

## RESEARCH ARTICLE

# Phenotypic flexibility of metabolic rate and evaporative water loss does not vary across a climatic gradient in an Afrotropical passerine bird

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## ABSTRACT

Small birds inhabiting northern temperate and boreal latitudes typically increase metabolic rates during cold winters or acclimation to low air temperatures ( $T_{\text{accl}}$ ). Recent studies suggest considerable variation in patterns of seasonal metabolic acclimatization in birds from subtropical and tropical regions with milder winters, but there remains a dearth of acclimation studies investigating metabolic flexibility among lower-latitude birds. We used short-term thermal acclimation experiments to investigate phenotypic flexibility in basal metabolic rate (BMR), thermoneutral evaporative water loss (EWL) and summit metabolism ( $M_{\text{sum}}$ ) in three populations of white-browed sparrow-weavers (*Plocepasser mahali*) along a climatic and aridity gradient. We allocated individuals to one of three  $T_{\text{accl}}$  treatments (5, 20 and 35°C;  $n=11$  per population per  $T_{\text{accl}}$ ) for 28 days, and measured post-acclimation BMR, EWL and  $M_{\text{sum}}$  using flow-through respirometry. Our data reveal the expected pattern of lower BMR and EWL (~12% and 25% lower, respectively) in birds at  $T_{\text{accl}}=35^{\circ}\text{C}$  compared with cooler  $T_{\text{accl}}$  treatments, as observed in previous acclimation studies on subtropical birds. We found no variation in the reaction norms of BMR and EWL among populations in response to acclimation, suggesting previously documented differences in seasonal BMR acclimatization are the result of phenotypic flexibility. In contrast to higher-latitude species,  $M_{\text{sum}}$  did not significantly vary in response to thermal acclimation. These findings support the idea that factors other than enhancing cold tolerance may be driving patterns of metabolic variation in subtropical birds.

**KEY WORDS:** Adaptive plasticity, Reaction norm, Acclimation, Summit metabolism, Basal metabolic rate, Subtropical birds

## INTRODUCTION

Global variation in avian thermal physiology is correlated with climate, with several analyses identifying air temperature ( $T_a$ ) as the major correlate of mass-independent variation in avian metabolic rates (Jetz et al., 2008; Stager et al., 2016; White et al., 2007). Avian metabolic rates tend to be lower in tropical latitudes than in temperate-zone regions (Hail, 1983; Londoño et al., 2015; Stager et al., 2016; Weathers, 1979; Wiersma et al., 2007), and both metabolic rates and evaporative water loss (EWL) are reduced in

arid-zone compared with mesic-zone birds (Tieleman et al., 2002, 2003; Tieleman and Williams, 2000). There is evidence of similar patterns of metabolic variation within species (e.g. MacMillen and Hinds, 1998; Sabat et al., 2006), supporting the idea that the thermal physiology of endotherms is more flexible than previously thought (Angilletta et al., 2010).

Understanding the sources of intraspecific variation in avian thermal physiology is particularly important when interpreting thermoregulatory responses in the context of a changing climate (Boyles et al., 2011; Huey et al., 2012; Williams et al., 2008). Physiological differences among birds can arise from genotypic adaptation (hard-wired differences in response to different selection pressures) or phenotypic plasticity (Angilletta et al., 2010; Piersma and Drent, 2003), although the distinction between these categories may be blurred by epigenetic transmission and the associated transgenerational effects (e.g. Mariette and Buchanan, 2016). In recent years, increasing attention has been given to the role of phenotypic plasticity, which includes developmental plasticity (ontogenetic variation that becomes fixed on reaching maturity) and phenotypic flexibility (reversible changes throughout an individual's lifetime; Piersma and Drent, 2003; Pigliucci, 2001). Phenotypic flexibility is an important component of acclimatization and acclimation – adjustments in response to environmental conditions in the field or laboratory, respectively (Piersma and Drent, 2003; Schlichting and Pigliucci, 1998).

A frequently-cited example of phenotypic flexibility is seasonal metabolic acclimatization in small north-temperate birds, typically involving winter increases in basal metabolic rate (BMR; minimum resting metabolic rate in post-absorptive, non-reproductive individuals) and summit metabolism ( $M_{\text{sum}}$ ; maximum metabolic rate during acute cold exposure; reviewed by McKechnie et al., 2015; Swanson, 2010). In particular, a higher  $M_{\text{sum}}$  is associated with enhanced cold tolerance during the cold winters typical of higher latitudes (Cooper, 2002; Petit et al., 2017; Swanson, 2001; Swanson and Liknes, 2006), and both BMR and  $M_{\text{sum}}$  have been reported as flexible in response to short-term (days to weeks) fluctuations in  $T_a$  in several temperate species (Broggi et al., 2007; Dubois et al., 2016; Petit and Vézina, 2014; Swanson and Olmstead, 1999). Adjustments in BMR are typically associated with changes in the mass and metabolic intensity of the digestive and excretory organs, whereas adjustments in  $M_{\text{sum}}$  are associated with changes in the mass and metabolic intensity of the skeletal muscles, heart mass and supply of substrates and oxygen to muscles (Milbergue et al., 2018; Swanson, 2010; Swanson and Vézina, 2015; Zhang et al., 2015a).

Only relatively recently have workers investigated seasonal adjustments in BMR and  $M_{\text{sum}}$  of birds inhabiting lower latitudes with milder winters and hotter summers, but the limited available data suggest more variability in the magnitude and direction of

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**List of symbols and abbreviations**

BMR	basal metabolic rate
EWL	evaporative water loss
$M_b$	body mass
ME	metabolic expansibility
$M_{sum}$	summit metabolism
PIT tags	passive integrated transponder tags
RER	respiratory exchange ratio
$T_a$	air temperature
$T_{a,max}$	maximum air temperature
$T_{a,min}$	minimum air temperature
$T_{acc1}$	acclimation air temperature
$T_b$	body temperature
$T_{CL}$	helox temperature at cold limit
$\dot{V}_{CO_2}$	rate of CO <sub>2</sub> consumption
$\dot{V}_{O_2}$	rate of O <sub>2</sub> consumption

metabolic adjustments compared with that of their high-latitude counterparts (reviewed by McKechnie et al., 2015). Intraspecific variation in seasonal metabolic acclimatization has also been reported among populations of two subtropical species, *Euplectes orix* (van de Ven et al., 2013) and *Plocepasser mahali* (Noakes et al., 2017; Smit and McKechnie, 2010). A continuum between selection for cold tolerance or energy conservation has been suggested to drive patterns of avian metabolic adjustments in response to fluctuations in minimum  $T_a$  and food availability, respectively (Smit and McKechnie, 2010), and it is possible that the milder winters at lower latitudes permit greater flexibility in the direction and magnitude of seasonal metabolic acclimatization (McKechnie et al., 2015; Noakes et al., 2017). This does not necessarily mean subtropical and tropical birds have an inherently greater physiological flexibility than their temperate-zone counterparts, but rather that very low winter  $T_a$  at high latitudes results in demands for enhanced cold tolerance that simply overwhelm other factors affecting metabolic adjustments (Noakes et al., 2017).

Short-term thermal acclimation experiments are a powerful tool for quantifying the reaction norms (i.e. range of phenotypic traits a single genotype can produce) of phenotypically flexible traits, and can provide information on how phenotypic flexibility varies among and within species. Numerous acclimation studies have investigated flexibility in avian BMR and  $M_{sum}$  in temperate-zone species (McKechnie and Swanson, 2010), with higher metabolic rates typically reported for birds from colder acclimation  $T_a$  ( $T_{acc1}$ ) treatments (generally:  $T_{acc1} \leq 5^\circ\text{C}$ ; e.g. Barceló et al., 2017; Milbergue et al., 2018; Swanson et al., 2014; Vézina et al., 2017). Fewer studies have investigated metabolic flexibility in lower-latitude birds, with these tending to focus on flexibility of

BMR and thermoneutral EWL in response to relatively moderate  $T_{acc1}$  treatments (often  $15^\circ\text{C}$  versus  $30^\circ\text{C}$ ) and typically reporting lower BMR and EWL in birds acclimated to higher  $T_a$  (e.g. Cavieres and Sabat, 2008; Maldonado et al., 2009; Tieleman et al., 2003).

It has also been hypothesized that greater thermoregulatory flexibility confers adaptive benefits in unpredictable or variable environmental conditions (i.e. temperature, rainfall and/or food abundance; Cavieres and Sabat, 2008; Tieleman et al., 2003). Support for this pattern at the intraspecific level was reported for *Zonotrichia capensis* from Chile, as the magnitude of flexibility in BMR and EWL in response to thermal acclimation varied among three populations in relation to environmental variability (Cavieres and Sabat, 2008). To the best of our knowledge, no acclimation studies have investigated metabolic flexibility in response to relatively low  $T_{acc1}$  (i.e.  $<10^\circ\text{C}$ ) or in  $M_{sum}$  among subtropical birds.

In light of the paucity of studies investigating metabolic flexibility in response to thermal acclimation in lower-latitude birds, and the apparently greater flexibility in patterns of seasonal acclimatization in subtropical taxa, we investigated whether metabolic reaction norms vary across a climatic gradient in a subtropical passerine bird, the white-browed sparrow-weaver (*Plocepasser mahali*, hereafter sparrow-weaver). Sparrow-weavers were caught from three populations that differ in patterns of seasonal acclimatization in BMR and  $M_{sum}$  (Noakes et al., 2017), although it remains unclear whether these differences reflect interpopulation variation in metabolic reaction norms or simply acclimatization to local environmental conditions.

As typically reported for north-temperate species (McKechnie and Swanson, 2010), we predicted that BMR and  $M_{sum}$  would be highest in sparrow-weavers from the coldest acclimation treatment ( $T_{acc1}=5^\circ\text{C}$ ). We also expected lower BMR and EWL in birds acclimated to the hottest treatment ( $T_{acc1}=35^\circ\text{C}$ ), as reported for other subtropical species (e.g. Cavieres and Sabat, 2008; Maldonado et al., 2009; Tieleman et al., 2003). We predicted that metabolic reaction norms vary among populations according to the environmental variability of local climates (*sensu* Cavieres and Sabat, 2008), with greater flexibility in sparrow-weavers from the Kalahari Desert site (Askham) where there is more seasonal variation in temperature, as well as less predictable rainfall (Table 1).

**MATERIALS AND METHODS****Study sites**

We received permission to conduct research from the University of Pretoria Animal Ethics Committee (EC-054-15) and the permitting bodies for the Gauteng (CPF6-000206), Limpopo (ZA/LP/H0/2355), Northern Cape (FAUNA 929/2/2015, FAUNA 1389/2015) and Free State (JM 3286/2018) Provinces of South Africa. We captured adult sparrow-weavers, *Plocepasser mahali* Smith 1836,

**Table 1. Daily air temperature minimum ( $T_{a,min}$ ) and maximum ( $T_{a,max}$ ), and total rainfall, during summer and winter at three capture sites in South Africa**

Capture site	Daily $T_{a,min}$ ( $^\circ\text{C}$ )		Daily $T_{a,max}$ ( $^\circ\text{C}$ )		Total rainfall (mm)		Location	
	Summer	Winter	Summer	Winter	Summer	Winter	Study site	Weather station
Polokwane	15.9±0.9	4.9±1.2	27.4±0.8	21.4±1.1	233.2±66.1	1.6±0.7	23°56'S, 29°28'E	23°51'S, 29°27'E
Frankfort	14.5±1.0	-3.0±2.0	29.3±1.0	20.1±1.3	298.7±42.7	17.9±8.7	27°16'S, 28°29'E	27°16'S, 28°30'E
Askham	20.4±1.2	0.6±0.8	38.1±1.8	23.8±1.4	60.1±17.1	0.0±0.0	26°59'S, 20°51'E	26°28'S, 20°36'E (26°57'S, 20°42'E)

Mean±s.d. daily  $T_{a,min}$  and  $T_{a,max}$  are provided for the hottest summer months (January) and coldest winter months (July), and total rainfall over peak summer (December to February) and winter (June to August), for the period December 2012 to August 2017 at three capture sites in South Africa. Weather data were obtained from the closest South African Weather Service stations – there are weather stations in Polokwane and Frankfort; however, the closest weather station to our study site near Askham providing temperature data is ~62 km northwest and rainfall data ~9 km west (co-ordinates presented in parentheses). White-browed sparrow-weavers (*Plocepasser mahali*) were captured from these sites and transported to the University of Pretoria for acclimation experiments.

during the austral summer (10–20 February 2018) at three sites in South Africa across a climatic gradient: one arid site near Askham in the Kalahari Desert (Northern Cape Province) and two more mesic sites at Frankfort (Free State Province) and Polokwane (Limpopo Province; Table 1). We obtained climate data for each site from the closest weather stations of the South African Weather Service to quantify variation in seasonal temperature extremes and rainfall among these sites (Table 1). Sparrow-weavers were captured (34 birds over two nights at each site) and temporarily housed at capture sites as described by Noakes and McKechnie (2019). We transported birds by road in modified pet carriers back to the Small Animal Physiological Research Facility at the University of Pretoria (25°45'S, 28°15'E). Birds from Frankfort and Polokwane spent about 3 h in transit (~200 km and 260 km to Pretoria, respectively), whereas the trip from Askham to Pretoria occurred over 2 days (~6 h travelling per day, ~1000 km in total).

At the Small Animal Physiological Research Facility, we allocated sparrow-weavers to one of three climate-controlled rooms (~11 birds from each site per room), all initially set to  $T_a=25^\circ\text{C}$  and a photoperiod of 12 h:12 h light:dark cycle. We housed birds in cages (600×400×400 mm), typically with two birds per cage (a male and female from the same site, sex determined by bill colour; du Plessis, 2005) but with six of the 102 birds housed individually. Birds were provided with water and wild bird seed mix *ad libitum*, as well as mealworms and superworms (~4 of each per bird per day) during the entire period in captivity. We monitored body mass ( $M_b$ ) of individuals regularly using a Scout Pro Balance scale (SP602US, Ohaus, Pine Brook, NJ, USA) throughout the study period to ensure birds maintained similar values to capture  $M_b$ . Sparrow-weavers were released at their capture sites after the completion of experiments.

### Acclimation regimes

Three days after the arrival of the birds from the final capture site, we set rooms to  $T_a=20^\circ\text{C}$  so that all birds were first exposed to the intermediate  $T_{\text{accl}}$  regime for 4 days. Following this initial period, we adjusted  $T_a$  in two of the rooms to experimental  $T_{\text{accl}}$  values of 5 and  $35^\circ\text{C}$  over a 4 day period, changing  $T_a$  in  $5^\circ\text{C}$  increments every 2 days. An acclimation period of 28 days was allowed after experimental  $T_{\text{accl}}$  values were set in all rooms, after which post-acclimation metabolic measurements occurred over 63 days. Therefore, the duration of acclimation actually varied among individuals from 28 to 91 days, with 28 days near the upper end of the range of acclimation periods used in previous studies (e.g. 30 days: Cavieres and Sabat, 2008; 21 days: McKechnie et al., 2007; 17 days: McKechnie and Wolf, 2004; 28 days: Tieleman et al., 2003). Actual  $T_a$  in each room fluctuated by only a small amount around the setpoint  $T_{\text{accl}}$  values over the course of experiments (Table 2).

### Gas exchange and temperature measurements

Open flow-through respirometry was used to measure  $\text{O}_2$  consumption ( $\dot{V}_{\text{O}_2}$ ) and  $\text{CO}_2$  production ( $\dot{V}_{\text{CO}_2}$ ) in sparrow-weavers, using the same experimental setup and calibration procedures described by Minnaar et al. (2014) and Noakes et al. (2017), with modifications listed below. We used clear plastic containers as metabolic chambers, with volumes of 2.6 l (ClipFresh, Hong Kong, China; incurrent flow rate  $\sim 1 \text{ l min}^{-1}$ ) and 4 l (Lock & Lock, Seoul, South Korea; incurrent flow rate  $\sim 2 \text{ l min}^{-1}$ ) for BMR and  $M_{\text{sum}}$  measurements, respectively. We placed metabolic chambers in a temperature-controlled cabinet (KMF 720, Binder, Tuttlingen, Germany) for BMR measurements, and in a modified fridge-freezer (40 l; ARB, Kilsyth, VIC, Australia) for  $M_{\text{sum}}$  measurements.

We measured  $T_a$  in the metabolic chambers using thermistor probes (TC-100, Sable Systems, Las Vegas, NV, USA) during BMR measurements, and a Cu-Cn thermocouple and thermocouple reader (RDXL12SD, OMEGA Engineering, Norwalk, CT, USA) during  $M_{\text{sum}}$  measurements, inserted through a small hole in the chamber walls sealed with a rubber grommet. We measured core body temperature ( $T_b$ ) of sparrow-weavers using temperature-sensitive passive integrated transponder (PIT) tags (Destron Fearing, St Paul, MN, USA), and the tags were calibrated and implanted into the peritoneal cavity of individuals as described by Noakes et al. (2017). We monitored  $T_b$  using a racket antenna positioned next to metabolic chambers and connected to PIT tag readers (HPR Plus, Biomark, Boise, ID, USA) during BMR measurements, and a handheld PIT tag scanner (DTR-4, Destron Fearing) during  $M_{\text{sum}}$  measurements.

### Experimental protocol

In an attempt to control for the effects of habituation to metabolic chambers after repeated measurements (Jacobs and McKechnie, 2014), we measured  $M_{\text{sum}}$  within 12 h before BMR measurements for half of the individuals and during the 12 h after BMR measurements for the rest of the birds. For three individuals we could not measure BMR and  $M_{\text{sum}}$  within 12 h of each other, and thus did not calculate metabolic expansibility (ME) for these individuals. We randomized the order in which birds from different populations and acclimation treatments were measured. Our maximum sample sizes were 11 birds from each population per  $T_{\text{accl}}$  treatment for BMR and  $M_{\text{sum}}$  measurements; however, because of equipment malfunction, our actual sample sizes were often lower (Table 2). Sample sizes for each sex were approximately equal within populations and  $T_{\text{accl}}$  treatment (overall  $n=91$  birds, 47 males and 44 females).

We quantified BMR and thermoneutral EWL by measuring gas exchange values for four birds simultaneously at  $T_a \approx 30^\circ\text{C}$  (actual

**Table 2. Acclimation temperature ( $T_{\text{accl}}$ ) treatment and sample size for basal metabolic rate (BMR) and summit metabolism ( $M_{\text{sum}}$ ) measurements of white-browed sparrow-weavers (*P. mahali*) from different populations**

$T_{\text{accl}}$ ( $^\circ\text{C}$ )	$T_a$ ( $^\circ\text{C}$ )	Sample size BMR ( $T_b$ )			Sample size $M_{\text{sum}}$ (ME)		
		Askham	Frankfort	Polokwane	Askham	Frankfort	Polokwane
5	5.5±0.5	10 (9)	8 (7)	8 (5)	10 (10)	11 (8)	10 (8)
20	20.3±0.6	9 (5)	6 (6)	11 (8)	9 (7)	7 (5)	11 (11)
35	34.4±0.2	10 (8)	11 (10)	11 (7)	10 (8)	11 (11)	11 (11)

Birds were captured from three sites across South Africa (Askham, Frankfort and Polokwane) and divided among three climate-controlled rooms at the University of Pretoria Small Animal Physiological Research Facility. Each room was set to a different, constant  $T_{\text{accl}}$ , and the mean±s.d. value of actual air temperature ( $T_a$ ) in each room is provided for the study period. Thermoneutral evaporative water loss and body temperature ( $T_b$ ) were measured concurrently with BMR, but  $T_b$  sample sizes were lower as a result of intermittent PIT tag reception (in parentheses after BMR sample size). Sample sizes of metabolic expansibility (ME) were also lower as we only calculated these values for an individual if BMR and  $M_{\text{sum}}$  were measured within 12 h of each other (in parentheses after  $M_{\text{sum}}$  sample size).

$T_a=30.1\pm 0.5^\circ\text{C}$ ) throughout the night, as this  $T_a$  has been reported to be in the thermoneutral zone of sparrow-weavers (Noakes et al., 2017; Smit and McKechnie, 2010). Thermoneutral  $T_b$  was concurrently monitored during BMR measurements, but sample sizes for  $T_b$  were lower as a result of intermittent PIT tag reception (Table 2). Birds were placed into individual metabolic chambers just before sunset and removed after sunrise, with  $M_b$  measured on entry into and removal from the chambers. We sequentially subsampled air from the baseline channel and respective chambers, repeating a cycle lasting 100 min throughout the night: baseline air for 10 min, followed by air from chambers in sequence for 20 min each, and baseline air again for the last 10 min.

We used the sliding cold exposure method in a helox environment (Swanson et al., 1996) to measure  $M_{\text{sum}}$ , and the protocol described by Minnaar et al. (2014) and Noakes et al. (2017). We visually monitored  $\text{O}_2$  and  $\text{CO}_2$  traces throughout measurements for a plateau in increasing metabolic rate indicating that  $M_{\text{sum}}$  had been obtained, and terminated measurements when a distinct decrease in metabolic rate away from this plateau was observed. The  $T_b$  of birds was measured on removal from chambers to confirm hypothermia, and all individuals decreased  $T_b$  by  $\geq 3.7^\circ\text{C}$  from initial values upon entering the chamber (mean  $T_b$  decrease =  $6.2\pm 1.5^\circ\text{C}$ ).

### Data analyses

We calculated whole-animal metabolic rates (BMR and  $M_{\text{sum}}$ ), EWL and ME values as described by Noakes et al. (2017). The BMR of each individual represents the lowest 5 min average metabolic rate during overnight measurements (average EWL and  $T_b$  were calculated for the same time period), and  $M_{\text{sum}}$  is the highest 5 min average metabolic rate during sliding cold exposure measurements. Respiratory exchange ratio (RER;  $\dot{V}_{\text{CO}_2}/\dot{V}_{\text{O}_2}$ ) during measurements was often below the expected range of 0.71 to 1.00 (average RER =  $0.63\pm 0.16$ ), and as thermal equivalence data are not available at RER < 0.71 (Withers, 1992), RER = 0.71 was assumed to calculate metabolic rates in watts for measurements below this value. To confirm that this assumption was not a source of error in our results, we repeated statistical analyses with  $\dot{V}_{\text{O}_2}$  and  $\dot{V}_{\text{CO}_2}$  as the response variable. The helox temperature at which  $M_{\text{sum}}$  was reached was used as an estimate of the cold limit temperature for each bird ( $T_{\text{CL}}$ ). All values are presented as means  $\pm$  s.d.

We fitted linear models to our data using R 3.5.2 (<http://www.R-project.org/>), and checked the assumptions of all models by testing for multicollinearity between continuous predictor variables and inspecting model residual plots (normal Q-Q, residuals versus

fitted, scale-location and residuals versus leverage plots). The ‘lm’ base function was used to investigate variation in capture  $M_b$  among sexes and sites, as well as variation in post-acclimation  $M_b$ , BMR, thermoneutral EWL and  $T_b$ ,  $M_{\text{sum}}$ , ME and  $T_{\text{CL}}$  with predictor variables:  $T_{\text{accl}}$  treatment, population,  $M_b$  (except when  $M_b$  was the response variable) and Julian date (to account for varying lengths of acclimation period). The ‘dredge’ function from the *MuMIn* package (<https://CRAN.R-project.org/package=MuMIn>) was used to determine the combination of predictor variables (and interactions between them) that produced linear models that best explained variation in the respective response variables, and if multiple top models had a similar fit (Akaike information criterion differed by < 2), we chose the most parsimonious model. Sex was included as a predictor variable in initial models, but as it was never included in the top models explaining variation in thermoregulatory variables and as Noakes et al. (2017) also reported no sex differences in this species, sex was excluded from further analyses. If  $T_{\text{accl}}$  and/or population was a significant predictor of a response variable, we used *post hoc* tests of multiple comparisons of means (Tukey contrasts for linear models, *multcomp* package; Hothorn et al., 2008) to investigate variation among specific  $T_{\text{accl}}$  treatments (for  $M_b$ , BMR and EWL) and/or populations (for  $M_b$  and  $M_{\text{sum}}$ ).

## RESULTS

### Body mass

The initial  $M_b$  of sparrow-weavers (i.e. at capture) was significantly higher for males ( $41.9\pm 3.2$  g) than for females ( $40.4\pm 3.0$  g,  $F_{1,88}=6.413$ ,  $P=0.013$ ) across all populations, and varied significantly among populations ( $F_{2,88}=7.964$ ,  $P<0.001$ ). Initial  $M_b$  of birds from Askham ( $39.7\pm 2.9$  g) was significantly lower than that of birds from both Frankfort ( $42.5\pm 3.1$  g,  $P<0.001$ ) and Polokwane ( $41.4\pm 3.1$  g,  $P=0.034$ ), but did not differ between the last two populations ( $P=0.315$ ).

Following acclimation to  $T_a$  of 5, 20 or  $35^\circ\text{C}$  (i.e.  $T_{\text{accl}}$ ), the  $M_b$  of most individuals increased compared with values measured at capture ( $7.2\pm 8.6\%$  higher at the time of each individual’s metabolic measurements), and ranged from a minimum of 37.8 g (an Askham bird at  $T_{\text{accl}}=35^\circ\text{C}$ ) to a maximum of 53.0 g (a Polokwane bird at  $T_{\text{accl}}=5^\circ\text{C}$ ). Post-acclimation  $M_b$  did not significantly vary among sexes ( $F_{1,85}=1.957$ ,  $P=0.166$ ), but did vary among populations and acclimation treatments (Table 3). The  $M_b$  of birds from Askham was significantly lower than that of birds from Frankfort after acclimation ( $\sim 6\%$  lower), but Polokwane sparrow-weavers did not differ significantly from Askham or Frankfort birds (Table 4). Birds acclimated to  $T_{\text{accl}}=35^\circ\text{C}$  had significantly lower  $M_b$  than those

**Table 3. Statistical results from models fitted to data of white-browed sparrow-weavers (*P. mahali*) from different acclimation treatments and populations**

Variable	Acclimation treatment			Population			Acclimation duration			$M_b$		
	F-value	d.f.	P-value	F-value	d.f.	P-value	F-value	d.f.	P-value	F-value	d.f.	P-value
$M_b$	11.646	2,88	<b>&lt;0.001</b>	5.952	2,88	<b>0.004</b>						
BMR	5.109	2,78	<b>0.008</b>				31.084	1,78	<b>&lt;0.001</b>			
Thermoneutral EWL	10.194	2,78	<b>&lt;0.001</b>									
Thermoneutral $T_b$	(Null hypothesis model)											
$M_{\text{sum}}$				3.950	2,86	<b>0.023</b>				22.072	1,86	<b>&lt;0.001</b>
ME							17.412	1,62	<b>&lt;0.001</b>			
$T_{\text{CL}}$	(Null hypothesis model)											

Statistical results [F-value, degrees of freedom (d.f.) and P-value] are from top linear models fitted to data, and predictor variables were only included if model selection indicated they improved model fit. Bold values indicate significance. Potential predictor variables included population (capture sites: Askham, Frankfort and Polokwane),  $T_{\text{accl}}$  treatment (5, 20 and  $35^\circ\text{C}$ ), length of acclimation period (28–91 days), body mass ( $M_b$ ; except when  $M_b$  was the response variable), sex and relevant interactions between these terms. Response variables include  $M_b$ , BMR, thermoneutral evaporative water loss (EWL) and  $T_b$ ,  $M_{\text{sum}}$ , ME and helox temperature at cold limit ( $T_{\text{CL}}$ ; i.e. temperature at which  $M_{\text{sum}}$  was reached).

**Table 4. Metabolic rates and associated thermal variables for white-browed sparrow-weavers (*P. mahali*) from different  $T_{\text{accl}}$  treatments and populations**

Variable	$T_{\text{accl}}$ treatment ( $^{\circ}\text{C}$ )			Population		
	5	20	35	Askham	Frankfort	Polokwane
$M_b$ (g)	44.90 $\pm$ 3.41 <sup>A</sup>	45.42 $\pm$ 3.38 <sup>A</sup>	41.84 $\pm$ 3.25 <sup>B</sup>	42.66 $\pm$ 3.48 <sup>a</sup>	45.27 $\pm$ 3.39 <sup>b</sup>	44.13 $\pm$ 3.77 <sup>a,b</sup>
BMR (W)	0.579 $\pm$ 0.102 <sup>A</sup>	0.566 $\pm$ 0.084 <sup>A,B</sup>	0.512 $\pm$ 0.106 <sup>B</sup>	0.572 $\pm$ 0.100	0.556 $\pm$ 0.106	0.527 $\pm$ 0.096
Thermoneutral EWL (mg min <sup>-1</sup> )	1.587 $\pm$ 0.345 <sup>A</sup>	1.514 $\pm$ 0.411 <sup>A</sup>	1.212 $\pm$ 0.211 <sup>B</sup>	1.419 $\pm$ 0.428	1.312 $\pm$ 0.254	1.473 $\pm$ 0.370
Thermoneutral $T_b$ ( $^{\circ}\text{C}$ )	38.96 $\pm$ 0.75	38.89 $\pm$ 0.80	38.51 $\pm$ 0.49	38.85 $\pm$ 0.69	38.69 $\pm$ 0.73	38.76 $\pm$ 0.70
$M_{\text{sum}}$ (W)	2.910 $\pm$ 0.279	2.882 $\pm$ 0.379	2.705 $\pm$ 0.294	2.943 $\pm$ 0.314 <sup>a</sup>	2.814 $\pm$ 0.327 <sup>b</sup>	2.738 $\pm$ 0.317 <sup>b</sup>
ME	5.235 $\pm$ 1.258	5.192 $\pm$ 1.051	5.510 $\pm$ 1.512	5.294 $\pm$ 1.120	5.274 $\pm$ 1.399	5.397 $\pm$ 1.395
$T_{\text{CL}}$ ( $^{\circ}\text{C}$ )	-8.21 $\pm$ 5.55	-7.73 $\pm$ 5.68	-5.83 $\pm$ 4.42	-7.24 $\pm$ 5.71	-6.94 $\pm$ 5.62	-7.45 $\pm$ 4.64

Birds were captured from sites along a climatic gradient (populations: Askham, Frankfort and Polokwane) and divided among three  $T_{\text{accl}}$  treatments ( $T_{\text{accl}}$ =5, 20 and 35 $^{\circ}\text{C}$ ;  $n \approx 11$  per population per  $T_{\text{accl}}$ ; for sample sizes, see Table 2). After an initial acclimation period of 28 days, we measured  $M_b$ , BMR, thermoneutral EWL and  $T_b$ ,  $M_{\text{sum}}$ , ME and  $T_{\text{CL}}$  (i.e. temperature at which  $M_{\text{sum}}$  was reached). Mean $\pm$ s.d. values are presented for each  $T_{\text{accl}}$  treatment (regardless of population) and population (regardless of  $T_{\text{accl}}$ ), as linear models identified response variables varied significantly with  $T_{\text{accl}}$  ( $M_b$ , BMR and EWL) and/or populations ( $M_b$  and  $M_{\text{sum}}$ ), respectively (Table 4), but never with the  $T_{\text{accl}}$   $\times$  population interaction. *Post hoc* tests of multiple comparisons of means (Tukey contrasts) were fitted to investigate significant variation among  $T_{\text{accl}}$  treatments and populations (denoted by uppercase and lowercase letters, respectively;  $P < 0.05$ ).

acclimated to  $T_{\text{accl}}$ =5 and 20 $^{\circ}\text{C}$  (~8% and 7% lower, respectively), but  $M_b$  did not significantly differ between the last two treatments (Table 4).

### Basal metabolic rate and evaporative water loss

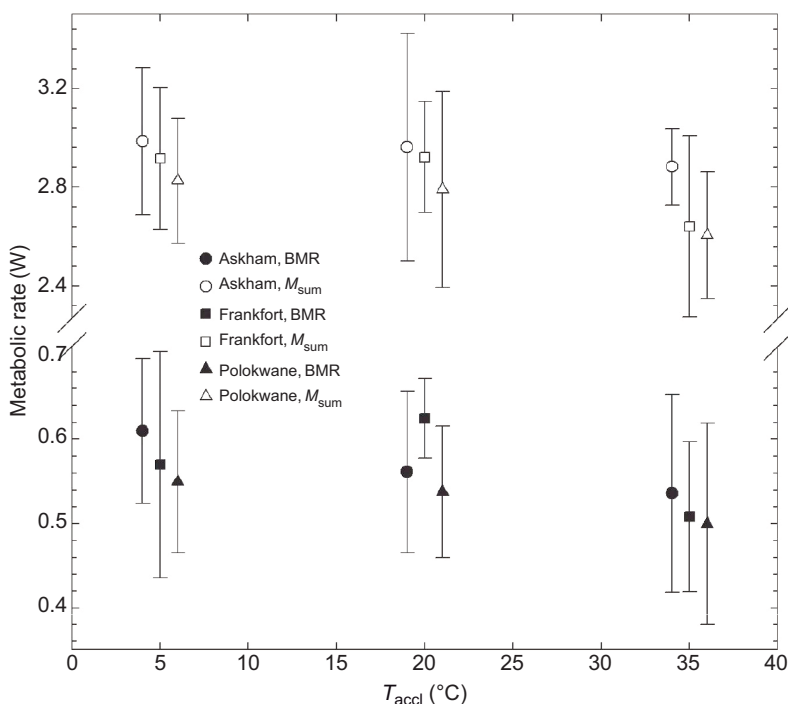
The BMR of sparrow-weavers after acclimation ranged from a minimum of 0.3 W (a Polokwane bird at  $T_{\text{accl}}$ =35 $^{\circ}\text{C}$ ) to a maximum of 0.7 W (an Askham bird at  $T_{\text{accl}}$ =35 $^{\circ}\text{C}$ ). The top model explaining variation in BMR included acclimation treatment and duration of acclimation for each individual, with BMR significantly decreasing at a rate of 3.5 mW per day after the start of the acclimation period (Table 3). BMR was significantly lower in birds acclimated to  $T_{\text{accl}}$ =35 $^{\circ}\text{C}$  than in those acclimated to  $T_{\text{accl}}$ =5 $^{\circ}\text{C}$  regardless of population (~12% lower), but birds at  $T_{\text{accl}}$ =20 $^{\circ}\text{C}$  did not significantly differ from either of the other acclimation treatments (Fig. 1, Table 4).

Thermoneutral EWL significantly varied among acclimation treatments regardless of population (Table 3), with lower EWL in

birds from  $T_{\text{accl}}$ =35 $^{\circ}\text{C}$  than in those from both  $T_{\text{accl}}$ =5 and 20 $^{\circ}\text{C}$  (~25% and 20% lower, respectively), but no significant difference between the last two treatments (Table 4). In contrast, the top model explaining variation in thermoneutral  $T_b$  was the null hypothesis (i.e. no predictor variables included; Tables 3 and 4). In summary, thermoregulatory responses measured at thermoneutrality did not vary significantly among populations after acclimation, with BMR and EWL lower in all birds from the hottest acclimation treatment compared with the cooler treatments. Repeating analyses using  $\dot{V}_{\text{O}_2}$  and  $\dot{V}_{\text{CO}_2}$  instead of BMR (W) as the response variable revealed the same patterns of variation.

### Summit metabolism, cold limit and metabolic expansibility

The  $M_{\text{sum}}$  of sparrow-weavers after acclimation ranged from a minimum of 2.0 W to a maximum of 3.8 W (both Askham individuals at  $T_{\text{accl}}$ =20 $^{\circ}\text{C}$ ). The top model explaining variation in  $M_{\text{sum}}$  included population and  $M_b$ , with  $M_{\text{sum}}$  increasing with



**Fig. 1. Basal metabolic rate (BMR) but not summit metabolism ( $M_{\text{sum}}$ ) of white-browed sparrow-weavers is flexible in response to thermal acclimation.** BMR did not vary significantly among populations (Askham, Frankfort and Polokwane) after an acclimation period of 30 days, but was significantly lower in birds acclimated to air temperature ( $T_{\text{accl}}$ )=35 $^{\circ}\text{C}$ , compared with those from  $T_{\text{accl}}$ =5 $^{\circ}\text{C}$  ( $P=0.015$ ). In contrast,  $M_{\text{sum}}$  was significantly higher in Askham birds than in Polokwane ( $P=0.004$ ) and Frankfort ( $P=0.031$ ) birds after acclimation, but did not significantly vary among acclimation treatments. Metabolic rate was measured using flow-through respirometry and mean $\pm$ s.d. values are presented for each population at each  $T_{\text{accl}}$  (points are jittered for illustrative purposes;  $n \approx 11$  per population per  $T_{\text{accl}}$ ; for sample sizes, see Table 2). Statistical results are from linear models and *post hoc* tests of multiple comparisons of means (Tukey contrasts; Tables 3 and 4).

increasing  $M_b$  at a rate of  $30 \text{ mW g}^{-1}$  (Table 3). Post-acclimation  $M_{\text{sum}}$  was significantly higher in Askham than in both Frankfort and Polokwane birds ( $\sim 5\%$  and  $8\%$  higher, respectively), but did not significantly differ between the last two populations (Fig. 1, Table 4).

The null hypothesis was the top model explaining variation in the  $T_{\text{CL}}$  (i.e. helox temperature at which  $M_{\text{sum}}$  was reached), and ME significantly increased with increasing length of acclimation at a rate of  $4.0 \times 10^{-2}$  per day (i.e. opposite pattern to that observed in BMR; Tables 3 and 4). In summary, responses during cold exposure did not vary significantly among acclimation treatments, but  $M_{\text{sum}}$  was higher in Askham birds than in the other populations after acclimation. Repeating analyses using  $\dot{V}_{\text{O}_2}$  and  $\dot{V}_{\text{CO}_2}$  instead of  $M_{\text{sum}}$  (W) as the response variable revealed the same patterns of variation.

## DISCUSSION

Sparrow-weavers showed phenotypic flexibility in  $M_b$ , BMR and EWL in response to thermal acclimation, but not in  $M_{\text{sum}}$ . In partial support of our predictions, BMR and EWL were lower in birds from the hottest acclimation treatment ( $T_{\text{accl}}=35^\circ\text{C}$  versus  $5$  and  $20^\circ\text{C}$ ), but  $M_{\text{sum}}$  did not significantly vary in response to acclimation. In contrast to our predictions, there were similar reaction norms for BMR and EWL in the three populations, although post-acclimation  $M_{\text{sum}}$  was higher in Askham birds regardless of  $T_{\text{accl}}$  treatment. These findings suggest the considerable variation previously reported for BMR of sparrow-weavers among populations and seasons is the result of phenotypic flexibility to local conditions (Noakes et al., 2017). The consistently higher  $M_{\text{sum}}$  of Askham birds, however, suggests that this trait could be fixed in this population as the result of genotypic adaptation, developmental plasticity or epigenetics (Noakes et al., 2017).

## Body mass

We found patterns of among-population variation in initial (i.e. capture)  $M_b$  similar to those previously reported for these sparrow-weaver populations (lowest in Askham, intermediate in Polokwane and highest in Frankfort birds; Noakes et al., 2016, 2017). After acclimation, however, we found no significant difference in  $M_b$  between Askham and Polokwane sparrow-weavers, suggesting  $M_b$  variation between these populations is the result of phenotypic flexibility (*sensu* Noakes and McKechnie, 2019), although higher  $M_b$  in Frankfort birds persisted regardless of  $T_{\text{accl}}$  treatment (Table 4). Frankfort is one of the coldest locations in the distribution of sparrow-weavers (daily winter minimum  $T_a$  regularly  $<0^\circ\text{C}$ ; Table 1) and thus a larger  $M_b$  may have an adaptive value for this population following Bergmann's rule (Bergmann, 1847).

Post-acclimation  $M_b$  was significantly lower in the hottest treatment ( $T_{\text{accl}}=35^\circ\text{C}$ ) than in the cooler treatments ( $T_{\text{accl}}=5$  and  $20^\circ\text{C}$ ; Table 4), a similar pattern to that reported among sparrow-weavers acclimated to three different  $T_a$  (i.e. lower  $M_b$  in  $T_{\text{accl}}=42^\circ\text{C}$  versus  $30$  and  $36^\circ\text{C}$ ; Noakes and McKechnie, 2019). Previous acclimation studies have typically interpreted avian  $M_b$  variation with  $T_{\text{accl}}$  as responses associated with adjustment to colder  $T_a$  (e.g. Barceló et al., 2017; Tieleman et al., 2003; Vézina et al., 2017; Williams and Tieleman, 2000), whereas lower  $M_b$  could also provide benefits for birds at hotter  $T_a$  (e.g. increased surface area available for passive heat dissipation relative to volume). It is unlikely that  $M_b$  variation is a major source of thermoregulatory differences in the present study, as  $M_b$  was only a significant predictor of  $M_{\text{sum}}$ , which did not significantly vary with  $T_{\text{accl}}$  (Table 3).

## Basal metabolic rate and evaporative water loss

An unexpected finding was that BMR of individuals significantly decreased with time since the onset of the acclimation period (post-acclimation measurements occurred over 63 days), despite an initial acclimation period (28 days) towards the upper end of the range typically used in previous studies ( $\sim 17$ – $30$  days; e.g. Cavieres and Sabat, 2008; McKechnie et al., 2007; McKechnie and Wolf, 2004; Tieleman et al., 2003). This finding questions the assumption that avian metabolic rates stabilize after a set period of time at a fixed  $T_{\text{accl}}$ , and many previous studies do not consider or provide the duration of post-acclimation measurement periods (e.g. Barceló et al., 2017; Cavieres and Sabat, 2008; Maldonado et al., 2009; Williams and Tieleman, 2000). To the best of our knowledge, only one previous study has included acclimation duration as a continuous variable in analyses of avian metabolic variation, finding no significant change in BMR or  $M_{\text{sum}}$  of *Poecile atricapillus* over 45 days of measurements (initial acclimation period=39 days; Milbergue et al., 2018). We are not certain why BMR decreased with acclimation duration in sparrow-weavers, although it does highlight the limited understanding of the rapidity of avian metabolic adjustments in the literature (McKechnie and Swanson, 2010). We consider it unlikely this trend influenced patterns of variation among  $T_{\text{accl}}$  treatments or populations in the present study, as the rate of decreasing BMR with time was consistent among all birds.

Post-acclimation BMR (Table 4) was similar to values measured in sparrow-weavers within 60 h of capture from the same populations during summer and winter (range of mean BMR:  $0.38$ – $0.64$  W; Noakes et al., 2017). Mean BMR of each population and  $T_{\text{accl}}$  treatment was consistently higher than values predicted using allometric equations for tropical ( $29$ – $42\%$  higher) and passerine ( $10$ – $18\%$  higher) birds (Table 5; Londoño et al., 2015). Higher than predicted metabolic rates appear to be typical in sparrow-weavers (Noakes et al., 2017; Noakes and McKechnie, 2020), which is unexpected as birds from lower latitudes are generally reported to have a 'slower pace of life' than higher-latitude counterparts (Hail, 1983; Weathers, 1979; Wiersma et al., 2007; Londoño et al., 2015).

Similar to findings in temperate-zone species (e.g. Barceló et al., 2017; Milbergue et al., 2018; Vézina et al., 2017), BMR was higher in birds from the coldest compared with the hottest acclimation treatment (i.e.  $T_{\text{accl}}=5^\circ\text{C}$  versus  $35^\circ\text{C}$ ); however, it did not significantly vary between the two cooler treatments (i.e.  $T_{\text{accl}}=5$  versus  $20^\circ\text{C}$ ; Fig. 1, Table 4). In support of our predictions, BMR and thermoneutral EWL were lower in birds at  $T_{\text{accl}}=35^\circ\text{C}$  compared with cooler treatments (Fig. 1, Table 4), which is consistent with studies on lower-latitude species (Cavieres and Sabat, 2008; Tieleman et al., 2003; Williams and Tieleman, 2000). For example, *Z. capensis* acclimated to  $30^\circ\text{C}$  had lower BMR and EWL compared with individuals acclimated to  $15^\circ\text{C}$  ( $\sim 16\%$  and  $17\%$  lower, respectively), but there was no seasonal variation in BMR or EWL in field-acclimatized birds from the same population in central Chile (Maldonado et al., 2009). In contrast, field-acclimatized sparrow-weavers displayed greater flexibility in BMR (ranging among populations from no seasonal change to  $\sim 59\%$  higher in winter), compared with variation among  $T_{\text{accl}}$  treatments ( $\sim 12\%$  lower BMR at  $T_{\text{accl}}=35$  versus  $5^\circ\text{C}$ ; Noakes et al., 2017).

We report no interpopulation variation in post-acclimation BMR or EWL, suggesting different patterns of seasonal BMR acclimatization among populations of sparrow-weavers reflects phenotypic flexibility in response to local environmental conditions (Noakes et al., 2017). This also supports the idea of no clear pattern

**Table 5. BMR and  $M_{\text{sum}}$  of white-browed sparrow-weavers (*P. mahali*) from different  $T_{\text{accl}}$  treatments and populations, expressed as a percentage of predicted metabolic values using allometry**

Variable	Category	$T_{\text{accl}}$ treatment ( $^{\circ}\text{C}$ )			Population		
		5	20	35	Askham	Frankfort	Polokwane
BMR	Tropical	140%	136%	129%	142%	134%	129%
	Passerine	118%	115%	110%	121%	113%	109%
$M_{\text{sum}}$	Tropical	124%	122%	122%	131%	119%	118%
	Oscine	105%	103%	102%	110%	101%	100%

Allometric equations for BMR of tropical and passerine birds were obtained from Londoño et al. (2015), and for  $M_{\text{sum}}$  from Wiersma et al. (2007) and Swanson and Bozinovic (2011) for tropical and oscine passerine birds, respectively. Predicted metabolic values were calculated for each population and  $T_{\text{accl}}$  treatment using the mean  $M_b$  for each of these categories.

of BMR and EWL variation among arid versus mesic populations of sparrow-weavers (Noakes et al., 2017), which contrasts with previous studies reporting lower BMR and/or EWL in arid compared with mesic populations of *Z. capensis* and *Haemorrhous mexicanus* (Cavieres and Sabat, 2008; MacMillen and Hinds, 1998; Sabat et al., 2006). Reduced BMR and EWL in arid-zone birds has been suggested to confer adaptive value by minimizing heat production and conserving water in hot, dry environments (Tieleman et al., 2002; Tieleman and Williams, 2000), and the reduced BMR and EWL in sparrow-weavers from the hottest  $T_{\text{accl}}$  treatment probably provided similar benefits.

#### Summit metabolism, cold limit and metabolic expansibility

Post-acclimation  $M_{\text{sum}}$  and  $T_{\text{CL}}$  were similar to values reported for field-acclimatized sparrow-weavers from the same populations during summer and winter (range of mean  $M_{\text{sum}}$ : 2.40–3.86 W; Noakes et al., 2017), and ME was within the range typically reported for birds (~3–8; Table 4; Swanson, 2010). As was the case for BMR and as reported previously in sparrow-weavers (Noakes et al., 2017; Noakes and McKechnie, 2020), mean  $M_{\text{sum}}$  values were higher than predicted using allometric equations for tropical birds (18–24% higher; Table 5; Wiersma et al., 2007). However, post-acclimation  $M_{\text{sum}}$  was generally similar to values predicted for oscine passerines (Table 5; Swanson and Bozinovic, 2011).

In contrast to what we expected,  $M_{\text{sum}}$  was not higher in sparrow-weavers at the coldest  $T_{\text{accl}}$ , and  $M_{\text{sum}}$ ,  $T_{\text{CL}}$  and ME did not vary in response to thermal acclimation (Fig. 1, Table 4). Higher BMR and  $M_{\text{sum}}$  have been reported in temperate birds from colder acclimation treatments (McKechnie and Swanson, 2010), but the coldest treatment in the current study ( $T_{\text{accl}}=5^{\circ}\text{C}$ ) is on the warmer end of the range typically used in previous experiments (cold  $T_{\text{accl}}$  range from  $-10$  to  $5^{\circ}\text{C}$ ; Barceló et al., 2017; Milbergue et al., 2018; Swanson et al., 2014; Vézina et al., 2017). However, two temperate-zone species (*Junco hyemalis* and *Calidris canutus islandica*) from cold-acclimation treatments similar to that of the current study ( $T_{\text{accl}}=3$  and  $5^{\circ}\text{C}$ , respectively) had ~16–32% higher BMR and/or  $M_{\text{sum}}$  than conspecifics from milder treatments ( $T_{\text{accl}}=24$  and  $25^{\circ}\text{C}$ , respectively; Swanson et al., 2014; Vézina et al., 2017). To the best of our knowledge, no previous acclimation studies have investigated flexibility in  $M_{\text{sum}}$ , or metabolic values in response to  $T_{\text{accl}} < 10^{\circ}\text{C}$ , in subtropical birds.

Increases in  $M_{\text{sum}}$  are associated with higher cold tolerance in small, north-temperate birds (Cooper, 2002; Petit et al., 2017; Swanson, 2001; Swanson and Liknes, 2006), and  $M_{\text{sum}}$  has been reported as flexible in response to short-term (days to weeks) fluctuations in minimum  $T_a$  in several higher-latitude species (Dubois et al., 2016; Petit and Vézina, 2014; Swanson and Olmstead, 1999). The lack of flexibility of  $M_{\text{sum}}$  in sparrow-weavers in response to thermal acclimation is consistent with the

idea that enhancing cold tolerance is of less importance at lower latitudes with comparatively milder winters. Metabolic values of Askham sparrow-weavers measured during the summers and winters over a 4 year period were never related to minimum  $T_a$ , but were significantly lower in seasons with lower food abundance (Noakes and McKechnie, 2020). This suggests that fluctuations in food availability in relation to energy conservation are an important driver of metabolic flexibility in subtropical birds, and constant food availability could explain the lack of significant  $M_{\text{sum}}$  variation among  $T_{\text{accl}}$  treatments. Moreover,  $M_{\text{sum}}$  can vary as a consequence of other constraints on muscle function; for example,  $M_{\text{sum}}$  is typically higher in birds with migratory disposition (Swanson, 2010), and exercise-training of *Passer domesticus* resulted in elevations in both maximal metabolic rates and  $M_{\text{sum}}$  (~19.7% and 15.5% higher, respectively; Zhang et al., 2015b). Another potential explanation for the lack of significant  $M_{\text{sum}}$  variation with  $T_{\text{accl}}$  in sparrow-weavers could be that  $M_{\text{sum}}$  is more coupled with activity levels rather than minimum  $T_a$  in this subtropical species.

Post-acclimation  $M_{\text{sum}}$  was higher in Askham birds than in the other populations regardless of  $T_{\text{accl}}$  treatment (Fig. 1, Table 4), which is consistent with interpopulation variation in field-acclimatized sparrow-weavers (Noakes et al., 2017). Higher  $M_{\text{sum}}$  could be a fixed trait in the Askham population associated with cold winters at this desert site (although Frankfort winters are colder; Table 1); however, we cannot identify whether this pattern is the result of genotypic adaptation, developmental plasticity or epigenetics. Moreover, higher  $M_{\text{sum}}$  in Askham birds was not associated with higher  $T_{\text{CL}}$  compared with the other populations, and variation in  $M_{\text{sum}}$  has never been directly associated with  $T_{\text{CL}}$  variation in sparrow-weavers (Noakes et al., 2017; Noakes and McKechnie, 2020), suggesting metabolic variation is not primarily associated with enhancing cold tolerance in this subtropical species.

#### Conclusion

It has been hypothesized that birds from environments with higher variability and/or unpredictability in climates may have greater flexibility in their thermal physiology (Schlichting and Pigliucci, 1998; Tieleman et al., 2003). Support for this pattern was reported at the intraspecific level among three populations of *Z. capensis*, as flexibility in BMR and EWL in response to  $T_{\text{accl}}=15$  and  $30^{\circ}\text{C}$  varied in relation to variability of local climates (Cavieres and Sabat, 2008). We thus predicted greater physiological flexibility in sparrow-weavers from Askham on account of considerable seasonality in  $T_a$  and less predictable rainfall (Table 1), but found similar reaction norms for BMR and EWL among populations and no  $M_{\text{sum}}$  variation with  $T_{\text{accl}}$ . The occurrence of similar reaction norms among sparrow-weaver populations was also reported for their capacity to cope with high  $T_a$  (i.e. thermoregulatory responses at  $\sim 38^{\circ}\text{C} \leq T_a \leq 54^{\circ}\text{C}$ ) during an acclimation study at different  $T_{\text{accl}}$

(30, 36 and 42°C; Noakes and McKechnie, 2019). However, reaction norms could vary in a manner outside the scope of what was measured during these studies, such as the upper/lower limits or the rate at which individuals adjust their physiology (McKechnie, 2008). Moreover, thermoregulatory reaction norms are not necessarily fixed in individuals; for example, flexibility in mammalian BMR has been reported to vary among and within individuals between seasons in *Phodopus sungorus* (Boratyński et al., 2016, 2017).

Little is known about the shape of avian physiological reaction norms as the majority of acclimation studies have only included two  $T_{\text{accl}}$  treatments (McKechnie, 2008). The reaction norm for BMR has been reported as approximately linear in *Streptopelia senegalensis* across  $T_{\text{accl}}$ =10, 22 and 35°C (McKechnie et al., 2007), as well as in field-acclimatized *Poecile atricapillus* at minimum  $T_a$  ranging from -20 to 30°C (Petit and Vézina, 2014). In contrast, the BMR reaction norm of sparrow-weavers does not appear to be precisely linear, as BMR was similar in birds from  $T_{\text{accl}}$ =5 and 20°C, but lower in individuals from  $T_{\text{accl}}$ =35°C (Table 4, Fig. 1). Petit and Vézina (2014) reported the reaction norm of  $M_{\text{sum}}$  in field-acclimatized *Poecile atricapillus* was approximately linear between lower and upper limits (i.e. -10 and 24°C, respectively), whereas the present study gives no information on the shape of the  $M_{\text{sum}}$  reaction norm of sparrow-weavers (Table 4, Fig. 1).

We found phenotypic flexibility in BMR and thermoneutral EWL of sparrow-weavers in response to short-term thermal acclimation, with similar reaction norms for BMR and EWL among populations along a climatic gradient. BMR and EWL were lower in birds from the hottest  $T_{\text{accl}}$  treatment as previously reported for lower-latitude species (Cavieres and Sabat, 2008; Maldonado et al., 2009; Tieleman et al., 2003). In contrast to studies on higher-latitude birds (McKechnie and Swanson, 2010),  $M_{\text{sum}}$  did not vary in response to thermal acclimation. This suggests that factors other than minimum  $T_a$  and enhancing cold tolerance may drive patterns of metabolic variation in this subtropical species (e.g. food availability; Noakes and McKechnie, 2020). To the best of our knowledge, this is the first acclimation experiment investigating phenotypic flexibility of  $M_{\text{sum}}$ , as well as metabolic rates in response to low  $T_{\text{accl}}$  (i.e. <10°C), in a subtropical bird, and more studies are required to determine how avian metabolic flexibility varies with latitude.

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#### Competing interests

The authors declare no competing or financial interests.

#### Author contributions

Conceptualization: M.J.N., A.E.M.; Methodology: M.J.N., A.E.M.; Formal analysis: M.J.N.; Investigation: M.J.N.; Resources: A.E.M.; Data curation: M.J.N.; Writing - original draft: M.J.N.; Writing - review & editing: M.J.N., A.E.M.; Visualization: M.J.N.; Supervision: A.E.M.; Project administration: M.J.N., A.E.M.; Funding acquisition: A.E.M.

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