Seasonal Metabolic Acclimatization Varies in Direction and Magnitude among Populations of an Afrotropical Passerine Bird*

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ABSTRACT

Avian metabolic responses demonstrate considerable diversity under fluctuating environmental conditions, a well-studied example being the seasonal upregulation of basal metabolic rate (BMR) and summit metabolism (M_{sum}) in temperate species experiencing harsh winters. Fewer studies have examined seasonal metabolic acclimatization in subtropical or tropical species. We investigated seasonal metabolic variation in an Afrotropical ploceid passerine, the white-browed sparrow-weaver (*Plocepasser mahali*; ~47 g), at three sites along a climatic gradient of approximately 7°C in winter minimum air temperature (T_a). We measured M_{sum} ($n \geq 10$ per site per season) in a helox atmosphere, BMR of the same birds at thermoneutrality ($T_a \approx 30$°C), and resting metabolic rates at 5°C $\leq T_a \leq$ 20°C. Patterns of seasonal adjustments in BMR varied among populations in a manner not solely related to variation in seasonal T_a extremes, ranging from BMR ~52% higher in winter than in summer to no seasonal difference. Greater cold tolerance was found in a population at a colder desert site, manifested as higher M_{sum} (~25% higher) and lower helox temperature at cold limit values compared with a milder, mesic site. Our results lend support to the idea that greater variance in the pattern of seasonal metabolic responses occurs in subtropical and tropical species compared with their temperate-zone counterparts and that factors other than T_a extremes (e.g., food availability) may be important in determining the magnitude and direction of seasonal metabolic adjustments in subtropical birds.

Keywords: acclimatization, hypothermia, metabolic diversity, summit metabolism, basal metabolic rate, subtropical birds.

Introduction

Comparative analyses of the lower and upper limits of avian resting metabolic rate (basal metabolic rate [BMR] and summit metabolism [M_{sum}; also referred to as cold-induced peak metabolic rate], respectively) have revealed several broad patterns of variation in resting energy turnover. One such pattern involves lower BMR and M_{sum} in species from tropical regions compared with those inhabiting higher latitudes (Weathers 1979; Hails 1983; Wiersma et al. 2007; Londoño et al. 2015). Another concerns lower BMR in arid-zone birds compared with their counterparts in more mesic habitats (Tieleman and Williams 2000; Tieleman et al. 2002, 2003a; Sabat et al. 2006). The latter pattern is thought to reflect the evolution of low maintenance energy demands under conditions of unpredictable rainfall and food availability (Louw and Seely 1982; Williams and Tieleman 2005), although recent analyses suggest that avian BMR is more strongly correlated with air temperature (T_a) than primary productivity (White et al. 2007; Jetz et al. 2008).

Phenotypic flexibility is a category of phenotypic plasticity that involves short-term, reversible adjustments of an individual's phenotype (Piersma and Drent 2003; Piersma and van Gils 2010). Increasing evidence suggests that phenotypic flexibility is an important process contributing to observed avian physiological variation (Garland and Adolph 1991) both within and among species (e.g., Swanson 1990; Battley et al. 2001; Tieleman et al. 2003b; McKechnie et al. 2007). A well-documented example involves winter increases in BMR and/or M_{sum} as a component of seasonal acclimatization in species that are year-round residents in north temperate regions characterized by long, cold winters (reviewed by McKechnie 2008; McKechnie and Swanson 2010; Swanson 2010). Seasonal metabolic adjustments—in particular, the increases in M_{sum} of 10%–50% compared with summer values—are thought to provide the thermogenic basis for enhanced cold tolerance (primarily determined by shivering thermogenic capacity in birds) and the defense of normothermic body temperature during harsh winter conditions (Swanson 2010).

The physiological mechanisms driving phenotypic flexibility in M_{sum} include adjustments in the mass of the pectoralis muscles and changes in the transport capacities for O_2 and metabolic substrates, whereas seasonal adjustments in BMR reflect changes in organ masses, mass-independent metabolic intensities of spe-

cific organs, and the activity levels of metabolic enzymes (Swanson 2010; Peña-Villalobos et al. 2014; Petit and Vézina 2014; Zhang et al. 2015). Recent studies suggest that the functional link between BMR and M_{sum} is not as clear as one might expect, an idea that is supported by the difference in physiological mechanisms determining flexibility in these variables (Swanson et al. 2012; Petit et al. 2013; Petit and Vézina 2014).

Compared with north temperate regions, avian seasonal metabolic acclimatization in warmer tropical and subtropical climates is less well understood. A number of authors have reported lower avian BMR in winter than in summer (Maddocks and Geiser 2000; Bush et al. 2008; Smit and McKechnie 2010), a pattern in the direction opposite to that typical of north temperate species. Smit and McKechnie (2010) reported significant winter reductions in BMR among five species in the Kalahari Desert of southern Africa and argued that this pattern reflects selection for winter energy conservation during cool, dry periods, in contrast to the enhanced cold tolerance required by temperate-zone species that experience substantially harsher winter conditions. However, some recent studies of subtropical species have documented winter increases in BMR quantitatively similar to those characteristic of north-temperate species (Chamane and Downs 2009; Lindsay et al. 2009a, 2009b; Wilson et al. 2011; reviewed by McKechnie et al. 2015), revealing that patterns of avian seasonal metabolic acclimatization in subtropical latitudes are more variable than suggested by Smit and McKechnie (2010).

Recent evidence also suggests that seasonal metabolic adjustments in birds inhabiting subtropical regions can vary substantially among conspecific populations. Van de Ven et al. (2013) reported contrasting patterns of seasonal variation in M_{sum} and BMR between two populations of southern red bishops (Euplectes orix) in southeastern South Africa. Bishops from a warmer coastal site showed seasonally stable BMR but significantly lower M_{sum} during winter (~8% lower than summer values), whereas individuals at a colder inland site increased both M_{sum} (~15%) and BMR (~58%) in winter.

The variability of seasonal metabolic adjustments among subtropical species—together with the between-population variation in E. orix—offers further support for the notion of adaptive thermoregulation, which argues that the thermal physiology of endotherms is far more flexible than previously recognized and predicts fine-scale variation among populations inhabiting different climatic conditions (Garland and Adolph 1991; Angilletta et al. 2010; Glanville et al. 2012; Smit et al. 2013). To explore this notion further, we hypothesized that avian seasonal metabolic adjustments vary among conspecific populations in a manner that reflects a continuum between winter energy conservation versus enhanced cold tolerance. We predicted that (1) populations at colder sites show more pronounced winter upregulation of BMR and M_{sum} compared with populations at warmer sites; and (2) arid-zone populations show lower overall metabolic rates and winter reductions in BMR and M_{sum}, reflecting selection for energy conservation during cool, dry winters in regions with low primary productivity (Smit and McKechnie 2010).

To test these predictions, we measured BMR, M_{sum}, and associated variables in summer and winter in three populations of

white-browed sparrow-weavers (Plocepasser mahali), a ploceid passerine that occurs over much of southern Africa. The three sites we selected span a gradient of approximately 7°C in winter minimum temperatures and include an arid-zone site in the Kalahari Desert as well as two more mesic sites in the eastern half of South Africa. We also included data from an earlier study, which revealed lower BMR in winter than summer in a P. mahali population at a site in the eastern Kalahari Desert (Smit and McKechnie 2010).

Methods

Study Species and Sites

The white-browed sparrow-weaver (Plocepasser mahali) is an ~47-g ploceid passerine that is widespread across southern Africa (du Plessis 2005). We examined thermoregulation in sparrow-weavers during winter (July–August 2013 and 2014) and summer (January–February 2014) at three study sites in South Africa that vary in seasonal temperature extremes; one arid site near Askham in the Kalahari Desert (Northern Cape Province; 26° 59′S, 20°51′E) and two more mesic sites at Frankfort (Free State Province; 27°16′S, 28°29′E) and Polokwane (Limpopo Province; 23°56′S, 29°28′E; fig. 1). We compared the BMR of these three populations to that of a fourth sparrow-weaver population at Molopo Nature Reserve in the Kalahari Desert (North West Province; 25°47′S, 22°56′E) for which data were collected by Smit and McKechnie (2010) during winter (June–August 2007) and summer (January–March 2008). These sites are all within the distributional range of the subspecies P. mahali mahali (du Plessis 2005). Ethical approval for this project was obtained from the Animal Ethics Committee of the University of Pretoria (protocol EC030-13).

Climate data were obtained from the South African Weather Service using the weather station closest to each study site. There are weather stations at Polokwane (~9.1 km north of our study site; 23°51′S, 29° 27′E) and Frankfort (~1.4 km away; 27°16′S, 28° 29′E), but the nearest station to Askham is at Twee Rivieren (~62 km northwest; 26°28′S, 20°36′E). For each site, we extracted mean daily temperature minimum (T_{min}) and maximum (T_{max}) values over the hottest summer month (January) and coldest winter month (July) during the season we collected data (fig. 1). The lowest T_{min} values occurred at Frankfort, but the greatest seasonal variation in T_{min} and T_{max} values occurred at Askham (fig. 1). In contrast, Polokwane has relatively mild summers and winters (fig. 1). Mean daily T_{min} and T_{max} values for Molopo were obtained from Smit and McKechnie (2010) and represent mean values for the entire periods that data were collected (~3 mo).

Sparrow-weavers were typically caught at night using two small nets mounted on the end of aluminium poles to cover the entrances of roost nests. A few birds were caught during the day using mist nets or spring traps baited with mealworms. To avoid trapping reproductive individuals, we did not catch sparrow-weavers over the peak egg-laying period of this species (November–December; du Plessis 2005) and avoided catching birds from breeding nests. Physiological data were collected at the various
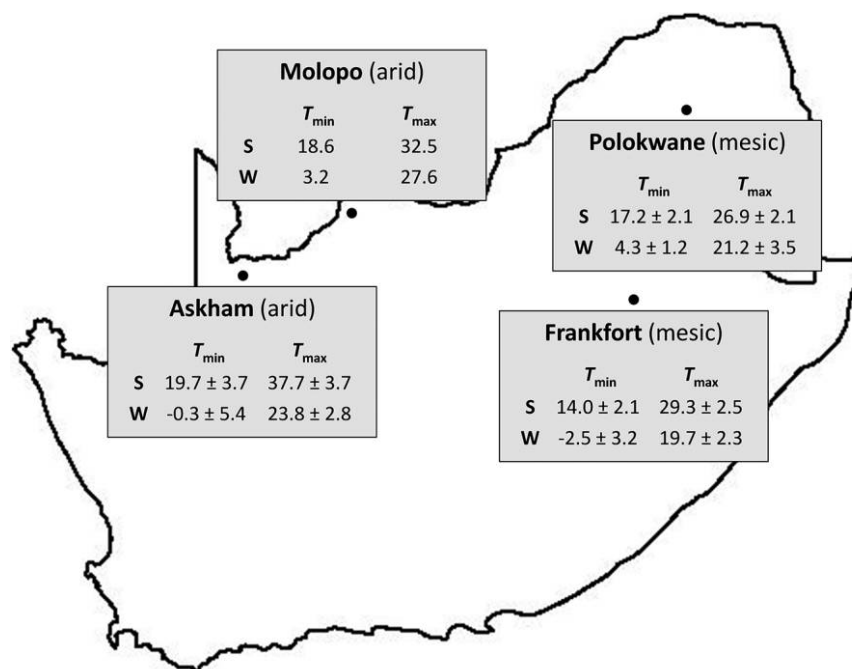
Figure 1. Mean daily air temperature minimum (T_{min}) and maximum (T_{max}) during the hottest summer (S) month (January) and coldest winter (W) month (July) at four study sites in South Africa. Mean $\pm$ SD values for Askham, Polokwane, and Frankfort were calculated from weather data obtained from the South African Weather Service, using the closest weather station to each site. Mean daily T_{min} and T_{max} values for Molopo Nature Reserve were obtained from Smit and McKechnie (2010; SD values were not presented).

study sites using a field respirometry system, and birds were housed in cages constructed of plastic mesh and shade cloth ($\sim$1.5 m³) for no more than 48 h before measurements, except for the M_{sum} measurements of two individuals that were conducted within 60 h of capture.

The natural diet of sparrow-weavers includes insects, seeds, fruits, and fleshy leaves, and in the Kalahari Desert, it consists predominantly of insects ($\sim$80%; du Plessis 2005). Birds kept in captivity were provided with water, mealworms, and a wild bird seed mix. Individuals were sexed according to the color of their beaks (du Plessis 2005) and released at the site of capture after data collection.

Gas Exchange Measurements

An open flow-through respirometry system was used to estimate metabolic rates, by measuring O_2 consumption ($\dot{V}O_2$; mL min^{-1}), CO_2 production ($\dot{V}CO_2$; mL min^{-1}), and total evaporative water loss (TEWL; mg min^{-1}), using an experimental setup similar to that described by Minnaar et al. (2014), with modifications listed below. We used airtight respirometry chambers constructed from 4-L clear plastic containers (Lock and Lock, Anaheim, CA) and elevated birds about 10 cm above a layer of mineral oil ($\sim$1 cm; to prevent evaporation of excreta influencing TEWL estimates), using a plastic mesh platform.

For overnight (rest phase) measurements of TEWL and resting metabolic rate (RMR; including BMR and RMR at 5°C $\leq T_a \leq$ 20°C), the chambers were placed in a modified ice chest ($\sim$75 L)

with a darkened interior, within which T_a was controlled by a Peltier device (AC-162, TE Technology, Traverse City, MI) and a custom-built digital controller. Atmospheric air was supplied by an air pump (model DAA-V515-ED, Gast Air Pumps, Benton Harbor, MI) and dried using scrubber columns of silica gel followed by Drierite (WA Hammond Drierite, Xenia, OH). For M_{sum} measurements, the chambers were placed within a modified portable fridge/freezer (40 L; ARB, Kilsyth, Victoria, Australia), and helox (21% O_2, 79% He) was supplied; a helox atmosphere increases the heat loss rate of birds about threefold without impairing gas exchange, thus allowing M_{sum} to be reached at relatively moderate T_a values (Rosenmann and Morrison 1974; Holloway and Geiser 2001).

Incurrent atmospheric air or helox was split into baseline and experimental channels, and baseline flow rate was maintained at $\sim$1.5 L min^{-1} with a needle valve (Swagelok, Solon, OH). Flow rates into the chambers were maintained at $\sim$1.2 L min^{-1} for overnight RMR measurements, using Alicat mass flow controllers (MC-10SLPM-D/5m, Alicat Scientific, Tuscon, AZ), and at $\sim$3.4 mL min^{-1} for M_{sum} measurements, using an Omega mass flow controller (FMA5520, Omega Engineering, Bridgeport, NJ). Excurrent air or helox from the chambers and from the baseline channel was alternately subsampled and pulled through an O_2 analyzer (FC-10A, Sable Systems, Las Vegas, NV) and a CO_2/H_2O analyzer (LI-840A, LI-COR, Lincoln, NE) at $\sim$200 mL min^{-1} by a custom-built pump with a rotameter (Omega, Stamford, CT). All mass flow controllers and analyzers were calibrated regularly, using the protocol described by Minnaar et al. (2014).

Air and Body Temperature Measurements

The T_a (°C) within each chamber and core body temperature (T_b; °C) were measured as described by Minnaar et al. (2014), except that a Cu-Cn thermocouple (IT-18, Physitemp Instruments, Clifton, NJ) and temperature recorder (RDXL12SD, Omega) was used to monitor T_a during M_{sum} measurements. Temperature-sensitive passive integrated transponder (PIT) tags (Destron Fearing, St. Paul, MN) were injected into the abdominal cavities of birds to measure T_b, after they were calibrated against a Cu-Cn thermocouple in a temperature-controlled circulating water bath (F34-ME, Julabo, Seelbach, Germany). A handheld PIT tag scanner (DTR-4, Destron Fearing) was used to monitor T_b during M_{sum} measurements.

Experimental Protocol

To quantify rest-phase RMR (including BMR and RMR at 5°C $\leq$ $T_a \leq$ 20°C) and TEWL, we measured gas exchange rates of two sparrow-weavers simultaneously within separate chambers throughout the night. Birds were placed into chambers before sunset and removed just after sunrise, and body mass (M_b; g) was measured on entering and after removal from chambers, using a Scout Pro balance scale (SP602US, Ohaus, Pine Brook, NJ). BMR and thermoneutral TEWL measurements were collected at a constant $T_a \approx$ 30°C for the entire rest-phase period (n = 10 per site per season, except Polokwane in winter [n = 11]), because this T_a is within the thermoneutral zone of this species (Smit and McKechnie 2010). Further overnight RMR measurements occurred at T_a values of 5°, 10°, 15°, and 20°C in a random sequence, using birds different from those used during BMR and M_{sum} measurements (n = 6 per T_a per site per season, except Kalahari in summer [n = 8 at 15°C and n = 4 at 20°C] and Frankfort in winter [n = 10] and summer [n = 80], at all T_a values). During these measurements, birds were exposed to a constant T_a for half the night and then a second T_a value for the remainder of the night. On two occasions (i.e., four individuals), birds were exposed to three T_a values within one night, but it was ensured that individuals spent at least 4 h at each T_a. Each bird was exposed to this protocol only once in order to avoid habituation to repeated metabolic measurements (Jacobs and McKechnie 2014); thus, measurements at different T_a do not necessarily involve the same individuals. We sequentially subsampled air from the baseline channel and respective chambers in cycles lasting 80 min repeated throughout the night: baseline air for 10 min, followed by air from the chambers in sequence for 30 min each, and finally baseline air again for the last 10 min.

To quantify M_{sum} in sparrow-weavers (using the same individuals as those for BMR measurements), gas exchange rates were measured individually during the daytime, using the sliding cold exposure method (Swanson et al. 1996) and a similar protocol to that described by Minnaar et al. (2014), with modifications listed below. M_{sum} was measured within 12 h before BMR measurements for about half of the sparrow-weavers and during the 12 h after for the rest of the birds, in order to control for the effect of habituation during repeated metabolic measurements

(Jacobs and McKechnie 2014). A baseline of helox was obtained for at least 5 min with the fridge/freezer set to 10°C, after which we switched to the experimental channel and set the fridge/freezer to −15°C so that T_a decreased at a relatively constant rate, and we manually recorded T_a at regular intervals (∼1°C decrease). We visually monitored [O_2] and [CO_2] traces, and if we suspected that a bird was hypothermic (sudden decrease in $\dot{V}O_2$, $\dot{V}CO_2$, and TEWL), we opened the fridge/freezer (but not the chamber) to determine the T_b value measured by the PIT tag. Hypothermia was assumed and birds were removed when T_b decreased by ≥4.0°C from initial values on entering the chamber (mean decrease in T_b: 8.7° ± 2.1°C), because hypothermia strongly indicates that maximal levels of heat production were obtained (Swanson et al. 1996). However, if birds were not hypothermic, the fridge/freezer was closed, the time was noted (to ensure M_{sum} was not calculated from active birds), and measurements were continued until hypothermia was elicited. After removal from chambers, baseline values were again recorded for at least 5 min.

Data Analyses

Data were corrected for drift in [O_2], [CO_2], and [H_2O] baselines using the relevant algorithms in Expedata Data Acquisition and Analysis Software (Sable Systems). To calculate RMR (including BMR) and TEWL values of each bird from overnight runs, the lowest 5-min sample period of average $\dot{V}O_2$ and $\dot{V}CO_2$ at each T_a was used. This sample period always occurred after birds had been in chambers for at least 120 min, and we are thus confident that values represent postabsorptive individuals because the predicted mean digesta retention time for a 47-g bird is ∼67.5 min (Karasov 1990). To calculate M_{sum}, the highest 5-min sample period of average $\dot{V}O_2$ and $\dot{V}CO_2$ was used to represent the maximum metabolism during cold exposure, and the helox T_a at which M_{sum} was reached (i.e., T_a at cold limit; T_{CL}) was estimated for each bird. In all calculations, it was ensured that the sample period was after equilibrium was reached within the chamber, estimated using the equation from Lasiewski et al. (1966).

$\dot{V}O_2$, $\dot{V}CO_2$, and TEWL values were calculated using equations [9.4]–[9.6] from Lighton (2008; assuming 0.803 mg H_2O per mL of water vapor for TEWL calculations) and using an excurrent flow rate estimated from equation [9.3] in all calculations. Respiratory exchange ratios (RERs) were calculated as $\dot{V}CO_2/\dot{V}O_2$, and gas exchange rates were converted into whole-animal metabolic rates (W), using thermal equivalence data from table 4.2 of Withers (1992). RER for BMR, M_{sum}, and RMR (at 5°C $\leq T_a \leq$ 20°C) data averaged 0.63 ± 0.10, 0.63 ± 0.08, and 0.66 ± 0.09, respectively; because there are no published thermal equivalence data outside the expected range of 0.71 $\leq$ RER $\leq$ 1.00 (Withers 1992), RER = 0.71 was assumed for the calculation of metabolic rates if the RER were <0.71. For each sparrow-weaver, metabolic expansibility (ME) was calculated as M_{sum}/BMR (also referred to as factorial aerobic scope). For BMR analyses, data collected by Smit and McKechnie (2010) from sparrow-weavers (n = 6 per season) at Molopo Nature Reserve were included as a fourth population. All values are presented as means ± SD.

Linear models and linear mixed-effects models (nlme package; Pinheiro et al. 2009) were fitted to the data using R 3.1.1 (R Development Core Team 2014). All models were initially run including a set of potential predictor variables and interactions between these variables. Models were then refined by removing nonsignificant interactions and predictor variables in a stepwise fashion, and second-order Akaike information criterion (AIC) values (MuMIn package) were compared to determine which combination of predictor variables and interactions produced models that best fit the data sets tested. Sex was initially included as a predictor variable, but because physiological response variables never varied significantly with sex (all $P > 0.05$) and because its removal either improved or did not affect model fit (i.e., decrease or no change in second-order AIC values), it was excluded from the final models on physiological variables. The assumptions of all models (including normality, homogeneity of variance, and multicollinearity) as well as model fit (residuals, leverage, and Cook's D values) were checked, using the appropriate tests described by Logan (2010).

A linear model was fitted to investigate how M_b varied among sites, seasons, and sexes, and the first M_b measurement after capture for each bird was used. Linear models were also used to investigate how T_{CL} and whole-animal BMR, thermoneutral TEWL (i.e., at $T_a \approx 30°C$), M_{sum}, and metabolic expansibility varied with M_b and among sites and seasons. Post hoc tests of multiple comparisons of means (Tukey contrasts for linear models; multcomp package; Hothorn et al. 2008) were used to investigate variation among sites for M_b and M_{sum} values and among site × season groups for BMR and T_{CL}, because these values significantly varied with the site × season interaction. Analyses for BMR, TEWL, and M_{sum} were repeated using mass-specific data as well as $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ data for BMR and M_{sum}.

A linear mixed-effects model was fitted to investigate how RMR at $5°C \leq T_a \leq 20°C$ varied with T_a, site, season, and M_b, with individual included as a random effect because there were repeated measurements within individuals at multiple T_a values. Because RMR varied significantly with site × season, separate linear regressions models were fitted within each site × season group in order to compare the respective relationships between T_a and RMR, and ANCOVAs and post hoc tests were fitted to investigate how the slopes and y-intercepts of these regressions varied among site × season groups.

Results

Body Mass

The M_b of sparrow-weavers varied significantly with site (linear model, $F_{2, 259} = 71.638, P < 0.001$) but not between seasons (linear model, $F_{1, 259} = 3.120, P = 0.080$) or with site × season (linear model, $F_{2, 257} = 1.774, P = 0.172$). The M_b of birds at all three sites differed significantly from each other: birds at Frankfort (a mesic site) were the largest ($n = 96$), followed by Polokwane (also mesic; $n = 81$), and birds at Askham (desert site) were the smallest ($n = 87$; table 1). The mean M_b of Molopo (also desert) birds was similar to Askham birds (table 1), but

because the sample size was substantially smaller than those of our study sites ($n = 12$; Smit and McKechnie 2010), we excluded data from this population in the statistical model for M_b. The M_b of males (43.6 ± 4.7 g; $n = 164$) was significantly larger than that of females (41.8 ± 4.3 g; $n = 100$) across all sites and seasons (linear model, $F_{2, 257} = 18.648, P < 0.001$).

Basal Metabolic Rate

Whole-animal BMR (measured at $T_a = 30.0° \pm 0.2°C$) of sparrow-weavers varied significantly with M_b (linear model, $F_{1, 65} = 7.167, P = 0.009$), site (linear model, $F_{3, 65} = 36.605, P < 0.001$), season (linear model, $F_{1, 65} = 47.150, P < 0.001$), and site × season (linear model, $F_{3, 65} = 14.026, P < 0.001$; table 1). No significant interactions between M_b and the other predictor variables were found (all $P > 0.05$), suggesting that BMR variation among site × season groups does not purely reflect M_b differences among sites. Post hoc tests indicated that BMR was significantly higher in winter than in summer at both Askham and Polokwane (~52% higher) but was similar between seasons at Frankfort and Molopo (table 1). During winter, BMR of Molopo sparrow-weavers was significantly lower than Askham, Frankfort, and Polokwane birds (on average ~52% lower), but there was no significant variation among the latter three populations (table 1). During summer, Frankfort sparrow-weavers had significantly higher BMR values than Askham, Polokwane, and Molopo birds (on average ~50% higher), but there was no significant variation among the latter three populations (table 1). The same general patterns of BMR variation were observed when analyses were repeated using mass-specific, $\dot{V}_{O_2}$, and $\dot{V}_{CO_2}$ values.

Whole-animal TEWL at thermoneutrality ($T_a \approx 30°C$) did not vary significantly with M_b (linear model, $F_{1, 56} = 2.203, P = 0.144$), site (linear model, $F_{2, 56} = 2.341, P = 0.106$), season (linear model, $F_{1, 56} = 1.126, P = 0.293$), or site × season (linear model, $F_{2, 54} = 2.828, P = 0.068$; table 1). No patterns of significant thermoneutral TEWL variation were found when analyses were repeated using mass-specific values.

Summit Metabolism and Metabolic Expansibility

Whole-animal M_{sum} of sparrow-weavers varied significantly with M_b (linear model, $F_{1, 56} = 27.242, P < 0.001$) and site (linear model, $F_{2, 56} = 6.708, P < 0.001$). M_{sum} was significantly higher in winter than in summer (linear model, $F_{1, 56} = 5.105, P < 0.001$) but did not vary with site × season (linear model, $F_{2, 54} = 1.449, P = 0.244$; table 1). The nonsignificance of the latter interaction term as a predictor means that post hoc tests of seasonal variation within each population are not statistically justified, but when we conducted post hoc tests regardless, they indicated no significant winter versus summer differences within any of the three populations ($P > 0.05$). No significant interactions between M_b and the other predictor variables were found ($P > 0.05$). Post hoc tests indicated that the M_{sum} of Askham sparrow-weavers was significantly higher than Polokwane birds (~25% higher), whereas Frankfort birds

Table 1: Data for white-browed sparrow-weavers (*Plocepasser mahali*) measured during summer and winter at three sites in South Africa that differ in minimum winter air temperature (T_{min}) by ~7°C

| | Site × season groups (daily T_{min}) | | | | | | | |
| | Askham | | Frankfort | | Polokwane | | Molopo | |
Variable	Summer (19.7° ± 3.7°C)	Winter (−0.3° ± 5.4°C)	Summer (14.0° ± 2.1°C)	Winter (−2.5° ± 3.2°C)	Summer (17.2° ± 2.1°C)	Winter (4.3° ± 1.2°C)	Summer (18.6°C)	Winter (3.2°C)
M_b (g)	39.1 ± 3.7[A]	41.0 ± 3.3[A]	46.3 ± 4.0[B]	46.3 ± 3.5[B]	40.6 ± 8.1[C]	42.2 ± 3.5[C]	40.4 ± 2.7	40.6 ± 3.4
BMR (W)	.42 ± .06[A]*	.64 ± .10[a]*	.57 ± .09[B]	.60 ± .05[a]	.38 ± .06[A]*	.57 ± .06[a]*	.35 ± .04[A]	.29 ± .05[b]
TEWL (mg min⁻¹)	1.76 ± .51[A]	1.86 ± .71[A]	1.98 ± .41[A]	1.91 ± .87[A]	1.82 ± .39[A]	1.35 ± .19[A]		
M_{sum} (W)	3.13 ± .56[A]	3.85 ± .66[A]	3.85 ± .58[AB]	3.86 ± .63[AB]	2.40 ± .44[B]	3.14 ± .79[B]		
ME	6.46 ± 1.09[A]	6.66 ± 1.56[A]	6.80 ± 1.17[A]	6.40 ± .93[A]	6.45 ± 1.49[A]	5.57 ± 1.77[A]		
T_{CL} (°C)	−2.26 ± 2.85[AB]	−5.96 ± 3.95[A]	−6.78 ± 3.33[AB]	−6.00 ± 5.41[AB]	−.19 ± 2.50[AB]	−.36 ± 4.99[B]		

Note. Data were collected for Askham, Frankfort, and Polokwane birds using an open flow-through respirometry system. Body mass (M_b) and basal metabolic rate (BMR) data for Molopo sparrow-weavers were obtained from Smit and McKechnie (2010; M_b sample sizes for Molopo were too small compared with the other sites to include in statistical analyses). Mean daily T_{min} are shown for each site during each season (obtained from the South African Weather Service and Smit and McKechnie [2010]). Uppercase letters indicate significant differences among sites regardless of season for M_b, summit metabolism (M_{sum}), total evaporative water loss (TEWL), metabolic expansibility (ME), and helox temperature at cold limit (T_{CL}; all $P < 0.01$), because there were no seasonal differences in these variables at any of the sites. However, because there was significant seasonal variation in BMR at two sites (indicated by an asterisk; both $P < 0.001$), uppercase letters indicate significant BMR differences during summer ($P < 0.05$), and lowercase letters indicate differences during winter ($P < 0.001$). Significance values are from linear models and post hoc tests of multiple comparisons of means (Tukey contrasts). M_b was included as a covariate in all analyses, and statistical results suggest that patterns of variation among sites do not purely reflect M_b differences.

had M_{sum} values similar to both of these populations (table 1). In contrast to the pattern for M_{sum}, metabolic expansibility (i.e., M_{sum}/BMR) did not vary significantly with M_b (linear model, $F_{1,56} = 0.527$, $P = 0.471$), site (linear model, $F_{2,56} = 1.318$, $P = 0.276$), or site × season (linear model, $F_{2,54} = 2.335$, $P = 0.485$) but was significantly lower (~13%) in winter than in summer when considering data from all populations (linear model, $F_{1,56} = 7.088$, $P = 0.010$; table 1). The same general patterns of M_{sum} and metabolic expansibility variation were observed when analyses were repeated using mass-specific, $\dot{V}O_2$, and $\dot{V}CO_2$ values.

The T_{CL} of sparrow-weavers varied significantly with M_b (linear model, $F_{1,51} = 10.956$, $P = 0.002$), site (linear model, $F_{2,51} = 4.820$, $P = 0.012$), and site × season (linear model, $F_{2,51} = 3.227$, $P = 0.048$) but not between seasons (linear model, $F_{1,51} = 0.132$, $P = 0.267$; table 1). Post hoc tests indicated that there was no significant among-population variation in T_{CL} during summer, but during winter Askham sparrow-weavers had significantly lower T_{CL} than Polokwane birds (table 1).

Resting Metabolic Rate

The RMR values at $5°C \leq T_a \leq 20°C$ increased significantly with decreasing T_a (linear mixed-effects model, $F_{1,82} = 372.993$, $P < 0.001$) and increasing M_b (linear mixed-effects model, $F_{1,82} = 20.299$, $P < 0.001$) and varied significantly with site (linear mixed-effects model, $F_{2,78} = 12.541$, $P < 0.001$), season (linear mixed-effects model, $F_{1,78} = 14.157$, $P < 0.001$), and site × season (linear mixed-effects model, $F_{2,78} = 8.758$,

$P < 0.001$; fig. 2). Separate linear regressions of $5°C \leq T_a \leq 20°C$ versus RMR were thus fitted within each site × season group (fig. 2). The slopes of these regressions did not vary significantly with site × season (linear mixed-effects model, $F_{1,161} = 0.395$, $P = 0.852$), but the y-intercepts did differ (linear mixed-effects model, $F_{1,161} = 11.686$, $P < 0.001$), reflecting variation in RMR values at any given T_a between 5° and 20°C (fig. 2).

Post hoc tests indicated that RMR at $5°C \leq T_a \leq 20°C$ was significantly higher in winter than summer within the Polokwane population ($P < 0.001$) but did not differ significantly between seasons at Askham or Frankfort ($P > 0.125$; fig. 2). No significant among-population variation during winter was found ($P > 0.09$), but during summer the only significant variation involved higher RMR values in the Frankfort population than both Askham and Polokwane birds ($P < 0.001$; fig. 2).

Discussion

Our data reveal significant intraspecific seasonal and spatial variation in the metabolic rates of a widespread Afrotropical passerine bird. Patterns of seasonal acclimatization in BMR and M_{sum} (also referred to as cold-induced peak metabolic rate) were not consistent with our hypothesis that interpopulation variation in seasonal metabolic adjustments reflects a continuum between winter energy conservation versus enhanced cold tolerance. However, patterns of seasonal adjustments in BMR did vary widely among populations, supporting the idea that patterns of metabolic acclimatization are more variable among
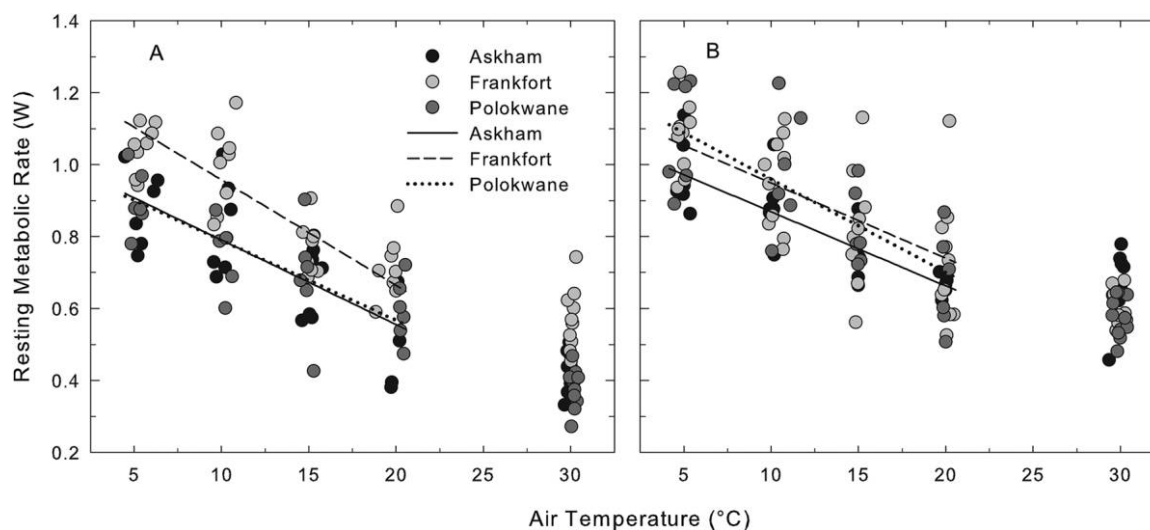

Figure 2. Resting metabolic rates of white-browed sparrow-weavers (*Plocepasser mahali*) were measured using open flow-through respirometry during summer (*A*) and winter (*B*) at three sites that differ in minimum winter temperature (T_{min}) by ~7°C ($n \geq 6$ at each air temperature [T_a] per site per season, except for $T_a = 20°C$ at Askham during summer [$n = 4$]). Lines indicate significant (all: $P < 0.001$) linear mixed-effects regression models fitted at $5°C \leq T_a \leq 20°C$. Summer: Askham, $y = -0.02x + 1.03$ ($t_{1,11} = -6.272$); Frankfort, $y = -0.03x + 1.25$ ($t_{1,15} = -12.930$); Polokwane, $y = -0.02x + 1.01$ ($t_{1,10} = -8.864$). Winter: Askham, $y = -0.02x + 1.07$ ($t_{1,12} = -10.794$); Frankfort, $y = -0.02x + 1.16$ ($t_{1,19} = -6.639$); Polokwane, $y = -0.03x + 1.2$ ($t_{1,11} = -7.329$).

subtropical/tropical birds than in their temperate-zone counterparts. Our results provide further evidence for considerable intraspecific variation in multiple traits related to the thermal physiology of sparrow-weavers. This variation includes significant between-population differences in T_b and the temperature dependence of heat dissipation behaviors, such as panting (Smit et al. 2013), differences in daily energy expenditure among free-ranging individuals of two arid-zone populations (Smit and McKechnie 2015), as well as interpopulation variation in heat tolerance and evaporative cooling capacity (Noakes et al. 2016).

An unexpected observation in this study was that RER across all measurements averaged below the expected range of 0.71–1.00 (Withers 1992). Since thermal equivalence values do not exist for RER < 0.71, we assumed RER $= 0.71$ for metabolic calculations when RER was below this value, thereby introducing a potential source of error into our metabolic rate estimates. To test the sensitivity of our results and overall conclusions, we repeated all statistical analyses using $\dot{V}O_2$ and $\dot{V}CO_2$ values. These analyses revealed the same overall patterns of variation as those found for BMR and M_{sum}, confirming that our conclusions are not affected by these unexpectedly low RER values. Previous studies have also reported RER values below the expected range (e.g., Walsberg and Wolf 1995; Minnaar et al. 2014; Thompson et al. 2015).

Basal Metabolic Rate

The mean BMR of sparrow-weaver populations varied from values higher than those predicted for tropical birds and passerines (Frankfort: ~39% and 18% higher, respectively) to lower (Molopo: ~18% and 30% lower, respectively; calculated using mean M_b of each site × season group; Londoño et al. 2015). During summer, BMR of the Askham and Polokwane populations was similar to values predicted for tropical birds and passerines (83%–110% of predicted values), but seasonal upregulation of BMR resulted in greater than predicted values in both of these populations during winter (121%–163%; Londoño et al. 2015). The only other published BMR estimate for sparrow-weavers of which we are aware is 0.45 W, reported by Ferguson et al. (2002) for sparrow-weavers during winter at Bloemhof Dam (semiarid site).

In contrast to our predictions, our data revealed no clear overall patterns of BMR and TEWL differences between desert versus mesic populations of sparrow-weavers (table 1). These results contrast with those for another small Neotropical passerine, the rufous-collared sparrow (Zonotrichia capensis; ~21 g), where lower BMR and TEWL values were observed in populations from more arid habitats (Sabat et al. 2006; Cavieres and Sabat 2008), although the much smaller size of Z. capensis potentially confounds this comparison. Lower BMR and TEWL in desert birds have been suggested to have an adaptive significance by minimizing heat production and conserving water in birds inhabiting hot environments with low primary productivity and scarce drinking water (Tieleman and Williams 2000; Tieleman et al. 2002).

The magnitude and direction of seasonal acclimatization of BMR varied among sparrow-weaver populations (table 1), making this the second Afrotropical species for which marked interpopulation differences in seasonal metabolic acclimatization have been observed (van de Ven et al. 2013). The lack of significant seasonal variation in BMR at Frankfort and Molopo (milder, arid site) is qualitatively similar to that documented in other subtropical species: Z. capensis (Maldonado et al. 2009) and a coastal population of Euplectes orix (van de Ven et al. 2013). In contrast, the winter increases in BMR by ~52% at both Askham and Polokwane (table 1) are near the upper end of the range reported for temperate-zone species (<10%–64%; Pohl 1971; Dawson et al. 1983; Battley et al. 2001; Arens and Cooper 2005) and are substantially greater than those reported for several other subtropical species (18%–28%; Pycnonotus sinensis, Onychognathus morio, and Tauraco corthaix; Zheng et al. 2008; Chamane and Downs 2009; Wilson et al. 2011). The lack of significant seasonal BMR variation in Molopo sparrow-weavers in this analysis contrasts with the original findings of significantly lower BMR values in winter (Smit and McKechnie 2010). We suspect that this difference reflects our statistical model for BMR incorporating M_b as a continuous predictor and the inclusion of data for multiple populations.

Summit Metabolism

The M_{sum} of all three sparrow-weaver populations was generally higher than values predicted for tropical birds (115%–176% of predicted values; Wiersma et al. 2007) and oscine passerines (120%–147% of predicted values, except Polokwane birds during summer [~96% of predicted M_{sum}]; Swanson and Bozinovic 2011). In all three populations, M_{sum} was equivalent to ~6 × BMR (i.e., metabolic expansibility), within the typical avian range of 3–8 × BMR (Swanson 2010). A correlation between M_{sum} and cold tolerance exists in temperate species (Swanson 2001; Swanson and Liknes 2006), and the fact that the significantly higher M_{sum} of Askham compared with Polokwane birds was associated with significantly lower winter T_{CL} values is consistent with the notion that cold tolerance is directly related to maximum capacity for resting heat production.

The lack of significant seasonal variation in T_{CL} in any of our study populations, however, makes the functional significance of the winter increases in M_{sum} unclear (table 1). The absence of significant seasonal T_{CL} variation could be an artifact of our use of a helox atmosphere to elicit M_{sum} (Rosenmann and Morrison 1974; Holloway and Geiser 2001); cold limits measured in air would be proportionally further apart. Fewer studies have investigated seasonal variation in M_{sum} in subtropical/tropical species compared with their temperate counterparts, but winter decreases have been reported in seven tropical species (12%–35% lower than summer; Wells and Schaeffer 2012), as well as winter decreases (~8%) and increases (~15%) in the M_{sum} of subtropical coastal and inland populations of E. orix, respectively (van de Ven et al. 2013).

Intraspecific Variation in Seasonal Metabolic Acclimatization

Contrary to our predictions, differences in seasonal BMR variation among sparrow-weaver populations appear not to be directly related to T_{min} values. BMR was upregulated in winter at the Askham site where low daily T_{min} values occur but was also upregulated at Polokwane, the site with the mildest winter T_{min} values, and the latter population was the only one to increase winter RMR at $5°C \leq T_a \leq 20°C$ (figs. 1, 2; table 1). Seasonal differences in insulation could potentially be responsible for the higher winter RMR in Polokwane sparrow-weavers; however, we did not measure thermal conductance to evaluate this possibility. No significant seasonal metabolic variation was observed in Frankfort sparrow-weavers, the coldest site in this study, although this population did have significantly higher BMR compared with the other populations during summer and thus had winter BMR values similar to those of the other populations (table 1). This is similar to the pattern of M_{sum} variation reported among populations of temperate house finches (*Carpodacus mexicanus*), where birds from Michigan (cold site) upregulated M_{sum} during winter, and the M_{sum} of finches from Colorado (cold site) and California (warm site) was seasonally stable; however, Colorado finches maintained M_{sum} values consistently higher than the California population and similar to those of Michigan birds during winter (Dawson et al. 1983; O'Connor 1996).

Upregulation of metabolic rates during winter in temperate-zone species is thought to be functionally related to enhanced cold tolerance (McKechnie 2008; Swanson 2010), with Smit and McKechnie (2010) reporting a correlation between T_{min} values and seasonal metabolic upregulation at a global scale. However, patterns of seasonal metabolic acclimatization in subtropical/tropical birds now appear generally more variable than those of temperate-zone birds both within and among species (Dawson et al. 1983; Cooper and Swanson 1994; O'Connor 1995, 1996; Maddocks and Geiser 2000; Sharbaugh 2001; Bush et al. 2008; Zheng et al. 2008; Chamane and Downs 2009; Smit and McKechnie 2010; Wilson et al. 2011; Wells and Schaeffer 2012; van de Ven et al. 2013), suggesting that factors other than T_a may influence the magnitude and direction of seasonal physiological adjustments. For instance, Smit and McKechnie (2010) argued that at subtropical/tropical latitudes lower food availability during winter may result in a prioritization of energy conservation over cold tolerance. However, recent studies demonstrating variable patterns of seasonal metabolic variation in subtropical/tropical birds have called this notion into question at the broad subtropical/tropical versus temperate level (Zheng et al. 2008; Chamane and Downs 2009; Maldonado et al. 2009; Wilson et al. 2011; van de Ven et al. 2013), although food availability could still be an important proximate factor in determining the magnitude and direction of seasonal variation among and within species (McKechnie et al. 2015).

Differences in seasonal BMR variation between arid versus mesic sparrow-weaver populations were not consistent with the prediction that metabolic variation should reflect a greater demand for winter energy conservation in arid habitats. However, this notion could potentially explain the difference in seasonal BMR responses between the two Kalahari Desert sparrow-weaver populations (Askham and Molopo; table 1). The winter data for Molopo sparrow-weavers were collected following a relatively dry rainy season, a period during which food availability was likely low (Smit and McKechnie 2010), whereas the Askham winter data were collected following a summer with above-average rainfall. We thus speculate that energy conservation may have been relatively more important for Molopo birds on account of the weather conditions preceding that particular study. Moreover, our finding that the BMR of Molopo sparrow-weavers during winter was significantly lower than that of other populations—whereas no such difference occurred in summer—supports Smit and McKechnie's (2010) argument that seasonal BMR changes in sparrow-weavers at Molopo are functionally related to energy conservation during winter.

The contrasting seasonal adjustments of BMR in sparrow-weavers from two arid-zone sites in the Kalahari Desert (Askham and Molopo) highlight the possibility that the direction and magnitude of seasonal metabolic variation may not be fixed within populations but may respond to year-to-year fluctuations in environmental variables (McKechnie et al. 2015). If this is the case, it is important to distinguish between ultimate (predictable, long-term seasonal patterns; e.g., photoperiod) and proximate (less predictable, short-term patterns; e.g., food availability) factors that determine seasonal adjustments, because understanding the relative importance of these factors is vital for understanding the determinants of avian seasonal metabolic responses (Swanson and Olmstead 1999; Swanson and Vézina 2015).

Collectively, the data currently available on seasonal metabolic adjustments among species from tropical and subtropical latitudes suggest that the winter conditions experienced in these lower latitudes permit a greater degree of flexibility in terms of the direction and magnitude of acclimatization responses than is the case for temperate-zone species. However, this variation does not necessarily mean that low-latitude species possess inherently greater physiological flexibility than high-latitude species in terms of matching metabolic output to prevailing conditions. Rather, we suspect that the very low winter temperatures at high latitudes result in demands for cold tolerance that simply overwhelm other environmental variables—such as food availability—to which avian metabolic machinery might otherwise be responsive. More studies (particularly for M_{sum}) are needed to investigate metabolic adjustments in subtropical/tropical species, because limited data are a serious constraint in current analyses seeking environmental correlates of patterns of avian seasonal metabolic acclimatization (McKechnie and Swanson 2010). Future studies involving metabolic measurements spanning multiple years while simultaneously monitoring fluctuations in potential determinants of metabolic variation (e.g., photoperiod, food availability, and T_a) are required to address this question.

Intraspecific variation in avian metabolic traits supports the idea of adaptive thermoregulation, defined as any thermoregulatory pattern used to counteract an environmental stressor, regardless of whether it is the result of phenotypic plasticity (developmental plasticity or acclimatization) or genotypic adaptation (by natural selection across generations; Angilletta et al. 2010). Relatively few studies have investigated the contributions

of phenotypic plasticity versus genotypic adaptation to avian physiological variation (Garland and Adolph 1991), although common garden experiments have been used to demonstrate a genetic basis for metabolic differences among tropical versus temperate populations of African stonechats (*Saxicola torquata*; Wikelski et al. 2003) as well as between two populations of great tits (*Parus major*; Broggi et al. 2004, 2005). This study, in which we simply described intraspecific patterns of metabolic variation in sparrow-weavers, should be considered as an initial step toward identifying a model species suitable for research studies designed to tease apart the roles of local adaptation versus phenotypic plasticity in determining interpopulation metabolic differences, using common garden and short-term thermal acclimation experiments.

Conclusions

Our results do not support the hypothesis that avian seasonal metabolic adjustments vary among conspecific populations in a manner that reflects a continuum between winter energy conservation versus enhanced cold tolerance. However, patterns of seasonal adjustments in BMR varied spatially among sparrow-weaver populations, lending support to the idea that greater variance in seasonal metabolic responses occur in subtropical/tropical versus temperate species (McKechnie et al. 2015). Moreover, greater cold tolerance was associated with a population at a colder, desert site (Askham) than a milder, mesic site (Polokwane; table 1). Future studies are required to investigate the factors determining patterns of metabolic variation, and in particular, food availability may be an important proximate factor in this regard.

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