased incrementally by 4°C to $T_{air} = 40$°C and thereafter increased incrementally by 2°C until the birds reached their thermal endpoints, following Freeman et al. (2022). For the humid protocol, following habituation at 28°C, T_{air} was gradually increased to 34°C, above which setpoints were increased incrementally by 2°C until the birds reached their thermal endpoints. Although the initial T_{air} setpoints differed between dry (28°C) and humid (34°C) protocols, the initial habituation periods at $T_{air} = 28$°C prior to the commencement of data collection were identical and rates of heating similar between protocols. For these reasons, we do not think these differences between protocols had any effect on observed patterns of thermoregulation, particularly at T_{air} approaching the upper limits of thermoregulation. Transitions between successive T_{air} setpoints took 10–15 min. At each setpoint T_{air}, the birds were exposed to stable T_{air} and humidity for a minimum of 15–20 min until concentrations of CO_2 and H_2O were stable for at least 5 min. We used the stepped respirometry protocol involving brief (15–20 min) exposure to each T_{air} setpoint, which has been shown to yield patterns of evaporative water loss, metabolic rate, and T_b similar to those using a steady-state protocol where birds experience each T_{air} setpoint for several hours (Short et al., 2022).

Gas exchange measurements

Evaporative water loss and carbon dioxide production ($\dot{V}_{CO_2}$) were measured using an open flow-through respirometry system, with our setup identical to that described by Freeman et al. (2020, 2022) and described in full in Appendix S1: Section S1 of the supplementary material.

During dry air measurements, flow rates were adjusted to minimize water vapor pressure within the metabolic chamber (mean chamber humidity across sites = 1.07 ± 0.84 g H_2O m^{-3}) and varied between 3 and 24 L min^{-1}. Humidity measurements were made following methods similar to the dry protocol, with modifications to the respirometry setup permitting the manipulation of in-chamber humidity (see Appendix S1: Figure S1 and Section S1 for a detailed description). During our humidity measurements, mean chamber absolute humidity was 19.21 ± 1.20 g H_2O m^{-3} and varied by <1.5 g H_2O m^{-3} between sites.

Data analyses

Sample sizes (n) for dry or humidity treatments were generally $n = 10$ individuals per species, but lower for a few (Appendix S1: Table S1). All data were analyzed in the R version 4.0.5 (R Core Team, 2018) environment, using R Studio version 1.1.463 (RStudio, Inc.). For each species, the respective inflection T_{air} values above which T_{b}, evaporative water loss, the ratio of evaporative heat loss to metabolic heat production (EHL/MHP), and metabolic rate increased rapidly were identified using the R package segmented.lme (Muggeo, 2016), with individual identity included as a random effect to account for measurements at multiple T_{air} values per individual to avoid pseudoreplication. All response variables, including T_{b}, evaporative water loss, and metabolic rate, were analyzed above and below inflection points separately using linear mixed-effects models in the R package nlme (Pinheiro et al., 2015). Slopes for the relationships of thermoregulatory response variables were estimated as functions of T_{air}.

We used the "dredge" function in the MuMIn package to undertake model selection (Barton, 2019). The standardized model used for within-species analysis included T_{air} (or $T_{\mathrm{air}} - T_{\mathrm{b}}$), M_{b}, T_{air}–M_{b} interaction, and Bird ID (individual) as a random factor. The model with the highest rank among competing models was selected using Akaike information criterion values corrected for small sample size ($\mathrm{AIC_c}$) as well as the Akaike weights (Burnham & Anderson, 2002). If competing models were within $\Delta\mathrm{AIC_c} < 2$, we selected the most parsimonious model. Significance was assessed at $\alpha < 0.05$ and values are presented as mean ± SD.

Interspecific analyses

We accounted for the effect of phylogenetic nonindependence in observed patterns of our thermoregulatory response variables by constructing a maximum-likelihood tree including all study species using Mesquite (Maddison & Maddison, 2014). Making use of the Hackett phylogeny as a backbone (Hackett et al., 2008), we downloaded 100 phylogenies from www.birdtree.org (Jetz et al., 2012). Determining the necessary branch-length transformations was achieved by comparing an Ornstein-Uhlenbeck model (Martins & Hansen, 1997) with a Brownian motion model of trait evolution (Grafen, 1989) using AIC values. Lower AIC values were attained for the Brownian motion model, and it was therefore retained. We used Pagel's λ (Pagel, 1999) to test for phylogenetic signal in the residual error of our phylogenetic least squares (PGLS) regression models while simultaneously estimating regression parameters (Revell, 2010) and rescaled our models using the estimates of λ. When testing for λ we included mean M_{b} for species to account for the known allometric scaling of physiological traits such as basal metabolic rate (McKechnie & Wolf, 2004; McNab, 2002) and HTL (van Jaarsveld et al., 2021). We detected a significant phylogenetic signal for HTL ($\lambda = 0.50$), metabolic rate ($\lambda = 0.95$), evaporative water loss ($\lambda = 0.85$), and EHL_MHP ($\lambda = 0.47$) across climatic study sites, and we therefore present results from our PGLS regression analysis and phylogenetically informed post hoc tests (PhylANOVA). The results of conventional analysis (i.e., phylogenetic nonindependence not controlled for) are available in the supplementary material (Appendix S1: Table S11). Conventional analyses are mostly consistent with our findings following phylogenetic correction.

The R package caper (Orme et al., 2012), along with the "pgls" function, was used to conduct phylogenetic regression analyses. To detect patterns and quantify differences in HTLs between climatic study areas and determine which physiological variables were predictors of HTL patterns, we constructed a phylogenetically informed linear mixed-effects model (Appendix S1: Table S12). The MuMIn package and "dredge" function were again used to detect which model selection procedure best explained observed patterns of HTLs under humid conditions (Barton, 2019). Model selection was conducted using $\mathrm{AIC_c}$ values and weights. In addition to model selection, we ran analyses to detect autocorrelation among predictor variables (Appendix S1: Table S13, Durbin Watson test) and assessed the normality of residuals using a Shapiro–Wilk test. Model 328 (HTL ~ Climate + MaxEHL/MHP+ EvapScope + T_{b}max + MaxEHL/MHP: climate; Appendix S1: Table S12) was selected.

The "anova.pgls" function in the R package caper (Orme et al., 2012) was applied to our model output to determine the significance of predictor variables and assess whether the response variable differed significantly among study localities (Appendix S1: Table S11). We conducted post hoc multiple comparison tests taking into account phylogenetic relatedness using the "PhylANOVA" function in the R package phytools (Revell, 2012) to obtain pairwise differences in both response and predictor variables between climatic areas. The "PhylANOVA" function conducts a simulation-based phylogenetic ANOVA and performs all post hoc comparisons of means among groups providing a p-value by phylogenetic simulation (Garland et al., 1993).

RESULTS

HTLs and body temperature

Among the 30 species included in the study, HTLs in humid air (19 g H_2O m^{-3}) ranged from 42.3 to 49.5°C ($\bar{x} = 45.88 \pm 1.81$°C), whereas HTLs in dry air (~1 g H_2O m^{-3}) ranged from 43.3 to 50.9°C ($x = 48.02 \pm 1.55$°C) (Appendix S1: Figure S2). HTLs were significantly higher under dry compared to humid air conditions (t-test: $t = 8.71$, df = 31, $p < 0.001$). Our top model for HTL_{humid} (Appendix S1: Table S12; PGLS: $F_{7,24} = 20.89$, $p < 0.001$, $R^2 = 0.82$) revealed that climate ($F_{2,24} = 36.58$, $p < 0.001$), $T_b$max ($F_{1,24} = 8.74$, $p = 0.007$), maxEHL/MHP ($F_{1,24} = 34.50$, $p < 0.001$), EvapScope ($F_{1,24} = 19.29$, $p < 0.001$), and the interaction between climate and maxEHL/MHP ($F_{2,24} = 5.24$, $p = 0.013$) were significant predictors of HTLs under humid conditions.

Humid HTLs were significantly higher for lowland birds ($\bar{x} = 47.11 \pm 1.44$°C) compared to arid-zone ($\bar{x} = 44.37 \pm 1.50$°C; PhylANOVA: $t = 4.58$, df = 29, $p = 0.003$) or montane ($\bar{x} = 45.45 \pm 1.30$°C; PhylANOVA: $t = 2.67$, df = 29, $p = 0.012$) species, but they did not differ between arid and montane birds (PhylANOVA: $t = -1.72$, df = 29, $p = 0.071$) (Figure 1B and Appendix S1: Table S11). Changes in HTLs associated with humidity (i.e., $HTL_{humid} - HTL_{dry}$) were largest for arid-zone species ($\bar{x} = -3.13 \pm 1.12$°C), significantly greater than for lowland birds ($\bar{x} = -1.23 \pm 1.34$°C; PhylANOVA: $t = -3.71$, df = 29, $p = 0.003$), but not montane birds ($\bar{x} = -2.44 \pm 1.0$°C; PhylANOVA: $t = -1.21$, df = 29, $p = 0.212$) (Figure 1A). Humidity-associated decreases in HTLs were significantly smaller among lowland birds compared to montane birds (PhylANOVA: $t = 2.37$, df = 29, $p = 0.030$).

Phylogenetic analysis (PGLS: $F_{3,28} = 11.37$, $p < 0.001$, $R^2 = 0.55$) revealed a significant positive relationship for humid HTL ~$T_b$max ($F_{1,28} = 16.60$, $p = 0.003$; Figure 1A). Humid $T_b$max was significantly higher among lowland ($\bar{x} = 46.26 \pm 0.48$°C; PhylANOVA: $t = 4.10$, df = 29, $p = 0.003$) and montane species ($\bar{x} = 46.19 \pm 0.92$°C [excluding *Quelea quelea*, $\bar{x} = 45.88 \pm 0.07$°C]; PhylANOVA: $t = 3.43$, df = 29, $p = 0.003$) compared to arid-zone species ($\bar{x} = 45.23 \pm 0.24$°C). Lowland and montane humid $T_b$max did not differ significantly (PhylANOVA: $t = 0.32$, df = 29, $p = 0.773$) even when

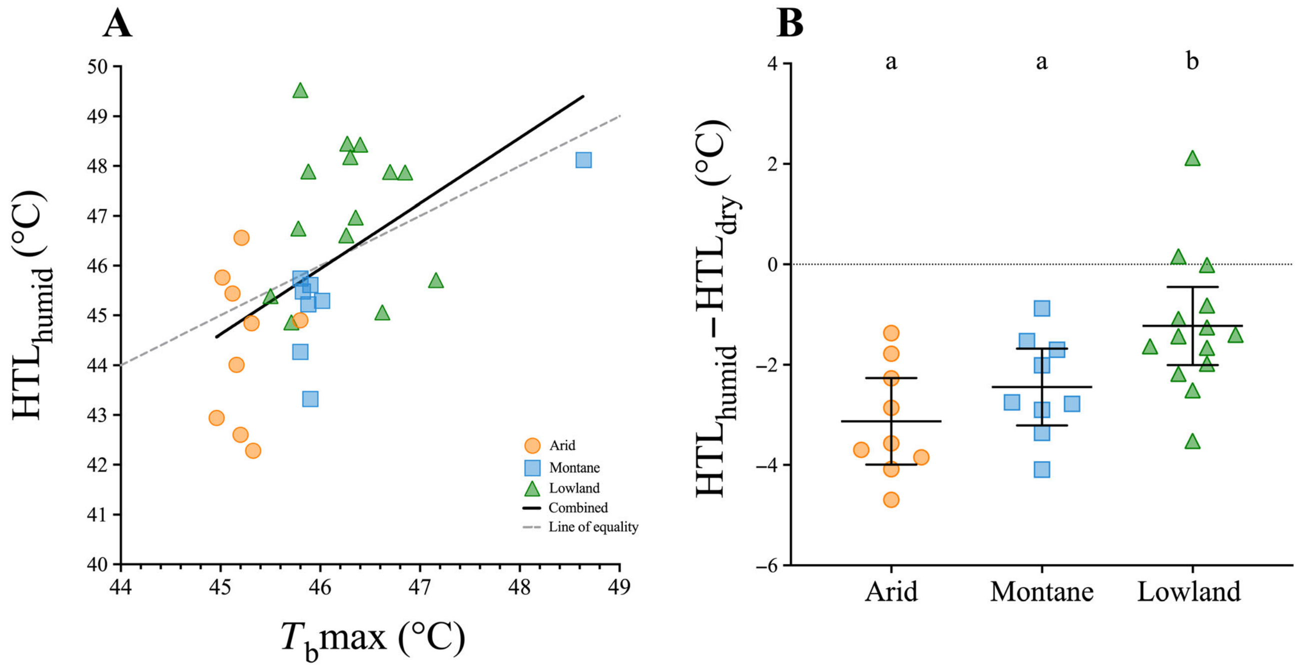

FIGURE 1 (A) Phylogenetic least squares (PGLS) regression analysis of the relationships between heat tolerance limit (HTL—highest T_{air} tolerated before onset of severe hyperthermia) and $T_b$max (maximum T_b before rapid declines in performance) under humid conditions (~19 g H_2O m^{-3}) among 30 southern African bird species. Phylogenetic regression analysis revealed a positive relationship between HTL and $T_b$max under humid conditions and a significant interaction between climatic area and $T_b$max. (B) Phylogenetic comparative analysis for differences in HTL (humid–dry) for birds subjected to a stepped respirometry protocol under dry (~1 g H_2O m^{-3}) or humid (~19 g H_2O m^{-3}) conditions in three climatically distinct regions. Climate categories are hot arid (orange circles, $n = 9$), mesic montane (blue squares, $n = 9$), and humid lowland (green triangles, $n = 14$). Horizontal lines represent mean values, and vertical lines show 95% CIs. Letters above plots denote significant differences ($\alpha < 0.05$) in HTL (humid–dry) between climatic areas. Decreases in HTL in response to humidity were significantly less among lowland birds compared to species from the mesic montane and arid climatic areas.

excluding *Q. quelea* from the montane data set (PhylANOVA: $t = 0.32$, df = 28, $p = 0.740$).

The difference in normothermic T_b (i.e., $T_b\text{norm}_{(\text{humid})} - T_b\text{norm}_{(\text{dry})}$) for lowland species ($\bar{x} = 40.31 \pm 0.43°\text{C}$) was significantly lower than for arid ($\bar{x} = 41.48 \pm 0.43°\text{C}$; PhylANOVA: $t = -6.11$, df = 29, $p = 0.003$) or montane ($\bar{x} = 41.80 \pm 0.49°\text{C}$; PhylANOVA: $t = -7.81$, df = 29, $p = 0.003$) species. The difference in normothermic T_b among arid-zone and montane birds did not differ significantly (PhylANOVA: $t = 1.53$, df = 29, $p = 0.106$) (Appendix S1: Figure S3 and Table S11). Fractional changes (i.e., humid/dry) in the slope of T_b change above thermoneutrality (i.e., T_bSlope) at our arid (34% increase; $\bar{x} = 1.34 \pm 0.16°\text{C}\ T_b °\text{C}\ T_{air}^{-1}$), montane (28% increase; $\bar{x} = 1.28 \pm 0.32\ T_b °\text{C}\ T_{air}^{-1}$), and lowland sites (26% increase; $\bar{x} = 1.26 \pm 0.24\ T_b °\text{C}\ T_{air}^{-1}$) did not vary significantly (Appendix S1: Figure S4 and Table S11).

Evaporative water loss

Maximum evaporative water loss decreased substantially among arid-zone (~26% decrease), montane (~39% decrease), and lowland birds (~37% decrease) under humid compared to dry conditions (Figure 2A). However, fractional differences (humid/dry) in maximum evaporative water loss did not vary significantly among arid-zone ($\bar{x} = 0.74 \pm 0.19$), montane ($\bar{x} = 0.61 \pm 0.13$; PhylANOVA: $t = 1.58$, df = 29, $p = 0.249$), and lowland ($\bar{x} = 0.62 \pm 0.15$; PhylANOVA: $t = 1.63$, df = 29, $p = 0.342$) species (Appendix S1: Table S11). Similarly, fractional changes in EvapScope (maximum evaporative water loss/minimum evaporative water loss) decreased among arid (~19% decrease; $\bar{x} = 0.81 \pm 0.24$), montane (~16% decrease; $\bar{x} = 0.84 \pm 0.28$), and lowland birds (~30% decrease; $\bar{x} = 0.70 \pm 0.26$) during the humidity protocol but did not vary significantly between our three climatic study sites (Appendix S1: Table S11). Changes in the rate of evaporative water loss (i.e., EWL slope) with increasing T_{air} associated with humidity were significantly smaller among arid-zone birds ($\bar{x} = 0.92 \pm 0.23$ [8% decrease]) compared to montane ($\bar{x} = 0.61 \pm 0.18$ [39% decrease]; PhylANOVA: $t = 3.50$, df = 29, $p = 0.003$) and lowland birds ($\bar{x} = 0.57 \pm 0.15$ [43% decrease]; PhylANOVA: $t = 4.25$, df = 29, $p = 0.004$) (Figure 2C).

Under humid conditions, compared with arid-zone birds ($\bar{x} = 0.06 \pm 1.20°\text{C}$), a significant change in T_{air} associated with the onset of panting occurred for montane ($\bar{x} = -1.98 \pm 1.24°\text{C}$; PhylANOVA: $t = -3.54$, df = 29, $p = 0.003$) and lowland ($\bar{x} = -1.70 \pm 0.70°\text{C}$; PhylANOVA: $t = -3.32$, df = 29, $p = 0.012$) species, but no significant difference was detected between lowland and montane birds (PhylANOVA: $t = 0.59$, df = 29, $p = 0.562$). Differences in T_b associated with the onset of panting between dry and humid treatments for arid-zone species ($\bar{x} = 0.24 \pm 0.57°\text{C}$) did not differ significantly from those of montane ($\bar{x} = 0.50 \pm 0.51°\text{C}$; PhylANOVA: $t = -0.79$, df = 29, $p = 0.408$) or lowland birds ($\bar{x} = -0.31 \pm 0.45°\text{C}$; PhylANOVA: $t = 2.38$, df = 29, $p = 0.112$). However, panting commenced at significantly lower T_b among lowland species compared to montane species (PhylANOVA: $t = -3.24$, df = 29 $p = 0.003$) (Appendix S1: Figure S7 and Table S11).

Maximum resting metabolic rate

Under humid conditions, maximum metabolic rate decreased among species from our arid (~22% decrease; $\bar{x} = 0.78 \pm 0.21$ W), montane (~27% decrease; $\bar{x} = 0.73 \pm 0.14$ W), and lowland (~21% decrease; $\bar{x} = 0.79 \pm 0.17$ W) sites. Changes in maximum metabolic rate did not differ significantly between arid and montane (PhylANOVA: $t = 0.58$, df = 29, $p = 1.000$) or lowland (PhylANOVA: $t = -0.14$, df = 29, $p = 1.000$) species. Similarly, changes in maximum metabolic rate were similar between lowland and montane (PhylANOVA: $t = 0.78$, df = 29, $p = 1.000$) species.

Differences in metabolic rate slope (humid/dry—Figure 2D) for our arid (~1% decrease; $\bar{x} = 0.99 \pm 0.36\ \text{W}\ °\text{C}^{-1}$), montane (~4% increase; $\bar{x} = 1.04 \pm 0.70\ \text{W}\ °\text{C}^{-1}$), and lowland (~8% decrease; $\bar{x} = 0.92 \pm 0.43\ \text{W}\ °\text{C}^{-1}$) species were small and did not vary significantly with climate (Appendix S1: Table S11). Differences in MetabCost for arid (~3% increase; $\bar{x} = 1.03 \pm 0.34$), montane (~3% increase; $\bar{x} = 1.03 \pm 0.18$), and lowland (~1% increase; $\bar{x} = 1.01 \pm 0.21$) birds were negligible and did not differ significantly among study areas (Appendix S1: Figure S6C and Table S11).

Maximum evaporative cooling efficiency

Maximum humid EHL/MHP did not differ significantly between species from our arid ($\bar{x} = 1.18 \pm 0.19$) and montane ($\bar{x} = 1.31 \pm 0.12$; PhylANOVA: $t = -1.16$, df = 29, $p = 0.678$) or lowland sites ($\bar{x} = 1.19 \pm 0.34$; PhylANOVA: $t = -0.19$, df = 29, $p = 0.876$). No significant difference in humid MaxEHL/MHP was detected between lowland and montane sites (PhylANOVA: $t = -1.09$, df = 29, $p = 0.620$). Fractional differences in MaxEHL/MHP (humid/dry) revealed decreases under humid conditions for arid (~16% decrease; $\bar{x} = 0.84 \pm 0.13$), lowland (~18% decrease; $\bar{x} = 0.82 \pm 0.24$), and montane (~17% decrease; $\bar{x} = 0.83 \pm 0.09$) species (Figure 3A). Subsequent decreases in MaxEHL/MHP did not differ significantly between climatic areas (Appendix S1: Table S11).

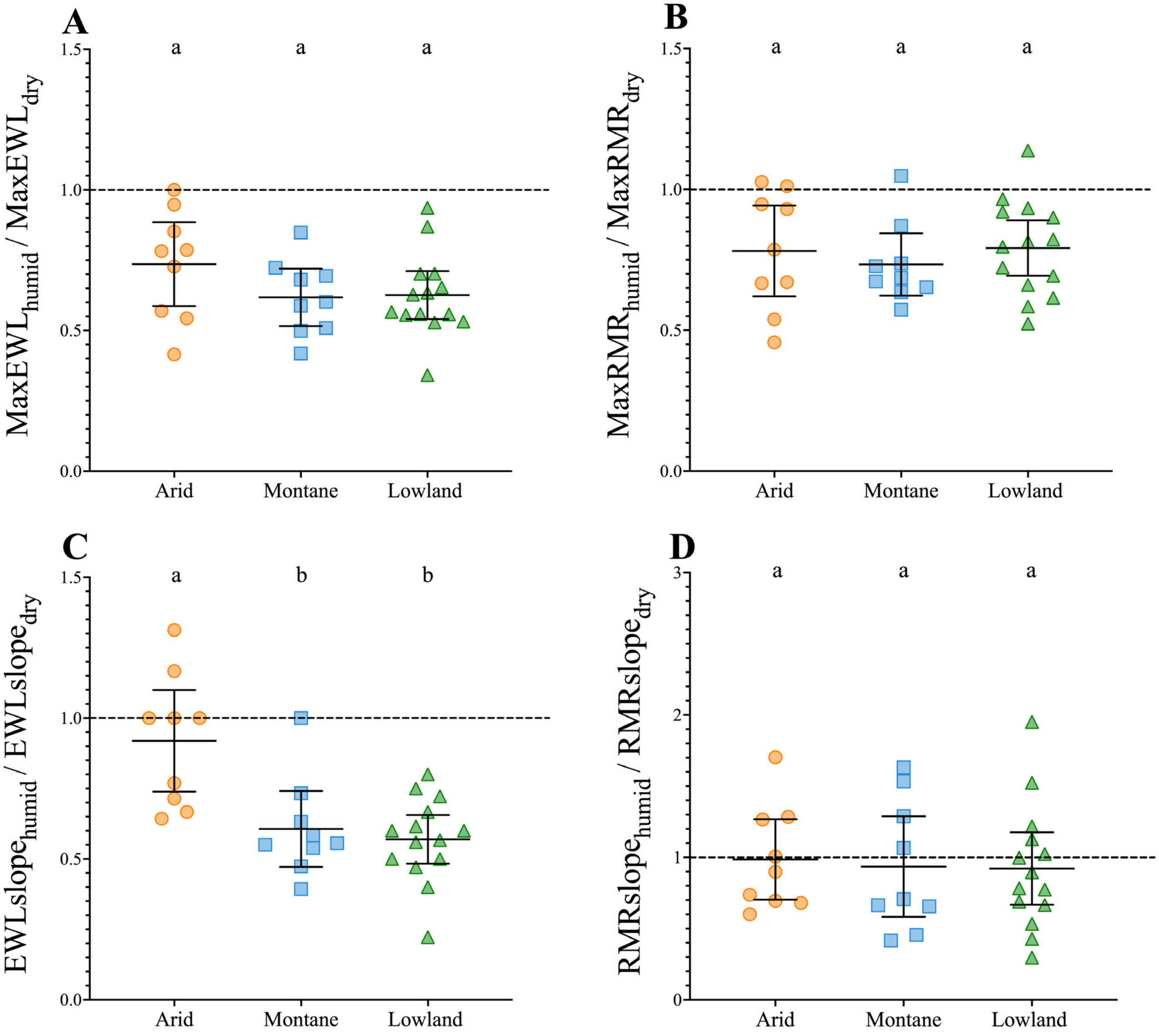

FIGURE 2 (A–D) Proportional changes (humid/dry) in maximum evaporative water loss (MaxEWL) (A), maximum resting metabolic rate (MaxRMR) (B), rate of change in evaporative water loss above inflection (EWLslope) (C), and rate of change in resting metabolic rate above thermoneutral values (RMRslope) (D) among 30 South African bird species inhabiting hot arid (orange circles, $n = 9$), mesic montane (blue squares, $n = 9$), or humid lowland (green triangles, $n = 14$) climates. Horizontal lines represent mean values, and vertical lines show 95% CIs. Letters above plots denote significant differences ($\alpha = 0.05$) as identified using phylogenetic ANOVA post hoc multiple comparison assessments. Maximum evaporative water loss and metabolic rate decreased across climatic areas under humid conditions, but differences in values under dry conditions and humid conditions did not differ significantly between climatic areas. Changes in rates of evaporative water loss under humid conditions were significantly lower for arid birds compared to lowland and montane birds, which did not differ. No significant differences were detected in proportional changes in rates of metabolic rate and slopes were similar between dry and humid assessments (A–D). RMR, resting metabolic rate.

DISCUSSION

Our data reveal that thermoregulatory performance during acute heat exposure is less affected by high atmospheric humidity among birds occupying humid lowlands than among species inhabiting environments characterized by drier air. Consistent with our predictions, the effects of raised humidity on heat tolerance were modest among lowland birds relative to their arid counterparts. The reduced sensitivity of lowland species arises primarily from a greater scope for increasing T_b above normothermic levels, supporting Weathers' (1997) hypothesis that birds in hot, humid environments have evolved pronounced hyperthermia tolerance to compensate for

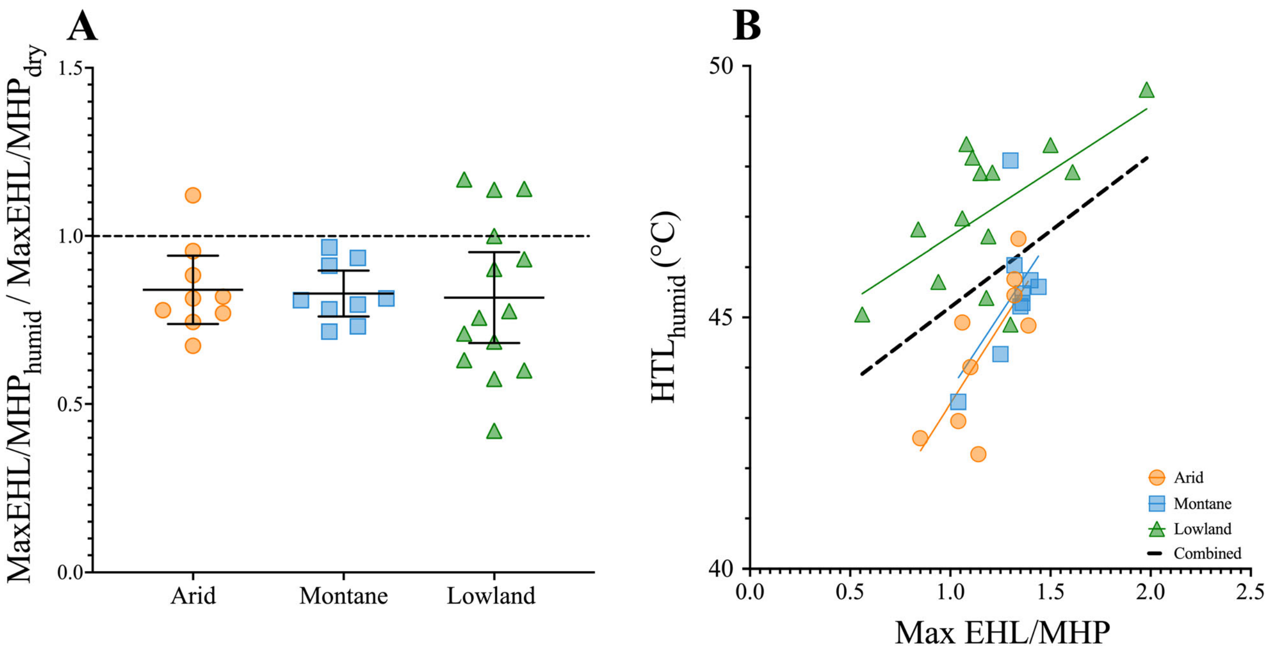

FIGURE 3 Proportional changes (humid/dry) in the maximum ratio of evaporative heat loss (EHL) and metabolic heat production (MHP) (MaxEHL/MHP) (A) among 30 southern African bird species. MaxEHL/MHP decreased in all climatic areas but differences did not differ significantly between sites. Horizontal lines represent mean values and vertical lines 95% CIs. Letters above plots denote significant differences ($\alpha < 0.05$) in MaxEHL/MHP values between sampling localities. Significant differences are derived from phylogenetic analysis of variance post hoc multiple comparison assessments and conventional Tukey multiple comparison assessments regressions. (B) Phylogenetic least squares regression analysis of relationships between heat tolerance limit (HTL) and $T_b$max under humid conditions (~19 g H_2O m^{-3}). Under humid conditions increases in MaxEHL/MHP were associated with an increase in HTL.

constraints on evaporative heat dissipation. More pronounced hyperthermia, combined with lower normothermic body temperatures, minimized the impact of humidity on HTLs of lowland birds. In contrast, arid-zone birds performed relatively poorly under humid conditions, with HTLs reduced by >3°C compared to dry air (Figure 1B).

Across all three bird assemblages we sampled, high humidity was associated with large reductions in both maximum evaporative water loss (27%–38% decrease) and maximum metabolic rate (21%–27% decrease). The latter observation contrasts with a recent study revealing large increases in metabolic rate (~40% at $T_{air} = 40$°C) under humid conditions in an arid-zone passerine (van Dyk et al., 2019). Instead, the blunting of evaporative water loss under humid conditions we observed (Figure 2A) was similar to patterns documented previously (e.g., Gerson et al., 2014; Powers, 1992) As a consequence of the reductions in both evaporative water loss and metabolic rate at high humidity, decreases in maximum EHL/MHP (16%–18%) across all three study sites were unexpectedly modest. Moreover, fractional increases in the slope of T_b as a function of T_{air} under humid conditions were similar across study sites, further underscoring the functional importance of adaptive variation in hyperthermia tolerance as the primary mechanism underlying the observed differences in the effect of humidity on HTLs.

Our data also suggest the capacity for anticipatory responses to high humidity may vary with climate. The smaller decreases in the slope of evaporative water loss as a function of T_{air} among arid-zone species compared to montane or lowland species (Figure 2C) appeared to be driven by inflections at lower T_{air} in montane (~2.7°C lower) and lowland (~1.2°C lower) species under humid conditions, whereas inflections for evaporative water loss in arid-zone species remained virtually unchanged (~0.1°C higher). The among-site variation slope of evaporative water loss corresponds to changes in T_{air} associated with the onset of panting, where montane ($\bar{x} = 1.97 \pm 1.24$°C lower) and lowland ($\bar{x} = 1.70 \pm 0.70$°C lower) species commenced panting at significantly lower T_{air} compared to arid birds ($\bar{x} = 0.06 \pm 1.20$°C higher) under humid conditions (Appendix S1: Figure S8). In other words, an anticipatory response involving the commencement of panting at lower T_{air} under humid conditions was evident among lowland and montane birds but absent in arid-zone birds. Initiating panting and maximizing evaporative cooling at lower T_{air} under humid conditions may delay increases in T_b and, hence, the onset of a hyperthermic state, likely maximizing thermal safety margins under conditions where evaporative heat dissipation is impeded. This notion is further supported by lowland birds initiating panting at significantly lower T_b than montane birds, delaying increases in T_b for as long as possible. Such differences may also explain why changes in HTLs between humid and dry conditions were smaller among lowland birds compared to montane birds, which also displayed pronounced hyperthermia tolerance relative to arid-zone birds. Moreover, differences between lowland and montane birds in terms of changes in HTLs between humid and dry conditions may also result from reduced normothermic T_b among lowland birds compared to their montane or arid-zone counterparts.

Previous authors suggested that lowered basal metabolic rate was advantageous for birds occupying hot and humid environments (Bartholomew et al., 1962; Weathers, 1979), with reduced endogenous heat production among tropical species thought to extend foraging periods when exposed to high operative temperatures (Weathers, 1997). In our study, reductions in minimum ([~24% decrease] [Appendix S1: Figure S8]) and maximum metabolic rate ([~23% decrease] [Figure 2B]) were observed under humid conditions across climatic areas. Reduced metabolic rate under humid conditions, particularly at thermoneutral T_{air}, raises the possibility of active metabolic suppression (Weathers, 1979, 1981). Whereas suppression of avian metabolism at hyperthermic T_b remains poorly understood, findings such as O'Connor et al.'s (2017) observation that in rufous-cheeked nightjars (*Caprimulgus rufigena*) an increase in T_b of 2.2°C was associated with only a 5% increase in metabolic rate (compared to the expected 17%–28% increase if $Q_{10} = 2–3$) highlight the potential thermoregulatory benefits of this phenomenon. Among small mammals, metabolic suppression during heat exposure appears important in hot tropical environments (e.g., Lovegrove et al., 2014; Reher & Dausmann, 2021).

Although pronounced hyperthermia tolerance and lower normothermic body temperature provide lowland species with a wider thermal safety margin, recent and anticipated future increases in T_{air} nevertheless pose a serious thermal challenge (IPCC, 2021). Exposure to increasingly frequent heat events is exacerbated by the rapid loss of shady natural vegetation in tropical landscapes (Jewitt et al., 2015; Pinto et al., 2010; Walsberg, 1993), reducing the availability of cool microrefugia to which animals can retreat during extreme heat (Kim et al., 2022). Moreover, many lowland species are year-round residents and cannot move to higher elevations (Şekercioğlu et al., 2008). It appears unlikely that many endotherms will have sufficient time to adapt to rapidly changing climates (Loarie et al., 2009), and increasing T_{air} coupled with raised humidity may render large areas uninhabitable (Sherwood & Huber, 2010). Elevated humidity is thought to have contributed to recent heat-related mass mortality events involving both birds and bats (McKechnie et al., 2021; Ratnayake et al., 2019; Welbergen et al., 2008b). Humidity and heat may also indirectly alter activity and behavioral regimes (Speakman & Król, 2010) of endotherms, with consequences for breeding success and fitness (Walsberg, 1993). For a given T_{air}, mortality events are more likely under humid conditions. Although arid-zone species are unlikely to experience prevailing combinations of humidity and T_{air} similar to experimental values in this study, even lower humidities well below our upper experimental value coupled with very hot weather have the potential to prove lethal. For example, a bat and bird mass mortality event in South Africa involved humidity of 12.4 g H_2O m^{-3} and $T_{air} = {\sim}45°C$ (McKechnie et al., 2021). Previous analyses assessing the risk of high T_{air} within arid-zones, however, focused solely on dry air conditions and did not consider the effects of raised humidity for both chronic and acute heat exposure (Conradie et al., 2019; Ma et al., 2023; Riddell et al., 2019).

There are several limitations to our study. First, comparing thermoregulatory responses of just one very low humidity setpoint (~1 g H_2O m^{-3}) to one relatively high value (~19 g H_2O m^{-3}) could potentially mask important nonlinear effects of humidity on evaporative cooling. Our experimental humidity treatment of ~19 g H_2O m^{-3} was characteristic of summer conditions at our humid lowland site (~18.4 g H_2O m^{-3}—mean for January/February), similar to the highest monthly averages for regions such as the Amazon (~21.6 g H_2O m^{-3}—May) and Congo (~19.3 g H_2O m^{-3}—April) basins, Southeast Asia lowlands (~21.9 g H_2O m^{-3}—May), and Panama (~20.2 g H_2O m^{-3}—July) (Fick & Hijmans, 2017). A second limitation is that most species in our data set were passerines, which rely on panting as their primary avenue of evaporative heat dissipation. Data on the effects of humidity on species from other orders that make use of more efficient evaporative cooling mechanisms such as gular flutter (e.g., Czenze et al., 2021) are needed to evaluate phylogenetic variation in sensitivity to extreme T_{air} coupled with humidity.

Conclusions

The pronounced hyperthermia tolerance, lowered normothermic body temperature, and limited effects of humidity on the upper limits of heat tolerance among lowland species and, to a lesser extent, montane species contrast with the limited hyperthermia tolerance and large reductions in HTLs among arid-zone species, reinforcing the idea that past climate has shaped avian thermal physiology, including T_b (Freeman et al., 2022). The lack of physiological adjustments to accommodate humidity effects at high T_{air} among arid-zone species suggests thermal specialization, whereas lowland and montane species displayed traits indicative of thermal generalization (e.g., hyperthermia tolerance, earlier onset of panting). These findings support the idea of a continuum from thermal specialization to thermal generalization among endotherms (Angilletta et al., 2010; Boyles et al., 2011; Freeman et al., 2022). Instead of being a nonadaptive constant (Scholander et al., 1950), avian T_b appears instead to have been shaped by selection in similar ways to rates of metabolism and evaporative water loss. Our results also reiterate the need for empirical data on inter- and intraspecific variation in species physiological limits for predicting species vulnerability to climate change, including the parameterization of biophysical models (Briscoe et al., 2023; Wolf & McKechnie, 2023).

AUTHOR CONTRIBUTIONS

All authors contributed critically to the drafts and gave final approval for publication. M. T. F. and A. E. M. designed the study. M. T. F., B. C., J. C. S., C. A. N., and M. O. M. collected data. M. T. F. analyzed data. M. T. F. and A. E. M. led the writing of the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data (Freeman et al., 2024) are available in Dryad at https://doi.org/10.5061/dryad.pg4f4qrxb.

ETHICS STATEMENT

This work was approved by the Animal Ethics Committee of the University of Pretoria (protocol NAS141/2020) and the Research and Scientific Ethics Committee of the South African National Biodiversity Institute (SANBI NZG/RES /P19/13). Birds were captured under Permit JM 8057/2019 from the Free State province’s Department of Economic, Small Business Development, Tourism and Environmental. At our lowland site, research was conducted and birds were captured under Permit OP42-2022 from the EZEMVELO KZN wildlife office. Also, at our arid site, research was conducted under authorization from the Northern Cape government (Permit No. FAUNA 0010/2021).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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