Seasonal and geographical variation in heat tolerance and evaporative cooling capacity in a passerine bird

Matthew J. Noakes[a]
Blair O. Wolf [b]
Andrew E. McKechnie[a,*]

[a] DST-NRF Centre of Excellence at the Percy FitzPatrick Institute, Department of Zoology and Entomology, University of Pretoria, Pretoria 0002, South Africa
[b] UNM Biology Department, University of New Mexico, MSC03-2020, Albuquerque, NM 87131-0001, USA

[*] Corresponding author:
aemckechnie@zoology.up.ac.za, tel: +27124203232, fax: +27123625242

Abstract

Intraspecific variation in avian thermoregulatory responses to heat stress has received little attention, despite increasing evidence that endothermic animals show considerable physiological variation among populations. We investigated seasonal (summer *versus* winter) variation in heat tolerance and evaporative cooling in an Afrotropical ploceid passerine, the white-browed sparrow-weaver (*Plocepasser mahali*; ~ 47 g) at three sites along a climatic gradient with more than 10 °C variation in mid-summer maximum air temperatures (T_a). We measured resting metabolic rate (RMR) and total evaporative water loss (TEWL) using open flow-through respirometry, and core body temperature (T_b) using passive integrated transponder tags. Sparrow-weavers were exposed to a ramped profile of progressively higher T_a between 30 °C – 52 °C to elicit maximum evaporative cooling capacity ($n = 10$ per site per season); the maximum T_a birds tolerated before the onset of severe hyperthermia ($T_b \approx 44$ °C) was considered their hyperthermia threshold T_a (HTT_a). Our data reveal significant seasonal acclimatisation of heat tolerance, with a desert population of sparrow-weavers reaching significantly higher T_a in summer (49.5 ± 1.4 °C; i.e., higher HTT_a) than winter (46.8 ± 0.9 °C), reflecting enhanced evaporative cooling during summer. Moreover, desert sparrow-weavers had significantly higher heat tolerance and evaporative cooling capacity during summer compared to populations from more mesic sites (HTT_a = 47.3 ± 1.5 and 47.6 ± 1.3 °C). A better understanding of the contributions of local adaptation *versus* phenotypic plasticity to intraspecific variation in avian heat tolerance and evaporative cooling capacity is needed for modelling species' responses to changing climates.

Key-words: Acclimatisation, heat stress, hyperthermia, evaporative water loss, arid-zone birds

Summary statement

Heat tolerance and evaporative cooling capacity in a passerine bird varies among populations, with individuals at a hot arid site showing significant seasonal acclimatisation.

Introduction

Birds inhabiting hot environments where air temperature (T_a) regularly exceeds normothermic body temperature (T_b) face physiological and behavioural challenges related to the avoidance of lethal hyperthermia and maintenance of water balance (Cade, 1965; Dawson and Bartholomew, 1968; Williams and Tieleman, 2005). These challenges are often manifested as consequential trade-offs affecting behaviour, body condition and reproductive decisions (e.g., du Plessis et al., 2012; Smit et al., 2013; Tieleman et al., 2008), which are likely to be strongly affected by the increases in maximum T_a (T_{max}) and duration and frequency of extreme heat waves predicted to occur during the 21st century as a result of anthropogenic climate change (Intergovernmental Panel on Climate Change, 2012).

Reports of intraspecific variation in physiological traits are providing increasing evidence that the thermal physiology of endotherms is far more flexible than was previously recognised, and that conspecific populations may vary substantially in their thermoregulatory physiology, even over relatively small climatic gradients (e.g., Glanville et al., 2012; Smit et al., 2013). Understanding intraspecific variation in heat tolerance and evaporative cooling capacity, and the relative contributions of local adaptation *versus* phenotypic plasticity to -inter-population differences, is directly relevant to testing the assumption implicit in climate-envelope modelling studies that species cannot occupy habitats hotter than those within which they currently occur (Boyles et al., 2011; Pearson and Dawson, 2003).

Evaporative water loss (EWL) is the only avenue of heat dissipation in birds when T_a exceeds normothermic T_b, and many birds have the capacity to maintain T_b significantly below T_a when necessary (Crawford and Schmidt-Nielsen, 1967; Whitfield et al., 2015; Williams and Tieleman, 2005). The relative importance of respiratory and cutaneous evaporation for thermoregulation at high T_a varies among taxa, with increases in respiratory evaporative water loss by panting being the predominant mechanism in all passerine birds investigated to date (Ro and Williams, 2010; Tieleman and Williams, 2002; Wolf and Walsberg, 1996). Panting requires an increase in ventilation rate (Calder and Schmidt-Nielsen, 1967; Dawson, 1982), and as T_a increases above the upper critical limit of thermoneutrality (T_{uc}), a concomitant increase in metabolic rate is typically observed (e.g., Ambrose et al., 1996; Tieleman et al., 2002a; Trost, 1972; Williams, 1999).

Little is known about how heat tolerance and evaporative cooling capacity vary among and within avian species, as the majority of studies have focused on resting metabolic rate (RMR) and/or EWL at $T_a < T_b$ (e.g., Dawson, 1982; Tieleman et al., 2002a; Williams, 1996). For example, a review comparing the physiological responses of 102 avian species demonstrated that desert birds have lower EWL at moderate T_a than mesic species (Williams, 1996), a response thought to be adaptive by conserving water and reducing heat production in birds inhabiting hot environments with scarce drinking water and low primary productivity (Tieleman et al., 2002a; Williams and Tieleman, 2000). Recently, Whitfield et al. (2015) quantified variation in the upper limits of heat tolerance and evaporative cooling capacity among three ploceid passerines varying approximately four-fold in body mass (M_b). These authors found that the maximum T_a tolerated during acute heat exposure was positively related to M_b, ranging from ~ 48 °C in the 10-g scaly-feathered weaver (*Sporopipes squamifrons*) to ~ 54 °C in the 40-g white-browed sparrow-weaver (*Plocepasser mahali*). In one of the few studies to examine intraspecific variation in variables related to heat tolerance, Trost (1972) found that the physiological responses of desert and mesic horned larks (*Eremophila alpestris*) were indistinguishable at T_a < 45 °C, but at T_a = 45 °C the desert population had significantly lower total EWL (TEWL; ~ 18 % lower) and RMR (~ 32 % lower) than their mesic conspecifics.

Seasonal acclimatisation of physiological responses is a form of phenotypic flexibility (*sensu* Piersma and Drent, 2003) and has been well studied at thermoneutral and low T_a. In temperate-zone birds, well-documented examples involve the up-regulation of basal metabolic rate (BMR) and summit metabolism during winter, compared to summer (reviewed by McKechnie, 2008; McKechnie and Swanson, 2010; Swanson, 2010). In contrast, we are aware of only one study in which seasonal acclimatisation of avian physiological responses at $T_a > T_{uc}$ was reported (Tieleman et al., 2002b). These authors demonstrated that captive-bred houbara bustards (*Chlamydotis macqueenii*) had significantly higher RMR at T_a = 35 °C and 50 °C (~ 23 % higher), and TEWL at T_a = 35 °C (~ 46 % higher) during winter than in summer (Tieleman et al., 2002b). It has been hypothesised that greater phenotypic flexibility confers adaptive advantages to organisms that inhabit temporally heterogenous environments (Schlichting and Pigliucci, 1998). A number of studies involving short-term thermal acclimation experiments were designed to address whether a correlation exists between the magnitude of phenotypic flexibility of avian physiological responses and environmental aridity (Cavieres and Sabat, 2008; Tieleman et al., 2003b; Tieleman and Williams, 2002), but these focused on responses at thermoneutral T_a values.

The first step towards understanding the roles of local adaptation and phenotypic plasticity is to quantify variation among populations along climatic gradients. We therefore investigated seasonal and geographical variation in thermoregulatory responses to high T_a in a widespread southern African passerine bird, the white-browed sparrow-weaver

(*Plocepasser mahali*). Three populations were chosen along a climatic gradient ranging from areas where T_{max} are well below normothermic T_{b} (two mesic sites), to a desert site where T_{max} routinely exceeds normothermic T_{b} in summer. We hypothesised that heat tolerance and evaporative cooling capacity varies among populations in a manner correlated with T_{max}, and that seasonal acclimatisation of these physiological variables occurs at sites with pronounced seasonal variation in T_{max}. Specifically, we predicted that evaporative cooling is more efficient in individuals that routinely experience T_{a} exceeding normothermic T_{b} than in those that do not, with greater evaporative cooling efficiency associated with more gradual increases in T_{b}, EWL and/or RMR at $T_{\mathrm{a}} > T_{\mathrm{b}}$. We define the efficiency of evaporative cooling as the ratio of evaporative heat loss to metabolic heat production (EHL / MHP). We also predicted that sparrow-weavers from hot regions tolerate higher T_{a} during acute heat exposure compared to conspecifics from cooler sites.

Materials and methods

Study species and sites

The white-browed sparrow-weaver (*Plocepasser mahali*; hereafter, sparrow-weaver) is a ploceid passerine widespread across southern Africa (du Plessis, 2005). We examined thermoregulation in sparrow-weavers during the austral winter (July – August 2013 and 2014) and summer (January – February 2014) at three study sites in South Africa that vary in seasonal temperature extremes: one arid site near Askham in the Kalahari Desert (Northern Cape Province), and two mesic sites at Frankfort (Free State Province) and Polokwane (Limpopo Province; Table 1). These sites were all within the distributional range of the subspecies *P. mahali mahali* (du Plessis, 2005).

Climate data were obtained from the South African Weather Service using the weather station closest to each study site. There are weather stations at Polokwane (~ 9.1 km north of our study site) and Frankfort (~ 1.4 km away), but the nearest station to Askham is at Twee Rivieren (~ 62 km northwest; Table 1). For each site, we extracted mean daily T_{max} and temperature minimum (T_{min}) values over the hottest summer month (January) and coldest winter month (July) during the season we collected data (Table 1). The highest T_{max} values occurred at Twee Rivieren (~ Askham; ~ 10 °C higher than Polokwane), as well as the most pronounced seasonal variation in T_{min} and T_{max} values (~ 14 °C higher T_{max} in summer than winter; Table 1). There was also pronounced seasonal variation in T_{a} extremes at Frankfort, but with comparatively milder summer T_{max} values (Table 1). In contrast, Polokwane had relatively mild summers and winters (Table 1).

Table 1. Mean daily air temperature minimum (T_{min}) and maximum (T_{max}; mean ± SD) during the hottest summer month (January) and coldest winter month (July) at three study sites in South Africa, calculated from weather data obtained from South African Weather Service stations.

Study sites	**Daily T_{min} (°C)**		**Daily T_{max} (°C)**		**Location**	
	Summer	**Winter**	**Summer**	**Winter**	**Study site**	**Weather Station**
Askham	19.7 ± 3.7	-0.3 ± 5.4	37.7 ± 3.7	23.8 ± 2.8	26° 59′ S, 20° 51′ E	26° 28′ S, 20° 36′ E
Frankfort	14.0 ± 2.1	-2.5 ± 3.2	29.3 ± 2.5	19.7 ± 2.3	27° 16′ S, 28° 29′ E	27° 16′ S, 28° 29′ E
Polokwane	17.2 ± 2.1	4.3 ± 1.2	26.9 ± 2.1	21.2 ± 3.5	23° 56′ S, 29° 28′ E	23° 51′ S, 29° 27′ E

Sparrow-weavers were typically caught at night using two small nets mounted on aluminium poles placed over the entrances of roost nests. A few birds were caught during the day using mist nets or spring traps baited with mealworms. To avoid trapping reproductive individuals, we did not catch birds over the peak egg-laying period for *P. mahali* (November – December; du Plessis, 2005), and avoided catching sparrow-weavers from breeding nests. Physiological data were collected at the various study sites, and birds were housed in cages constructed of plastic mesh and shade cloth (~ 1.5 m^3) for no more than 48 hr prior to measurements.

The diet of *P. mahali* includes insects, seeds, fruits and fleshy leaves, and in the Kalahari Desert they eat mostly insects (~ 80 %; du Plessis, 2005). While in captivity, birds were provided with water and a wild bird seed mix *ad lib.*, as well as giant mealworms (~ 5 per bird per day). Individuals were sexed by bill colour (du Plessis, 2005), and a Scout *Pro* Balance scale (SP602US, Ohaus, Pine Brook NJ, USA) was used to measure M_b to 0.01 g. Sparrow-weavers were released at their site of capture after data collection.

Gas exchange and temperature measurements

We measured CO_2 production (ml min^{-1}) and TEWL (mg min^{-1}) using an open flow-through respirometry system, and core T_b of sparrow-weavers using temperature-sensitive passive integrated transponder tags, using the same experimental setup as Whitfield et al. (2015). All sparrow-weavers were placed individually in airtight respirometry chambers constructed from 4-L clear plastic containers (Lock & Lock, Seoul, South Korea). Relatively high flow rates (2 – 20 L min^{-1}) were used, and were continuously monitored and adjusted during data collection to ensure that water vapour partial pressure within the chambers always remained low (< 0.31 kPa), while also maintaining differences in [CO_2] and [H_2O] between incurrent and excurrent air sufficient for accurate measurements. All equipment was calibrated and passive integrated transponder tags injected into birds as described by Whitfield et al. (2015).

Experimental protocol

To quantify heat tolerance and maximum evaporative cooling capacity in *P. mahali* in a manner facilitating comparisons among and within populations, we measured gas exchange rates and T_b of sparrow-weavers individually during their active phase (day-time) using the standardised protocol described by Whitfield et al. (2015; modifications described below). We exposed birds to a ramped T_a profile of progressively higher T_a values (between 30 – 52 °C) in a stepwise fashion, with individuals being maintained at constant T_as for a period of ≥

10-min (mean exposure time per T_a: 15.2 ± 4.6 min, calculated from a subset of 42 of 240 data files), before increasing T_a to the next setpoint. Different individuals were used for measurements at $30\ °C \leq T_a \leq 38\ °C$ and $T_a \geq 40\ °C$ respectively, with a sample size of $n = 10$ per site per season for each of these two T_a ranges (each bird was only exposed to a given T_a once). In the lower T_a range, individuals were exposed to constant T_a values of 30 °C, 34 °C, 36 °C and 38 °C, and in the higher T_a range, data were collected from $T_a = 40$ ºC upwards in 2 ºC increments until birds became hyperthermic. The behaviour of birds during trials was monitored as described by Whitfield et al. (2015), but we did not present or analyse behavioural observations as there were seldom enough records of active birds to enable reliable comparisons, and because of the difficulty of quantifying and interpreting behavioural responses. Trials were ended when birds showed signs of distress (occurred in only three cases) or in calm birds when their T_b exceeded 44 °C. This value was chosen as Whitfield et al. (2015) found that $T_b = 44$ °C – 45 °C is close to the critical thermal maximum for three ploceid passerines (including *P. mahali*), and pilot studies demonstrated that sparrow-weavers at our more mesic sites became behaviourally distressed at $T_b > 44$ °C. The T_a at which each bird reached $T_b > 44$ °C (actual $T_b = 44.3$ °C after calibration) was thus considered the hyperthermia threshold T_a (HTT_a; °C) for that individual in the present study. If birds had to be removed earlier due to reasons other than severe hyperthermia (e.g., power outages or intermittent PIT tag reception), the data were excluded from HTT_a analyses.

Data analyses

Data were corrected for drift in [CO_2] and [H_2O] baselines using the relevant algorithms in Expedata Data Acquisition and Analysis Software (Sable Systems, Las Vegas NV, USA). For each bird, the 5-min sample period with the lowest average [CO_2] at each T_a was assumed to be representative of resting values, and behavioural observations were used to verify that birds were calm during this period. In a few cases, birds were not calm for a full 5-min at a given T_a, and thus all data from these birds at that T_a were discarded. Whole-animal $\dot{V}_{CO_2}$, RMR (W) and TEWL values were calculated as described in Whitfield et al. (2015), except that a respiratory exchange ratio of 0.85 was assumed (representative of a metabolic substrate consisting of a mix of carbohydrates and lipids), as we could not be certain that all birds were post-absorptive before being placed in the chambers. Evaporative heat loss (W; EHL) was calculated from TEWL using a latent heat of vaporisation of 2.4 J mg^{-1} H_2O (corresponding with T_a = 40 °C; Withers 1992), and the efficiency of evaporative cooling was calculated as the ratio of EHL to metabolic heat production (EHL / MHP) was calculated.

All values are presented as mean ± standard deviation (SD). Linear models (LM) and linear mixed effects models (LME; *nlme* package; Pinheiro et al., 2009) were fitted to data using R 3.1.1 (R Development Core Team, 2014). The assumptions of all models (including normality, homogeneity of variance and multicollinearity), as well as model fit (residuals, leverage and Cook's D values), were checked using the appropriate tests described in Logan (2010). An initial LM was fitted to M_b data, with site, season and sex as predictor variables, and as significant M_b variation was found, we included M_b in further analyses on physiological variables.

Little is known about the physiological processes responsible for among- and within-species differences in avian heat tolerance, and for this reason each response variable (T_b, TEWL, RMR and EHL / MHP) was analysed separately. All models were initially run including a set of potential predictor variables (M_b, site, season and sex) and interactions among these variables, and models were refined by comparing second-order Akaike Information Criterion values (AICc, *MuMIn* package) to determine which combination of predictor variables and interactions produced models that best fitted the datasets tested. Sex was initially included as a predictor variable, but as response variables never varied significantly with sex (all $p > 0.05$), and its removal either improved or did not affect model fit (i.e., decrease or no change in AICc values), it was excluded from the final models on physiological variables.

We could not calculate inflection points representing the T_{uc} of individuals on account of too few data points to fit a segmented linear regression model (only four points per individual at 30 °C ≤ T_a ≤ 38 °C), and thus considered $T_a \approx 30$ °C (actual $T_a = 30.1 \pm 0.2$ °C; $n = 10$ per site per season) to be representative of thermoneutrality, as Smit and McKechnie (2010) found this T_a to be within the TNZ of sparrow-weavers. LMs were fitted to T_b, TEWL, RMR and EHL / MHP data at $\mathrm{T_a} \approx 30$ °C, as well as to HTT_a data. *Post hoc* tests of multiple comparisons of means (Tukey Contrasts for linear models; *multcomp* package; Hothorn et al., 2008) were used to identify between which sites, or site × season groups, significant differences occurred.

LMEs were fitted to data (T_b, TEWL, RMR and EHL / MHP) at $T_a \geq 40$ °C that included repeated measurements of birds at multiple T_as (n = 10 per site per season per T_a), and thus individual was included as a random effect. As T_b, TEWL and EHL / MHP varied significantly among site × season groups, separate linear regression models were fitted within each group to investigate the respective relationships between T_a and the response variables, and analyses of covariance and *post hoc* tests were used to investigate how the slopes and y-intercepts of these regressions varied among site × season groups. RMR did not vary among site × season groups and thus separate linear regression models could not be fitted within each group. Moreover, we could not fit *post hoc* tests to investigate RMR and EHL / MHP variation among sites at $T_a \geq 40$ °C, due to the significant T_a × site and T_a × site × season interactions respectively, and thus fitted LMs to examine how RMR and EHL / MHP at $T_a \approx 42$ °C (actual $T_a = 42.1 \pm 0.2$ °C; $n = 10$ per site per season) varied with predictor variables, as this was the highest T_a that was below the HTT_a of all individuals (i.e., the highest T_a all birds reached without becoming hyperthermic).

Results

Body mass

The M_b of sparrow-weavers varied significantly with site, but not between seasons, nor with site × season (Table 1). The M_b of birds at all three sites differed significantly from each other ($p < 0.05$): birds at Frankfort were the largest (46.3 ± 3.7 g, $n = 96$), followed by Polokwane (42.0 ± 4.0 g, $n = 81$), and birds at Askham the smallest (40.2 ± 3.7 g, $n = 87$). The M_b of males (43.6 ± 4.7 g; $n = 164$) was significantly greater than that of females (41.8 ± 4.3 g; $n = 100$) across all sites and seasons (LM, $F_{2,257} = 18.648$, $p < 0.001$).

Body temperature

The T_b of sparrow-weavers remained relatively stable at $T_a < 40$ °C, above which it increased linearly with increasing T_a (Fig. 1). The T_b of birds in their TNZ (actual $T_a = 30.1 \pm 0.2$ °C) was significantly lower (~ 0.7 °C) in summer than in winter, but did not vary significantly with M_b, among sites, nor with site × season (Table 2 and 3a). At $T_a \geq 40$ °C, T_b increased significantly with increasing T_a, and varied significantly with site, season and site × season, but not with M_b (Table 2; Fig. 1). Separate linear regressions of $T_a \geq 40$ °C and T_b were fitted within each site × season group, and T_b increased significantly with T_a in all groups (Fig. 1).

The slope of increasing T_b with $T_a \geq 40$ °C was significantly steeper in Polokwane birds during both seasons than for the Askham and Frankfort populations (LME, $F_{5,221} = 2.611$, $p = 0.025$; Fig. 1). Furthermore, Polokwane birds did not show significant seasonal variation in their T_b response to increasing $T_a \geq 40$ °C (slopes of increasing T_b with T_a: LME,

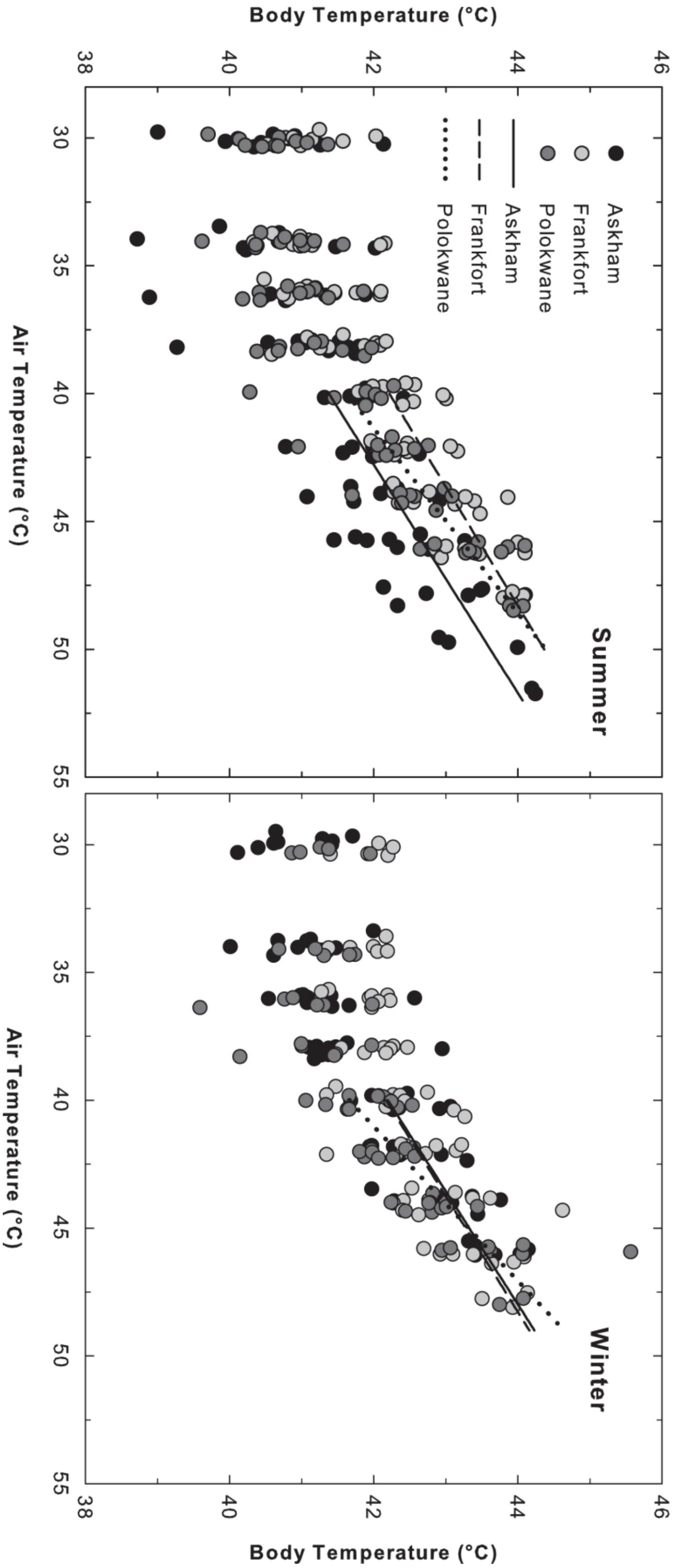
Summer
Winter
Askham
Frankfort
Polokwane
Askham
Frankfort
Polokwane
Body Temperature (°C)
Air Temperature (°C)
38
40
42
44
46
30
35
40
45
50
55

Fig. 1. Body temperature (T_b) of white-browed sparrow-weavers (*Plocepasser mahali*) during summer and winter at three sites in South Africa that vary in maximum summer air temperatures by ~ 10 °C**.** Different birds were exposed to 30 °C ≤ T_a ≤ 38 °C and for T_a ≥ 40 °C respectively (n = 10 per site per season per T_a range), and T_b measured using passive-integrated transponder tags. Lines indicate significant (all: $p < 0.001$) linear mixed effects regression models fitted to data at T_a ≥ 40 °C. During summer, Askham: y = 0.22 x + 22.42 ($t_{1,36}$ = 11.556), Frankfort: y = 0.22 x + 33.61 ($t_{1,29}$ = 10.907), and Polokwane: y = 0.28 x + 30.58 ($t_{1,24}$ = 10.512). During winter, Askham: y = 0.23 x + 33.15 ($t_{1,22}$ = 9.611), Frankfort: y = 0.22 x + 33.33 ($t_{1,30}$ = 9.077), and Polokwane: y = 0.33 x + 28.42 ($t_{1,27}$ = 11.627).

Table 2. Statistical results (*F*-value, *P*-value, degrees of freedom [df] and sample size [*n*]) from linear models fitted to response variables of white-browed sparrow-weavers (*Plocepasser mahali*) during summer and winter at three sites in South Africa that vary in maximum summer air temperatures (T_a) by ~ 10 °C ($n = 10$ per site per season), exposed to various T_a within laboratory setup: at $T_a \approx 30$ °C (in their thermoneutral zone; linear models), $T_a \geq 40$ °C (linear mixed effects models) and $T_a \approx 42$ °C (linear models). Response variables include: body mass (M_b), body temperature (T_b), total evaporative water loss (TEWL), resting metabolic rate (RMR), ratio of evaporative heat loss to metabolic heat production (EHL / MHP) and hyperthermia threshold air temperature (HTT_a; T_a at which $T_b \approx 44$ °C). RMR and TEWL were measured using open flow-through respirometry, and T_b using passive-integrated transponder tags. Predictor variables include: M_b (except when M_b was a response variable), site, season and the site × season interaction, as well as T_a for models at $T_a \geq 40$ °C. When a response variable varied significantly with a predictor variable, the *P*-value is presented in bold.

	T_a values (°C)	**T_a**			**M_b**			**Site**			**Season**			**Site × season**		
		F-value	df,*n*	*P*-value	*F*-value	df,*n*	*P*-value	*F*-value	df,*n*	*P*-value	*F*-value	df,*n*	*P*-value	*F*-value	df,*n*	*P*-value
M_b	-	-	-	-	-	-	-	71.638	2,259	< **0.001**	3.120	1,259	0.080	1.774	2,257	0.172
T_b	~ 30	-	-	-	0.040	1,43	0.842	2.529	2,43	0.092	11.475	1,43	**0.002**	1.256	2,41	0.296
	≥ 40	590.700	1,172	< **0.001**	1.700	1,172	0.193	4.800	2,53	**0.012**	8.900	1,53	**0.004**	1.700	2,53	**0.005**
EWL	~ 30	-	-	-	0.535	1,51	**0.036**	3.551	2,51	0.468	0.042	1,51	0.839	3.551	2,51	**0.036**
	≥ 40	1136.220	1,192	< **0.001**	8.382	1,192	**0.004**	63.617	2,54	< **0.001**	13.031	1,54	< **0.001**	18.744	2,54	< **0.001**
RMR	~ 30	-	-	-	0.277	1,53	0.601	47.716	2,53	< **0.001**	8.262	1,53	< **0.001**	1.191	2,51	0.511
	≥ 40	8.590	1,190	**0.004**	5.809	1,190	**0.017**	82.167	2,56	< **0.001**	16.728	1,56	< **0.001**	1.807	2,54	0.174
	~ 42	-	-	-	6.255	1,55	**0.015**	72.220	2,55	< **0.001**	11.502	1,55	**0.005**	1.211	2,53	0.306
EHL / MHP	~ 30	-	-	-	7.781	1,52	**0.007**	8.717	2,52	< **0.001**	8.101	1,52	**0.006**	1.686	2,50	0.196
	≥ 40	1083.017	1,187	< **0.001**	0.019	1,187	0.890	46.435	2,55	< **0.001**	80.628	1,55	< **0.001**	25.313	2,55	< **0.001**
	~ 42	-	-	-	0.068	1,53	0.796	21.448	2,53	< **0.001**	38.001	1,53	< **0.001**	6.503	2,53	**0.003**
HTT_a	-	-	-	-	0.033	1,51	0.089	3.771	2,51	**0.030**	10.998	1,51	**0.002**	6.502	2,51	**0.003**

Table 3.

Body temperature (T_b), total evaporative water loss (TEWL), resting metabolic rate (RMR), ratio of evaporative heat loss to metabolic heat production (EHL / MHP) and hyperthermia threshold air temperature (HTT_a; air temperature [T_a] at which $T_b \approx 44$ °C) of white-browed sparrow-weavers (*Plocepasser mahali*) were measured during summer and winter at three sites in South Africa that differ in maximum summer T_a (T_{max}) by ~ 10 °C. RMR and TEWL were measured using open flow-through respirometry and T_b using passive-integrated transponder tags, at various T_as including: $T_a \approx 30$ °C and $T_a \approx 42$ °C ($n = 10$ per site per season per T_a; mean ± SD). T_b, RMR and EHL / MHP at $T_a \approx 30$ °C, as well as RMR at $T_a \approx 42$ °C, did not vary with the site × season interaction, and thus these values are presented for each site and season separately (a); however, TEWL at $T_a \approx 30$ °C, EHL / MHP at $T_a \approx 42$ °C and HTT_a varied with site × season, and thus values are presented for each site × season group (b). Significance values (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$) of the differences among site and season categories (relationships shown by dashed lines) are from linear models and *post hoc* tests of multiple comparisons of means (Tukey Contrasts).

(a)	**Seasons**		**Sites**		
	Summer	**Winter**	**Askham**	**Frankfort**	**Polokwane**
T_b (°C) at $T_a \approx 30$ °C	40.7 ± 0.2	41.4 ± 0.5	40.8 ± 0.2	41.4 ± 0.7	40.9 ± 0.5
	└ **	┘			
RMR (W) at $T_a \approx 30$ °C	0.78 ± 0.43	1.00 ± 0.59	0.48 ± 0.12	0.75 ± 0.22	1.45 ± 0.51
	└ ***	┘		└ ***	┘
			└ ***	–	┘
RMR (W) at $T_a \approx 42$ °C	0.73 ± 0.41	0.85 ± 0.28	0.56 ± 0.15	0.72 ± 0.10	1.10 ± 0.22
	└ **	┘		└ ***	┘
			└ ***	–	┘
EHL / MHP at $T_a \approx 30$ °C	0.26 ± 0.05	0.22 ± 0.06	0.28 ± 0.04	0.22 ± 0.05	0.22 ± 0.06
	└ **	┘	└ ***	┘	
			└ ***	–	┘

(b)	**Site × season groups**					
	Askham		**Frankfort**		**Polokwane**	
	Summer	**Winter**	**Summer**	**Winter**	**Summer**	**Winter**
Daily T_{max} (°C)	37.7 ± 3.7	23.8 ± 2.8	29.3 ± 2.5	19.7 ± 2.3	26.9 ± 2.1	21.2 ± 3.5
TEWL (mg min^{-1}) at $T_a \approx 30$ °C	3.13 ± 1.03	3.48 ± 0.53	3.20 ± 0.57	4.77 ± 0.98	8.27 ± 2.14	7.02 ± 1.82
			└ ***	–	┘	
	└ ***	–	–	–	┘	
				└ **	–	┘
		└ ***	–	–	–	┘
EHL / MHP at $T_a \approx 42$ °C	1.39± 0.26	1.30 ± 0.08	1.25 ± 0.12	0.94 ± 0.12	1.32 ± 0.17	0.89 ± 0.06
			└ *	┘	└ ***	┘
		└ ***	–	┘		
		└ ***	–	–	–	┘
HTT_a (°C)	49.5 ± 1.4	46.8 ± 0.9	47.3 ± 1.5	47.3 ± 1.5	47.6 ± 1.3	46.7 ± 1.2
	└ ***	┘				
	└ **	–	┘			
	└ *	–	–	–	┘	

Table 4.

Linear mixed effects regression models revealed that body temperature (T_b) and total evaporative water loss (TEWL) of white-browed sparrow-weavers (*Plocepasser mahali*) during summer and winter at three sites in South Africa that vary in maximum air temperatures (T_{max}) by ~ 10 °C, significantly increased with air temperatures (T_a) ≥ 40 °C (n = 10 per site per season). TEWL was measured using open flow-through respirometry, and T_b using passive-integrated transponder tags. The y-intercepts of these regressions varied significantly reflecting variation in response variables at a given T_a ≥ 40 °C among site × season groups. We therefore presented T_b and TEWL values at $T_a \approx 42$ °C (mean ± SD) along with the results from *post hoc* tests of multiple comparisons of means (Tukey Contrasts) on the y-intercepts (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$; relationships shown by dashed lines).

	Site × season groups					
	Askham		**Frankfort**		**Polokwane**	
	Summer	**Winter**	**Summer**	**Winter**	**Summer**	**Winter**
Daily T_{max} (°C)	37.7 ± 3.7	23.8 ± 2.8	29.3 ± 2.5	19.7 ± 2.3	26.9 ± 2.1	21.2 ± 3.5
T_b (°C) at $T_a \approx 42$ °C	41.8 ± 0.5	42.5 ± 0.5	42.5 ± 0.4	42.5 ± 0.6	41.7 ± 0.7[a]	42.2 ± 0.3[a]
Y-intercepts of regressions at $T_a \geq 40$ °C	*** ***					
TEWL (mg min^{-1}) at $T_a \approx 42$ °C	15.8 ± 2.1	21.2 ± 3.3	20.4 ± 3.5	17.0 ± 1.7	33.3 ± 4.5	26.0 ± 5.9
Y-intercepts of regressions at $T_a \geq 40$ °C	*** ***	***	***		***	

a. Y-intercepts of Polokwane regressions could not be compared to those of Askham and Frankfort, as the slopes of the Polokwane regressions significantly differed from the other two sites

$F_{1,69}$ = 0.358, p = 0.552; y-intercepts: LME, $F_{1,70}$ = 2.611, p = 0.082; Fig. 1; Table 4). The slope of increasing T_b with increasing $T_a \geq 40$ °C in Askham and Frankfort birds did not vary significantly with site × season (LME, $F_{3,152}$ = 0.335, p = 0.800), but the y-intercepts did vary significantly with site × season (LME, $F_{3,155}$ = 21.610, p < 0.001; Fig. 1; Table 4). This is reflected in variation of T_b values at a given $T_a \geq 40$ °C, and Askham birds maintained significantly lower T_b during summer than in winter (e.g., T_b was ~ 0.7 °C lower in summer at $T_a \approx 42$ °C; Fig. 1; Table 4). Moreover, during summer, T_b at a given $T_a \geq 40$ °C was significantly lower in the Askham population than in Frankfort birds (e.g., T_b was ~ 0.7 °C lower at Askham at $T_a \approx 42$ °C; Fig. 1; Table 4).

In summary, T_b within the TNZ did not vary significantly among populations (Table 3b), but at $T_a \geq 40$ °C several patterns of variation emerged (Fig. 1). Polokwane sparrow-weavers had significantly steeper slopes of increasing T_b with $T_a \geq 40$ °C than the other two populations (Fig. 1). Furthermore, Askham sparrow-weavers had significantly lower T_b in summer at $T_a \geq 40$ °C than in winter, and also had lower T_b values than Frankfort birds during summer (Fig. 1; Table 4).

Evaporative water loss rates

Rates of TEWL in all three populations remained relatively stable at lower T_a (30 °C – 34 °C), but increased linearly with higher T_a above an inflection point (Fig. 2). We could not calculate this inflection point (as explained in the data analysis section), but our limited behavioural observations indicated that birds started panting at T_a = 36.8 ± 2.7 °C. At $T_a \approx 30$ °C (in the TNZ), TEWL varied significantly with site and site × season (Table 3b), but not with season nor M_b (Table 2). There was no significant seasonal variation in TEWL in the TNZ within the three populations (Table 3b). However, during both seasons, the TEWL in the TNZ of sparrow-weavers was significantly higher at Polokwane than at Askham (~ 167 % and 100 % higher in summer and winter respectively) and Frankfort (~ 160 % and 46 % higher in summer and winter respectively; Table 3b).

Rates of TEWL at $T_a \geq 40$ °C increased significantly with increasing T_a and M_b, and also varied significantly with site, season and site × season (Table 2; Fig. 2). Separate linear regressions of TEWL *versus* $T_a \geq 40$ °C were fitted within each site × season group, and TEWL increased significantly with T_a in all groups (Fig. 2). The slopes of these regressions did not significantly vary with site × season (LME, $F_{1,242}$ = 0.816, p = 0.540), but the y-intercepts did significantly vary with site × season (LME, $F_{1,247}$ =104.400, p < 0.001), reflecting variation in TEWL at a given T_a value ≥ 40 °C (Fig. 2; Table 4). For example at T_a

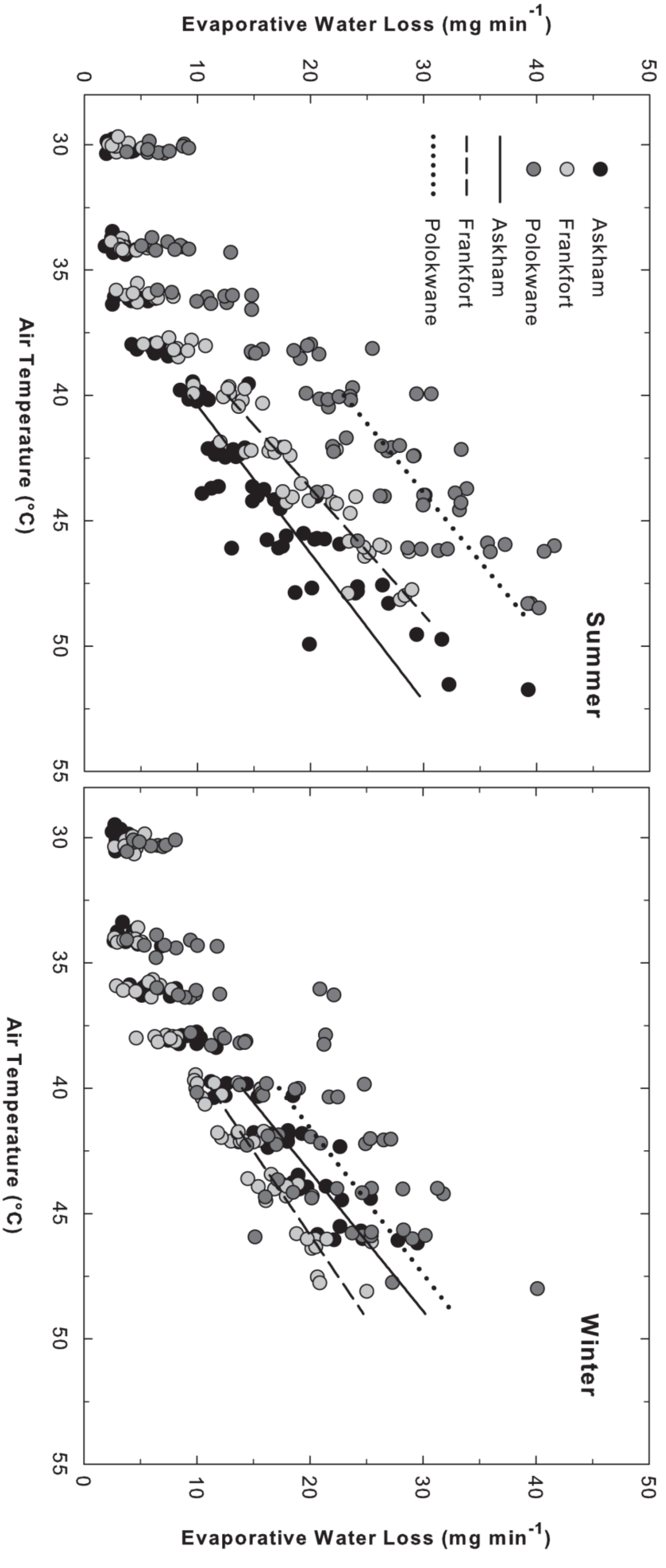
Summer
Winter
Askham
Frankfort
Polokwane
Askham
Frankfort
Polokwane
Evaporative Water Loss (mg min-1)
0
10
20
30
40
50
Air Temperature (°C)
30
35
40
45
50
55

Fig. 2. Total evaporative water loss rates (TEWL) of white-browed sparrow-weavers (*Plocepasser mahali*) during summer and winter at three sites in South Africa that vary in maximum summer air temperatures (T_a) by ~ 10 °C**.** Different birds were measured at 30 °C $\leq T_a \leq$ 38 °C and for $T_a \geq$ 40 °C respectively (n = 10 per site per season per T_a range), and TEWL measured using open flow-through respirometry. Lines indicate significant (all: $p < 0.001$) linear mixed effects regression models fitted to data at $T_a \geq$ 40 °C. During summer, Askham: y = 1.70 x – 58.54 ($t_{1,41}$ = 16.967), Frankfort: y = 1.99 x – 66.77 ($t_{1,30}$ = 17.618), and Polokwane: y = 1.82 x – 49.74 ($t_{1,32}$ = 11.763). During winter, Askham: y = 1.80 x – 58.51 ($t_{1,26}$ = 13.615), Frankfort: y = 1.50 x – 48.78 ($t_{1,30}$ = 16.505), and Polokwane: y = 1.72 x – 51.45 ($t_{1,29}$ = 10.885).

≈ 42 °C (actual T_a = 42.1 ± 0.2 °C) Polokwane and Frankfort sparrow-weavers had higher TEWL in summer compared to winter (~ 28 % and 20 % higher respectively), whereas the Askham population had lower TEWL in summer than in winter (~ 25 % lower; Table 4).

In summary, TEWL in the TNZ of birds did not significantly differ between seasons, but did at $T_a \geq 40$ °C (Fig. 2; Tables 3b and 4). At both $T_a \approx 30$ °C and $T_a \geq 40$ °C, Polokwane birds had significantly higher TEWL than the other two populations, regardless of season (Fig. 2; Tables 3b and 4). The same general patterns of significant TEWL variation were observed when analyses were repeated using mass-specific values.

Resting metabolic rate

The relationship between whole-animal RMR and T_a was less clear than those between T_a and other physiological variables (Fig. 3). In the TNZ of sparrow-weavers ($T_a \approx 30$ °C), RMR was significantly lower in summer than in winter (~ 22 % lower), and also varied significantly among sites, but not with site × season nor M_b (Table 2). Polokwane sparrow-weavers had significantly higher RMR in their TNZ than both Frankfort and Askham birds (~ 93 % and 202 % higher respectively; Table 3a).

At $T_a \geq 40$ °C, RMR was significantly lower in summer than in winter, increased significantly with increasing T_a and M_b (Table 2), and varied significantly with site (Table 2) and T_a × site (LME, $F_{1,54} = 18.744$, $p = 0.008$). However, RMR did not vary significantly with site × season (Table 2), so we could not fit separate regression models within each site × season groups (hence the absence of regression lines; Fig. 3). Moreover, *post hoc* tests to investigate variation among sites could not be fitted due to the significant T_a × site interaction, thus RMR values at $T_a \approx 42$ °C were analysed instead. At $T_a \approx 42$ °C, RMR was also significantly lower in summer than in winter (~ 14 % lower), and varied significantly with M_b and site, but not with site × season (Table 2). The Polokwane population had significantly higher RMR at $T_a \approx 42$ °C compared to both Frankfort and Askham birds (~ 53 % and 96 % higher respectively; Table 3a).

In summary, RMR in the TNZ of birds, at $T_a \geq 40$ °C and at $T_a \approx 42$ °C was significantly higher in winter than in summer (Fig. 3; Table 3a). Furthermore, RMR at both $T_a \approx 30$ °C and $T_a \approx 42$ °C was significantly higher in the Polokwane population than Askham and Frankfort sparrow-weavers (Table 3a). The same general patterns of significant RMR variation were observed when analyses were repeated using mass-specific values.

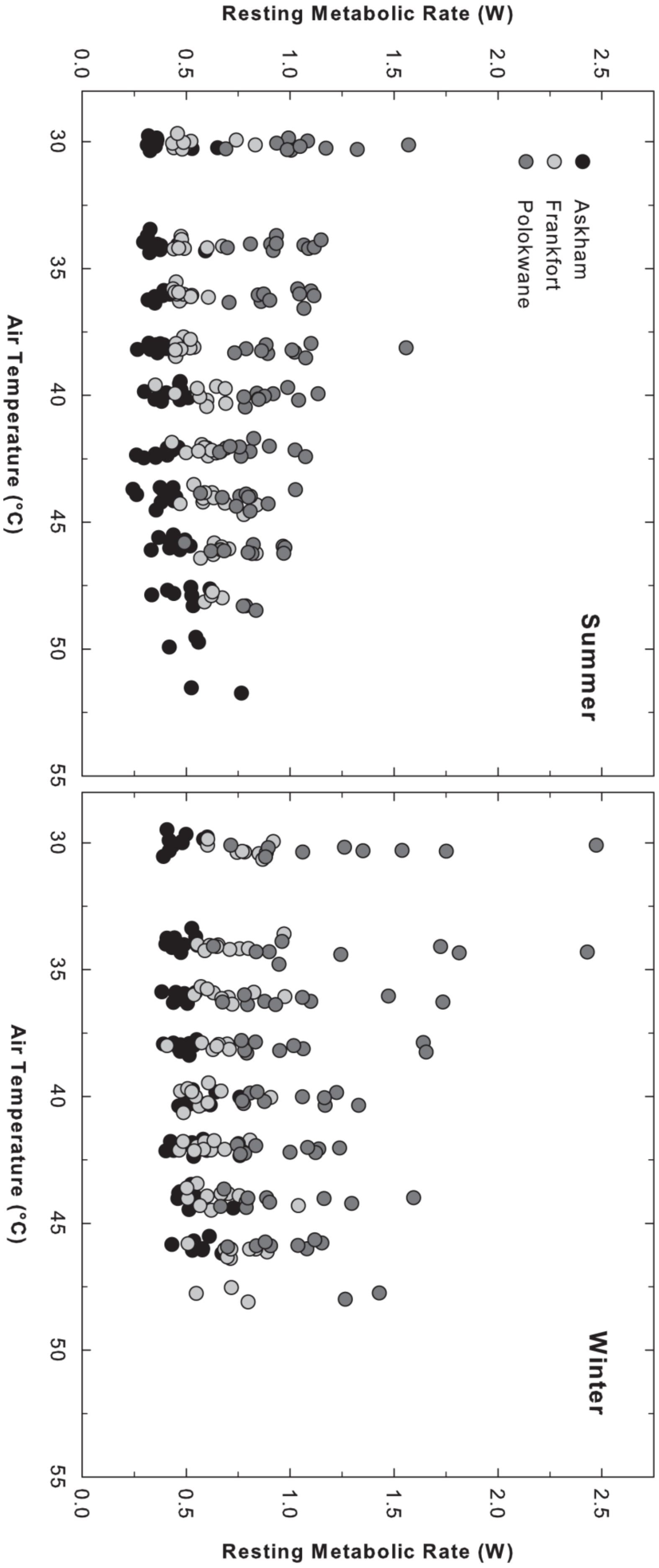
Resting Metabolic Rate (W)
0.0
0.5
1.0
1.5
2.0
2.5
Air Temperature (°C)
30
35
40
45
50
55
Askham
Frankfort
Polokwane
Summer
Winter

Fig. 3. Resting metabolic rate (RMR) of white-browed sparrow-weavers (*Plocepasser mahali*) during summer and winter at three sites in South Africa that vary in maximum summer air temperatures (T_a) by ~ 10 °C**.** Different birds were measured at 30 °C ≤ T_a ≤ 38 °C and for T_a ≥ 40 °C respectively (*n* = 10 per site per season per T_a range), and RMR measured using open flow-through respirometry. RMR increased significantly with increasing T_a ≥ 40 °C and also varied significantly among sites and seasons. As RMR did not significantly vary among site × season groups and the T_a × site interaction was significant, we did not fit separate regression models or *post hoc* tests to investigate variation among sites at T_a ≥ 40 °C, and thus RMR values at T_a ≈ 42 °C were analysed instead.

Ratio of evaporative heat loss to metabolic heat production

The ratio of EHL / MHP remained relatively stable at lower T_a (30 °C – 34 °C), but increased linearly with higher T_a above an inflection point (Fig. 4). During winter, EHL / MHP of the Polokwane and Frankfort population reached a plateau at higher T_as (~ 44 °C and 46 °C respectively), suggesting maximum evaporative capacity for heat dissipation had been reached (Fig. 4). In the TNZ of sparrow-weavers ($T_a \approx 30$ °C), EHL / MHP was significantly higher in summer compared to winter, and also varied significantly among sites and M_b, but not with site × season (Table 2). Askham sparrow-weavers had significantly higher EHL / MHP at $T_a \approx 30$ °C than both Frankfort and Polokwane birds (Table 3a).

At $T_a \geq 40$ °C, EHL / MHP increased significantly with increasing T_a, and varied significantly with site, season, site × season and T_a × site × season (LME, $F_{1,187} = 11.855$, $p < 0.001$; Fig. 4), but not with M_b (Table 2). *Post hoc* tests to investigate variation among site × season groups could not be fitted due to the significant T_a × site × season interaction, thus RMR values at $T_a \approx 42$ °C were analysed instead. At $T_a \approx 42$ °C, EHL / MHP varied significantly with site, season and site × season, but not with M_b (Table 2). Polokwane and Frankfort sparrow-weavers had higher EHL / MHP at $T_a \approx 42$ °C in summer than in winter (~ 49 % and 21 % higher respectively), whereas no significant seasonal variation occurred in the Askham population (Table 3b). During summer, no variation in EHL / MHP occurred among the three populations, but during winter Askham birds had higher EHL / MHP than both Polokwane and Frankfort sparrow-weavers (Table 3b).

In summary, EHL / MHP ratios in the TNZ were significantly higher in Askham birds than the other two populations (Fig. 4; Table 3a). At $T_a \approx 42$ °C, seasonal variation of EHL / MHP occurred within the Polokwane and Frankfort populations, but not in Askham sparrow-weavers, and the latter population therefore had higher EHL / MHP at $T_a \approx 42$ °C than the other two sites during winter (Fig. 4; Table 3b).

Hyperthermia threshold air temperature

The HTT_a of sparrow-weavers (i.e., T_a at which $T_b \approx 44$ °C; actual $T_b = 44.3$ °C after calibration) varied significantly among seasons and sites, and with site × season, but not with M_b (Table 2). Askham sparrow-weavers had significantly higher HTT_a (~ 2.7 °C higher) in summer compared to winter, but there was no significant seasonal variation in HTT_a within the other two populations (Table 3b). During summer, sparrow-weavers at Askham exhibited significantly higher HTT_a values than conspecifics at Polokwane and Frankfort (~ 1.9 °C and

2.2 °C higher respectively); however, HTT_a did not vary significantly among the three populations during winter (Table 3b).

Discussion

We found significant intraspecific seasonal and spatial variation in the evaporative cooling capacity and heat tolerance of *P. mahali*, manifested as significant differences in the maximum T_a birds were able to tolerate before the onset of severe hyperthermia. As predicted, sparrow-weavers at the hot desert site with the greatest seasonal variation in T_{max} values tolerated significantly higher T_a during summer than conspecifics at cooler sites, and were the only population to show significant seasonal acclimatisation in HTT_a (Table 3b). Askham sparrow-weavers demonstrated thermoregulatory responses broadly similar to those reported for this species by Whitfield et al. (2015) at two climatically similar sites, Wildsgenot Game Ranch (27° 04′ S, 21° 23′ E) and Leeupan Ranch (26° 58′ S, 21° 50′ E).

Thermoneutral T_b values were indistinguishable among sparrow-weaver populations, as was the case for populations of *E. alpestris* (Trost, 1972) and *Passer domesticus* (Hudson and Kimzey, 1966). However, significant T_b variation among populations emerged at $T_a \geq 40$ °C, with Polokwane sparrow-weavers increasing T_b with increasing $T_a \geq 40$ °C at a faster rate than in the Askham and Frankfort populations. The latter two populations had slopes of increasing T_b with increasing $T_a \geq 40$ °C similar to those previously reported for Kalahari sparrow-weavers during summer at 40 °C $< T_a <$ 48 °C (Whitfield et al., 2015).

The seasonal acclimatisation in T_b at $T_a \geq 40$ °C in desert sparrow-weavers is a novel finding, with the only other study of seasonality of heat tolerance at high T_a of which we are aware finding no significant acclimatisation in captive-reared *C. macqueenii* (Tieleman et al., 2002b). However, the validity of this comparison is questionable as *C. macqueenii* (1200 g) is a substantially larger bird than *P. mahali*, and it is also unclear whether captivity could have had an impact on the physiological responses of these birds (Tieleman et al., 2002b). The reduction of T_b during summer at $T_a \geq 40$ °C that was observed in Askham sparrow-weavers, resulted in this population also having significantly lower T_b than conspecifics from a more mesic population in summer (Frankfort; ~ 0.7 °C difference in T_b at $T_a = 42$ °C; Table 4b). This pattern of among-population variation in T_b is similar to that observed in desert and mesic populations of *E. alpestris* at $T_a = 45$ °C (~ 1.7 °C difference in T_b; Trost, 1972). Moreover, these patterns are consistent with our predictions, as sparrow-weavers from the hot desert site increased T_b more slowly with increasing $T_a \geq 40$ °C compared to conspecifics from a mesic site (Polokwane), and maintained lower T_bs at a given $T_a \geq 40$ °C compared to birds from the other mesic site (Frankfort).

Facultative hyperthermia is thought to contribute to the ability of birds to survive in hot environments by decreasing the thermal gradient between their surface and the environment, thereby conserving water by reducing EWL (Dawson, 1958; Trost, 1972; Weathers, 1981). Nord and Williams (2015), for instance, estimated that incubating greater hoopoe larks (*Alaemon alaudipes*) can reduce TEWL by 15 – 20 % at a T_a of 40 °C by increasing T_b from 42 °C to 45 °C. However, the notion that the capacity for facultative hyperthermia may be greater in desert birds than in mesic species is not supported by the finding that the magnitude of hyperthermic responses did not differ between desert and non-desert birds at $T_a = 45$ °C (Tieleman and Williams, 1999). It is also possible that maintaining lower T_b has adaptive value by providing a greater capacity for heat storage before lethal T_b limits are reached (McNab and Morrison, 1963; Tieleman et al., 2002a).

Smit et al. (2013) found that in two free-ranging populations of *P. mahali* in the Kalahari Desert, the T_b set-point was significantly higher in a desert (41.5 ± 0.2 °C) *versus* semi-desert population (40.2 ± 0.2 °C), but that the desert population did not have a greater capacity for hyperthermia (i.e., $T_b >$ modal T_b values). In contrast to these free-ranging populations, our laboratory data for *P. mahali* (present study) and that of Trost (1972) on *E. alpestris*, reveal lower T_b in desert than in mesic populations at high T_a. Moreover, free-ranging sparrow-weavers commenced panting at $T_a > 28$ °C (Smit et al. 2013), whereas our limited behavioural observations indicated that birds in the laboratory started panting at a substantially higher T_a across all sites (mean T_a at onset of panting = 36.8 ± 2.7 °C; lowest T_a = 32.0 °C). The latter differences likely reflect the very low chamber humidities in the present study and the effects of solar radiation on the operative temperatures experienced by free-ranging sparrow-weavers. Differences in physiological responses to acute heat stress between natural habitats and artificial conditions largely preclude the extrapolation of laboratory data to free-ranging birds; however, the goal of the present study was to quantify intraspecific variation in physiological responses to high T_a in a manner allowing for direct comparisons among populations.

At $T_a \approx 30$ °C and at $T_a \geq 40$ °C, TEWL in the desert population (Askham) was similar to that reported by Whitfield et al. (2015) for Kalahari sparrow-weavers during summer (~ 2.69 mg min^{-1} at 25 °C < T_a < 35 °C and ~ 11.71 mg min^{-1} at $T_a = 42$ °C). The reduction in both TEWL and T_b during summer compared to winter in desert sparrow-weavers, suggests that these birds can enhance evaporative cooling to conserve water and cope with high summer T_{max} (Table 1). Seasonal adjustments in TEWL have also been demonstrated in *C. macqueenii*, with reduced summer TEWL at T_a = 35 °C (~ 32 % lower compared to winter values), but not at T_a = 50 °C (Tieleman et al., 2002b).

Many arid-zone birds have been found to have lower TEWL at thermoneutrality compared to mesic-zone species (Tieleman et al., 2002a; Tieleman et al., 2003a; Williams, 1996), and some studies suggest that a similar variation may exist at the intraspecific level (MacMillen and Hinds, 1998; Sabat et al., 2006). Our results provide only limited support for this idea, as TEWL in the TNZ was significantly higher in a mesic population (Polokwane) compared to both the desert (Askham) and other mesic population (Frankfort; Table 3b). However, at $T_a \geq 40$ °C variation in TEWL was consistent with our predictions, with the desert population having significantly lower TEWL than either mesic population; a difference similar to that observed between desert and mesic populations of *E. alpestris* (~ 32 % lower in the desert population at T_a = 45 °C; Trost, 1972). The adaptive value of lower TEWL in desert birds is thought to concern water conservation (Tieleman et al., 2002a; Williams, 1996; Williams and Tieleman, 2000), and our results suggest that this pattern may become more pronounced with increasing $T_a > T_b$ (Dawson and Whittow, 2000; Williams, 1999).

The lack of an obvious increase in RMR with increasing T_a, despite the obvious increase of T_b and TEWL, is puzzling. However, this relationship between RMR and T_a is consistent with previous work at similar T_a ranges on birds in the Kalahari desert, including *P. mahali* and two other ploceid passerines (Whitfield et al. 2015), and three columbids (*Oena capensis*, *Spilopelia senegalensis* and *Streptopelia capicola*; M.C. Whitfield, B. Smit, A.E. McKechnie and B.O. Wolf, unpublished data). The RMR of the Askham population was similar to those previously observed in Kalahari sparrow-weavers during summer (Whitfield et al., 2015). A number of studies have demonstrated lower metabolic rates in desert compared to mesic birds at moderate temperatures (Sabat et al., 2006; Tieleman et al., 2002a; Tieleman et al., 2003a; Tieleman and Williams, 2000), but we could only find one study demonstrating lower RMR at $T_a > T_b$ in a desert compared to a mesic population (~ 32 % lower at T_a = 45 °C in *E. alpestris*; Trost, 1972). In contrast to our predictions, there was no clear pattern of RMR variation among desert *versus* mesic populations in the present study; corresponding variation in basal metabolic rate is also absent in *P. mahali* (i.e., BMR; M.J. Noakes, B.O. Wolf and A.E. McKechnie, unpublished data).

Sparrow-weavers in the present study had EHL / MHP ratios ranging from ~ 0.20 at thermoneutrality to maximum values of ~ 1.00 – 2.31. These maximum EHL / MHP ratios are similar to the value reported previously for Kalahari sparrow-weavers during summer

(1.93; Whitfield et al., 2015), and other avian species (Lasiewski et al., 1966; Lasiewski and Seymour, 1972; Trost, 1972). The lack of seasonal variation in EHL / MHP ratios at $T_a \approx 42$ °C in desert (Askham) sparrow-weavers contrasts with the reduction in both TEWL and T_b during summer in this population.

The mechanisms allowing birds to adjust TEWL as a component of seasonal acclimatisation have received less attention than those underlying seasonal adjustments in metabolic variables such as basal and summit metabolism. Several mechanisms have been proposed as drivers of lower TEWL in arid-zone birds compared to their mesic counterparts, including an increased capacity for facultative hyperthermia (Dawson, 1958; Trost, 1972; Weathers, 1981), countercurrent heat exchange in the nasal passages (Geist, 2000; Schmidt-Nielsen et al., 1970), and a reduction in cutaneous evaporative water loss (CEWL) by adjustments in the lipid composition of the epidermis (Menon et al., 1989; Tieleman and Williams, 2002; Webster and Bernstein, 1987; Williams, 1996). As discussed above, lower summer T_b in desert sparrow-weavers implies that facultative hyperthermia is not the mechanism responsible for reduced summer TEWL in this population during acute heat exposure. Furthermore, seasonal variation in TEWL was only significant at $T_a \geq 40$ °C, suggesting this is not the result of countercurrent heat exchange in the nasal passages nor reduced CEWL; both of these mechanisms should be more efficient at moderate T_a, as passerines typically respond to increasing $T_a > T_b$ by an increase in respiratory evaporation via panting (see also: Geist, 2000; Sabat et al., 2006; Schmidt-Nielsen et al., 1970; Tieleman et al., 1999; Tieleman and Williams, 2002). Alternately, adjustments in respiratory variables may enhance the evaporative efficiency of panting. Although adjustments in the rate of EWL are well-studied (Dawson, 1982; Richards, 1970; Wolf and Walsberg, 1996), mechanisms that could potentially increase the energy dissipated evaporatively per unit energy expended on muscle contractions during panting remain unclear. One potential mechanism concerns changes in the elastic properties of avian respiratory systems to increase the resonant frequency of respiration during summer, thereby resulting in an increase in EHL without an associated change in MHP (Crawford and Kampe, 1971; Richards, 1970). However, such changes would be reflected as increased EHL / MHP in summer, whereas no such increases were evident in the Askham sparrow-weavers.

Patterns of variation in the maximum T_a that sparrow-weavers could tolerate before the onset of severe hyperthermia (i.e., HTT_a; $T_b > 44$ °C) were closely linked to the T_{max} experienced by each population, with seasonal acclimatisation of HTT_a observed only in the desert population. The greater heat tolerance of Askham sparrow-weavers appears to result from enhanced evaporative cooling at $T_a \geq 40$ °C during summer compared to winter, as at a given $T_a \geq 40$ °C, these birds lost less water by TEWL during summer than in winter, but could still maintain lower T_b values during summer, allowing them to conserve water and reach higher T_a values before the onset of severe hyperthermia. To ensure that the upper thermoregulatory limit was quantified, Whitfield et al. (2015) allowed birds in the Kalahari Desert to reach substantially higher T_bs (max $T_b = 45.5 \pm 0.1$ °C) than in the present study, and as a result the maximum T_a values in the latter study were as high as $T_a \approx 54$ °C. However, the aim of the present study was to use a standardised protocol to compare the heat tolerance and evaporative cooling capacity of *P. mahali* populations between seasons and among populations, and as sparrow-weavers from the more mesic sites displayed clear signs of behavioural and physiological stress at $T_b > 44$ °C, we used this T_b value as a cut-off during all measurements. The HTT_a is a novel metric for comparative analyses, and moreover is probably the most ecologically significant variable in this study, as it quantifies the combined effect of the thermoregulatory variables (TEWL, RMR and T_b) that resulted in the up-regulation of summer heat tolerance and evaporative cooling capacity compared to winter in the desert population.

We quantified thermoregulatory variation among *P. mahali* populations along a climatic gradient, but our data do not permit us to infer the processes responsible for these phenotypic differences. The patterns of physiological variation we observed could arise from local adaptation, but might also reflect phenotypic plasticity through acclimatisation to current conditions or developmental plasticity to conditions experienced during development (Piersma and Drent, 2003; Pigliucci, 2001). Moreover, the small number of populations we examined also constrains our ability to make any inferences in this regard (Garland and Adolph, 1994; Hurlbert, 1984). The present study should instead be seen as an initial step towards identifying a model species suitable for experimental work designed to tease apart the roles of local adaptation *versus* phenotypic plasticity in determining these among-population differences, using common-garden and short-term thermal acclimation experiments. We argue that *P. mahali* is a suitable model for such studies, as intraspecific variation in thermoregulatory responses to high T_a have been demonstrated under both free-ranging (Smit et al., 2013) and laboratory conditions (present study).

Increasing evidence of fine-scale intraspecific physiological variation lends further support to the idea of adaptive thermoregulation and the thermal physiology of endotherms being more flexible than previously recognised (Angilletta et al., 2010; Glanville et al., 2012; Smit et al., 2013). A number of studies have demonstrated seasonal acclimatisation in avian basal and summit metabolism in relation to cold tolerance (reviewed by McKechnie and Swanson, 2010), but to the best of our knowledge this is the first study to reveal distinct seasonal acclimatisation in avian evaporative cooling capacity and heat tolerance. Pronounced seasonal adjustments in heat tolerance occurred in the population that experienced the most pronounced variation in climate (Table 1), which suggests that birds could have the potential to show adaptive physiological responses when faced with changing climates. Future studies are required to establish whether seasonal acclimatisation in heat tolerance and evaporative cooling capacity is widespread among birds inhabiting hot environments, and to investigate the evolutionary processes driving intraspecific variation in avian thermoregulatory responses to high T_a.

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List of symbols and abbreviations

Air temperature (T_a)

Basal metabolic rate (BMR)

Body mass (M_b)

Body temperature (T_b)

Cutaneous evaporative water loss (CEWL)

Evaporative heat loss (EHL)

Evaporative water loss (EWL)

Hyperthermia threshold air temperature (HTT_a)

Linear mixed effects model (LME)

Linear model (LM)

Maximum air temperature (T_{max})

Metabolic heat production (MHP)

Minimum air temperature (T_{min})

Resting metabolic rate (RMR)

Second-order Akaike information criterion (AICc)

Standard deviation (SD)

Thermoneutral zone (TNZ)

Total evaporative water loss (TEWL)

Upper critical air temperature (T_{uc})

Competing interests

The authors declare that there are no competing interests.

Author contributions

A.E.M. and B.O.W. conceived and designed the study. M.J.N. performed the experiments and analysed the data with guidance from A.E.M. and B.O.W. M.J.N. and A.E.M. wrote the manuscript, and B.O.W. provided editorial comments and advice.

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