

RESEARCH ARTICLE

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

Matthew J. Noakes¹ and Andrew E. McKechnie^{1,2,*}

ABSTRACT

Small birds inhabiting northern temperate and boreal latitudes typically increase metabolic rates during cold winters or acclimation to low air temperatures (T_{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_{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_{accl} treatments (5, 20 and 35°C; $n=11$ per population per T_{accl}) for 28 days, and measured post-acclimation BMR, EWL and M_{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_{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_{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_{sum} ; maximum metabolic rate during acute cold exposure; reviewed by McKechnie et al., 2015; Swanson, 2010). In particular, a higher M_{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_{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_{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_{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

¹DST-NRF Centre of Excellence at the FitzPatrick Institute, Department of Zoology and Entomology, University of Pretoria, Pretoria 0001, South Africa. ²South African Research Chair in Conservation Physiology, South African National Biodiversity Institute, Pretoria 0001, South Africa.

*Author for correspondence (andrew.mckechnie@up.ac.za)

 M.J.N., 0000-0002-1270-4422; A.E.M., 0000-0002-1524-1021

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 ₂ consumption
\dot{V}_{O_2}	rate of O ₂ 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°C versus 30°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_{accl} regime for 4 days. Following this initial period, we adjusted T_a in two of the rooms to experimental T_{accl} values of 5 and 35°C over a 4 day period, changing T_a in 5°C increments every 2 days. An acclimation period of 28 days was allowed after experimental T_{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_{accl} values over the course of experiments (Table 2).

Gas exchange and temperature measurements

Open flow-through respirometry was used to measure O_2 consumption (\dot{V}_{O_2}) and CO_2 production (\dot{V}_{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_{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_{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_{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_{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_{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_{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_{accl} treatment for BMR and M_{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_{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_{accl}) treatment and sample size for basal metabolic rate (BMR) and summit metabolism (M_{sum}) measurements of white-browed sparrow-weavers (*P. mahali*) from different populations

T_{accl} ($^\circ\text{C}$)	T_a ($^\circ\text{C}$)	Sample size BMR (T_b)			Sample size M_{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_{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_{sum} were measured within 12 h of each other (in parentheses after M_{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_{sum} , and the protocol described by Minnaar et al. (2014) and Noakes et al. (2017). We visually monitored O_2 and CO_2 traces throughout measurements for a plateau in increasing metabolic rate indicating that M_{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_{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_{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 ± 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}_{O_2} and \dot{V}_{CO_2} as the response variable. The helox temperature at which M_{sum} was reached was used as an estimate of the cold limit temperature for each bird (T_{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_{sum} , ME and T_{CL} with predictor variables: T_{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_{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_{accl} treatments (for M_b , BMR and EWL) and/or populations (for M_b and M_{sum}).

RESULTS

Body mass

The initial M_b of sparrow-weavers (i.e. at capture) was significantly higher for males (41.9 ± 3.2 g) than for females (40.4 ± 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 ± 2.9 g) was significantly lower than that of birds from both Frankfort (42.5 ± 3.1 g, $P<0.001$) and Polokwane (41.4 ± 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°C (i.e. T_{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	<0.001	5.952	2,88	0.004						
BMR	5.109	2,78	0.008				31.084	1,78	<0.001			
Thermoneutral EWL	10.194	2,78	<0.001									
Thermoneutral T_b	(Null hypothesis model)											
M_{sum}				3.950	2,86	0.023				22.072	1,86	<0.001
ME							17.412	1,62	<0.001			
T_{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_{accl} treatment (5, 20 and 35°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_{sum} , ME and helox temperature at cold limit (T_{CL} ; i.e. temperature at which M_{sum} was reached).

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

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

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

acclimated to T_{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_{accl} =35 $^{\circ}\text{C}$) to a maximum of 0.7 W (an Askham bird at T_{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_{accl} =35 $^{\circ}\text{C}$ than in those acclimated to T_{accl} =5 $^{\circ}\text{C}$ regardless of population (~12% lower), but birds at T_{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_{accl} =35 $^{\circ}\text{C}$ than in those from both T_{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}_{O_2} and \dot{V}_{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_{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_{accl} =20 $^{\circ}\text{C}$). The top model explaining variation in M_{sum} included population and M_b , with M_{sum} increasing with

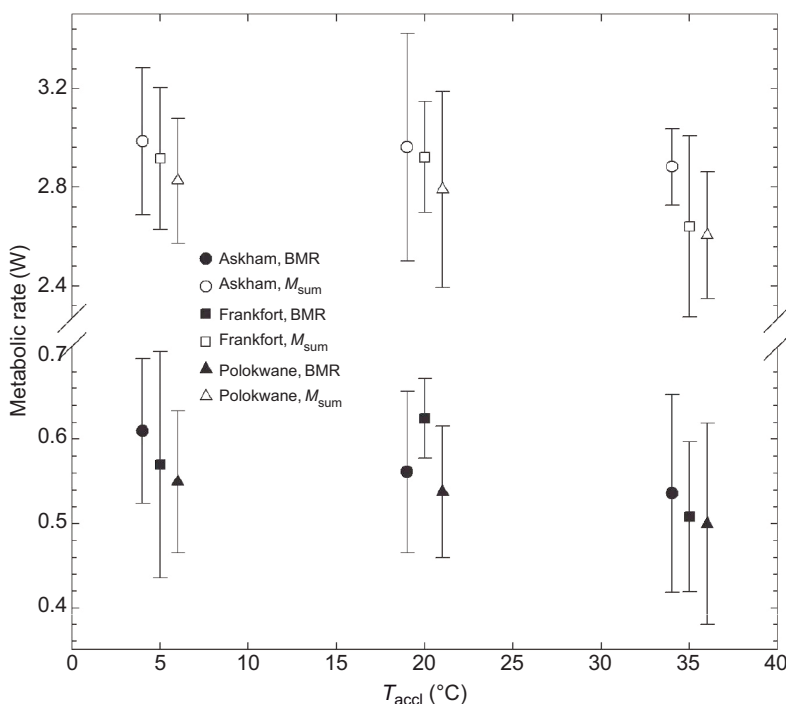


Fig. 1. Basal metabolic rate (BMR) but not summit metabolism (M_{sum}) of white-browed sparrow-weavers is flexible in response to thermal acclimation. BMR did not vary significantly among populations (Askham, Frankfort and Polokwane) after an acclimation period of 30 days, but was significantly lower in birds acclimated to air temperature (T_{accl})=35 $^{\circ}\text{C}$, compared with those from T_{accl} =5 $^{\circ}\text{C}$ ($P=0.015$). In contrast, M_{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±s.d. values are presented for each population at each T_{accl} (points are jittered for illustrative purposes; $n \approx 11$ per population per T_{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 mW g^{-1} (Table 3). Post-acclimation M_{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_{CL} (i.e. helox temperature at which M_{sum} was reached), and ME significantly increased with increasing length of acclimation at a rate of 4.0×10^{-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_{sum} was higher in Askham birds than in the other populations after acclimation. Repeating analyses using \dot{V}_{O_2} and \dot{V}_{CO_2} instead of M_{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_{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°C), but M_{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_{sum} was higher in Askham birds regardless of T_{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_{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_{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°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°C ; Noakes and McKechnie, 2019). Previous acclimation studies have typically interpreted avian M_b variation with T_{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_{sum} , which did not significantly vary with T_{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 (~ 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_{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_{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_{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_{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°C); however, it did not significantly vary between the two cooler treatments (i.e. $T_{\text{accl}}=5$ versus 20°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°C had lower BMR and EWL compared with individuals acclimated to 15°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_{accl} treatments ($\sim 12\%$ lower BMR at $T_{\text{accl}}=35$ versus 5°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_{sum} of white-browed sparrow-weavers (*P. mahali*) from different T_{accl} treatments and populations, expressed as a percentage of predicted metabolic values using allometry

Variable	Category	T_{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_{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_{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_{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_{accl} treatment probably provided similar benefits.

Summit metabolism, cold limit and metabolic expansibility

Post-acclimation M_{sum} and T_{CL} were similar to values reported for field-acclimatized sparrow-weavers from the same populations during summer and winter (range of mean M_{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_{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_{sum} was generally similar to values predicted for oscine passerines (Table 5; Swanson and Bozinovic, 2011).

In contrast to what we expected, M_{sum} was not higher in sparrow-weavers at the coldest T_{accl} , and M_{sum} , T_{CL} and ME did not vary in response to thermal acclimation (Fig. 1, Table 4). Higher BMR and M_{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_{accl} range from -10 to 5°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°C , respectively) had ~16–32% higher BMR and/or M_{sum} than conspecifics from milder treatments ($T_{\text{accl}}=24$ and 25°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_{sum} , or metabolic values in response to $T_{\text{accl}} < 10^{\circ}\text{C}$, in subtropical birds.

Increases in M_{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_{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_{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_{sum} variation among T_{accl} treatments. Moreover, M_{sum} can vary as a consequence of other constraints on muscle function; for example, M_{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_{sum} (~19.7% and 15.5% higher, respectively; Zhang et al., 2015b). Another potential explanation for the lack of significant M_{sum} variation with T_{accl} in sparrow-weavers could be that M_{sum} is more coupled with activity levels rather than minimum T_a in this subtropical species.

Post-acclimation M_{sum} was higher in Askham birds than in the other populations regardless of T_{accl} treatment (Fig. 1, Table 4), which is consistent with interpopulation variation in field-acclimatized sparrow-weavers (Noakes et al., 2017). Higher M_{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_{sum} in Askham birds was not associated with higher T_{CL} compared with the other populations, and variation in M_{sum} has never been directly associated with T_{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°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_{sum} variation with T_{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_{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_{acc1} treatments (McKechnie, 2008). The reaction norm for BMR has been reported as approximately linear in *Streptopelia senegalensis* across T_{acc1} =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_{acc1} =5 and 20°C, but lower in individuals from T_{acc1} =35°C (Table 4, Fig. 1). Petit and Vézina (2014) reported the reaction norm of M_{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_{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_{acc1} 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_{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_{sum} , as well as metabolic rates in response to low T_{acc1} (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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References

- Angilletta, M. J., Jr, Cooper, B. S., Schuler, M. S. and Boyles, J. G. (2010). The evolution of thermal physiology in endotherms. *Front. Biosci.* **E2**, 861-881. doi:10.2741/e148
- Barceló, G., Love, O. P. and Vézina, F. (2017). Uncoupling basal and summit metabolic rates in white-throated sparrows: digestive demand drives maintenance costs, but changes in muscle mass are not needed to improve thermogenic capacity. *Physiol. Biochem. Zool.* **90**, 153-165. doi:10.1086/689290
- Bergmann, C. (1847). Über die verhältnisse der wärmeökonomie der thiere zu ihrer grösse. *Göttinger Studien* **3**, 595-708.
- Boratyński, J. S., Jefimow, M. and Wojciechowski, M. S. (2016). Phenotypic flexibility of energetics in acclimated Siberian hamsters has a narrower scope in winter than in summer. *J. Comp. Physiol. B* **186**, 387-402. doi:10.1007/s00360-016-0959-3
- Boratyński, J. S., Jefimow, M. and Wojciechowski, M. S. (2017). Individual differences in the phenotypic flexibility of basal metabolic rate in Siberian hamsters are consistent on short- and long-term timescales. *Physiol. Biochem. Zool.* **90**, 139-152. doi:10.1086/689870
- Boyles, J. G., Seebacher, F., Smit, B. and McKechnie, A. E. (2011). Adaptive thermoregulation in endotherms may alter responses to climate change. *Integr. Comp. Biol.* **51**, 676-690. doi:10.1093/icb/ict053
- Broggi, J., Hohtola, E., Koivula, K., Orell, M., Thompson, R. L. and Nilsson, J.-Å. (2007). Sources of variation in winter basal metabolic rate in the great tit. *Funct. Ecol.* **21**, 528-533. doi:10.1111/j.1365-2435.2007.01255.x
- Cavieres, G. and Sabat, P. (2008). Geographic variation in the response to thermal acclimation in rufous-collared sparrows: are physiological flexibility and environmental heterogeneity correlated? *Funct. Ecol.* **22**, 509-515. doi:10.1111/j.1365-2435.2008.01382.x
- Cooper, S. J. (2002). Seasonal metabolic acclimatization in mountain chickadees and Juniper titmice. *Physiol. Biochem. Zool.* **75**, 386-395. doi:10.1086/342256
- du Plessis, M. A. (2005). White-browed Sparrow-Weaver (*Plocepasser mahall*). In *Roberts Birds of Southern Africa* (ed. P.A.R. Hockey, W.R.J. Dean and P.G. Ryan), pp. 1006-1007. Cape Town, South Africa: Trustees of the John Voelcker Bird Book Fund.
- Dubois, K., Hallot, F. and Vézina, F. (2016). Basal and maximal metabolic rates differ in their response to rapid temperature change among avian species. *J. Comp. Physiol. B* **186**, 919-935. doi:10.1007/s00360-016-1001-5
- Hail, C. J. (1983). The metabolic rate of tropical birds. *Condor* **85**, 61-65. doi:10.2307/1367889
- Hothorn, T., Bretz, F. and Westfall, P. (2008). Simultaneous inference in general parametric models. *Biometrical J.* **50**, 346-363. doi:10.1002/bimj.200810425
- Huey, R. B., Kearney, M. R., Krockenberger, A., Holtum, J. A. M., Jess, M. and Williams, S. E. (2012). Predicting organismal vulnerability to climate warming: roles of behaviour, physiology and adaptation. *Phil. Trans. R. Soc. B* **367**, 1665-1679. doi:10.1098/rstb.2012.0005
- Jacobs, P. J. and McKechnie, A. E. (2014). Experimental sources of variation in avian energetics: estimated basal metabolic rate decreases with successive measurements. *Physiol. Biochem. Zool.* **87**, 762-769. doi:10.1086/676933
- Jetz, W., Freckleton, R. P. and McKechnie, A. E. (2008). Environment, migratory tendency, phylogeny and basal metabolic rate in birds. *PLOS ONE* **3**, e3261.
- Londoño, G. A., Chappell, M. A., del Rosario Castañeda, M., Jankowski, J. E. and Robinson, S. K. (2015). Basal metabolism in tropical birds: latitude, altitude, and the 'pace of life'. *Funct. Ecol.* **29**, 338-346. doi:10.1111/1365-2435.12348
- MacMillen, R. E. and Hinds, D. S. (1998). Water economy of granivorous birds: California house finches. *Condor* **100**, 493-503. doi:10.2307/1369715
- Maldonado, K. E., Cavieres, G., Veloso, C., Canals, M. and Sabat, P. (2009). Physiological responses in rufous-collared sparrows to thermal acclimation and seasonal acclimatization. *J. Comp. Physiol. B* **179**, 335-343. doi:10.1007/s00360-008-0317-1
- Mariette, M. M. and Buchanan, K. L. (2016). Prenatal acoustic communication programs offspring for high posthatching temperatures in a songbird. *Science* **353**, 812-814. doi:10.1126/science.aaf7049
- McKechnie, A. E. (2008). Phenotypic flexibility in basal metabolic rate and the changing view of avian physiological diversity: a review. *J. Comp. Physiol. B* **178**, 235-247. doi:10.1007/s00360-007-0218-8
- McKechnie, A. E. and Wolf, B. O. (2004). Partitioning of evaporative water loss in white-winged doves: plasticity in response to short-term thermal acclimation. *J. Exp. Biol.* **207**, 203-210. doi:10.1242/jeb.00757
- McKechnie, A. E., Chetty, K. and Lovegrove, B. G. (2007). Phenotypic flexibility in the basal metabolic rate of laughing doves: responses to short-term thermal acclimation. *J. Exp. Biol.* **210**, 97-106. doi:10.1242/jeb.02615
- McKechnie, A. E. and Swanson, D. L. (2010). Sources and significance of variation in basal, summit and maximal metabolic rates in birds. *Curr. Zool.* **56**, 741-758. doi:10.1093/czoolo/56.6.741
- McKechnie, A. E., Noakes, M. J. and Smit, B. E. (2015). Global patterns of seasonal acclimatization in avian resting metabolic rates. *J. Ornithol.* **156**, 367-376. doi:10.1007/s10336-015-1186-5
- Milbergue, M. S., Blier, P. U. and Vézina, F. (2018). Large muscles are beneficial but not required for improving thermogenic capacity in small birds. *Sci. Rep.* **8**, 14009. doi:10.1038/s41598-018-32041-w

- Minnaar, I. A., Bennett, N. C., Chimimba, C. T. and McKechnie, A. E.** (2014). Summit metabolism and metabolic expansibility in Wahlberg's epauletted fruit bats (*Epomophorus wahlbergi*): seasonal acclimatisation and effects of captivity. *J. Exp. Biol.* **217**, 1363-1369. doi:10.1242/jeb.098400
- Noakes, M. J. and McKechnie, A. E.** (2019). Reaction norms for heat tolerance and evaporative cooling capacity do not vary across a climatic gradient in a passerine bird. *Comp. Biochem. Physiol. A* **236**, 110522. doi:10.1016/j.cbpa.2019.06.022
- Noakes, M. J. and McKechnie, A. E.** (2020). Seasonal metabolic acclimatization varies in direction and magnitude among years in two arid-zone passerines. *Physiol. Biochem. Zool.* **93**, 140-152. doi:10.1086/707679
- Noakes, M. J., Wolf, B. O. and McKechnie, A. E.** (2016). Seasonal and geographical variation in heat tolerance and evaporative cooling capacity in a passerine bird. *J. Exp. Biol.* **219**, 859-869. doi:10.1242/jeb.132001
- Noakes, M. J., Wolf, B. O. and McKechnie, A. E.** (2017). Seasonal metabolic acclimatization varies in direction and magnitude among populations of an Afrotropical passerine bird. *Physiol. Biochem. Zool.* **90**, 178-189. doi:10.1086/689030
- Petit, M. and Vézina, F.** (2014). Reaction norms in natural conditions: how does metabolic performance respond to weather variations in a small endotherm facing cold environments? *PLoS ONE* **9**, e113617. doi:10.1371/journal.pone.0113617
- Petit, M., Clavijo-Baquet, S. and Vézina, F.** (2017). Increasing winter maximal metabolic rate improves intrawinter survival in small birds. *Physiol. Biochem. Zool.* **90**, 166-177. doi:10.1086/689274
- Piersma, T. and Drent, J.** (2003). Phenotypic flexibility and the evolution of organismal design. *Trends Ecol. Evol.* **18**, 228-233. doi:10.1016/S0169-5347(03)00036-3
- Pigliucci, M.** (2001). *Phenotypic Plasticity: Beyond Nature and Nurture*. Baltimore, USA: Johns Hopkins University Press.
- R Core Team** (2018). *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing. URL <https://www.R-project.org/>.
- Sabat, P., Cavieres, G., Veloso, C. and Canals, M.** (2006). Water and energy economy of an omnivorous bird: population differences in the rufous-collared sparrow (*Zonotrichia capensis*). *Comp. Biochem. Phys. A* **144**, 485-490. doi:10.1016/j.cbpa.2006.04.016
- Schlichting, C. D. and Pigliucci, M.** (1998). *Phenotypic Evolution: A Reaction Norm Perspective*. Sunderland, UK: Sinauer Associate.
- Smit, B. E. and McKechnie, A. E.** (2010). Avian seasonal metabolic variation in a subtropical desert: Basal metabolic rates are lower in winter than in summer. *Funct. Ecol.* **24**, 330-339. doi:10.1111/j.1365-2435.2009.01646.x
- Stager, M., Pollock, H. S., Benham, P. M., Sly, N. D., Brawn, J. D. and Cheviron, Z. A.** (2016). Disentangling environmental drivers of metabolic flexibility in birds: the importance of temperature extremes versus temperature variability. *Ecography* **39**, 787-795. doi:10.1111/ecog.01465
- Swanson, D. L.** (2001). Are summit metabolism and thermogenic endurance correlated in winter acclimatized passerine birds? *J. Comp. Physiol. B* **171**, 475-481. doi:10.1007/s003600100197
- Swanson, D. L.** (2010). Seasonal metabolic variation in birds: functional and mechanistic correlates. *Curr. Ornithol.* **17**, 75-129. doi:10.1007/978-1-4419-6421-2_3
- Swanson, D. L. and Bozinovic, F.** (2011). Metabolic capacity and the evolution of biogeographic patterns in oscine and suboscine passerine birds. *Physiol. Biochem. Zool.* **84**, 185-194. doi:10.1086/658291
- Swanson, D. L. and Liknes, E. T.** (2006). A comparative analysis of thermogenic capacity and cold tolerance in small birds. *J. Exp. Biol.* **209**, 466-474. doi:10.1242/jeb.02024
- Swanson, D. L. and Olmstead, K. L.** (1999). Evidence for a proximate influence of winter temperature on metabolism in passerine birds. *Physiol. Biochem. Zool.* **72**, 566-575. doi:10.1086/316696
- Swanson, D. L. and Vézina, F.** (2015). Environmental, ecological and mechanistic drivers of avian seasonal metabolic flexibility in response to cold winters. *J. Ornithol.* **156**, S377-S388. doi:10.1007/s10336-015-1192-7
- Swanson, D. L., Drymalski, M. W. and Brown, J. R.** (1996). Sliding vs static cold exposure and the measurement of summit metabolism in birds. *J. Therm. Biol.* **21**, 221-226. doi:10.1016/0306-4565(96)00005-8
- Swanson, D. L., Zhang, Y. F., Liu, J.-S., Merkord, C. L. and King, M. O.** (2014). Relative roles of temperature and photoperiod as drivers of metabolic flexibility in dark-eyed juncos. *J. Exp. Biol.* **217**, 866-875. doi:10.1242/jeb.096677
- Tieleman, B. I. and Williams, J. B.** (2000). The adjustment of avian metabolic rates and water fluxes to desert environments. *Physiol. Biochem. Zool.* **73**, 461-479. doi:10.1086/317740
- Tieleman, B. I., Williams, J. B. and Buschur, M. E.** (2002). Physiological adjustments to arid and mesic environments in larks (Alaudidae). *Physiol. Biochem. Zool.* **75**, 305-313. doi:10.1086/341998
- Tieleman, B. I., Williams, J. B., Buschur, M. E. and Brown, C. R.** (2003). Phenotypic variation of larks along an aridity gradient: are desert birds more flexible? *Ecol.* **84**, 1800-1815. doi:10.1890/0012-9658(2003)084[1800:PVOLAA]2.0.CO;2
- van de Ven, T. M. F. N., Mzilikazi, N. and McKechnie, A. E.** (2013). Seasonal metabolic variation in two populations of an Afrotropical euplectid bird. *Physiol. Biochem. Zool.* **86**, 19-26. doi:10.1086/667989
- Vézina, F., Gerson, A. R., Guglielmo, C. G. and Piersma, T.** (2017). The performing animal: causes and consequences of body remodeling and metabolic adjustments in red knots facing contrasting thermal environments. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **313**, R120-R131. doi:10.1152/ajpregu.00453.2016
- Weathers, W. W.** (1979). Climatic adaptation in Sivan standard metabolic rate. *Oecologia* **42**, 81-89. doi:10.1007/BF00347620
- White, C. R., Blackburn, T. M., Martin, G. R. and Butler, P. J.** (2007). The basal metabolic rate of birds is associated with habitat temperature and precipitation, not productivity. *Proc. R. Soc. Lond. B. Biol.* **274**, 287-293. doi:10.1098/rspb.2006.3727
- Wiersma, P., Muñoz-García, A., Walker, A. and William, J. B.** (2007). Tropical birds have a slow pace of life. *Proc. Natl. Acad. Sci. USA* **104**, 9340-9345. doi:10.1073/pnas.0702212104
- Williams, J. B. and Tieleman, B. I.** (2000). Flexibility in basal metabolic rate and evaporative water loss among hoopoe larks exposed to different environmental temperatures. *J. Exp. Biol.* **203**, 3153-3159.
- Williams, S. E., Shoo, L. P., Isaac, J. L., Hoffmann, A. A. and Langham, G.** (2008). Towards an integrated framework for assessing the vulnerability of species to climate change. *PLoS Biol.* **6**, e325. doi:10.1371/journal.pbio.0060325
- Withers, P. C.** (1992). *Comparative Animal Physiology*. Fort Worth, USA: Saunders College Publishing.
- Zhang, Y., King, M. O., Harmon, E. and Swanson, D. L.** (2015a). Summer-to-winter phenotypic flexibility of fatty acid transport and catabolism in skeletal muscle and heart of small birds. *Physiol. Biochem. Zool.* **88**, 535-549. doi:10.1086/682154
- Zhang, Y., Eyster, K., Liu, J.-S. and Swanson, D. L.** (2015b). Cross-training in birds: cold and exercise training produce similar changes in maximal metabolic output, muscle masses and myostatin expression in house sparrows (*Passer domesticus*). *J. Exp. Biol.* **218**, 2190-2200. doi:10.1242/jeb.121822