Phenotypic and genotypic sources of variation in the thermal physiology of a passerine bird

by

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Submitted in partial fulfilment of the requirements for the degree

PhD in Zoology

in the

Department of Zoology and Entomology

Faculty of Natural and Agricultural Sciences

University of Pretoria

January 2020

SUMMARY

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Degree: PhD in Zoology

Keywords: phenotypic flexibility, acclimatization, acclimation, reaction norms, summit metabolism, basal metabolic rate, pectoral muscle, organs, heat tolerance, heat stress, evaporative water loss, passerine, subtropical birds

There is considerable variation in avian thermal physiology, with traits such as metabolic rates correlated with climate at both inter- and intraspecific levels. However, it is often unclear whether these correlations result from genotypic adaptation, developmental plasticity or phenotypic flexibility. Seasonal acclimatization is a frequently cited example of phenotypic flexibility, with small, temperate-zone birds typically increasing metabolic rates in response to low winter air temperatures (T_a). Recent studies suggest considerable variation in patterns of seasonal acclimatization in birds inhabiting lower latitudes with milder winters, with variation also reported among populations of subtropical species such as the white-browed sparrow-weaver (*Plocepasser mahali*). To gain insights into the sources of intraspecific variation, I investigated phenotypic flexibility in the thermal physiology of Afrotropical passerine birds, using sparrow-weavers as a model species.

I measured seasonal variation in basal metabolic rate (BMR) and summit metabolism (M_{sum}) over a four-year period in arid-zone populations of sparrow-weavers and scaly-feathered weavers (*Sporopipes squamifrons*; chapter 1). Patterns of acclimatization varied substantially among years in both species, for example, sparrow-weaver BMR ranged from ~ 20 % lower to 68 % higher during winter compared to summer. In contrast to higher-latitude species, interannual metabolic variation was never related to minimum T_{a} or enhanced cold tolerance, but metabolic rates were significantly lower in seasons with lower food abundance.

I also investigated seasonal changes in the body composition of sparrow-weavers, focusing on traits commonly correlated with BMR and M_{sum} in temperate-zone birds (chapter 2). As expected, a lack of seasonal variation in BMR during the study period was associated with no significant variation in the dry mass of digestive and excretory organs. However, despite significant winter increases in pectoral muscle (~ 9 % higher; primary muscle for avian shivering thermogenesis) and heart mass (~ 18 % higher), there was a winter decrease in M_{sum}. These findings support the idea that factors other than enhanced cold tolerance drive patterns of metabolic variation in subtropical birds.

I used short-term (~ 30 days) thermal acclimation experiments to investigate whether phenotypic flexibility in thermoregulatory responses of sparrow-weaver varies among populations across a climatic gradient (~ 8 °C and 11 °C in mean daily winter minimum and summer maximum T_{a} respectively). I measured BMR and M_{sum} after acclimating individuals to one of three acclimation T_{a} (T_{accl}) treatments (5 °C, 15 °C or 35 °C; chapter 3). There was ~ 12 %

lower BMR and 25 % lower evaporative water loss in birds at the hottest T_{accl} compared to cooler treatments, with no interpopulation variation after acclimation (i.e., similar reaction norms). In contrast to the findings of most studies on temperate-zone birds, M_{sum} did not vary with T_{accl} regardless of population.

I also investigated flexibility in heat tolerance and evaporative cooling capacity of sparrow-weavers, measuring their ability to handle high T_{a} (~ 38 °C to 54 °C) after acclimation to one of three treatments (day-time T_{accl} = 30 °C, 36 °C or 42 °C; chapter 4). Birds at the hottest T_{accl} coped better with high T_{a} than those at milder T_{accl}, maintaining lower body temperatures and reaching $T_{\mathrm{a}} \approx 2$ °C higher before the onset of severe hyperthermia. There was no variation among populations after acclimation, suggesting similar reaction norms for heat tolerance and evaporative cooling capacity. These findings suggest previously documented interpopulation differences in sparrow-weaver thermal physiology is largely the result of phenotypic flexibility rather than local adaptation.

In conclusion, I found considerable flexibility in the thermal physiology of an Afrotropical passerine bird, both within and among populations. My data support the idea that enhancing winter cold tolerance is less important in subtropical birds compared to higher-latitude species. Moreover, similar thermoregulatory reaction norms among populations highlights the importance of phenotypic flexibility as a source of variation in avian physiology.

RESEARCH OUTPUTS

Journal article

Noakes M.J. and A.E. McKechnie. 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.

Noakes M.J. and A.E. McKechnie. In press. Seasonal metabolic acclimatization varies in direction and magnitude among years in two arid-zone passerines. Physiol Biochem Zool.

Manuscripts in review

Noakes M.J., W.H. Karasov, and A.E. McKechnie. Seasonal variation in body composition in an Afrotropical passerine bird: increases in pectoral muscle mass are, unexpectedly, associated with lower thermogenic capacity. Submitted to: J Comp Physiol B.

Noakes M.J. and A.E. McKechnie. Phenotypic flexibility of metabolic rate and evaporative water loss does not vary across a climatic gradient in an Afrotropical passerine bird. Submitted to: J Exp Biol.

Conference oral presentations

Noakes, M.J. and McKechnie, A.E. Phenotypic flexibility in the heat tolerance and evaporative cooling capacity of a model Afrotropical passerine bird in response to short-term thermal acclimation. 39th Congress of the Zoological Society of Southern Africa, 7-10 July 2019, Skukuza, South Africa.

Noakes, M.J., Wolf, B.O. and McKechnie, A.E. Seasonal metabolic variation in desert populations of two southern African passerine species. 27th International Ornithological Congress, 18-26 August 2018, Vancouver, Canada.

Noakes, M.J., McKechnie, A.E. and Wolf, B.O. Seasonal metabolic variation in desert populations of two southern African passerine species. Learn About Birds Conference (co-hosted by: BirdLife South Africa and Fitzpatrick Institute of African Ornithology), 10-11 March 2016, Skukuza, South Africa.

LIST OF ABBREVIATIONS

BMR	Basal metabolic rate
EHL	Evaporative heat loss
EWL	Evaporative water loss
M_b	Body mass
ME	Metabolic expansibility
MHP	Metabolic heat production
M_{sum}	Summit metabolism
PIT tags	Passive integrated transponder tags
RER	Respiratory exchange ratio
RMR	Resting metabolic rate
SFW	Scaly-feathered weavers
T_a	Air temperature
$T_{a,HT}$	Hyperthermia threshold air temperature
$T_{a,min}$	Minimum air temperature
$T_{a,max}$	Maximum air temperature
T_{accl}	Acclimation air temperature
T_b	Body temperature
T_{CL}	Helox temperature at cold limit
$\dot{V}_{O_2}$	Rate of O_2 consumption
$\dot{V}_{CO_2}$	Rate of CO_2 consumption
WBSW	White-browed sparrow-weaver

DECLARATION

I, Matthew J. Noakes, declare that the thesis, which I hereby submit for the degree PhD in Zoology at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

ETHICS STATEMENT

I, Matthew J. Noakes, have obtained the applicable research ethics approval for the research described in this work (protocol numbers: EC-054-15, EC040-15 and EC088-15). I declare that I have observed the ethical standards required in terms of the University of Pretoria's Code of Ethics for Researchers and the Policy guidelines for responsible research.

I also obtained approval for this research work from the relevant permitting bodies of the Gauteng (CPF6-000206), Limpopo (ZA/LP/H0/2355), Northern Cape (FAUNA 929/2/2015, FAUNA 1389/2015), North-West (HQ 13/12/16-340 NW) and Free State (JM 3286/2018) Provinces of South Africa.

Matthew Jason Noakes

31 January 2020

ACKNOWLEDGEMENTS

I would like to first thank my supervisor, Prof Andrew McKechnie, for his consistent guidance and active input, without which this PhD research would not have been possible. His unwavering support for both this work and in developing me as a researcher, has been invaluable during this first stage of my academic career.

I thank Prof William Karasov for his input and guidance in developing the methods and writing the second chapter of this thesis, and am grateful to Prof Blair Wolf for the use of his portable ultrasound system. I also thank four anonymous journal reviewers who provided constructive comments on manuscripts based on chapters from this thesis. Monica Leitner and Louwtjie Snyman provided invaluable advice in developing the methodology for the food abundance sampling used in chapter 1, and Caitlyn Nauschutz spent many hours processing and identifying the arthropods caught in pit fall traps (assisted by: Johan Saayman, Alex Nepomuceno, Paula-Jean Jardin, Bernard Olivier and Annerine Venter).

I thank the Rossouw family of Murray Guest farm near Askham for their ongoing hospitality, and for allowing me to conduct research work on their property. I also thank the manager (Sampie van der Merwe) and staff of Barberspan Bird Sanctuary for their hospitality and assistance with catching birds, and the de Klerk family of Pecan Grove Bed and Breakfast in Frankfort for allowing us to catch birds on their property. I am grateful to the South African Weather Service for providing weather data for each study site.

My original office mates, Michelle Thompson and Ryan O’Connor, were a part of this journey from the beginning, and I appreciate all the long days, late nights and online chats we shared in the process. I also thank the following special people for their continual love and support: my partner, Mervyn Uys, and dear friends, Natasha Lombard, Tegan Carpenter-Kling and Samantha Naidoo.

Finally, I am grateful to the many assistants, colleagues and friends that helped with field work, laboratory work and maintaining animals, including those mentioned above, as well as Sekgwari Malematja, Gabriel Foley, Alicia Fick, Andries van Vuuren, Janca Nortjé and Chuma Mateza.

This work would not have been possible without the bursary and research funding from the DST-NRF Centre of Excellence at the FitzPatrick Institute and the National Research Foundation of South Africa (grant number 110506 to Prof Andrew McKechnie). Any opinions, findings and conclusions or recommendations expressed in this material do not necessarily reflect the views of the National Research Foundation.

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CHAPTER 1:

Seasonal metabolic acclimatization varies in direction and magnitude among years in two arid-zone passerines

Published in *Physiological and Biochemical Zoology*

1.1 Abstract

There is increasing evidence of adaptive plasticity in avian thermal physiology, with a well-studied example being metabolic upregulation during cold winters in small birds inhabiting temperate and boreal latitudes. Recent studies have revealed greater variation in the direction and magnitude of seasonal metabolic adjustments among subtropical/tropical birds experiencing milder winters compared to higher-latitude counterparts, suggesting that patterns could vary among years within populations. I quantified seasonal metabolic variation (summer *vs* winter) in Kalahari Desert populations of two Afrotropical passerines, the white-browed sparrow-weaver (WBSW; *Plocepasser mahali*; ~ 40 g) and scaly-feathered weaver (SFW; *Sporopipes squamifrons*, ~ 10 g) over subsequent years (2014 to 2017). I used flow-through respirometry to measure basal metabolic rate (BMR) and summit metabolism (M_{sum}; maximum cold-induced resting metabolic rate), and quantified seasonal fluctuations in air temperature (T_a) and food abundance (arthropod and grass seed abundance) at the study site. The data reveal the direction and magnitude of seasonal metabolic acclimatization varies among years in both species, with the winter BMR of WBSWs ranging from ~ 20 % lower to 68 % higher compared to summer. In contrast to higher-latitude species, M_{sum} was not related to the cold limit temperature of birds or to winter minimum T_a at the study site, but interannual variation in BMR and M_{sum} was

significantly lower in seasons with lower food abundance in both WBSWs and SFWs. The data support the idea that patterns of seasonal acclimatization are more variable in birds from lower latitudes, and that there is considerable phenotypic flexibility in avian thermal physiology.

1.2 Introduction

There is considerable variation in endothermic thermal physiology, with traits such as metabolic rates correlated with climate at both inter- and intraspecific levels (e.g., Weathers 1979; MacMillen and Hinds 1998; Sabat et al. 2006; Londoño et al. 2015). Differences among species or populations of a species can confer adaptive value, although it is often unclear whether these differences result from adaptation via natural selection or adaptive plasticity (Gotthard and Nylon 1995; Angilletta et al. 2010). Adaptive plasticity occurs within individuals via adjustments in the phenotype expressed by a genotype in response to environmental conditions (i.e., phenotypic plasticity), and includes both developmental plasticity (ontogenic variation that becomes fixed on reaching maturity) and phenotypic flexibility (reversible changes within individuals; Via et al. 1995; Piersma and van Gils 2010; Kelly et al. 2012). The beneficial acclimation hypothesis (BAH) posits that acclimation to an environment enhances the performance or fitness of an individual within that particular environment (Leroi et al. 1994). Testing the BAH in the context of thermal physiology has almost entirely been restricted to ectotherms (Huey et al. 1999; Wilson and Franklin 2002; Marais and Chown 2008), primarily on account of the difficulty of quantifying fitness-related performance metrics over a range of body temperature (T_b) in endotherms.

Increasing attention is being given to the role of phenotypic flexibility as a source of intraspecific variation in the metabolic rates of endotherms, which can occur via acclimatization or acclimation in the field or laboratory respectively (Piersma and Drent 2003). A frequently-cited example of phenotypic flexibility is seasonal acclimatization in basal metabolic rate (BMR; minimum resting metabolic measured in post-absorptive, non-reproductive individuals during their rest-phase) and summit metabolism (M_{sum}; maximum metabolic rate measured during cold exposure; reviewed for birds by McKechnie et al. 2015).

Seasonal metabolic acclimatization has been well studied in small north-temperate birds, with the typical pattern involving upregulation of BMR and M_{sum} in winter compared to summer, presumably to enhance cold tolerance during cold winters (reviewed by Swanson 2010; McKechnie et al. 2015). Seasonal adjustments of BMR are primarily driven by changes in organ masses and/or tissue metabolic intensities (Chappell et al. 1999; Petit et al. 2014; Vézina et al. 2017), whereas M_{sum} is adjusted primarily through changes in the size of skeletal muscles (particularly pectoral muscle), cellular metabolic intensities, and/or the transport capacities of O_2 and metabolic substrates (Petit and Vézina 2014a; Zhang et al. 2015; Barceló et al. 2017). Recent studies suggest an uncoupling of adjustments in BMR and M_{sum} (Swanson et al. 2012; Petit et al. 2013; Dubois et al. 2016; Barceló et al. 2017), and several authors have reported differing patterns of seasonal variation for these two variables (Ambrose and Bradshaw 1988; O’Connor 1996; van de Ven et al. et al. 2013; Noakes et al. 2017). The uncoupling of BMR and M_{sum} questions the relevance of using metabolic expansibility (ME; i.e., metabolic scope, calculated as the ratio of M_{sum} to BMR) as a measure of thermogenic capacity (Petit et al. 2013).

Only relatively recently have workers investigated avian seasonal metabolic acclimatization in subtropical and tropical regions with generally milder winters and hotter summers (e.g., Maddocks and Geiser 2000; Smit and McKechnie 2010; van de Ven et al. 2013), and consequently patterns of metabolic variation remain less well understood among birds from lower latitudes. Smit and McKechnie (2010) analyzed global variation in seasonal changes of avian BMR, and proposed the existence of a continuum between enhanced cold tolerance via upregulated BMR in higher-latitude regions with long, cold winters, to winter energy conservation via reductions in BMR at lower-latitudes with milder, dry winters. However, recent studies have reported winter increases in BMR and M_{sum} in subtropical birds, with these increases similar in magnitude to those typical of north-temperate species (e.g., Lindsay et al. 2009 a,b; Wilson et al. 2011). There thus appears to be greater variability in the patterns of seasonal metabolic adjustments in tropical and subtropical species, with BMR ranging from ~ 35 % lower to 60 % higher during winter compared to summer (McKechnie et al. 2015; Pollock et al. 2019). Variation in seasonal metabolic adjustments has also been reported among populations of subtropical species; for example, patterns of seasonal acclimatization in BMR and M_{sum} varies from no seasonal change to winter upregulation among populations of southern red bishops (*Euplectes orix*; van de Ven et al. 2013) and white-browed sparrow-weavers (WBSW; *Plocepasser mahali*; Smit and McKechnie 2010; Noakes et al. 2017).

The relative roles of proximate cues (less predictable, short-terms trends; e.g., variation in daily temperatures and food abundance) versus ultimate cues reflecting predictable, long-term trends (e.g., seasonal variation in climate and photoperiod) as determinants of seasonal acclimatization in avian metabolic rates remains unclear (Swanson and Vézina 2015). There is, however, evidence that avian metabolic rates are adjusted over shorter time scales, and seasonal variation may reflect physiological responses to conditions over days to weeks (Swanson and Olmstead 1999; Broggi et al. 2007; Petit and Vézina 2014b; Dubois et al. 2016). For example, data for several north-temperate birds reveal that BMR and M_{sum} is more closely related to short- (0 to 5 days; *Spizella arborea*) and medium-term (14 to 30 days; *Poecile atricapillus* and *Junco* *hyemalis*) fluctuations in winter minimum temperatures than long-term trends (Swanson and Olmstead 1999). Moreover, two arid-zone WBSW populations in the Kalahari Desert of southern Africa (Molopo and Askham) had contrasting patterns of seasonal BMR variation (no seasonal change and ~ 52 % higher BMR in winter compared to summer respectively; Smit and McKechnie 2010; Noakes et al. 2017), suggesting that the direction of seasonal metabolic adjustments may not be fixed in populations.

In light of a) the variability in observed seasonal metabolic adjustments in subtropical species, and b) the evidence for a proximate effect of temperature on intra-winter metabolic rates in north-temperate passerines, I hypothesized that variation in patterns of seasonal metabolic acclimatization in subtropical species are modulated along a cold tolerance / energy conservation continuum in response to environmental conditions. Specifically, I predicted patterns of seasonal variation in BMR and M_{sum} in two passerines would vary over the study period, with an upregulation in metabolic rates during colder winters with high food abundance, and downregulation during milder winters with low food abundance. During seasons with contrasting environmental selection pressures (e.g., a cold winter with low food abundance), I expected to find either no seasonal metabolic change or seasonal patterns prioritizing the more pressing environmental factor.

To investigate flexibility in the magnitude and direction of seasonal metabolic variation in Afrotropical passerine birds, I measured seasonal variation in BMR and M_{sum} over a three-year period in Kalahari Desert populations of two ploceid passerine species with a four-fold variation in body mass (M_{b}), the WBSW (~ 40 g, diet: insects and grass seeds) and scaly-feathered weaver (SFW; *Sporopipes squamifrons*; ~ 10 g, diet: predominantly grass seeds). I simultaneously quantified variation in potential ecological correlates of metabolic acclimatization: daily minimum and maximum T_a ($T_{a,min}$ and $T_{a,max}$), rainfall and food abundance (arthropod and grass seed abundance). For WBSWs, I included metabolic data collected during 2014 by Noakes et al. (2017), providing a four year data set on seasonal variation in BMR and M_{sum} in this species.

1.3 Materials and methods

1.3.1 Study site

I examined thermoregulation in WBSWs and SFWs during winter (July/August) and summer (January/February) over a three year period (2015 to 2017) at Murray Guest Farm near Askham in the Kalahari Desert (Northern Cape Province, South Africa; 26° 59′ S, 20° 51′ E). For WBSWs, I included data from a previous study that recorded seasonal metabolic variation at the same study site during 2014 (Noakes et al. 2017). I caught WBSWs from their nests at night using two small nets mounted on aluminium poles, with a few birds being caught during the day using mistnets or spring traps baited with mealworms. I caught SFWs using mistnets, except for five individuals I found roosting in a WBSW nest. I housed WBSWs and SFWs in cages constructed of plastic mesh and shade cloth (~ 1.5 m^3), and provided birds with *ad libitum* water, wild bird seed mix and mealworms. The natural diet of WBSWs includes arthropods and grass seeds (~ 30 % arthropods; Ferguson 1988; du Plessis 2005), and for adult SFWs consists of grass seeds (juveniles supplemented with arthropods; Herremans 1997; Dean 2005). Individual WBSWs were sexed according to the colour of their beaks (du Plessis 2005), however, I did not sex SFWs as they are not sexually dimorphic (Dean 2005). Physiological data were collected using a field respirometry system within 60 hr of capture, after which all birds were released at the site of capture.

1.3.2 Seasonal variation in temperature and food abundance

To investigate whether patterns of seasonal metabolic acclimatization in WBSWs and SFWs vary with environmental factors, I quantified seasonal fluctuations in T_a extremes and food abundance (arthropod and grass seed abundance) during each year I collected gas exchange data. Daily $T_{a,min}$ and $T_{a,max}$, and rainfall data were obtained from the South African Weather Service using the weather stations closest to Askham (T_a: Twee Rivieren, ~ 62 km northwest, 27° 28′ S, 20° 36′ E; rainfall: Witdraai Police Station, ~ 9 km west, 26° 57′ S, 20° 42′ E). I calculated mean daily $T_{a,min}$ and $T_{a,max}$, as well as total rainfall, over the hottest summer months (December to January) and coldest winter months (June to July) of each year, which also included the month directly before gas exchange data were collected.

Five locations at my study site where I frequently observed WBSWs and SFWs foraging were chosen to serve as replicates for food abundance measurements (conducted concurrently with metabolic measurements), with replicates being > 300 m apart and < 120 m from trees with active WBSW colonies (WBSWs typically forage 60 m to 120 m from their colony; Lewis 1982). Arthropod abundance was estimated using pit fall traps, which are likely to provide reliable estimates of availability for WBSW as they are ground-foraging birds (Collias and Collias 1978; Lewis 1982; Ferguson 1988). Each replicate consisted of two grids of traps (< 100 m apart), one in the dune valley and another about halfway up the dune slope. Each grid consisted of 10 traps arranged in two rows of five traps spaced 10 m apart (thus 20 traps per replicate, 100 traps in total per season). I used plastic cups (200 ml) as pit fall traps. The cups were buried in the ground and filled up to one-third with a liquid soap (Body Wash, Clicks, Cape Town, South Africa) and water mixture (ratio: ~ 1 part soap to 100 parts water) to impede the evaporation of liquid. Traps were collected and replaced every second or third day, with a total sampling period of seven days per season. After collection, the soap-water mixture was drained from traps, and arthropod samples stored in resealable plastic bags containing 70 % ethanol to preserve specimens. Arthropods were classified to order level, and the data for orders that are part of the WBSW's diet (du Plessis 2005) were pooled for each season to provide an estimate of arthropod abundance in the summer and winter of each year (see appendix for the respective abundances of each order).

Percentage grass cover was used as an estimate of seasonal variation in grass seed abundance over the study period. I did step-counts at the same five replicates used for pit fall trapping, again in the dune valley and on the slope. Each step-count involved walking 50 steps (thus 100 steps per replicate, 500 steps in total per season) and recording whether live grass or dead grass/bare ground was stepped on to provide an estimate of live grass density per area. The grass species most commonly identified were *Stipagrostis* spp. (positively identified: *S. ciliata* and *S. amabilis*) and *Schmidtia kalahariensis*, which flower throughout most of the year (*Schmidtia kalahariensis*: January to December, *Stipagrostis amabilis*: August to May, *Stipagrostis ciliata*: August to October and February to June; Fish et al. 2015).

1.3.3 Gas exchange and temperature measurements

I measured O_2 consumption ($\dot{V}_{O_2}$) and CO_2 production ($\dot{V}_{CO_2}$) using open flow-through respirometry, and 4-L and 1.2-L clear plastic containers (Lock & Lock, Seoul, South Korea) as metabolic chambers for WBSWs and SFWs respectively. For BMR measurements, I placed metabolic chambers in a modified ice chest (~ 75 L) and regulated T_a using a Peltier device (AC-162, TE Technology Inc, Traverse City MI, USA) and custom-built digital controller. An air pump (model DAA-V515-ED, Gast Air Pumps, Benton Harbour MI, USA) supplied atmospheric air into the system, and incurrent air was dried using scrubber columns of silica gel and Drierite (WA Hammond DRIERITE Co, Xenia OH, USA). For M_{sum} measurements, I placed metabolic chambers in a modified fridge-freezer (40 L; ARB, Kilsyth, VIC, Australia), and helox (21 % O_2, 79 % He) was supplied into the system. A helox environment increases the heat loss rates of birds, allowing M_{sum} to be measured at relatively moderate T_{a} values (Rosenmann and Morrison 1974; Holloway and Geiser 2001). Mass flow controllers were used to maintain flow rates of ~ 1 L min^{-1} for WBSWs and ~ 0.5 L min^{-1} for SFWs during BMR measurements (MC-10SLPM-D/5m, Alicat Scientific Inc., Tuscon AZ, USA), and ~ 2 L min^{-1} for WBSWs and ~ 1.4 L min^{-1} for SFWs during M_{sum} measurements (FMA5520, Omega Engineering, Bridgeport NJ, USA). Excurrent air or helox from the chambers and baseline channels was alternately subsampled, and pulled through a CO_2 / H_2O analyser (LI-840A, LI-COR, Lincoln NE, USA) and O_2 analyser (FC-10A, Sable Systems, Las Vegas NV, USA) at ~ 200 ml min^{-1} by a custom-built pump with a rotameter (Omega, Stamford CT, USA).

I 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, USA) during M_{sum} measurements, which were inserted into the chambers through a small hole sealed with a rubber grommet. I measured core T_{b} in WBSWs using temperature-sensitive passive integrated transponder (PIT) tags (Destron Fearing, St. Paul, USA) injected into the peritoneal cavities of birds. T_{b} was monitored using a handheld PIT tag scanner (DTR-4, Destron Fearing, St Paul MN, USA). I did not implant tags into SFWs as they are substantially smaller than WBSWs, and thus measured cloacal T_{b} directly after M_{sum} measurements using a Cu-Cn thermocouple and thermocouple reader. All equipment was calibrated as described by Minnaar et al. (2014) and Noakes et al. (2017).

1.3.4 Experimental protocol

To measure BMR of WBSWs and SFWs, I exposed birds to $T_a \approx 30$ °C (actual $T_a = 30.0 \pm 0.4$ °C) for the entire night, measuring the gas exchange rates of two individuals simultaneously in separate metabolic chambers ($n = 10$ for each species per season per year, except winter 2016 for WBSWs: $n = 14$ and winter 2017 for SFWs: $n = 11$). A T_a of 30 °C was chosen as this T_a is within the thermoneutral zone of WBSWs (Smit and McKechnie 2010; Noakes et al. 2017) and SFWs (Whitfield et al. 2015), although metabolic measurements for SFWs were recorded from birds at rest during the daytime (active-phase; current study was during the rest-phase). I also conducted overnight resting metabolic rate (RMR) measurements at 5 °C ≤ T_a ≤ 35 °C (at 5 °C increments, two to three T_a per night) to confirm $T_a = 30$ °C represented thermoneutrality for WBSWs and SFWs during every season of data collection ($n \approx 6$ individuals per species per T_a). The M_b of individuals was measured on entering (just prior to sunset) and after removal (after sunrise) from chambers using a Scout *Pro* Balance scale (SP602US, Ohaus, Pine Brook NJ, USA). Air was sequentially sampled from the baseline channels and chambers in an 80-min cycle throughout the night: 10-min baseline air, then 30-min per chamber respectively, and baseline air again for the last 10-min.

I quantified M_{sum} in WBSWs and SFWs during the daytime within 12-h of the BMR measurement for each individual (alternating whether BMR or M_{sum} was measured first for each bird), and used the sliding cold exposure method in a helox environment (Swanson et al. 1996), ($n = 10$ for each species per season per year, except the winters of 2016 and 2017 for WBSWs: n

= 11 and 13 respectively, and the winter 2017 for SFWs $n = 11$). To habituate birds to the experimental setup, I initially provided atmospheric air and regulated chamber T_a at ~ 10 °C and 20 °C for ~ 10-min for WBSWs and SFWs respectively – a higher initial T_a was used for SFWs to avoid individuals becoming hypothermic too early during measurements. After the habituation period, I switched to providing helox into the system and collected a 5-min baseline of helox air. The fridge-freezer was then set to -15 °C to allow a constant decrease in T_a, and excurrent air was sampled from the chamber. I visually monitored O_2 and CO_2 traces during measurements for a plateau in increasing metabolic rate indicating that maximum thermogenic capacity (i.e., M_{sum}) had been elicited. I terminated measurements when O_2 and CO_2 traces indicated a distinct decrease in metabolic rate away from this plateau indicative of M_{sum}, and measured T_b of birds on removal from chambers to confirm hypothermia. Hypothermia was assumed in WBSWs if T_b decreased by ≥ 5.0 ºC from initial values upon entering the chamber (mean decrease in T_b: 9.4 ± 2.3 °C). I did not have initial T_b values for SFWs but assumed hypothermia if $T_b \leq 36.0$ ºC after removal from the chamber (mean T_b after measurements: 33.0 ± 1.7 °C). All individuals met these criteria for hypothermia, and thus all data were included in analyses.

1.3.5 Data analysis

Expedata Data Acquisition and Analysis Software (Sable Systems, Las Vegas, NV) was used to correct for drift in the data between respective [O_2], [CO_2] and [H_2O] baselines. $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ values were calculated using equations 9.4 and 9.5 respectively in Lighton (2008), using an excurrent flow rate estimated from equation 9.3 in all calculations. Gas exchange ratios were converted into whole animal metabolic rates (BMR and M_{sum}) using thermal equivalence data from table 4.2 in Withers (1992), with BMR representing the lowest 5-min metabolic rate measured overnight for each individual and M_{sum} the highest 5-min metabolic rate obtained during sliding cold exposure measurements. The average respiratory exchange ratios (RER; $\dot{V}_{CO_2}/\dot{V}_{O_2}$) during measurements was 0.60 ± 0.13, which is below the expected range of 0.71 to 1.00 (Withers 1992). As thermal equivalence data is not available for RER < 0.71, I assumed an RER of 0.71 to calculate metabolic rates in watts for measurements below this value, and repeated statistical analyses with $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ as the response variable to confirm this was not a source of error in the results. I used the helox temperature at which M_{sum} was reached as an estimate of the cold limit temperature for each bird (T_{CL}). ME was calculated as the ratio of M_{sum} to BMR for each individual. All values are presented as mean ± standard deviation.

I used R 3.5.1 (R Core Team 2018) to fit linear models to the data and assumptions of all models were checked by inspecting model residual plots (residuals *vs* fitted, normal Q-Q, scale-location and residuals *vs* leverage plots). I used the "lm" base function to investigate how BMR, M_{sum}, ME and T_{CL} varied among years, seasons, and with M_{b}, and how M_{b} varied among years and seasons in each species. Sex was initially included as a predictor variable in models for WBSWs, but as it was never included in the top models explaining variation in response variables and Noakes et al. (2017) also reported no sex differences in this species, I excluded sex from further analyses. I used the "dredge" function from the *MuMIn* package (Bartoń 2018) to determine the combination of predictor variables that produced linear models that best explained variation in response variables. *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 year × season groups for each response variable.

I also fitted linear models to investigate interannual variation in BMR, M_{sum} and M_{b} in summer and winter respectively in relation to environmental variables: T_{a} extremes (mean $T_{\mathrm{a,max}}$

for summer and $T_{a,min}$ for winter) and food abundance. I used percentage grass cover as an estimate of grass seed abundance for the food abundance of SFWs (Herremans 1997), and for WBSWs I calculated standardized residuals for arthropod and grass abundance for each season ([observed abundance of season – mean abundance of all seasons] / standard deviation of mean abundance of all seasons) and combined these values into one metric for food abundance using diet proportions reported by Ferguson (1988; 30 % arthropods and 70 % seed). Rainfall was correlated with percentage grass cover, and thus was excluded from these models.

1.4 Results

1.4.1 Interannual variation in temperature and food abundance

There was consistent seasonal variation in rainfall, $T_{a,min}$ and $T_{a,max}$ at the study site over the study period, with summers being considerably hotter and wetter compared to winters (figure 1c). There was no rain during any of the winters (June to July) over the study period, and the summer of 2015 (December 2014 to January 2015) had substantially lower total rainfall (15.0 mm) compared to the summers of other years (70.0 mm for both summers 2014 and 2016, 42.5 mm for summer 2017). The winter $T_{a,min}$ were similar across years of the study period (1.4 °C range in mean values), but there was greater variation in the summer $T_{a,max}$ (range of 2.5 °C in mean values), with the summer of 2016 being hotter than the other years (figure 1c). The coldest $T_{a,min}$ recorded was -9.4 °C (winter 2014) and the hottest $T_{a,max}$ was 45.4 °C (summer 2016).

There was considerable variation in food abundance (arthropod and grass seed abundance) from 2015 to 2017, but no consistent pattern of seasonal differences (figure 1d). There was a ten-fold range in the total number of arthropods caught in the traps during respective seasons, with the highest abundance occurring in winter 2016 and summer 2017, and the lowest abundance in winter 2015 (figure 1d). Variation in percentage grass cover (an estimate of grass seed abundance) reflected variation in summer rainfall, ranging from a minimum of 22 % cover in summer 2015 to a maximum of 90 % cover in summer 2016 (figure 1d).

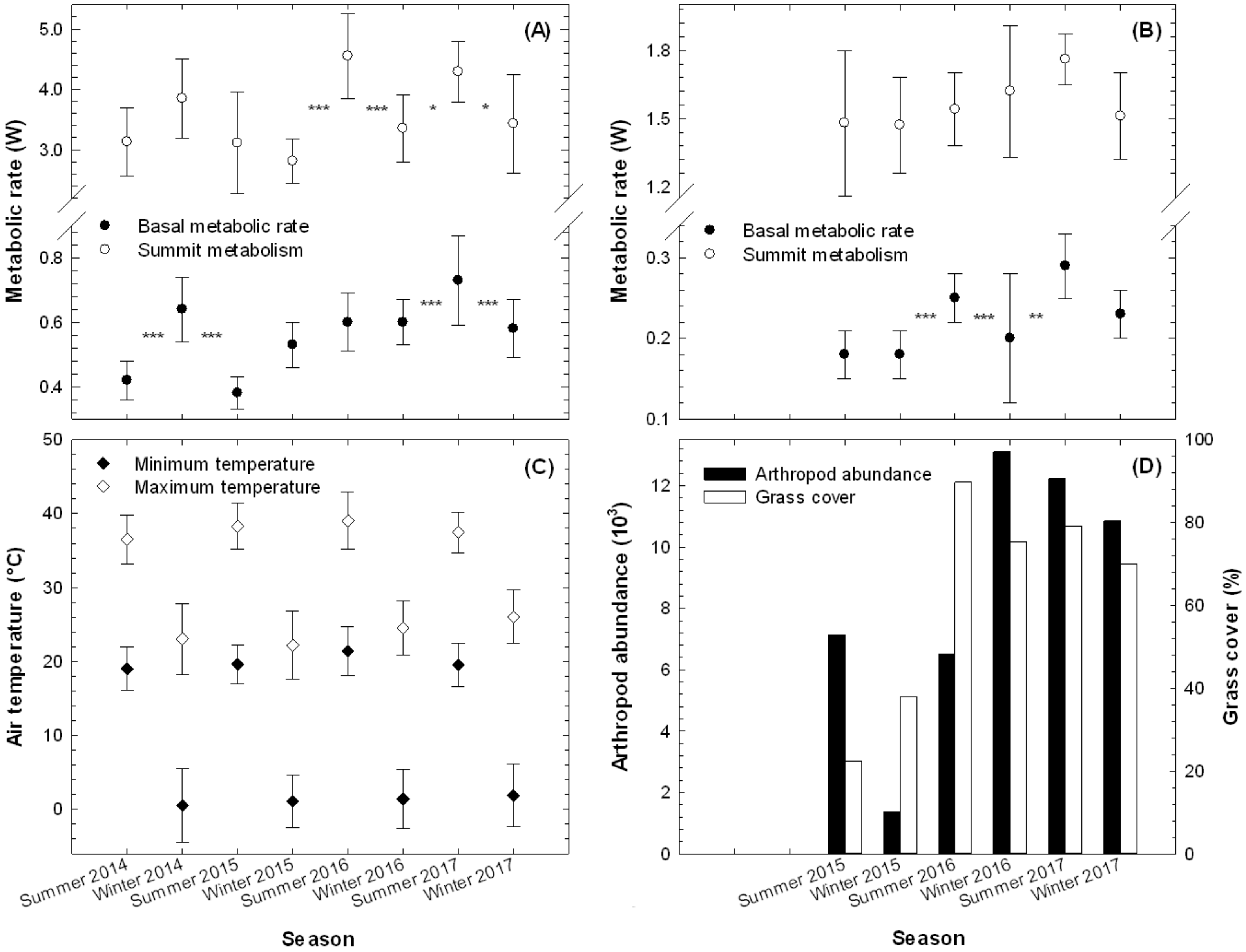
(A)
Metabolic rate (W)
5.0
4.0
3.0
0.8
0.6
0.4
Basal metabolic rate
Summit metabolism
***
***
***
***
*
*
***
***
(B)
Metabolic rate (W)
1.8
1.5
1.2
0.3
0.2
0.1
Basal metabolic rate
Summit metabolism
***
***
**
(C)
Air temperature (°C)
50
40
30
20
10
0
Minimum temperature
Maximum temperature
Summer 2014
Winter 2014
Summer 2015
Winter 2015
Summer 2016
Winter 2016
Summer 2017
Winter 2017
Season
(D)
Arthropod abundance (10^3)
12
10
8
6
4
2
0
Grass cover (%)
100
80
60
40
20
0
Arthropod abundance
Grass cover
Summer 2015
Winter 2015
Summer 2016
Winter 2016
Summer 2017
Winter 2017
Season

Figure 1.

Figure 1. Basal metabolic rate and summit metabolism of two arid-zone passerine birds, white-browed sparrow-weavers (*Plocepasser mahali*, A) and scaly-feathered weavers (*Sporopipes squamifrons*, B), were measured during summer and winter over a three-year period (2015- 2017; $n \approx 10$ for each species per season per year; mean ± standard deviation values are presented). Data were also included from a previous study for *P. mahali* in 2014 (Noakes et al. 2017). Significant differences between adjacent summers and winters are denoted by asterisks (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$; values obtained from linear models and *post hoc* tests of multiple comparisons of means, Tukey Contrasts). Interannual variation is also presented for seasonal temperature extremes (C), and food abundance (arthropod and grass seed abundance, D). Mean ± standard deviation of daily minimum and maximum air temperatures, are presented for the hottest summer months (December-January) and coldest winter months (June-July) using data obtained from the South African Weather Service. Arthropod abundance was estimated using pit fall trapping and values represent the total number of arthropods caught per season for all orders that are part of the diet of *P. mahali* (diet: 30 % arthropods, 70 % seed). Grass cover (%) was used as an estimate of grass seed abundance and was measured using grass steps counts during each season (diet of *S. squamifrons* consists of grass seeds). There were no estimates of food abundance during the summer and winter of 2014.

1.4.2 White-browed sparrow-weavers

The M_b of WBSWs varied among individuals during the study period from a minimum of 33.2 g to a maximum of 46.4 g (both values from summer 2015). The top model explaining variation in the M_b of WBSWs included only season (table 1), with birds being ~ 1.5 g heavier in winter compared to summer (table 2). The top model explaining interannual variation in M_b in relation to fluctuations in environmental variables was the model supporting the null hypothesis (i.e., no environmental variables included as predictors). BMR and M_{sum} increased significantly with increasing M_b, and T_{CL} decreased significantly with increasing M_b (table 1). There was also significant variation in BMR, M_{sum} and T_{CL} with the year × season interaction (table 1), but no interactions between M_b and the other predictor variables were included in the top models for any response variables of WBSWs, suggesting that variation among years and seasons does not solely reflect M_b differences.

The BMR of indiviudal WBSWs varied from a minimum of 0.3 W (summer 2015) to a maximum of 1.0 W (summer 2017). The direction and magnitude of seasonal acclimatization in BMR differed among years, including significantly higher BMR in winter compared to summer (~ 52 % and 68 % higher in winter 2014 *vs* summers 2014 and 2015 respectively), no significant seasonal variation in 2015 and 2016, and significantly lower BMR in winter compared to summer (~ 20 % lower in winters 2016 and 2017 *vs* summer 2017; figure 1a; table 2). Food abundance (estimated assuming WBSW's diet: 30 % arthropods and 70 % seeds; Ferguson 1988) was the only predictor variable included in the top models investigating interannual variation in BMR, and BMR significantly decreased with decreasing food abundance among summers ($F_{1,28} = 43.913$, $p < 0.001$) and winters ($F_{1,32} = 5.817$, $p = 0.022$; figure 1).

The M_{sum} of individual WBSWs varied from a minimum of 2.3 W (winter 2015) to a maximum of 5.4 W (summer 2016). Patterns of seasonal acclimatization in M_{sum} differed among years, ranging from no significant seasonal variation in 2014 and 2015 to significantly lower M_{sum} in winter compared to summer (~ 20 % to 38 % lower in winters 2015, 2016 and 2017 *vs* summers 2016 and 2017; figure 1a; table 2). M_{sum} significantly decreased with decreasing food abundance during among summers ($F_{1,28} = 23.004$, $p < 0.001$) and winters ($F_{1,32} = 6.427$, $p = 0.016$; figure 1). Patterns of seasonal variation in T_{CL} also differed among years but was not related to variation in M_{sum}, including significantly lower T_{CL} in winter compared to summer (reaching T_a ~ 7.3 °C lower in winter 2015 *vs* summers 2015 and 2016), no significant seasonal variation, and significantly higher T_{CL} in winter compared to summer (~ 8.5 °C higher in winter *vs* summer 2017; table 2). In contrast, the top model for variation in ME (i.e., the ratio of BMR to M_{sum}) of WBSWs only included season (table 1), with ME being ~ 1.3 lower in winter compared to summer (table 2).

In summary, patterns of seasonal acclimatization in the BMR, M_{sum} and T_{CL} of WBSWs varied among years, but variation in patterns were not consistent among variables (table 2). BMR and M_{sum} significantly decreased with decreasing food abundance among summers and winters over the study period (figure 1; table 2). Repeating analyses using $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ instead of metabolic rate (W) as the response variable revealed the same patterns of variation for BMR and M_{sum} of WBSWs.

Table 1: Statistical results from models fitted to data from two arid-zone passerine birds, white-browed sparrow-weavers (*Plocepasser mahali*, WBSW) and scaly-feathered weavers (*Sporopipes squamifrons*, SFW)

		Body mass			Year			Season			Year × Season		
Species	Variable	*F*-value	df	*p*-value	*F*-value	df	*p*-value	*F*-value	df	*p*-value	*F*-value	df	*p*-value
WBSW	M_{b}							65.601	1,86	**0.006**			
	BMR	16.467	1,75	<**0.001**	21.764	3,75	<**0.001**	8.589	1,75	**0.004**	16.195	3,75	<**0.001**
	M_{sum}	7.261	1,75	**0.009**	10.120	3,75	<**0.001**	10.346	1,75	**0.002**	9.374	3,75	<**0.001**
	ME							17.265	1,77	<**0.001**			
	T_{CL}	5.541	1,75	**0.021**	8.742	3,75	<**0.001**	0.514	1,75	0.476	12.714	3,75	<**0.001**
SFW	M_{b}				2.146	2,56	0.127	0.007	1,56	0.980	10.154	2,56	<**0.001**
	BMR	37.500	1,54	<**0.001**	28.720	2,54	<**0.001**	18.823	1,54	<**0.001**	2.753	2,54	0.073
	M_{sum}	49.849	1,56	<**0.001**	4.173	2,56	**0.020**	2.054	1,56	0.157			
	ME	0.005	1,51	0.942	10.830	2,51	<**0.001**	9.060	1,51	**0.004**	4.419	2,51	**0.017**
	T_{CL}	32.871	1,59	<**0.001**									

Note: Predictor variables were only included in the final models for each response variable if model selection indicated they improved model fit. Response variables for each species include body mass (M_{b}), basal metabolic rate (BMR), summit metabolism (M_{sum}), metabolic expansibility (ME) and helox temperature at cold limit (T_{CL}; i.e., temperature that M_{sum} was reached). Statistical results (*F*-value, *p*-value, degrees of freedom [df]) are from the top linear models fitted to data. Data were collected in the summer and winter of each year over the time period 2014-2017, and predictor variables included body mass (except when body mass was the response variable), year, season and the year × season interaction.

Table 2: Data for two arid-zone passerine birds, white-browed sparrow-weavers (*Plocepasser mahali*, WBSW) and scaly-feathered weavers (*Sporopipes squamifrons*, SFW) during summer and winter over a four year period

		2014		2015		2016		2017	
Species	Variable	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter
WBSW	M_b (g)	38.51 ± 2.54	40.30 ± 2.87	36.85 ± 4.07	40.28 ± 2.28	38.66 ± 2.95	40.03 ± 3.14	39.57 ± 2.67	39.99 ± 2.59
	BMR (W)	.42 ± 0.06^{A*}	.64 ± 0.10^{a*}	.38 ± 0.05^{A}	.53 ± 0.07^{b}	.60 ± 0.09^{B}	.60 ± 0.07^{ab*}	.73 ± 0.14^{C*}	.58 ± 0.09 ^{ab}
	M_{sum} (W)	3.13 ± 0.56^{A}	3.85 ± 0.66^{a}	3.11 ± 0.84^{A}	2.81 ± 0.36^{b*}	4.55 ± 0.70^{B*}	3.35 ± 0.56^{ab*}	4.29 ± 0.51^{B*}	3.43 ± 0.81^{ab}
	ME	7.44 ± 1.14	6.12 ± 1.56	8.25 ± 2.71	5.47 ± 1.11	7.79 ± 1.55	5.48 ± 1.25	6.17 ± 1.68	6.18 ± 1.64
	T_{CL} (°C)	-2.26 ± 2.85^{A}	-5.96 ± 3.95^{ab}	-2.39 ± 3.25^{A*}	-9.72 ± 4.07^{a*}	-2.52 ± 3.82^{A}	-3.74 ± 5.21^{b}	-4.45 ± 5.10^{A*}	4.00 ± 4.54^{c}
SFW	M_b (g)			10.05 ± 0.67^{A}	10.66 ± 0.51^{a}	9.83 ± 0.47^{A}	10.29 ± 0.83^{a}	10.99 ± 0.56^{B*}	10.00 ± 0.70^{a}
	BMR (W)			.18 ± 0.03^{AB}	.18 ± 0.03^{ab*}	.25 ± 0.03^{A*}	.20 ± 0.08^{a*}	.29 ± 0.04^{B}	0.23 ± 0.03^{b}
	M_{sum} (W)			1.48 ± 0.32^{A}	1.47 ± 0.21^{A}	1.54 ± 0.16^{B}	1.62 ± 0.29^{B}	1.76 ± 0.11^{AB}	1.51 ± 0.19^{AB}
	ME			8.29 ± 1.59^{A}	8.44 ± 1.87^{a*}	6.16 ± 0.65^{B*}	8.63 ± 1.76^{a*}	6.10 ± 0.71^{B}	6.62 ± 0.63^{b}
	T_{CL} (°C)			12.37 ± 2.37	10.45 ± 3.7	12.36 ± 3.17	12.55 ± 3.71	8.84 ± 3.24	9.71 ± 4.74

Note. Body mass (M_b), basal metabolic rate (BMR), summit metabolism (M_{sum}), metabolic expansibility (ME) and helox temperature at cold limit (T_{CL}) were measured during summer and winter over a three-year period (2015- 2017; n ≈ 10 for each species per season per year; mean ± standard deviation presented). Data were also included from a previous study for WBSWs in 2014 at the same study site in the Kalahari Desert (Noakes et al. 2017). Statistical results are from linear models and *post hoc* tests of multiple comparisons of means (Tukey Contrasts). If the year × season interaction was a significant predictor of response variables (WBSW: BMR, M_{sum} and T_{CL}; SFW: M_b, BMR and ME) I conducted *post hoc* tests to investigate variation between pairs of different seasons - asterisks denote significant seasonal variation between adjacent summers and winters (placed after the mean value of the earlier season of each pair), and letters denote patterns of significant variation among the summers (upper case) and winters (lower case) respectively (all $p < 0.05$). Year but not year × season was a significant predictor of the M_{sum} of SFW, and thus patterns of significant variation among years are denoted by uppercase letters regardless of season.

1.4.3 Scaly-feathered weavers

The M_b of individual SFWs varied from a minimum of 8.4 g during the summer of 2015 to a maximum of 12.0 g during the summer of 2017. M_b varied significantly with the year × season interaction (table 1), and was significantly higher in the summer of 2017 compared to the winter of 2017, as well as compared to all other summers (table 2). Interannual variation in M_b in each season respectively was significantly related to variation in T_a extremes, decreasing with increasing $T_{a,\text{max}}$ among summers ($F_{1,28} = 20.323$; $p = 0.035$) and increasing with decreasing $T_{a,\text{min}}$ among winters ($F_{1,30} = 4.855$; $p < 0.001$). BMR and M_{sum} increased significantly with increasing M_b, and T_{CL} decreased significantly with increasing M_b (table 1). ME significantly varied with the year × season interaction and the effect of year × season on BMR was marginally not significant, whereas year but not season was a significant predictor of M_{sum} (table 1). Interaction terms between M_b and the other predictor variables were not included in the top models for any response variables of SFWs, suggesting that variation among years and seasons does not reflect M_b differences.

The BMR of individual SFWs varied from a minimum of 0.1 W during the winter of 2016 to a maximum of 0.4 W during the summer of 2017. Patterns of seasonal acclimatization in BMR differed among years from no seasonal variation to lower BMR in winter compared to summer (~ 20 % to 31 % lower in winter 2015 *vs* summer 2016, and winter 2016 *vs* summers 2016 and 2017; figure 1b; table 2). The top models explaining interannual variation in BMR among summers and winters respectively, revealed summer BMR significantly decreased with increasing $T_{a,max}$ ($F_{1,27} = 7.817$, $p = 0.009$) and decreasing food abundance (i.e., grass seed abundance; $F_{1,27} = 48.086$, $p < 0.001$), and winter BMR significantly increased with increasing $T_{a,min}$ ($F_{1,27} = 48.086$, $p < 0.001$; figure 1).

The M_{sum} of individual SFWs varied from a minimum of 0.8 W during the summer of 2015 to a maximum of 2.1 W during the winter of 2016. There was no seasonal variation in M_{sum} but there was variation among years (table 1), with significantly higher M_{sum} in 2015 compared 2016 (~ 7 % higher), and M_{sum} in 2017 did not significantly differ from 2015 or 2016 (figure 1b; table 2). The top model explaining interannual variation in summer M_{sum} revealed a significant decrease with increasing $T_{a,max}$ ($F_{1,27} = 5.293$, $p = 0.029$), and also a decrease with decreasing food abundance although this effect was marginally not significant ($F_{1,27} = 3.897$, $p = 0.059$; figure 1). In contrast, the model supporting the null hypothesis (i.e., including no environmental variables) was the top model for interannual variation in winter M_{sum} (figure 1). There was no variation in T_{CL} with year or season (table 1). Seasonal variation of ME of SFWs followed the same patterns of seasonal acclimatization as BMR over the study period, ranging from no seasonal variation to lower ME in winter compared to summer (~ 2.3 to 2.5 lower in winters; table 2).

In summary, patterns of seasonal acclimatization in the BMR and ME of SFWs varied among years, with the same patterns of seasonal variation for BMR and ME (table 2). Summer BMR and M_{sum} decreased with increasing $T_{a,max}$ and decreasing food abundance over the study period (figure 1). Repeating analyses using $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ instead of metabolic rate (W) as the response variable revealed the same patterns of variation for BMR and M_{sum} of SFW.

1.5 Discussion

The data reveal considerable flexibility in the thermal physiology of two species of subtropical passerines, highlighting the importance of phenotypic plasticity as a source of intraspecific variation in endotherm thermal physiology. We found significant variation in the magnitude and direction of seasonal acclimatization of BMR and M_{sum}, supporting the idea that patterns of seasonal metabolic variation are more variable among subtropical and tropical birds compared to north-temperate counterparts (McKechnie et al. 2015). Seasonal metabolic changes ranged from winter downregulation in some years to winter upregulation in others, with magnitudes similar to those observed in species from cold higher-latitude regions (McKechnie et al. 2015). The results partly support my prediction that patterns of seasonal acclimatization vary among years in a manner reflecting a trade-off between energy conservation and cold tolerance during winter (Smit and McKechnie 2010). Food abundance appears to be an important proximate factor determining interannual variation in metabolic rates of WBSWs and SFWs, as both BMR and M_{sum} decreased with decreasing food abundance, and metabolic rates were never related to winter $T_{\mathrm{a,min}}$ in a manner associated with enhancing cold tolerance. The results suggest that food abundance is an important driver of metabolic adjustments in these two subtropical species, but unlike their temperate-zone counterparts, enhancing cold tolerance by metabolic increases is less important during milder subtropical winters.

1.5.1 White-browed sparrow-weavers

Metabolic rates of WBSWs in the current study were generally higher than values predicted using allometric equations for tropical and passerine birds (table 3), which is unexpected as lower-latitude species are generally considered to have a “slower pace of life” than their temperate counterparts (Weathers 1979; Hail 1983; Londõno et al. 2015). Mean BMR values for each season were higher than values predicted for tropical (37 % to 90 % higher) and passerine birds (16 % to 40 % higher), except during the summers of 2014 and 2015 when BMR was similar to predicted values (table 3; Londõno et al. 2015) and significantly lower than the other summers during the study period (figure 1a; table 2). Mean M_{sum} values were higher than values predicted for tropical birds (31 % to 119 % higher; Wiersma et al. 2007) and for oscine passerines (24 % to 80 % higher), except during the winter of 2015 when M_{sum} was downregulated by the greatest magnitude and thus similar to the value predicted for oscine passerines (table 2, 3; Swanson and Bozinovic 2011).

Mean BMR of WBSWs during each season (table 2) were similar to values previously reported among four WBSW populations across a climatic gradient of ~ 7 °C in winter $T_{\mathrm{a,min}}$ and ~ 11 °C in summer $T_{\mathrm{a,max}}$ (mean BMR range: 0.38 W to 0.64 W; Noakes et al. 2017). The magnitude and direction of seasonal acclimatization of BMR in WBSWs varied substantially among years in the current study (from ~ 20 % lower to 68 % higher during winter compared to summer; figure 1a), surpassing variation among populations in WBSWs (no seasonal change to 52 % higher in winter; Noakes et al. 2017), and is also comparable to the range of variability in seasonal patterns across all subtropical species for which data are available (35 % lower to 60 % higher in winter *vs* summer; McKechnie et al. 2015). As far as I am aware the increase of BMR by 68 % in winter compared to summer, is the largest seasonal metabolic change recorded in a field-acclimatized bird (i.e., measured within three days of capture), with the greatest difference previously reported being 64 % higher BMR during winter in temperate *Passer domesticus* in Wisconsin USA (Arens and Cooper 2005).

Mean M_{sum} of WBSWs during each season (table 2) were similar to values reported for interpopulation variation in Noakes et al. (2017; mean M_{sum} range: 2.40 W to 3.86 W). The ME of WBSWs (table 2) approached the upper end of the typical avian range for ME (~ 3 to 8; Swanson 2010; highest reported avian ME = 9, *Pa. domesticus*; Arens and Cooper 2005). I found variation in the magnitude and direction of seasonal acclimatization of M_{sum} in WBSWs (from no seasonal change to 38 % lower in winter compared to summer; figure 1a), whereas no seasonal variation in M_{sum} were reported in any populations by Noakes et al. (2017). Downregulation in M_{sum} during winter compared to summer has been reported in other birds from lower latitudes (e.g., winter decreases of 12 % to 35 % in seven tropical species; Wells and Schaeffer 2012; McKechnie et al. 2015). In contrast to north-temperate birds, higher M_{sum} in WBSWs did not appear to be associated with enhancements in cold tolerance (Swanson 2001; Cooper 2002; Swanson and Liknes 2006); for example, during the winter of 2015 I found both the lowest mean T_{CL} as well as the lowest mean M_{sum}.

I also report interannual metabolic variation in relation to environmental factors, with BMR and M_{sum} of WBSWs decreasing with decreasing food availability among seasons but never influenced by fluctuations in winter $T_{\mathrm{a,min}}$. Moreover, metabolic rates generally mirrored fluctuations in food abundance during the study period regardless of season, being lower and less seasonally variable during years with lower food abundance (figure 1). These interannual patterns partly support my prediction of a cold tolerance / energy conservation continuum driving patterns of seasonal metabolic variation in subtropical birds, as food abundance is highlighted as an important determinant of metabolic rates, but metabolic variation was never related to winter $T_{\mathrm{a,min}}$. The results for WBSWs reveal considerable variation in seasonal metabolic responses, adding to the growing evidence of considerable flexibility in the thermal physiology of this species (Smit and McKechnie 2010, 2015; Smit et al. 2013; Noakes et al. 2016, 2017; Noakes and McKechnie 2019).

Table 3: Basal metabolic rate (BMR) and summit metabolism (M_{sum}) of white-browed sparrow-weavers (*Plocepasser mahali*, WBSW) and scaly-feathered weavers (*Sporopipes squamifrons*, SFW) during summer and winter over a four year period, expressed as percentages of allomaetrically predicted metabolic rates

			2014		2015		2016		2017	
Species	Variable	Category	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter
WBSW	BMR	Tropical	111 %	165 %	103 %	137 %	158 %	155 %	190 %	150 %
		Passerine	95 %	140 %	88 %	116 %	135 %	132 %	162 %	128 %
	M_{sum}	Tropical	151 %	179 %	156 %	131 %	219 %	157 %	203 %	161 %
		Oscine	124 %	149 %	127 %	108 %	180 %	130 %	167 %	133 %
SFW	BMR	Tropical			105 %	102 %	148 %	115 %	161 %	135 %
		Passerine			94 %	91 %	133 %	103 %	143 %	121 %
	M_{sum}	Tropical			214 %	202 %	226 %	229 %	236 %	219 %
		Oscine			133 %	128 %	141 %	144 %	150 %	137 %

Note. Allometric equations for BMR were obtained from Londõno et al. (2015) for tropical and passerine birds, and equations for M_{sum} were obtained from Wiersma et al. (2007) for tropical birds and from Swanson and Bozinovic (2011) for oscine passerines. Predicted values of BMR and M_{sum} for each season was calculated using the mean body mass for that season for each species.

1.5.2 Scaly-feathered weavers

Metabolic rates of SFWs were also generally higher than those predicted for tropical and passerine birds using allometric equations (table 3), suggesting that higher-than-predicted metabolic rates are the norm rather than the exception for both WBSWs and SFWs. Mean BMR values were higher than predicted for tropical birds (35 % to 61 % higher) and for passerine birds (21 % to 33 % higher) during the summers of 2016 and 2017 and winter of 2017, but similar to predicted values during the summer of 2015 and winters of 2015 and 2016 (table 3; Londõno et al. 2015). Mean M_{sum} values for each season were consistently greater than double the values predicted for tropical birds (102 % to 136 % higher; Wiersma et al. 2007), as well as 28 % to 50 % higher than values predicted for oscine passerines (Swanson and Bozinovic 2011; table 3).

As far as I am aware, no previous studies have measured BMR or M_{sum} of SFWs, although Lubbe et al. (2018) investigated the effect of communal roosting and nest use on RMR at -5 °C ≤ T_{a} ≤ 20 °C of SFWs caught at the same site in the Kalahari Desert as the current study. These authors reported lower RMR values at T_{a} = 20 °C for individuals roosting in groups of two and four birds within a nest (RMR = 0.16 ± 0.05 W and 0.15 ± 0.07 W respectively; Lubbe et al. 2018), compared to the BMR values of SFWs during the current study (table 2). This raises the potential importance of huddling in quantifying BMR in species that roost communally, as it is possible that solitary SFWs during the present study had elevated metabolic rates due to higher stress levels (*sensu* Chappell et al. 2016). However, SFWs used by Lubbe et al. (2018) were acclimated to 25 °C for ~ 30 d before the onset of metabolic measurements, and previous studies have demonstrated reductions in metabolic rates in wild-caught birds after periods of captivity (Piersma et al. 1996; Al-Mansour 2005; McKechnie et al. 2007; Noakes and McKechnie 2019, chapter 4).

The magnitude and pattern of seasonal acclimatization of BMR in SFWs varied among years (from no seasonal change to ~ 31 % lower during winter), but not as substantially as for WBSWs (figure 1b). Winter reductions in BMR have been reported for several other subtropical species (McKechnie et al. 2015); for example, BMR of *Zosterops lateralis* (Maddocks and Geiser 2000) and *Falco tinnunculus* (Bush et al. 2008) were ~ 18 % and 12 % lower in winter compared to summer respectively. Similar to the ME of WBSWs, the ME of SFWs (table 2) also approached the upper end of the avian range for ME (Swanson 2010). There was no significant seasonal variation in the M_{sum} or T_{CL} of SFWs over the study period, however, there were the same patterns of seasonal variation in ME as there were for BMR (table 2). This suggests that unlike the pattern typical for higher-latitude species (Stager et al. 2016), adjustments in the ME of SFWs were driven by changes in BMR rather than M_{sum}, supporting the idea that ME is not necessarily a good indicator of avian thermogenic capacity (Petit et al. 2013).

Interannual metabolic variation in SFWs were also related to variation in environmental variables, but there were different patterns for BMR and M_{sum} during each season respectively. Among summers, BMR and M_{sum} of SFWs decreased with decreasing food abundance and decreased significantly with increasing $T_{\mathrm{a,max}}$, and downregulating metabolic rates during hotter summers could have an adaptive value by reducing heat production. Among winters, neither BMR nor M_{sum} varied with food abundance but BMR increased significantly with increasing winter $T_{\mathrm{a,min}}$, which contrasts with the pattern reported in higher-latitude birds that typically increase both BMR and M_{sum} during colder periods (Swanson and Olmstead 1999; Broggi et al. 2007; Petit et al. 2013; Petit and Vézina 2014b). As for WBSWs, metabolic rates of SFW were generally lower and less seasonally variable during years with lower food abundance, with metabolic variation mirroring fluctuations in food abundance regardless of season (figure 1). Thus patterns of interannual metabolic variation in SFWs also partly supports the enhanced cold tolerance / energy conservation hypothesis as food availability was identified as an important determinant of BMR and M_{sum} during summer, but metabolic variation was never related to winter $T_{\mathrm{a,min}}$ in a manner reflecting changes in cold tolerance.

1.5.3 Global variation in patterns of seasonal acclimatization

Studies on small, higher-latitude birds have frequently reported upregulation in BMR and M_{sum} during winter, associated with increased cold tolerance, colder climates and lower intra-winter mortality (Swanson 2010; McKechnie et al. 2015; Petit et al. 2017). At lower latitudes with comparatively milder winters, there appears to be greater variation in the magnitude and direction of seasonal metabolic responses, a pattern reported at both the interspecific and interpopulation level (McKechnie et al. 2015; Noakes et al. 2017). The present study extends this pattern to the intrapopulation level, as I found considerable interannual variation in the magnitude and direction of seasonal adjustments in BMR and M_{sum} of two subtropical species. This does not necessarily imply lower-latitude birds possess an inherently greater physiological flexibility compared to higher-latitude counterparts, but could rather reflect the extreme winter T_{a} at higher latitudes overwhelming other environmental factors that could drive patterns of avian seasonal metabolic variation (Noakes et al. 2017).

In contrast to higher-latitude birds, seasonal metabolic variation in WBSWs and SFWs was not related to enhancing winter cold tolerance, and M_{sum} of both species was never significantly higher in winter compared to summer. Moreover, interannual variation in BMR and M_{sum} were never related to winter $T_{\mathrm{a,min}}$ in a manner reflecting changes in cold tolerance. The lowest daily winter T_{a} recorded during the study period was -9.4 °C, and extrapolating the relationship between RMR and T_{a} below the thermoneutral zone from previous studies, suggest that M_{sum} in both WBSWs and SFWs (table 2) is about three- and two-fold higher respectively than the RMR required to defend T_b at this T_a (1.26 W for Askham WBSWs during winter: Noakes et al. 2017; 0.83 W for solitary SFWs in the absence of a nest: Lubbe et al. 2018). It is therefore conceivable that metabolic upregulation to enhance cold tolerance is not required during milder winters at lower latitudes (McKechnie et al. 2015).

It has been suggested that ecological factors other than winter $T_{a,min}$ could also drive patterns of seasonal acclimatization in birds, a potential candidate being food availability as birds may downregulate metabolism to conserve energy if food is in short supply (Smit and McKechnie 2010). This notion is supported by interannual variation in the BMR and M_{sum} of WBSWs and SFWs, which decreased with decreasing food abundance, suggesting a proximate effect of food availability on metabolic rates in these species. BMR and M_{sum} of WBSWs and SFWs were typically higher than values predicted using allometric equations for tropical and passerine birds (table 3), which could increase the pressure to downregulate metabolic rates during periods of low food abundance. Studies on north-temperate birds have reported a proximate effect of short-term (days to weeks) fluctuations in $T_{a,min}$ on BMR in *Parus major* (Broggi et al. 2007), and M_{sum} in *Poecile atricapillus*, *Junco hyemalis* and *Spizella americana* (Swanson and Olmstead 1999; Petit et al. 2013; Petit and Vézina 2014b). These contrasting patterns of proximate factors driving metabolic variation in birds are reminiscent of the different patterns of mammalian seasonal metabolic changes between small (M_b < 100-g, reduced BMR for energy conservation) and intermediate-sized species (0.1 < M_b < 10-kg, increased thermogenic capacity; Lovegrove 2005), although all birds in the above examples are considered small (M_b < 50-g). During the current study, there was substantial variation in food abundance among seasons and low variability in mean $T_{a,min}$ among winters (~ 1.4 °C range; figure 1c, d), and thus it is possible that patterns of avian seasonal metabolic acclimatization are driven by a cold tolerance / energy conservation continuum, but that food abundance was the more limiting factor during the milder winters of the Kalahari Desert.

In conclusion, my data reveal considerable flexibility in the thermal physiology of two Afrotropical passerine species, reiterating the importance of phenotypic plasticity as a source of intraspecific variation in endotherms. The magnitude and direction of seasonal metabolic variation varied among years in both species, supporting the idea that patterns of seasonal acclimatization are more variable in subtropical and tropical birds compared to their temperate-zone counterparts (McKechnie et al. 2015). In contrast to higher-latitude birds, patterns of BMR and M_{sum} adjustments were not associated with enhancing cold tolerance in either species, but food abundance was a significant proximate factor driving interannual variation in metabolic rates in relation to energy conservation. Further studies are required to compare flexibility in BMR and M_{sum} in birds from higher and lower latitudes, to understand if subtropical birds possess greater physiological plasticity, or if milder winters simply provide more opportunities for variation in seasonal metabolic responses.

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1.7 Appendix

Table A1. Interannual and seasonal variation in the abundance (count data) of the arthropod orders that form part of white-browed sparrow-weavers (*Plocepasser mahali*) diet

	2015		2016		2017	
Arthropod abundance	Summer	Winter	Summer	Winter	Summer	Winter
Blattodea	75	15	158	356	321	73
Coleoptera	1068	148	3663	446	2764	869
Hemiptera	65	429	24	3218	3453	578
Hymenoptera	5635	773	2541	9417	5539	9289
Orthoptera	305	7	123	19	155	39
Total	7148	1372	6509	13101	12232	10848

Note. Arthropods were collected using pit fall traps at the study site near Askham in the Kalahari Desert, with equal sampling effort during each season.

CHAPTER 2:

Seasonal variation in body composition in an Afrotropical passerine bird: increases in pectoral muscle mass are, unexpectedly, associated with lower thermogenic capacity

2.1 Abstract

Phenotypic flexibility in avian metabolic rates and body composition have been well-studied in high-latitude species, which typically increase basal metabolic rate (BMR) and summit metabolism (M_{sum}) when acclimatized to winter conditions. Patterns of seasonal metabolic acclimatization are more variable in lower-latitude birds that experience milder winters, with fewer studies investigating adjustments in avian organ and muscle masses in the context of metabolic flexibility in these regions. I quantified seasonal variation (summer *vs* winter) in the masses of organs and muscles frequently associated with changes in BMR (gizzard, intestines and liver) and M_{sum} (heart and pectoral muscles), in white-browed sparrow-weavers (*Plocepasser mahali*). I also measured pectoral muscle thickness using a portable ultrasound system to determine whether I could non-lethally estimate muscle size. There was no seasonal variation in the dry masses of the gizzard, intestines or liver of sparrow-weavers, and during the same period, BMR did not vary seasonally. I found significantly higher heart (~ 18 % higher) and pectoral muscle (~ 9 % higher) dry mass during winter, although ultrasound measurements did not detect seasonal changes in pectoral muscle size. Despite winter increases in pectoral muscle mass, M_{sum} was ~ 26 % lower in winter compared to summer. To the best of my knowledge, this is the first study to report an increase in avian pectoral muscle mass but a concomitant decrease in thermogenic capacity.

2.2 Introduction

Phenotypic flexibility is a subcategory of phenotypic plasticity whereby individual organisms reversibly adjust phenotypes in response to fluctuating environments, often with adaptive benefits (Piersma and Drent 2003; Piersma and van Gils 2010). A well-studied example of phenotypic flexibility is avian seasonal metabolic acclimatization, with the typical pattern among temperate-zone birds being an increase in basal metabolic rate (BMR) and summit metabolism (M_{sum}) during cold northern winters (reviewed by Swanson 2010; McKechnie et al. 2015). A better understanding of the adaptive consequences of phenotypic flexibility requires us to not only investigate the patterns of these adjustments, but also the underlying physiological basis for these phenotypic changes (Piersma and Drent 2003; Swanson and Vézina 2015).

Basal metabolic rate is the minimum normothermic resting metabolic rate of an individual, and reflects the maintenance energy requirements of organs (McNab 1997; Swanson 2010). Adjustments in avian BMR are commonly associated with changes in the mass of the digestive and excretory organs (e.g., gizzard, intestines and liver; Chappell et al. 1999; Williams and Tieleman 2000; Petit et al. 2014), with a winter increase in BMR interpreted as greater support costs of organs to facilitate higher daily food consumption during cold winters (Vézina et al. 2011; McWilliams and Karasov 2014). Some studies have also associated variation in avian BMR with changes in tissue mass-specific metabolic intensities (Vézina and Williams 2005; Vézina et al. 2017; Milbergue et al. 2018), as well as increased support costs of the skeletal muscles involved in shivering thermogenesis (Petit et al. 2014; Vézina et al. 2017; Milbergue et al. 2018).

Avian M_{sum} represents the ceiling of an individual's thermogenic capacity, measured as the maximum metabolic rate during cold exposure, and is positively correlated with cold tolerance in temperate-latitude birds (e.g., Swanson 2001; Cooper 2002; Swanson and Liknes

2006; Petit et al. 2017). Avian thermogenesis occurs primarily via shivering of the skeletal muscles, with the pectoral muscles being the largest (~ 25 % of total body mass [M_b] in a ploceid passerine; Hartman 1961) and most important site for shivering thermogenesis (Hohtola 1982; Marsh and Dawson 1989; Hohtola et al. 1998; Petit and Vézina 2014a). Winter upregulation of M_{sum} is typically associated with an increase in pectoral muscle mass (Swanson 2010; Swanson and Vézina 2015), although there are cases of M_{sum} upregulation in the absence of such increases (e.g., Swanson et al. 2014a, 2014b; Milbergue et al. 2018). Avian M_{sum} has also been associated with changes in the mass of the heart, lungs and/or leg muscles, cellular metabolic intensity of muscles, and the supply of substrates and O_2 to muscles (e.g., Petit et al. 2014; Stager et al. 2015; Zhang et al. 2015; Barceló et al. 2017; Milbergue et al. 2018).

Seasonal metabolic variation has been less comprehensively studied among birds inhabiting lower latitudes experiencing milder winters and hotter summers, but the available data suggest greater variation in the direction and magnitude of seasonal metabolic variation in these regions (reviewed by McKechnie et al. 2015). Patterns of seasonal metabolic acclimatization can also vary intraspecifically in subtropical birds, with studies reporting adjustments in BMR and M_{sum} ranging from a winter increase to no seasonal change among populations of both *Euplectes orix* (van de Ven et al. 2013) and white-browed sparrow-weavers (*Plocepasser mahali*, hereafter sparrow-weavers; Smit and McKechnie 2010; Noakes et al. 2017). It is possible that the typically milder winters at lower latitudes may allow for a greater degree of flexibility in the patterns of seasonal metabolic acclimatization (Smit and McKechnie 2010; McKechnie et al. 2015; Noakes et al. 2017). Fewer studies have investigated seasonal changes in avian body composition in relation to metabolic variation at lower latitudes compared to temperate regions, although a winter increase in BMR as well as the mass of digestive and excretory organs has been reported for two subtropical passerines in

China (Zheng et al. 2014a, 2014b). To the best of my knowledge, no previous studies have investigated variation in body composition in the context of maximum thermogenic capacity (M_{sum}) in tropical or subtropical birds.

Traditional methods for measuring organ and muscles masses involve euthanasia of birds and excising these tissues, but advancements in visualization techniques such as ultrasound scanning, can provide non-lethal alternatives (e.g., Newton 1993; Dietz et al. 1999; Swanson et al. 2013). Ultrasonography has been validated to measure the thickness of pectoral muscles as an estimate of muscle size in birds ranging in M_{b} from ~ 11-g parids (Royer-Boutin et al. 2015) to 10-kg swans (Sears 1988). To the best of my knowledge, only two studies have investigated whether ultrasound measurements of pectoral muscle thickness are precise enough to detect adjustments in pectoral muscle size (Swanson and Merkord 2013; Royer-Boutin et al. 2015). Ultrasonography did detect seasonal changes in pectoral muscle size of *Passer domesticus*, although the percentage increase in muscle thickness (~ 9 %) was about half that of mass (~ 19 %; Swanson and Merkord 2013), but did not detect larger pectoral muscles in cold-acclimated *Poecile atricapillus* compared to warm-acclimated conspecifics (~ 12 % higher muscle mass; Royer-Boutin et al. 2015). Observer bias and the life-state of birds (i.e., alive or dead) have been identified as factors influencing the accuracy of ultrasound measurements (Royer-Boutin et al. 2015).

In the present study, I quantified seasonal variation in body composition in a Kalahari Desert population of sparrow-weavers, focusing on traits commonly associated with avian metabolic acclimatization (i.e., mass of gizzard, intestines, liver, heart and pectoral muscle). I also evaluated ultrasonography as a non-lethal alternative for estimating the size of pectoral muscles in sparrow-weavers (*sensu* Swanson and Merkord 2013; Swanson et al. 2013, Royer-Boutin et al. 2015). A concurrent study investigated patterns of seasonal metabolic acclimatization in the same population of sparrow-weavers during the same year (Noakes and

McKechnie in press, chapter 1; table 1), allowing us to compare patterns of metabolic variation with body composition changes at the population level. I predicted that seasonal variation in BMR and M_{sum} would be associated with similar changes in organ and muscle masses as commonly observed in temperate species. Specifically, I expected higher BMR to be associated with an increase in the mass of digestive and excretory organs (Swanson 2010; Barceló et al. 2017; Petit et al. 2014), and higher M_{sum} associated with increases in the mass of pectoral muscles and potentially the heart (Swanson 2010; Swanson and Vézina 2015).

Table 1. Seasonal variation in the basal metabolic rate, summit metabolism and cold limit temperature of a southern African arid-zone passerine bird, as well as environmental variables at the study site

Category	Variable	Summer	Winter
Plocepasser mahali	Basal metabolic rate (W)	0.60 ± 0.09	0.60 ± 0.07
	Summit metabolism (W)	4.55 ± 0.70*	3.35 ± 0.42*
	Cold limit temperature (°C)	-2.52 ± 3.82	-3.74 ± 5.21
Climatic variables	Minimum temperature (°C)	21.4 ± 3.3	1.4 ± 4.0
	Maximum temperature (°C)	39.0 ± 3.9	24.5 ± 3.7
	Total rainfall (mm)	70	0
Food availability	Total arthropod abundance	6509	13101
	Grass cover (%)	89.8	75.4

Note: Data are from the summer and winter of 2016 (Noakes and McKechnie in press, chapter 1), with seasonal variation in basal metabolic rate, summit metabolism and the cold limit temperature (helox temperature where summit metabolism was reached) presented for white-browed sparrow-weavers (*Plocepasser mahali*; $n = 10$ per season, metabolic rates measured using respirometry). Statistical results are from linear models fitted to metabolic data, and asterisks denote significant seasonal variation ($p < 0.05$). I also present variation in weather variables for the hottest summer months (December to January) and coldest winter months (June to July): mean ± standard deviation values of the daily minimum and maximum temperatures, and total rainfall. I present seasonal fluctuations in food availability: arthropod abundance measured using pitfall trapping, and grass seed abundance estimated using grass step counts to measure percentage grass cover.

2.3 Materials and methods

2.3.1 Study species and site

I examined body composition in white-browed sparrow-weavers (*Plocepasser mahali*; hereafter: sparrow-weavers) during the summer (mid-January) and winter (mid-July) of 2016, at Murray Guest Farm near Askham in the Kalahari Desert (Northern Cape Province, South Africa; 26° 59′ S, 20° 51′ E). A concurrent study measured seasonal variation in BMR and M_{sum} in sparrow-weavers at the same study site and during the same year as the present study (i.e., different individuals from the same population; table 1; Noakes and McKechnie in press, chapter 1). I caught sparrow-weavers at night using two small nets mounted on the end of aluminium poles to cover the entrances of roost nests. Birds were housed in cages constructed of plastic mesh and shade cloth (~ 1.5 m^3), and all body composition data were collected during the following day (< 24 h after capture). The natural diet of sparrow-weavers includes arthropods and grass seeds (~ 30 % arthropods, 70 % seeds; Ferguson 1988; du Plessis 2005), and I provided captive birds with water, mealworms and a wild bird seed mix. To avoid trapping reproductive individuals, I did not catch sparrow-weavers from breeding nests or over the peak egg-laying period (November to December; du Plessis 2005). Individual sparrow-weavers were sexed according to the colour of their beaks (du Plessis 2005).

2.3.2 Ultrasound measurements of pectoral muscle thickness

I validated the use of portable ultrasonography to estimate the mass of pectoral muscles of sparrow-weavers. The M_b of individuals was measured (to the nearest 0.01-g) prior to ultrasound measurements using a Scout Pro Balance scale (SPU402, Ohaus, Pine Brook NJ, USA). I measured the thickness of pectoral muscles during summer (n = 32) and winter (n =

31) using a SonoSite Titan portable ultrasound system and Titan L38 5-10 MHz 38 mm linear array transducer (FUJIFILM SonoSite, Inc, Bothell WA, USA). A previous study did not detect significant differences between the left and right pectoral muscles in *Cinclus cinclus* (Newton 1993), and thus I took measurements of only the left pectoral muscle to reduce handling time. During the first season (summer), two observers worked together and alternated taking measurements, whereas in the second season a single observer took measurements using the same procedure.

Birds were held with their back and closed wings against the palm of the hand, the thumb, ring and little finger closed around the front of the bird's body, and the head in between the index and middle finger. A cloth bag was placed over the head of birds to calm them but allow unimpeded ventilation. I parted the breast feathers and applied ultrasound transmission gel (NCCB-0234, Ningbo Medelast Co, Ltd, Ningbo, China) over the left pectoral muscle. I set the ultrasound system to a depth of 24 mm and a frequency of 10 MHz, and placed the transducer transversely across the left pectoral muscle perpendicular to the keel, being careful to not apply pressure with the transducer as this could distort ultrasound images. I used the scanner software to freeze ultrasound images and measure the width of the muscle (i.e., thickness) to the nearest 0.1 mm, from the base of the keel to the top of the muscle along the short axis (*sensu* Dietz et al. 1999; Swanson et al. 2013; Swanson and Merkord 2013). I obtained three thickness measurements per individual, and used the average value for further analyses.

2.3.3 Dissection and removal of muscle and organs

All mass measurements described in this section were measured to the nearest 0.01 g using the Scout Pro Balance scale. Directly after the completion of ultrasound measurements for the first 15 individuals during each season, I euthanized birds via cervical dislocation and measured the total M_b of the carcass. I then excised the liver, intestines (from gizzard to vent, i.e., gut tube), gizzard, heart and both pectoral muscles still attached to the keel and ribs (hereafter, pectoral muscles-keel complex), and rinsed and temporarily stored samples in saline solution while dissection continued. I removed the intestines with the pancreas still attached, and thus mass values includes the pancreas. I also measured the length of the intestines (to the nearest 0.01 mm), but only have these measurements for a few individuals in the first season of data collection (summer: n = 5, winter: n = 15). I cut open and rinsed the intestines and gizzard in saline solution to remove digesta prior to mass measurements. After dissection was completed for each individual, I measured the wet mass of the organs and pectoral muscles-keel complex after blotting each dry with absorbent paper. After completion of measurements, I wrapped all tissues in tin foil, stored samples in resealable plastic bags along with the remaining carcass, and froze samples at -18 °C until dry mass measurements were performed. Prior to dry mass measurements, I defrosted the pectoral muscles-keel complex and dissected the left pectoral muscle from the keel and other bones to measure the wet mass of this muscle separately. I dissected the left pectoral muscle with the supracoracoideus muscle attached, and thus mass values includes the supracoracoideus muscle.

To measure the dry mass of organs and the left pectoral muscle of sparrow-weavers, I first defrosted samples, removed them from the tin foil, and placed them on baking trays in an oven (Ecotherm Economy, Labotec, Midrand, South Africa) set to 55 °C. I removed samples from the oven once a day to measure mass, and continued measurements until I obtained the same mass for three consecutive days. The final mass measured was considered the dry mass of each organ and pectoral muscle.

2.3.4 Data analyses

All values are presented as mean ± standard deviation. I used the “lm” base function in R 3.5.2 (R Core Team 2018) to fit linear models to the data, and checked the assumptions of all models by inspecting model residual plots (residuals *vs* fitted, normal Q-Q, scale-location and residuals *vs* leverage plots). Response variables included: M_b, wet and dry masses of the left pectoral muscle and organs (heart, liver, intestines and gizzard); and predictor variables: season, sex, M_b (except when M_b was a response variable) and tarsus length (only for organ and muscle masses). Tarsus length and M_b could not be included as predictor variables in the same models as they are correlated, but including M_b instead of tarsus length consistently produced models with better fit, and thus tarsus length was excluded from all final models. I used the “dredge” function from the *MuMIn* package (Bartoń 2018) to determine the combination of predictor variables that produced linear models that best explained variation in response variables, by selecting models with the lowest second-order Akaike Information Criterion values. If multiple top models had Akaike Information Criterion values that differed by < 2, I chose the most parsimonious model.

I also investigated whether the thickness of pectoral muscles measured by ultrasonography could be used as a non-lethal estimate of the mass of the pectoral muscle. I fitted linear regression models to determine the relationships between the thickness of the left pectoral muscle, and the wet and dry mass of the same muscle respectively. I refitted regression models to data from summer and winter respectively, to investigate whether the strength of the relationship between pectoral muscle thickness and mass differed between seasons as a result of observer bias (*sensu* Royer-Boutin et al. 2015). I used the equations from the regression models including data from both seasons to convert pectoral muscle thickness into estimates of the wet and dry masses, and fitted linear models as described previously to investigate how pectoral muscle thickness and mass estimates varied with predictor variables (i.e., season, sex and M_b).

2.4 Results

The top model explaining variation in the M_b of sparrow-weavers included only sex, with M_b being significantly higher in males (41.9 ± 3.0 g) compared to females (38.6 ± 1.9 g; $F_{1,26}$ = 10.928, p = 0.003). Sex was not included in the top models for wet or dry masses of pectoral muscles and all organs, although all tissue masses scaled significantly with M_b (except intestine dry mass; table 2).

Season was not included in the top models explaining variation in the dry masses of the digestive organs (gizzard and intestines), nor the wet or dry mass of the liver (table 2). However, contrasting patterns of significant seasonal variation were apparent for wet masses of the digestive organs, with ~ 17 % higher gizzard mass and ~ 12 % lower intestines mass in winter compared to summer (table 2, 3). I could not fit models to investigate seasonal variation in the length of the intestines as the summer sample was too small, but the mean length was longer in winter (162.9 ± 13.3 mm, n = 15) compared to summer (147.6 ± 3.4 mm, n = 5). Both the pectoral muscle (~ 7 % and 9 % higher respectively) and heart (~ 8 % and 18 % higher respectively) were significantly heavier (both wet and dry mass) during winter compared to summer (table 2, 3).

Pectoral muscle thickness measured by ultrasonography significantly increased with increasing wet and dry mass of the pectoral muscle, however, the correlation coefficients for these relationships were low (figure 1; table 4). When analyses were repeated for each season separately, the pectoral muscle thickness was not significantly related to the wet or dry mass during summer (two observers working together), and there were lower correlation coefficients for these relationships during this season (r^2 values < 0.2; table 4). However, pectoral muscle thickness was significantly related to wet and dry masses during winter (single observer), and correlation coefficients for these relationships were also higher during this season (r^2 values > 0.5; table 4). Season was not included as a predictor variable in the top models for pectoral muscle thickness (summer: 6.15 ± 0.58 mm, winter: 6.11 ± 0.64 mm), neither for wet and dry mass estimates calculated using the equation from the linear model including data from both seasons (figure 1; table 2, 3).

Table 2. Statistical results from models fitted to data of seasonal variation in organ and muscle masses of a southern African arid-zone passerine bird

		Predictor variables					
		Body mass			Season		
Category	Response variable	*F*-value	df	*p*-value	*F*-value	df	*p*-value
Wet mass	Gizzard	13.392	1,27	0.001	22.983	1,27	<0.001
	Intestines	7.899	1,27	0.009	6.999	1,27	0.013
	Liver	10.327	1,28	0.003			
	Heart	10.058	1,27	0.004	6.575	1,27	0.025
	Pectoral muscle	22.665	1,27	<0.001	5.716	1,27	0.024
	Pectoral muscle estimate	13.066	1,61	<0.001			
Dry mass	Gizzard	1.11	1,28	0.003			
	Intestines						
	Liver	10.422	1,28	0.003			
	Heart	18.104	1,27	<0.001	20.937	1,27	<0.001
	Pectoral muscle	28.532	1,27	<0.001	7.785	1,27	0.01
	Pectoral muscle estimate	13.031	1,61	<0.001			

Note: Statistical results (*F*-value, *p*-value, degrees of freedom [df]) are from the top linear models fitted to data, which included body mass and/or season (no statistical results indicates a predictor variable was not included in the top model). Data were collected during the summer and winter of 2016, and both dry and wet mass were measured for the gizzard, intestines (including pancreas), liver, heart, and left pectoral muscle (including supracoracoideus muscle) of white-browed sparrow-weaver (*Plocepasser mahali*; n = 15 per season). Estimates of the left pectoral muscle mass were calculated using equations for the relationship between the pectoral muscle thickness (measured using ultrasonography on live birds) and the actual wet and dry masses respectively (regression equations calculated from n = 15 per season, and estimates from: summer n = 32, winter n = 31).

Table 3. Seasonal variation in the body, organ and muscle masses of a southern African arid-zone passerine bird

Response variable	Wet mass		Dry mass	
	Summer	Winter	Summer	Winter
Body mass (g)	38.72 ± 2.50	40.27 ± 3.01		
Gizzard (g)	1.07 ± 0.09*	1.25 ± 0.15*	0.30 ± 0.03	0.32 ± 0.06
Intestines (g)	0.82 ± 0.10*	0.72 ± 0.14*	0.19 ± 0.05	0.18 ± 0.05
Liver (g)	0.91 ± 0.19	0.84 ± 0.16	0.23 ± 0.06	0.22 ± 0.05
Heart (g)	0.51 ± 0.05*	0.55 ± 0.06*	0.11 ± 0.01*	0.13 ± 0.01*
Pectoral muscle (g)	3.66 ± 0.42*	3.92 ± 0.37*	1.01 ± 0.11*	1.10 ± 0.13*
Pectoral muscle estimates (g)	3.76 ± 0.20	3.74 ± 0.22	1.04 ± 0.06	1.04 ± 0.07

Note: Data were collected during the summer and winter of 2016, and mean ± standard deviation values are presented for the body mass, and wet and dry masses of the gizzard, intestines (including pancreas), liver, heart, and left pectoral muscle (including supracoracoideus muscle) of white-browed sparrow-weavers (*Plocepasser mahali*; $n = 15$ per season). Estimates of the left pectoral muscle mass were calculated using equations for the relationship between the pectoral muscle thickness (measured using ultrasonography on live birds) and the actual wet and dry masses respectively (regression equations calculated from $n = 15$ per season, and estimates from: summer $n = 32$, winter $n = 31$). Statistical results are from linear models, and asterisks denote significant seasonal variation ($p < 0.05$).

Table 4. Statistical results from models fitted to investigate the relationship between pectoral muscle thickness and mass of a southern African arid-zone passerine bird

Response variable:		Predictor variable: pectoral muscle thickness					
Pectoral muscle	Season	*F*-value	df	*p*-value	r^2	*m*	*y*-int
Wet mass	Both	10.939	1,28	0.003	0.253	0.345	1.635
	Summer	4.208	1,13	0.061	0.186	0.384	1.235
	Winter	14.279	1,13	0.003	0.523	0.369	1.640
Dry mass	Both	11.301	1,28	0.002	0.262	0.106	0.392
	Summer	2.757	1,13	0.121	0.112	0.084	0.480
	Winter	17.192	1,13	0.001	0.569	0.134	0.271

Note: I investigated whether measuring the thickness of the pectoral muscle using portable ultrasonography, could be used to non-lethally estimate pectoral muscle mass of white-browed sparrow-weavers (*Plocepasser mahali*). Data were collected during the summer and winter of 2016 from ($n = 15$ per season), and linear regression models were fitted to data for both seasons together, and then for summer and winter separately. Statistical results include *F*-value, degrees of freedom (df), *p*-value and adjusted correlation coefficients (r^2), as well as the gradient (*m*) and *y*-intercept (*y*-int) of the equation from each model.

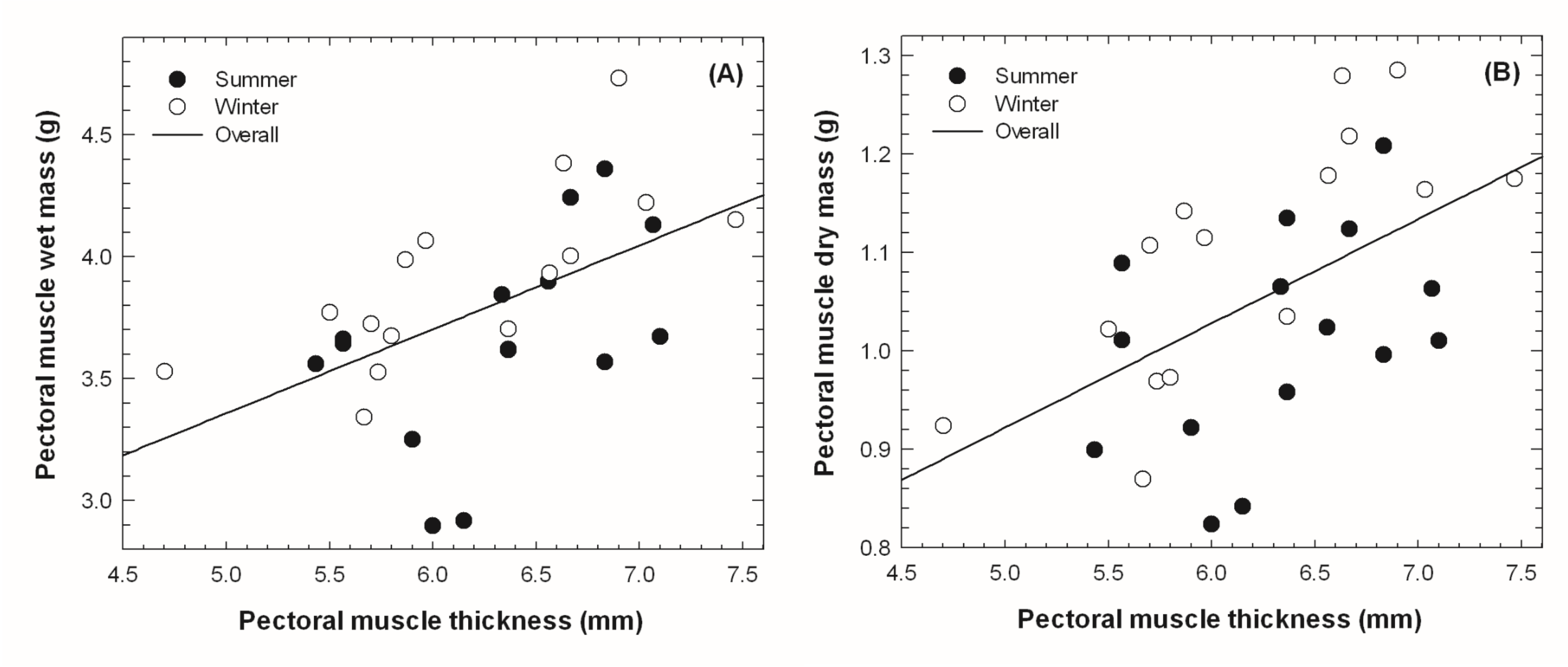

Figure 1. Pectoral muscle thickness measured using ultrasonography emerged as a weak predictor of the pectoral muscle mass of white-browed sparrow-weavers (*Plocepasser mahali*). Data were collected during both summer and winter, and data from both seasons are presented together (n = 15 per season). Solid lines represent linear regression models fitted to the thickness of the left pectoral muscle measured in live birds, and the wet (A; y = 0.345 x + 1.635, $r^2 = 0.253$, $F_{1,28} = 10.939$, $p = 0.003$) and dry (B; y = 0.106 x + 0.392, $r^2 = 0.262$, $F_{1,28} = 11.301$, $p = 0.002$) mass of the same muscle dissected from birds after euthanasia.

2.5 Discussion

The data reveal no significant seasonal change in the dry mass of the digestive and excretory organs of sparrow-weavers (table 3), which coincided with no seasonal BMR variation during the same year (table 1; measured for chapter 1, Noakes and McKechnie in press). These findings are consistent with previous studies suggesting that changes in avian organ masses and BMR are functionally linked (Swanson 2010; Petit et al. 2014; Barceló et al. 2017). I also found a significant winter increase in the mass of pectoral muscles and heart of sparrow-weavers (table 3), but a decrease in maximum thermogenic capacity (i.e., M_{sum}; table 1; Noakes and McKechnie in press, chapter 1), a finding that contrasts with previous studies associating winter increases in pectoral muscle and heart mass with a winter increase in M_{sum} in higher-latitude birds (Swanson 2010; Swanson and Vézina 2015). To the best of my knowledge, this is the first study to report an increase in avian pectoral muscle mass but a concomitant decrease in maximum thermogenic capacity.

The lack of seasonal variation in the digestive and organ masses as well as BMR of sparrow-weavers is consistent with my predictions and the results of previous studies reporting correlations between these traits in birds (e.g., Chappell et al. 1999; Williams and Tieleman 2000; Petit et al. 2014). The direction and magnitude of seasonal acclimatization of BMR vary interannually in the same Kalahari Desert population of sparrow-weavers (from ~ 20 % lower to 68 % higher in winter over a 4-year period; Noakes and McKechnie in press, chapter 1), and presumably variation in organ mass would mirror patterns of BMR variation as documented in other subtropical species (e.g., Chappell et al. 1999; Zheng et al. 2014b; Cavieres and Sabat 2008). For example, the BMR of hoopoe larks (*Alaemon alaudipes*) from the Arabian Desert was 42 % higher in winter compared to summer, with associated increases in the dry masses of the intestines, gizzard, liver and kidney (~ 43 %, 40 %, 43 % and 37 % higher in winter respectively; Williams and Tieleman 2000).

I found contrasting patterns of seasonal variation in the wet masses of the intestines and gizzard (~ 12 % higher and 16 % lower in winter respectively; table 3), and contrasting patterns in the small intestine and gizzard mass were also reported in *Py. sinensis* from Wenzhou, China (~ 43 % higher and 19 % lower dry masses in winter respectively; Zheng et al. 2014a). The majority of studies investigating adjustments in the mass of vital organs have focused on dry masses as they are more reliable than wet masses (e.g., Williams and Tieleman 2000; Vézina et al. 2017; Milbergue et al. 2018). The lack of seasonal changes in the dry masses of digestive organs of sparrow-weavers is likely more relevant in the context of BMR variation (table 1; Noakes and McKechnie in press, chapter 1).

An increase in avian BMR associated with cold, high-latitude winters is often attributed to greater maintenance requirements of larger digestive and excretory organs, to accommodate higher rates of food consumption in response to increased energy demands (Vézina et al. 2011; McWilliams and Karasov 2014; Barceló et al. 2017). More variable patterns of seasonal acclimatization of BMR among subtropical birds likely reflect milder winter minimum T_a and more modest increases in energy requirements (McKechnie et al. 2015), and it has been suggested that fluctuations in food availability could also be an important proximate factor driving avian metabolic adjustments in relation to energy conservation (Smit and McKechnie 2010; Noakes and McKechnie in press, chapter 1). This notion is supported by data for sparrow-weavers: summer and winter BMR decreased with decreasing food abundance over a four-year period (Noakes and McKechnie in press, chapter 1), suggesting BMR variation (and presumably organ mass) is related to rates of food consumption in sparrow-weavers as is the case for high-latitude species (Vézina et al. 2011; Barceló et al. 2017).

The data reveal significantly higher pectoral muscle and heart mass in winter compared to summer, but in contrast to predictions this was not reflected in a winter increase in M_{sum} of sparrow-weavers, but rather a ~ 26 % decrease (Noakes and McKechnie in press, chapter 1). To the best of my knowledge, this is the first study to report an increase in pectoral muscle mass without an associated increase in thermogenic capacity (Swanson 2010; Swanson and Vézina 2015), although the ~ 9 % increase in muscle dry mass was slightly below the range typically reported for temperate-zone birds during winter (~ 12 % to 18 % higher; e.g., Cooper 2002; Liknes and Swanson 2011; Vézina et al. 2017). This pattern is surprising considering that larger pectoral muscles should produce greater heat production (Hohtola 1982; Marsh and Dawson 1989; Milbergue et al. 2018). Moreover, higher heart mass has also been associated with increases in M_{sum} in temperate-zone birds (Swanson 2010; Swanson and Vézina 2015).

Some studies have reported an increase in M_{sum} in cold-acclimated/-acclimatized birds without significant variation in pectoral muscle mass (e.g., Swanson et al. 2014b; Stager et al. 2015; Milbergue et al. 2018). For example, *Zonotrichia albicollis* acclimated to T_a = -8 °C had ~ 19 % higher M_{sum} but no difference in pectoral muscle mass compared to conspecifics acclimated to T_a = 28 °C, however, M_{sum} was positively correlated with muscle mass in cold-acclimated but not warm-acclimated birds (Barceló et al. 2017). These authors suggested that, despite having similar pectoral muscle masses as warm-acclimated birds, cold-acclimated individuals had undergone changes at the cellular level (i.e., in the cellular metabolic intensity of muscles) that resulted in increased thermogenic capacity. Previous studies have also suggested a contribution of cellular metabolic intensity to variation in thermogenic capacity (e.g., Swanson et al. 2014b; Stager et al. 2015), and it is possible there was no M_{sum} increase in sparrow-weavers because pre-requisites at the cellular level were not met. Moreover, other studies have associated M_{sum} variation with changes in the mass of leg muscles and lungs, as well as the supply of substrates and O_2 to muscles (e.g., Petit et al. 2014; Zhang et al. 2015; Barceló et al. 2017).

Increase in M_{sum} and pectoral muscle mass in high-latitude birds enhances cold tolerance during winter (e.g., Swanson 2001; Cooper 2002; Swanson and Liknes 2006; Petit et al. 2017), and intra-winter M_{sum} varies with short-term fluctuations in minimum T_{a} in some species (Swanson and Olmstead 1999; Petit et al. 2013; Petit and Vézina 2014b). There was no winter increase in M_{sum} over a four-year period in sparrow-weavers (seasonal differences ranged from no variation to ~ 38 % lower in winter), and interannual variation in M_{sum} significantly varied with food availability but not winter minimum T_{a} (Noakes and McKechnie in press, chapter 1). Winters in the Kalahari Desert are relatively mild compared to higher latitudes (mean winter minimum $T_{\mathrm{a}} > 0$ °C during 2016; table 1) and thus a winter increase in thermogenic capacity may not be necessary for sparrow-weavers at this site (Noakes and McKechnie in press, chapter 1). Pectoral muscles are also the primary flight muscles of birds, and variation in muscle mass has been associated with changes in activity levels (Swanson 2010). An increase in pectoral muscle mass in *Po. atricapillus* has been suggested to facilitate more intensive foraging to meet greater energy demands during shorter and colder winter days (Milbergue et al. 2018). Higher pectoral muscle mass of sparrow-weavers could be related to greater activity levels, possibly in relation to foraging behaviours varying with unpredictable fluctuations in food availability in the Kalahari Desert (Noakes and McKechnie in press, chapter 1). However, an upregulation in pectoral muscle mass for long-distance flight (i.e., migration) was still associated with higher M_{sum} in several temperate species (Swanson 1995; Swanson and Dean 1999; Vézina et al. 2007).

Sparrow-weavers' pectoral muscle thickness measured using ultrasonography varied significantly with wet and dry pectoral muscle mass (figure 1), but correlation coefficients for these relationships were low (table 4) compared to most previous studies (typically: $r^2 = 0.66$ to 0.96; reviewed by Royer-Boutin et al. 2015). Moreover, ultrasound measurements did not detect the winter increase in pectoral muscle mass (no seasonal variation in pectoral muscle thickness or mass estimates; figure 1; table 2, 3), as was also reported for *Po. atricapillus* acclimated to T_a = 0 °C *vs* T_a = 30 °C (~ 12 % higher pectoral muscle mass in cold-acclimated birds but no variation in muscle thickness; Royer-Boutin et al. 2015). However, Swanson and Merkord (2013) reported significantly higher pectoral muscle mass (~ 18 % higher) and thickness (~ 9 % higher) in winter compared to summer for *Pa. domesticus* (Swanson and Merkord 2013). As muscle thickness is a linear measure of a 3-D structure, large sample sizes are likely required to detect adjustments in muscle size in small birds, although my sample sizes (summer: *n* = 32, winter: *n* = 31) were greater than those of Swanson and Merkord (2013; summer: *n* = 13, winter: *n* = 17).

Before modern advancements in ultrasonography allowing for measurements at higher frequencies, studies reporting high correlation coefficients between pectoral muscle thickness and mass were collected from relatively large birds (at frequencies of ≤ 10 MHz, $M_b \approx$ 120 g to 10 000 g, r^2 = 0.70 to 0.92; Sears 1988; Dietz et al. 1999; Battley et al. 2004). The ultrasound measurements were conducted at 10 MHz on sparrow-weavers ($M_b \approx$ 40-g), but more advanced systems are capable of higher frequency measurements allowing more accurate measurements in small birds (reviewed by Royer-Boutin et al. 2015). For example, measurements at a frequency of 25 MHz report high correlation coefficients between pectoral muscle thickness and mass in *Spinus tristis* ($M_b \approx$ 13-g, r^2 = 0.73; Swanson et al. 2013) and *Pa. domesticus* ($M_b \approx$ 28-g, r^2 = 0.74; Swanson and Merkord 2013). Royer-Boutin et al. (2015) found a considerable range in correlation coefficients (r^2 = 0.02 to 0.76) from measurements on *Po. atricapillus* ($M_b \approx$ 11-g, 12 MHz frequency), varying with the life-state of birds (alive or dead) and the observer conducting measurements. My findings also suggest observer bias can influence measurements, as separate analyses for each season revealed lower correlation coefficients in summer (first season, two observers working together) compared to winter (single observer; table 4). Ultrasound systems can be useful tools for estimating avian pectoral muscle size, but further studies are required to determine appropriate techniques, sample sizes and specifications of equipment relative to body size (e.g., frequencies required; Royer-Boutin et al. 2015).

In summary, I found no seasonal variation in the dry mass of digestive and excretory organs or the BMR of sparrow-weavers (Noakes and McKechnie in press, chapter 1), supporting the argument that changes in these avian traits are functionally linked (Swanson 2010; Petit et al. 2014; Barceló et al. 2017). Pectoral muscle and heart mass were significantly higher in winter compared to summer, but M_{sum} was lower during winter (Noakes and McKechnie in press, chapter 1), in contrast to previous studies associating higher pectoral muscle and heart mass with increased thermogenic capacity in higher-latitude birds (Swanson 2010; Swanson and Vézina 2015). To the best of my knowledge, this is the first study to report higher pectoral muscle mass without a concomitant increase in M_{sum}, and it is possible that changes at the cellular level did not occur in sparrow-weavers that function as pre-requisites for increased thermogenic capacity (Barceló et al. 2017). As far as I am aware, this is also the first study to investigate seasonal variation in pectoral muscle mass in the context of M_{sum} adjustments in a subtropical bird, and more studies are required to understand the mechanisms and factors driving metabolic flexibility in birds inhabiting lower latitudes experiencing milder winters.

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CHAPTER 3:

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

3.1 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. I used 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. I allocated individuals to one of three T_{accl} treatments (5 °C, 20 °C 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. The data reveal the expected pattern of lower BMR and EWL (~ 12 % and 25 % lower respectively) in birds at $T_{\text{accl}} = 35$ °C compared to cooler T_{accl} treatments, as observed in previous acclimation studies on subtropical birds. I 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.

3.2 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 (White et al. 2007; Jetz et al. 2008; Stager et al. 2016). Avian metabolic rates tend to be lower in tropical latitudes compared to temperate-zone regions (Weathers 1979; Hail 1983; Wiersma et al. 2007; Londoño et al. 2015; Stager et al. 2016), and both metabolic rates and evaporative water loss (EWL) is reduced in arid- compared to mesic-zone birds (Tieleman and Williams 2000; Tieleman et al. 2002, 2003). 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 changing climates (Williams et al. 2008; Boyles et al. 2011; Huey et al. 2012). Physiological differences among birds can arise from genotypic adaptation (hard-wired differences in response to different selection pressures) or phenotypic plasticity (Piersma and Drent 2003; Angilletta et al. 2010), 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) or phenotypic flexibility (reversible changes throughout an individual’s lifetime; Pigliucci 2001; Piersma and Drent 2003). Phenotypic flexibility is an important component of acclimatization and acclimation, adjustments in response to environmental conditions in the field or laboratory respectively (Schlichting and Pigliucci 1998; Piersma and Drent 2003).

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 during acute cold exposure; reviewed by Swanson 2010; McKechnie et al. 2015). In particular, higher M_{sum} is associated with enhanced cold tolerance during the cold winters typical of higher latitudes (Swanson 2001; Cooper 2002; Swanson and Liknes 2006; Petit et al. 2017), 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 (Swanson and Olmstead 1999; Broggi et al. 2007; Petit and Vézina 2014; Dubois et al. 2016). Adjustments in BMR are typically associated with changes in the mass and metabolic intensities of the digestive and excretory organs, whereas adjustments in M_{sum} are associated with changes in the mass and metabolic intensities of the skeletal muscles, heart mass, and supply of substrates and oxygen to muscles (Swanson 2010; Swanson and Vézina 2015; Zhang et al. 2015; Milbergue et al. 2018).

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 metabolic adjustments compared to 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* (Smit and McKechnie 2010; Noakes et al. 2017). 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 temperate-zone counterparts, but rather that very low winter T_a at high latitudes result 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 investigating 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_{accl}) treatments (generally: $T_{accl} \leq 5$ °C; e.g., Swanson et al. 2014; Barceló et al. 2017; Vézina et al. 2017; Milbergue et al. 2018). 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_{accl} treatments (often 15 °C *vs* 30 °C) and typically reporting lower BMR and EWL in birds acclimated to higher T_a (e.g., Tieleman et al. 2003; Cavieres and Sabat 2008; Maldonado et al. 2009).

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; Tieleman et al. 2003; Cavieres and Sabat 2008). 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 my knowledge, no acclimation studies have investigated metabolic flexibility in response to relatively low T_{accl} (i.e., < 10 °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, I 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), I predicted that BMR and M_{sum} would be highest in sparrow-weavers from the coldest acclimation treatment (T_{accl} = 5 °C). I also expected lower BMR and EWL in birds acclimated to the hottest treatment (T_{accl} = 35 °C), as reported for other subtropical species (e.g., Tieleman et al. 2003; Cavieres and Sabat 2008; Maldonado et al. 2009). I 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).

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

	Daily $T_{a,min}$ (°C)		Daily $T_{a,max}$ (°C)		Total rainfall (mm)		Location	
Capture site	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)

Note. Mean ± standard deviation 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 was obtained from the closest South African Weather Service stations – there are weather stations in Polokwane and Frankfort, however, the closest weather station to the study site near Askham providing temperature data is ~ 62 km northwest and rainfall data ~ 9 km west (co-ordinates presented in brackets). White-browed sparrow-weavers (*Plocepasser mahali*) were captured from these sites and transported to the University of Pretoria for acclimation experiments.

3.3 Methods

3.3.1 Study sites

I captured sparrow-weavers during the austral summer (10 to 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 site at Frankfort (Free State Province) and Polokwane (Limpopo Province, table 1). I 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, chapter 4). I transported birds by road in modified pet carriers back to the Small Animal Physiological Research 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 two days (~ 6 h travelling per day, ~ 1000 km in total).

At the Small Animal Physiological Research Facility, I allocated sparrow-weavers to one of three climate-controlled rooms (~ 11 birds from each site per room), all initially set to T_a = 25 °C and a photoperiod of 12: 12 h light: dark cycle. I housed birds in cages (600 × 400 × 400 mm), generally two birds per cage (a male and female from the same site, sex determined by bill colour; du Plessis 2005) but some birds were housed individually (six out of 102 birds). 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. I 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.

3.3.2 Acclimation regimes

Three days after the arrival of the birds from the final capture site, I set rooms to T_a = 20 °C so that all birds were first exposed to the intermediate T_{accl} regime. Following this initial period, I adjusted T_a in two of the rooms to experimental T_{accl} values of 5 °C and 35 °C over a four-day period, changing T_a in 5 °C increments every two 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., 28 days: Tieleman et al. 2003; 17 days: McKechnie and Wolf 2004; 21 days: McKechnie et al. 2007; 30 days: Cavieres and Sabat 2008). 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).

Table 2. Acclimation temperature treatments and sample sizes for basal metabolic rate (BMR) and summit metabolism (M_{sum}) measurements of white-browed sparrow-weavers (*Plocepasser mahali*) from different populations

Air temperature (°C)		Sample sizes for BMR (T_b)			Sample sizes for M_{sum} (ME)		
Set	Actual	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)

Note: 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 acclimation temperature, and the mean ± standard deviation value of actual air temperature in each room are 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 due to intermittent PIT tag reception (in brackets after BMR sample size). Sample sizes of metabolic expansibility (ME) were also lower as I only calculated these values for an individual if BMR and M_{sum} were measured within 12 h of each other (in brackets after M_{sum} sample size).

3.3.3 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 in chapter 1 (Noakes et al. in press), with modifications listed below. I used clear plastic containers as metabolic chambers, with volumes of 2.6-L (ClipFresh, Hong Kong, China; incurrent flow rates ≈ 1 L min^{-1}) and 4-L (Lock & Lock, Seoul, South Korea; incurrent flow rates ≈ 2 L min^{-1}) for BMR and M_{sum} measurements respectively. I 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.

I 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. I measured core body temperature (T_b) of sparrow-weavers using temperature-sensitive passive integrated transponder (PIT) tags (Destron Fearing, St. Paul MN, USA) injected into the peritoneal cavity of birds. I 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, St. Paul MN, USA) during M_{sum} measurements.

3.3.4 Experimental protocol

To control for the effects of habituation to metabolic chambers after repeated measurements (Jacobs and McKechnie 2014), I measured M_{sum} within 12 h before BMR measurements for half of the individuals and during the 12 h after for the rest of the birds. For three individuals I could not measure BMR and M_{sum} within 12 h of each other, and thus did not calculate metabolic expansibility (ME) for these individuals. I randomized the order in which birds from different populations and acclimation treatments were measured. The maximum sample sizes were 11 birds from each population per T_{accl} treatment for BMR and M_{sum} measurements, however, due to equipment malfunction the 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).

I quantified BMR and thermoneutral EWL by measuring gas exchange values for four birds simultaneously at $T_{\mathrm{a}} \approx 30$ °C (actual $T_{\mathrm{a}} = 30.1 \pm 0.5$ °C) throughout the night, as this T_{a} has been reported as in the thermoneutral zone of sparrow-weavers (Smit and McKechnie 2010; Noakes et al. 2017). Thermoneutral T_{b} was monitored concurrently during BMR measurements, but sample sizes for T_{b} were lower due to intermittent PIT tag reception (table 2). Birds were placed into individual metabolic chambers just before sunset and removed after sunrise, measuring M_{b} on entering and removal from chambers. I 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.

I used the sliding cold exposure method in a helox environment (Swanson et al. 1996) to measure M_{sum}, and the protocol described in chapter 1 (Noakes and McKechnie in press). I 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 ≥ 3.7 ºC from initial values upon entering the chamber (mean T_{b} decrease = 6.2 ± 1.5 °C).

3.3.5 Data analyses

I calculated whole animal metabolic rates (BMR and M_{sum}) and ME (M_{sum} / BMR) values as described in chapter 1 (Noakes and McKechnie in press). EWL was calculated using equation 9.6 in Lighton (2008), assuming 0.803 mg H_2O per ml of water vapour. 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 ratios (RER; $\dot{V}_{CO_2} / \dot{V}_{O_2}$) during measurements were often below the expected range of 0.71 to 1.00 (average RER = 0.63 ± 0.16), and as thermal equivalence data is 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 the results, I repeated statistical analyses with $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ as the response variable. The helox temperature that M_{sum} was reached was used as an estimate of the cold limit temperature for each bird (T_{CL}). All values are presented as mean ± standard deviation.

I fitted linear models to the data using R 3.5.2 (R Core Team 2018), and checked the assumptions of all models by testing for multicollinearity between continuous predictor variables and inspecting model residual plots (normal Q-Q, residuals *vs* fitted, scale-location and residuals *vs* 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 (Bartoń 2018) was used to determine the combination of predictor variables (and interactions between them) that produced linear models that best explained variation in respective response variables, and if multiple top models had similar fit (Akaike Information Criterion values differed by < 2), I 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 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, I 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}).

3.4 Results

3.4.1 Body mass

The initial M_b of sparrow-weavers (i.e., at capture) was significantly higher for males (41.9 ± 3.2 g) compared to 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 compared to those 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 latter two populations ($p = 0.315$).

Following acclimation to T_a of 5 °C, 20 °C or 35 °C (i.e., T_{accl}), the M_b of most individuals increased compared to values measured at capture (7.2 ± 8.6 % higher at the time of each individual's metabolic measurements), and ranged from minimum of 37.8 g (an Askham bird at T_{accl} = 35 °C) to a maximum of 53.0 g (a Polokwane bird at T_{accl} = 5 °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 compared to Frankfort after acclimation (~ 6 % lower), but Polokwane sparrow-weavers did not differ significantly from Askham or Frankfort (table 4). Birds acclimated to T_{accl} = 35 °C had significantly lower M_b compared to T_{accl} = 5 °C and 20 °C (~ 8 % and 7 % lower respectively), but M_b did not significantly differ between the latter two treatments (table 4).

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

	Acclimation treatment			Population			Acclimation duration			Body mass		
Variable	*F*-value	df	*p*-value	*F*-value	df	*p*-value	*F*-value	df	*p*-value	*F*-value	df	*p*-value
Body mass	11.646	2,88	< **0.001**	5.952	2,88	**0.004**						
Basal metabolic rate	5.109	2,78	**0.008**				31.084	1,78	< **0.001**			
Thermoneutral evaporative water loss	10.194	2,78	< **0.001**									
Thermoneutral body temperature	*(Null hypothesis model)*											
Summit metabolism				3.950	2,86	**0.023**				22.072	1,86	< **0.001**
Metabolic expansibility							17.412	1,62	< **0.001**			
Cold limit temperature	*(Null hypothesis model)*											

Note: Statistical results (*F*-value, degrees of freedom [df] 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. Potential predictor variables included population (capture sites: Askham, Frankfort and Polokwane), acclimation temperature treatment (5 °C, 20 °C and 35 °C), length of acclimation period (28 – 91 days), body mass (except when body mass was the response variable), sex and relevant interactions between these terms. Response variables include body mass, basal metabolic rate, thermoneutral evaporative water loss and body temperature, summit metabolism, metabolic expansibility and helox temperature at cold limit (i.e., temperature that summit metabolism was reached).

Table 4. Data for white-browed sparrow-weavers (*Plocepasser mahali*) from different acclimation temperature treatments and populations

	Acclimation temperature treatment			Population		
Variable	5 °C	20 °C	35 °C	Askham	Frankfort	Polokwane
Body mass (g)	44.896 ± 3.409^{A}	45.416 ± 3.380^{A}	41.837 ± 3.246^{B}	42.664 ± 3.480^{a}	45.274 ± 3.385^{b}	44.133 ± 3.772^{ab}
Basal metabolic rate (W)	0.579 ± 0.102^{A}	0.566 ± 0.084^{AB}	0.512 ± 0.106^{B}	0.572 ± 0.100	0.556 ± 0.106	0.527 ± 0.096
Thermoneutral evaporative water loss (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 body temperature (°C)	38.962 ± 0.747	38.885 ± 0.796	38.512 ± 0.491	38.849 ± 0.688	38.693 ± 0.728	38.759 ± 0.695
Summit metabolism (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}
Metabolic expansibility	5.235 ± 1.258	5.192 ± 1.051	5.510 ± 1.512	5.294 ± 1.120	5.274 ± 1.399	5.397 ± 1.395
Cold limit temperature (°C)	-8.207 ± 5.547	-7.726 ± 5.684	-5.831 ± 4.419	-7.241 ± 5.707	-6.938 ± 5.616	-7.450 ± 4.644

Note. Birds were captured from sites along a climatic gradient (populations: Askham, Barberspan and Polokwane) and divided among three acclimation temperature treatments (T_{accl} = 5 °C, 20 °C and 35 °C; n ≈ 11 per population per T_{accl}; sample sizes: table 2). After an initial acclimation period of 28 days, I measured body mass (M_{b}), basal metabolic rate (BMR), thermoneutral evaporative water loss (EWL) and body temperature, summit metabolism (M_{sum}), metabolic expansibility and helox temperature at cold limit (i.e., temperature that M_{sum} was reached). Mean ± standard deviation 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).

3.4.2 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 °C) to a maximum of 0.7 W (an Askham bird at T_{accl} = 35 °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 4). BMR was significantly lower in birds acclimated to T_{accl} = 35 °C compared to T_{accl} = 5 °C regardless of population (~ 12 % lower), but birds at T_{accl} = 20 °C did not significantly differ from either of the other acclimation treatments (figure 1; table 4).

Thermoneutral EWL significantly varied among acclimation treatments regardless of population (table 3), with lower EWL in birds from T_{accl} = 35 °C compared to both T_{accl} = 5 °C and 20 °C (~ 25 % and 20 % lower respectively), but no significant difference between the latter two treatments. In contrast, the top model explaining variation in thermoneutral T_{b} was the null hypothesis (i.e., no predictor variables included). 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 to 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.

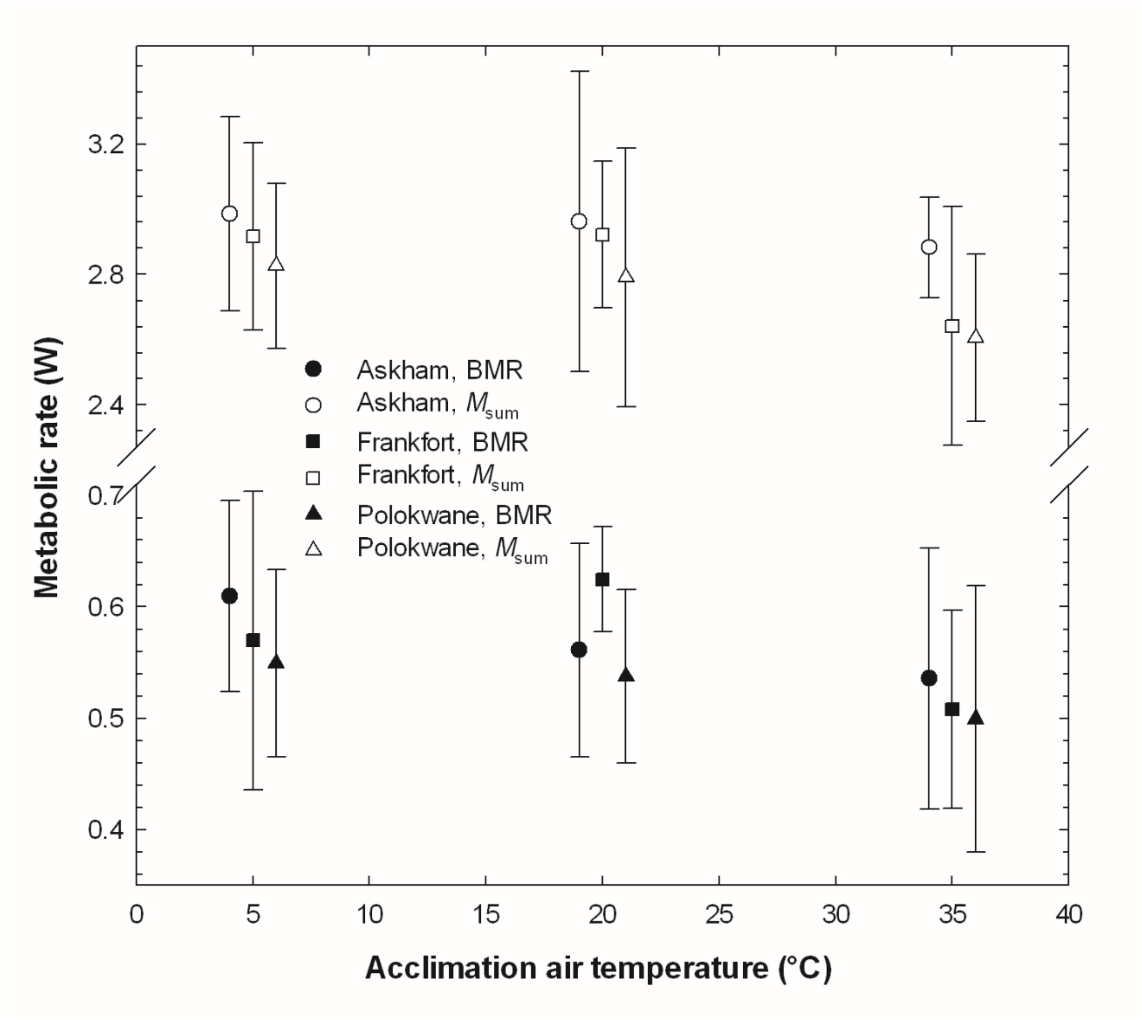

Figure 1. The basal metabolic rate (BMR) of white-browed sparrow-weavers did not vary significantly among populations (Askham, Frankfort and Polokwane) after an initial acclimation period of 28 days, but was significantly lower in birds acclimated to air temperature (T_{accl}) = 35 °C, compared to those from T_{accl} = 5 °C and 20 °C. In contrast, summit metabolism (M_{sum}) was significantly higher in Askham birds compared to Polokwane and Frankfort birds after acclimation, but did not significantly vary among acclimation treatments. Metabolic rate was measured using flow-through respirometry and mean ± standard deviation values are presented for each population at each T_{accl} (points are jittered for illustrative purposes). Statistical results are from linear models and *post hoc* tests of multiple comparisons of means (Tukey Contrasts; table 3, 4).

3.4.3 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 °C). The top model explaining variation in M_{sum} included population and M_{b}, with M_{sum} increasing with increasing M_{b} at a rate of 30 mW per g (table 3). Post-acclimation M_{sum} was significantly higher in Askham compared to both Frankfort and Polokwane birds (~ 5 % and 8 % higher respectively), but did not significantly differ between the latter two populations (figure 1; table 4).

The null hypothesis was the top model explaining variation in the T_{CL} (i.e., helox temperature that 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). In summary, responses during cold exposure did not vary significantly among acclimation treatments, but M_{sum} was higher in Askham birds compared to the other populations after acclimation. Repeating analyses using $\dot{\mathrm{V}}_{\mathrm{O}_2}$ and $\dot{\mathrm{V}}_{\mathrm{CO}_2}$ instead of M_{sum} (W) as the response variable revealed the same patterns of variation.

3.5 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 my predictions, BMR and EWL were lower in birds from the hottest acclimation treatment (T_{accl} = 35 °C *vs* 5 °C and 20 °C), but M_{sum} did not significantly vary in response to acclimation. In contrast to my 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, suggest that this trait could be fixed in this population as the result of genotypic adaptation, developmental plasticity or epigenetics (Noakes et al. 2017).

3.5.1 Body mass

I 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, I 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, chapter 4), 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 °C; table 1) and thus larger M_b may have adaptive value for this population following Bergmann's rule (Bergmann 1847).

Post-acclimation M_b was significantly lower in the hottest treatment (T_{accl} = 35 °C) compared to the cooler treatments (T_{accl} = 5 °C 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_{accl} = 42 °C *vs* 30 °C and 36 °C; Noakes and McKechnie 2019, chapter 4). Previous acclimation studies have typically interpreted avian M_b variation with T_{accl} as responses associated with adjustments to colder T_a (e.g., Williams and Tieleman 2000; Tieleman et al. 2003; Barceló et al. 2017; Vézina et al. 2017), 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).

3.5.2 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 to 30 days; e.g., Tieleman et al. 2003; McKechnie and Wolf 2004; McKechnie et al. 2007; Cavieres and Sabat 2008). 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., Williams and Tieleman 2000; Cavieres and Sabat 2008; Maldonado et al. 2009; Barceló et al. 2017). To the best of my 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). I am 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).

Post-acclimation BMR (table 4) were 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 W to 0.64 W; Noakes et al. 2017). Mean BMR of each population and T_{accl} treatment were consistently higher than values predicted using allometric equations for tropical (29 % to 42 % higher) and passerine (10 % to 18 % higher) birds (table 5; Londõno et al. 2015). Higher than predicted metabolic rates appear to be typical in sparrow-weavers (Noakes et al. 2017; Noakes and McKechnie in press, chapter 1), which is unexpected as birds from lower latitudes are generally reported to have a “slower pace of life” than higher-latitude counterparts (Weathers 1979; Hail 1983; Wiersma et al. 2007; Londõno et al. 2015).

Table 5. Basal metabolic rate and summit metabolism of white-browed sparrow-weavers (*Plocepasser mahali*) from different acclimation temperature treatments and populations, expressed as percentages of allomaetrically predicted metabolic rates

		Acclimation temperature treatment			Population		
Variable	Category	5 °C	20 °C	35 °C	Askham	Frankfort	Polokwane
Basal metabolic rate	Tropical	140 %	136 %	129 %	142 %	134 %	129 %
	Passerine	118 %	115 %	110 %	121 %	113 %	109 %
Summit metabolism	Tropical	124 %	122 %	122 %	131 %	119 %	118 %
	Oscine	105 %	103 %	102 %	110 %	101 %	100 %

Note. Allometric equations for basal metabolic rate of tropical and passerine birds were obtained from Londõno et al. (2015), and for summit metabolism 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 acclimation temperature treatment using the mean body mass for each of these categories respectively.

Similar to findings in temperate-zone species (e.g., Barceló et al. 2017; Vézina et al. 2017; Milbergue et al. 2018), BMR was higher in birds from the coldest compared to the hottest acclimation treatment (i.e., T_{accl} = 5 °C *vs* 35 °C), however, it did not significantly vary between the two cooler treatments (i.e., T_{accl} = 5 °C *vs* 20 °C; figure 1; table 4). In support of my predictions, BMR and thermoneutral EWL were lower in birds at T_{accl} = 35 °C compared to cooler treatments (figure 1; table 4), which is consistent with studies on lower-latitude species (Williams and Tieleman 2000; Tieleman et al. 2003; Cavieres and Sabat 2008). For example, *Z. capensis* acclimated to 30 °C had lower BMR and EWL compared to individuals acclimated to 15 °C (~ 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

~ 59 % higher in winter), compared to variation among T_{accl} treatments (~ 12 % lower BMR at T_{accl} = 35 °C *vs* 5 °C; Noakes et al. 2017).

I 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 of BMR and EWL variation among arid *vs* mesic populations of sparrow-weavers (Noakes et al. 2017), which contrasts with previous studies reporting lower BMR and/or EWL in arid compared to mesic populations of *Z. capensis* and *Haemorhous mexicanus* (MacMillen and Hinds 1998; Sabat et al. 2006; Cavieres and Sabat 2008). 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 and Williams 2000; Tieleman et al. 2002), and the reduced BMR and EWL in sparrow-weavers from the hottest T_{accl} treatment likely provided similar benefits.

3.5.3 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 W to 3.86 W; Noakes et al. 2017), and ME was within the range typically reported for birds (~ 3 to 8; table 4; Swanson 2010). As was the case for BMR and has been reported previously in sparrow-weavers (Noakes et al. 2017; Noakes and McKechnie in press, chapter 1), mean M_{sum} values were higher than predicted using allometric equations for tropical birds (18 % to 24 % higher; table 5; Wiersma et al. 2007). However, post-acclimation M_{sum} were generally similar to values predicted for oscine passerines (table 5; Swanson and Bozinovic 2011).

In contrast to what I expected, M_{sum} was not higher in sparrow-weavers at the coldest T_{accl}, and neither M_{sum}, T_{CL} nor ME varied in response to thermal acclimation (figure 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_{accl} = 5 °C) is on the warmer end of the range typically used in previous experiments (cold T_{accl} range from -10 °C to 5 °C; Swanson et al. 2014; Barceló et al. 2017; Vézina et al. 2017; Milbergue et al. 2018). However, two temperate-zone species (*Junco hyemalis* and *Calidris canutus islandica*) from cold-acclimation treatments similar to the current study (T_{accl} = 3 °C and 5 °C respectively) had ~ 16 % to 32 % higher BMR and/or M_{sum} than conspecifics from milder treatments (T_{accl} = 24 °C and 25 °C respectively; Swanson et al. 2014; Vézina et al. 2017). To the best of my knowledge, no previous acclimation studies have investigated flexibility in M_{sum}, nor metabolic values in response to T_{accl} < 10 °C, in subtropical birds.

Increases in M_{sum} are associated with higher cold tolerance in small, north-temperate birds (Swanson 2001; Cooper 2002; Swanson and Liknes 2006; Petit et al. 2017), 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 (Swanson and Olmstead 1999; Petit and Vézina 2014; Dubois et al. 2016). 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 four-year period were never related to minimum T_a, but were significantly lower in seasons with lower food abundance (Noakes and McKechnie in press, chapter 1). This suggests that fluctuations in food availability in relation to energy conservation is 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 dosmesticus* resulted in elevations in both maximal metabolic rates and M_{sum} (~ 19.7 % and 15.5 % higher respectively; Zhang et al. 2015b). This suggests that M_{sum} could vary as a byproduct of changes in activity levels, which is conceivable given that exercise and shivering capacity in birds are both predominantly determined by the flight muscles (McKechnie and Swanson 2010; Swanson 2010; Petit et al. 2017). Another potential explanation for the lack of significant M_{sum} variation with T_{accl} in sparrow-weavers could thus 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 compared to the other populations regardless of T_{accl} treatment (figure 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, I 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 to 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 in press, chapter 1), suggesting metabolic variation is not primarily associated with enhancing in cold tolerance in this subtropical species.

3.5.4 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_{accl} = 15 °C and 30 °C varied in relation to variability of local climates

(Cavieres and Sabat 2008). I 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}. Similar reaction norms among sparrow-weaver populations was also reported for their capacity to cope with high T_a (i.e., thermoregulatory responses at ~ 38 °C $\leq T_a \leq$ 54 °C) during an acclimation study at different T_{accl} (30 °C, 36 °C and 42 °C; Noakes and McKechnie 2019, chapter 4). 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_{accl} treatments (McKechnie 2008). The reaction norm for BMR has been reported as approximately linear in *Streptopelia senegalensis* across T_{accl} = 10 °C, 22 °C and 35 °C (McKechnie et al. 2007), as well as in field-acclimatized *Po. atricapillus* at minimum T_a ranging from -20 ˚C 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_{accl} = 5 ˚C and 20 ˚C, but lower in individuals from T_{accl} = 35 ˚C (table 4; figure 1). Petit and Vézina (2014) reported the reaction norm of M_{sum} in field-acclimatized *Po. atricapillus* was approximately linear between lower and upper limits (i.e., -10 °C 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; figure 1).

I found phenotypic flexibility in BMR and thermoneutral EWL of sparrow-weavers in response to 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_{accl} treatment as previously reported for lower-latitude species (Tieleman et al. 2003; Cavieres and Sabat 2008; Maldonado et al. 2009). In contrast to studies on higher-latitude birds (McKechnie and Swanson 2010), M_{sum} did not vary in response to thermal acclimation. Moreover, the magnitude of BMR variation with T_{accl} was considerably less than that of seasonal variation reported among and within sparrow-weaver populations (Noakes et al. 2017; Noakes and McKechnie in press, chapter 1). 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 in press, chapter 1). To the best of my knowledge, this is the first acclimation experiment investigating phenotypic flexibility of M_{sum}, as well as metabolic rates in response to low T_{accl} (i.e., < 10 °C), in a subtropical bird, and more studies are required to determine how avian metabolic flexibility varies with latitude.

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CHAPTER 4:

Reaction norms for heat tolerance and evaporative cooling capacity do not vary across a climatic gradient in a passerine bird

Published in *Comparative Biochemistry and Physiology Part A*

4.1 Abstract

There is increasing evidence for considerable phenotypic flexibility in endothermic thermal physiology, a phenomenon with far-reaching implications for the evolution of traits related to heat tolerance. Numerous studies have documented intraspecific variation in avian thermoregulatory traits, but few have revealed the shapes of thermoregulatory reaction norms or how these might vary among populations. I investigated phenotypic flexibility in the ability of a model Afrotropical passerine bird (the white-browed sparrow-weaver, *Plocepasser mahali*) to handle high air temperatures (T_a). I allocated birds from three sites varying by ~ 11 °C in mean daily summer maximum T_a to three acclimation temperature (T_{accl}) treatments (daytime $T_{accl} \approx 30$ °C, 36 °C or 42 °C respectively; $n \approx 10$ per site per T_{accl}). After an acclimation period of 30 days, heat tolerance and evaporative cooling capacity was quantified by exposing birds to progressively higher T_a until they approached severe hyperthermia (body temperature [T_b] = 44.5 °C; T_a range: 38 °C to 54 °C). I measured metabolic rate and evaporative water loss using open flow-through respirometry, and T_b using temperature-sensitive passive-integrated transponder tags. Hyperthermia threshold T_a ($T_{a,HT}$) was significantly higher and T_b significantly lower in birds acclimated to the hottest T_{accl} compared to those from milder acclimation treatments. Population (i.e., site of capture) was not a significant predictor of any thermoregulatory variables or hyperthermia threshold T_a

($T_{a,HT}$) after acclimation, revealing that the shape of reaction norms for heat tolerance and evaporative cooling capacity does not vary among these three populations.

4.2 Introduction

The thermal physiology of mammals and birds varies intraspecifically (e.g., Glanville et al. 2012; Smit et al. 2013; Noakes et al. 2016, 2017), supporting the idea that endothermic thermoregulation is far more flexible than previously thought (Angilletta et al. 2010). Adaptive thermoregulation is variation in the thermal physiology of an endotherm to counteract an environmental stressor, either via phenotypic flexibility (in response to acclimatization or acclimation), developmental plasticity or adaptation through natural selection (Piersma and Drent 2003; Angilletta et al. 2010). The notion that traits related to thermal physiology can vary over short time scales challenges the classical model of endothermic homeothermy, which assumes endothermic body temperature (T_b) is a fixed species-specific trait (Scholander et al. 1950). The concept of adaptive thermoregulation also challenges the central assumptions of the majority of climate envelope and correlative models predicting species' responses to anthropogenic climate change, as these models typically assume endotherms occur in a fixed climate space and/or cannot show adaptive physiological responses to changing climates (Boyles et al. 2011; Pearson and Dawson 2013; Milne et al. 2015).

Predicting how species will respond to rising air temperatures (T_a), particularly at lower latitudes, requires quantifying heat tolerance and evaporative cooling capacity, as well as an individuals' capacity to adjust these responses to different climatic conditions (Williams et al. 2008; Boyles et al. 2011; Smit et al. 2013). Vital in this respect are studies of intraspecific variation in heat tolerance. I am aware of only four studies that have demonstrated intraspecific variation in the capacity of birds to tolerate high T_a, involving seasonal differences (Oswald et al. 2018: *Chaetops frenatus*), interpopulation variation (Trost 1972: *Eremophila alpestris*) or a combination thereof (Noakes et al. 2016: *Plocepasser mahali*; O'Connor et al. 2017: *Caprimulgus tristigma*). Noakes et al. (2016) investigated seasonal variation (summer *vs* winter) in heat tolerance and evaporative cooling capacity in white-browed sparrow-weaver (*P. mahali*; hereafter: sparrow-weaver) populations across a climatic and aridity gradient. Sparrow-weavers from a hot desert site had significantly higher heat tolerance and evaporative cooling capacities in summer compared to winter, as well as in comparison to two cooler, more mesic sites during summer (Noakes et al. 2016).

To accurately interpret intraspecific variation in avian tolerance of high T_a in the context of changing climates, it is important to understand the origin of such variation (Williams et al. 2008; Boyles et al. 2011; Huey et al. 2012). For example, if phenotypic plasticity [either developmental plasticity (non-reversible changes that occur during developmental stages of an individual; e.g., Mariette and Buchanan 2016) or phenotypic flexibility (reversible changes that occur as a component of acclimation or acclimatization; Pigliucci 2001; Piersma and Drent 2003)] is the source of the greater summer heat tolerance and evaporative cooling capacity of desert sparrow-weavers compared to their mesic conspecifics (Noakes et al. 2016), these patterns reflect the responses of individual birds to current environmental conditions (Pigliucci 2001; Piersma and Drent 2003). On the other hand, these differences among populations could also have evolved as hard-wired genotypic variation in response to different selection pressures associated with the harsh climate of the desert site compared to the more mesic sites (Boyles et al. 2011; Noakes et al. 2016). This distinction between phenotypic plasticity and genotypic adaptation may, however, be blurred by epigenetic transmission and the accompanying transgenerational effects (e.g., Mariette and Buchanan 2016).

Short-term acclimation experiments provide a powerful tool for testing predictions about the reaction norms of phenotypically flexible traits and are an important initial step in teasing apart phenotypic plasticity and genotypic adaptation as sources of physiological variation within species. Most previous acclimation experiments have focused on the flexibility of avian thermoregulatory responses at moderate T_a, with some studies suggesting birds from more temporally heterogenous environments have greater flexibility in their thermal physiology (Schlichting and Pigliucci 1998; Tieleman et al. 2003a; Cavieres and Sabat 2008). Studies of two columbids have revealed an increase in evaporative cooling efficiency after heat acclimation, although these focused on the plasticity of the partitioning of evaporative water loss (EWL) into cutaneous and respiratory avenues (*Columba livia*: Marder and Arieli 1988; *Zenaida asiatica mearnsii*: McKechnie and Wolf 2004). There have also been acclimation studies investigating the effect of acute heat stress on chickens, but these experiments were designed to determine the consequences for production rather than thermal physiology traits *per se* (e.g., Hutchinson and Sykes 1953; Sykes and Fataftah 1986; Abdelqader and Al-Fataftah 2014). To the best of my knowledge, no studies have investigated flexibility in the capacity of passerine birds to defend T_b at high T_a, the urgent need for which is underscored by recent studies suggesting passerines may be more sensitive to increasing T_a than some other avian taxa (Albright et al. 2017; Smith et al. 2017; Conradie et al. 2019).

In light of previous work showing considerable intraspecific variation in the thermal physiology of white-browed sparrow-weavers (*P. mahali*; Noakes et al. 2016, 2017; Smit and McKechnie 2010, 2015; Smit et al. 2013), I investigated variation in the flexibility of heat tolerance and evaporative cooling capacity among populations of this species. Specifically, I sought to establish whether the increased heat tolerance during summer evident in a population from a hot, desert site, but not in populations from cooler sites, arises from phenotypic flexibility or from other sources of variation, including developmental plasticity or genotypic adaptation. If sparrow-weavers from multiple populations along a climatic gradient demonstrate similar reaction norms for traits determining heat tolerance and evaporative cooling capacity following thermal acclimation, this would imply the interpopulation variation in these traits arises from acclimatization to different summer maximum T_a. On the other hand, if desert sparrow-weavers retain their higher evaporative cooling capacity compared to mesic conspecifics following acclimation, this would suggest the source of the interpopulation variation is either genetic adaption or developmental plasticity.

As numerous previous studies have reported considerable physiological flexibility in sparrow-weavers (Smit and McKechnie 2010, 2015; Smit et al. 2013; Noakes et al. 2016, 2017), I hypothesized that intraspecific variation in their capacity to handle high T_a arises from phenotypic flexibility, and hence that birds from different populations exhibit similar reaction norms for heat tolerance and evaporative cooling capacity in response to short-term thermal acclimation. I caught birds from three sites along a climatic and aridity gradient (arid: Askham, semi-arid: Barberspan, mesic: Polokwane) and acclimated them to three different T_a regimes (daytime T_a = 30 °C, 36 °C or 42 °C). I predicted that heat tolerance and evaporative cooling capacity is greatest in sparrow-weavers acclimated to the highest daytime T_a (42 °C; T_{accl}) and more modest in birds acclimated to the milder T_{accl} (30 °C and 36 °C), irrespective of site of capture. I also predicted that higher heat tolerance and evaporative cooling capacity in birds acclimated to hotter T_a is associated with reductions in EWL, resting metabolic rate (RMR) and T_b compared to the milder acclimation treatments, as previously observed in desert sparrow-weavers during summer compared to winter (Noakes et al. 2016).

4.3 Materials and methods

4.3.1 Study sites

I captured sparrow-weavers during the austral spring (25 September to 15 October 2017) at three sites in South Africa across a climatic and aridity gradient: one arid site near Askham in the Kalahari Desert (Northern Cape Province), a semi-arid site at Barberspan Bird Sanctuary near Delareyville (Northwest Province) and a more mesic site at Polokwane (Limpopo Province; table 1). I obtained climate data for each site from the closest weather stations of the South African Weather Service to quantify variation in seasonal temperature extremes among these sites (table 1).

At each site, I captured ~ 30 sparrow-weavers at night by covering the entrances of roost nests with small nets mounted on aluminium poles, and then flushing birds from nests. I caught sparrow-weavers over three nights at Askham, and over one night each at Barberspan and Polokwane. Birds were temporarily housed in cages (~ 1.5 m^3) constructed of plastic mesh and shade cloth, before being transported by road to the Small Animal Physiological Research at the University of Pretoria (25° 45’ S, 28° 15’ E) in modified pet carriers. Birds from Polokwane and Barberspan spent about 3 h in transit (~ 300 km from the capture sites to Pretoria), whereas the trip from Askham to Pretoria occurred over two days (~ 1000 km in total; ~ 6 h travelling per day). Birds were provided with water and wild bird seed mix and mealworms *ad libitum* during the capture and travel periods.

At the Small Animal Physiological Research Facility, each sparrow-weaver was allocated to one of three climate-controlled rooms (~ 10 birds from each site per room; table 2), all initially set to T_a = 30 °C. Birds were housed in cages (600 × 400 × 400 mm), with generally two birds per cage (male and female from the same site) and a small number (10 out of 92 birds) housed individually. Sex was determined by bill colour (du Plessis 2005).

Birds were provided with water and a wild bird seed mix *ad libitum*, as well as mealworms and superworms (~ 4 of each per bird per day). A Scout Pro Balance scale (SP602US, Ohaus, Pine Brook NJ, USA) was used to monitor the body mass (M_b) of individuals regularly throughout the study period. After completion of the experiments, sparrow-weavers were released at their capture sites.

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

Capture site	Daily $T_{a,min}$ (°C)		Daily $T_{a,max}$ (°C)		Location	
	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	23° 56′ S, 29° 28′ E	23° 51′ S, 29° 27′ E
Barberspan	16.6 ± 1.0	1.1 ± 1.5	32.7 ± 2.4	21.4 ± 0.8	26° 33' S, 25° 36' E	26° 49' S, 26° 01' E
Askham	20.4 ± 1.2	0.6 ± 0.8	38.1 ± 1.8	23.8 ± 1.4	26° 59′ S, 20° 51′ E	26° 28′ S, 20° 36′ E

Note: Mean (± standard deviation) $T_{a,min}$ and maximum $T_{a,max}$ are for the hottest summer months (January) and coldest winter months (July) over 2013 to 2017, calculated from weather data obtained from South African Weather Service stations. White-browed sparrow-weavers (*Plocepasser mahali*) were captured from these sites and transported to the University of Pretoria for acclimation experiments.

4.3.2 Acclimation regimes

Four days after the arrival of the birds from the final capture site, the T_a in each room was set to a 24 h cycle intended to approximate natural temperature variation, with night-time T_a set to 20 °C from 22:00 to 05:00 (seven hours) and an experimental day-time T_a (T_{accl}) from 10:00 to 17:00 (seven hours). Between these periods, T_a increased or decreased at a constant rate (table 2). I set the photoperiod in each the room to a 12: 12 h light: dark cycle. I initially set all rooms to T_{accl} = 33 °C for four days and then to 36 °C for eight days so that all birds were first exposed to the intermediate T_{accl} regime. Following this initial period, the three rooms were set to experimental T_{accl} values of 30 °C, 36 °C or 42 °C, respectively, for the remainder of the study. After the T_{accl} values were set, an acclimation period of 30 days was allowed, which is towards the longer end of the range of acclimation periods used in previous acclimation studies (e.g., 28 days: Tieleman et al. 2003a; 17 days: McKechnie and Wolf 2004; 21 days: McKechnie et al. 2007; 30 days: Cavieres and Sabat 2008). Thereafter, post-acclimation measurements of thermoregulatory variables took place over 45 days and thus the duration of acclimation actually varied among individuals from 30 to 75 days. Over the course of the experiments, actual T_a in each room fluctuated by only a small amount around the setpoint values (table 2).

Table 2. Acclimation air temperature (T_a) treatments and sample sizes of white-browed sparrow-weavers (*Plocepasser mahali*) from different populations

Set T_a (°C)		Actual T_a (°C)		T_a rate of change (°C h^{-1})	Sample sizes		
Day	Night	Day	Night		Askham	Barberspan	Polokwane
30	20	30.1 ± 0.2	20.4 ± 0.2	2.0	10	9	11
36	20	35.2 ± 0.4	20.8 ± 0.6	3.1	10	10	11
42	20	41.2 ± 0.2	20.4 ± 0.2	4.4	11	9	11

Note: Mean (± standard deviation) T_a during the day (10:00 to 17:00) and night (22:00 to 5:00), and the rate of change between day and night T_a over three hours, of three climate-controlled rooms at the University of Pretoria Small Animal Physiological Research Facility. Sparrow-weavers from three different capture sites (Askham, Barberspan and Polokwane) were divided among the rooms.

4.3.4 Gas exchange and temperature measurements

I used an open flow-through respirometry system to measure CO_2 production (ml min^{-1}) and EWL (mg min^{-1}), using the same experimental setup described in chapter 1 (Noakes and McKechnie in press). Sparrow-weavers were measured individually in air-tight respirometry chambers constructed from 4-L clear plastic containers (Lock&Lock, Seoul, South Korea).

Flow rates were frequently adjusted (2 to 12 L min^{-1}) during data collection to ensure that water vapour partial pressure within the chambers remained low (< 0.49 kPa), and I ensured that all measurements obtained were after the 95 % equilibrium time estimated for each flow rate using the equation in Lasiewski et al. (1966). I implanted temperature-sensitive passive integrated transponder tags intraperitoneally into sparrow-weavers to measure core T_b, and monitored T_b during measurements using a racket antennae and passive integrated transponder tag reader (HPR Plus, Biomark, Boise ID, USA). I calibrated mass flow controllers, transponder tags and gas analysers as Whitfield et al. (2015).

4.3.5 Experimental protocol

I quantified heat tolerance and maximum evaporative cooling capacity in *P. mahali* during their active phase (day-time) using the standardized protocol described by Whitfield et al. (2015). The only difference between my protocol and that of the latter study was that I exposed birds to a ramped T_a profile of progressively higher T_a values, starting at $T_a \approx 38$ °C and going upwards in 2 ºC increments until birds became distressed or severely hyperthermic ($T_b = 44.5$ °C; maximum T_a reached ≈ 54 °C). Individuals were kept at each setpoint T_a for a period of ≥ 10-min, or until gas exchange values had levelled off, before increasing T_a to the next setpoint. Baseline gas exchange values were measured at the start and at regular intervals throughout measurements, typically when T_a was increasing to the next setpoint. I removed food from the cages about two hours prior to measurements to ensure that birds were post-absorptive (mean gut retention time for a 47-g birds is 67.5 min; Karasov 1990), and measured the M_b of indiviudals before placing them in the chamber using a Scout *Pro* Balance scale (SP602US, Ohaus, Pine Brook NJ, USA).

I collected data from about three sparrow-weavers per day over a 45 day period, and thus included the date in statistical models to account for varying durations of acclimation. I randomized the order in which birds from different acclimation treatments and populations were measured. To quantify variation in heat tolerance among sparrow-weavers from different acclimation treatments and populations, I considered the T_a at which each bird reached T_b = 44.0 °C to be the hyperthermia threshold T_a ($T_{a,HT}$; °C) for that individual. A T_b of 44.0 °C was chosen as Whitfield et al. (2015) and Noakes et al. (2016) found that this T_b was close to the critical thermal maximum for *P. mahali*.

4.3.6 Data analyses

For each bird, the 5-min sample period with the lowest average [CO_2] at each T_a was used in calculations, and behavioural observations were checked to verify that individuals were calm during this period. I calculated whole-animal RMR and EWL as described in chapter 1 (Noakes and McKechnie in press) and chapter 3 respectively, assuming a respiratory exchange ratio value of 0.71 representative of post-absorptive birds (Withers 1992). Evaporative heat loss (EHL) was calculated from EWL using a latent heat of vaporisation of 2.4 J mg^{-1} H_2O (corresponding with T_a = 40 °C; Withers 1992), and the efficiency of evaporative cooling as the ratio of EHL to metabolic heat produced (EHL / MHP). All mean values presented are in the format: mean ± standard deviation. I used R 3.5.1 (R Core Team 2018) to fit linear models and linear mixed effects models (*nlme* package; Pinheiro et al. 2018) to the data, and tested that model assumptions were met (i.e., no multicollinearity between continuous predictor variables, or obvious deviations from normality and homogeneity of variance in model residuals plots).

I investigated variation in thermoregulatory variables (RMR, EWL, T_b and EHL/MHP) with predictor variables T_a, M_b, sex, population, T_{accl} and date (to account for varying durations of acclimation). I used the “dredge” function from the *MuMIn* package (Bartoń 2018) to determine the combination of predictor variables that produced linear models that best explained variation in thermoregulatory variables, as described by Noakes et al. (2016). If models indicated that T_{accl} was a significant predictor of thermoregulatory variables, I repeated analyses for birds within each acclimation treatment separately. I used a Davies Test to determine whether there were significant inflection points in the relationship between each thermoregulatory variable and T_a, and fitted broken stick linear regressions where appropriate to determine the T_a value of inflection points (*segmented* package; Muggeo 2003, 2008). If significant inflection points were identified, I repeated analyses separately for data on either side of each inflection. For the final models on variation in thermoregulatory variables, I fitted linear mixed effects models to each dataset with individual specified as a random predictor. The statistical results reported are those obtained from the models of best fit, and the equations plotted in the figures for the relationship between each thermoregulatory variable and T_a are those obtained from the final linear mixed effects models. To confirm that converting CO_2 production into RMR in watts was not a source of error, I repeated statistical analyses with CO_2 production as the response variable.

I also fitted linear models to investigate variation in M_b and $T_{a,HT}$ among sparrow-weavers from different acclimation regimes and populations. *Post hoc* tests of multiple comparisons of means were conducted to investigate between which acclimation regimes significant differences were present (Tukey contrasts for linear models; *multcomp* package; Hothorn et al. 2008).

4.4 Results

4.4.1 Body mass

Following acclimation to daytime T_a of 30 °C, 36 °C or 42 °C (i.e., T_{accl}), sparrow-weavers' M_b did not vary significantly among the three populations ($F_{2,81} = 2.641$; $P = 0.077$), and was significantly higher in males (44.4 ± 2.9 g) compared to females (42.3 ± 3.0 g ; $F_{1,81} = 13.131$; $P < 0.001$). There was, however, significant M_b variation among the acclimation treatments ($F_{2,81} = 10.104$; $P < 0.001$). The M_b of sparrow-weavers exposed to $T_{accl} = 42$ °C (41.0 ± 2.7 g) was significantly lower than those exposed to $T_{accl} = 30$ °C (44.6 ± 3.4 g; $P < 0.001$) and $T_{accl} = 36$ °C (43.7 ± 2.8 g; $P = 0.014$), but did not vary significantly between the latter two treatments ($P = 0.277$).

4.4.2 Hyperthermia threshold temperatures

The top model for variation in the T_a that sparrow-weavers approached severe hyperthermia ($T_{a,HT}$) included T_{accl} ($F_{2,88} = 9.343$; $P < 0.001$). $T_{a,HT}$ was significantly higher in birds exposed to $T_{accl} = 42$ °C than those exposed to $T_{accl} = 30$ °C (~ 2 °C higher; $P < 0.001$) and 36 °C (~ 1.3 °C; $P = 0.014$), but did not vary significantly among the latter two treatments ($P = 0.355$; figure 1). $T_{a,HT}$ was the only response variable that included the duration of acclimation as a predictor in the top model, with a significant decline in $T_{a,HT}$ over the 45 day period of measurements at a consistent rate among birds from all acclimation treatments (~ 0.06 °C per day; $F_{1,88} = 23.115$; $P < 0.001$).

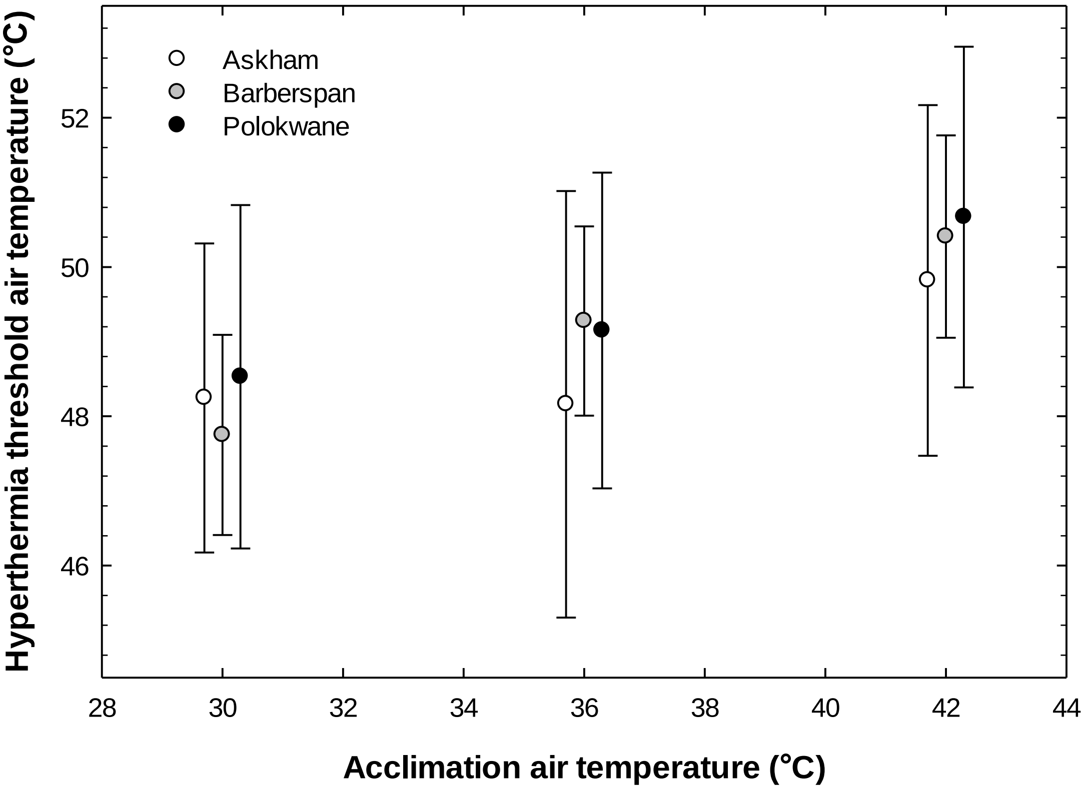


Figure 1. The hyperthermia threshold air temperature (HTT_a) of white-browed sparrow-weavers (*Plocepasser mahali*) was determined by exposing birds to progressively higher $T_a \geq 38$ °C until birds became severely hyperthermic (body temperature = 44 °C). Sparrow-weavers were caught from three populations along a climatic gradient (Askham, Barberspan and Polokwane), and split among three acclimation temperature treatments (T_{accl} = 30 °C, 36 °C and 42 °C; $n \approx 10$ per population per T_{accl}). HTT_a did not significantly vary among populations after acclimation (points are jittered for illustrative purposes), but was significantly higher in birds from the hottest compared to the milder acclimation treatments. Mean values are presented and error bars represent standard deviation.

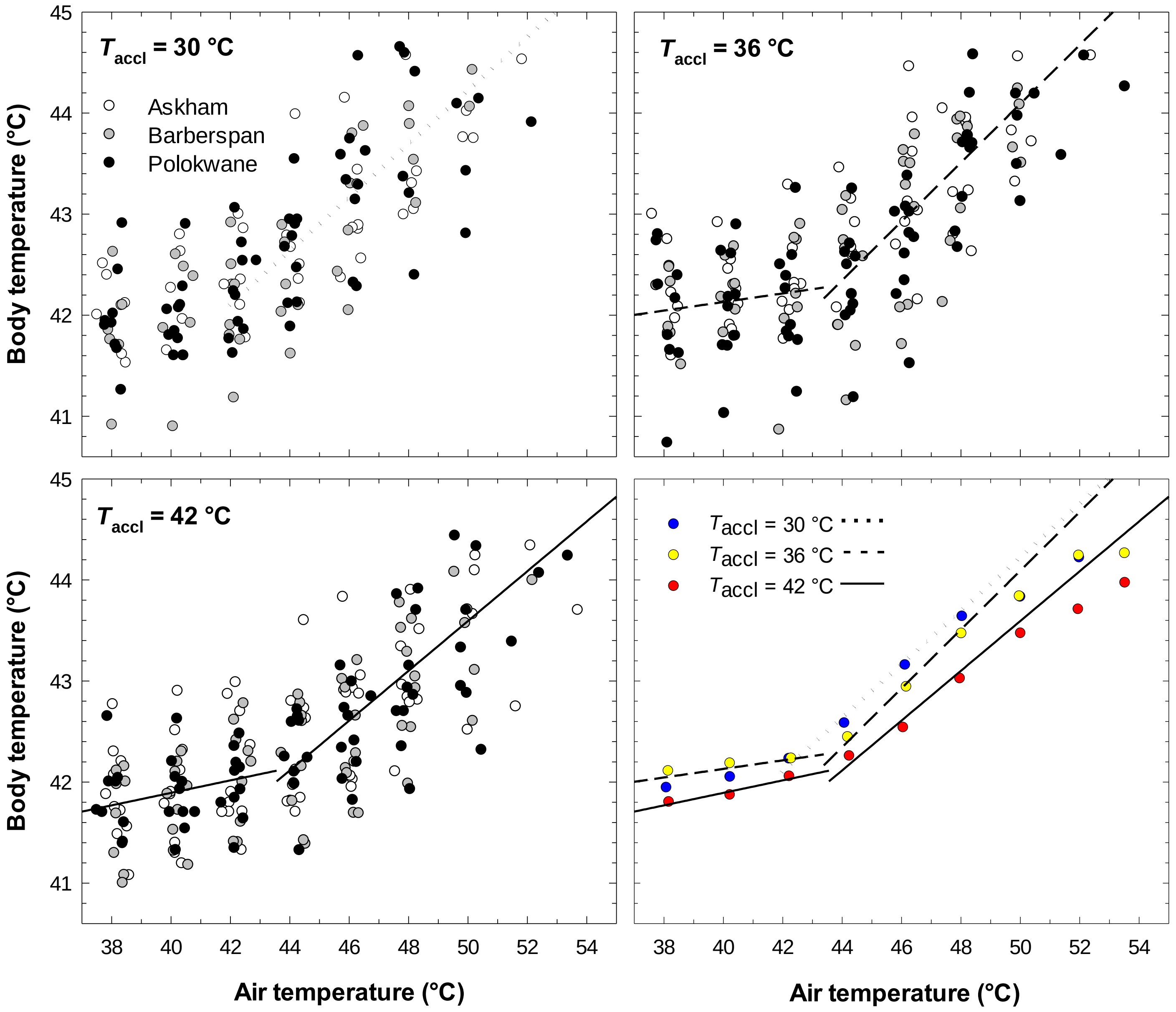
4.4.3 Body temperature

The T_b of sparrow-weavers was the only thermoregulatory variable significantly predicted by T_{accl} ($F_{1,416} = 5.000$, $P = 0.007$). I therefore investigated the relationship between T_b and T_a separately for birds from each acclimation treatment (figure 2).

Birds from all acclimation treatments showed a significant inflection in the relationship between T_a and T_b. However, this inflection was lower for birds exposed to T_{accl} = 30 °C (inflection T_a = 42.0 °C; $P = 0.022$) than those from the rooms set to T_{accl} = 36 °C and 42 °C [inflection T_a = 43.4 °C ($P < 0.001$) and 43.6 °C ($P = 0.002$] respectively; figure 2]. Below the inflection T_a, the null model was the top model explaining variation in T_b of sparrow-weavers exposed to T_{accl} = 30 °C, and the mean T_b was 42.0 ± 0.4 °C in this range (i.e., ~ 38 °C < T_a < 42 °C). In contrast, T_b increased significantly with increasing T_a for birds exposed to T_{accl} = 36 °C ($F_{1,46} = 9.740$, $P = 0.003$) and 42 °C ($F_{1,55} = 19.090$, $P < 0.001$; figure 2).

Above the inflection T_a, the top models consistently included T_a during thermoregulatory measurements as a predictor variable, and T_b increased with increasing T_a at a similar rate for birds from all acclimation treatments: T_{accl} = 30 °C ($F_{1,69} = 347.860$, $P < 0.001$), 36 °C ($F_{1,64} = 299.970$, $P < 0.001$) and T_{accl} = 42 °C ($F_{1,78} = 553.430$, $P < 0.001$; figure 2). The rate of increasing T_b with increasing T_a was faster above the inflection point compared to below the inflection for sparrow-weavers exposed to T_{accl} = 36 °C (~ 6.9 × faster) and 42 °C (~ 4.0 × faster; figure 2).

The T_b of sparrow-weavers exposed to T_{accl} = 42 °C appeared lower than birds from the other acclimation treatments (figure 2). This was particularly evident when comparing birds from T_{accl} = 30 °C and 42 °C above the inflection points (i.e., at T_a > 43 °C) where there is a consistent difference of ~ 0.6 °C between the regression lines (figure 2). However, I could not test this variation statistically because of different inflection points in the relationships between T_a and T_b for birds from each acclimation treatment.



T_{accl} = 30 °C
Askham
Barberspan
Polokwane
T_{accl} = 36 °C
T_{accl} = 42 °C
T_{accl} = 30 °C
T_{accl} = 36 °C
T_{accl} = 42 °C
Body temperature (°C)
Body temperature (°C)
41
42
43
44
45
38
40
42
44
46
48
50
52
54
Air temperature (°C)
Air temperature (°C)

Figure 2.

Figure 2. The body temperature (T_b) of white-browed sparrow-weavers (*Plocepasser mahali*) was measured over an air temperature (T_a) range of ~ 38 to 54 °C. Sparrow-weavers were caught from three populations along a climatic gradient (Askham, Barberspan and Polokwane), and split among three acclimation temperature treatments (T_{accl} = 30 °C, 36 °C and 42 °C; first three panels). The bottom right panel is of mean T_b values of birds from each acclimation treatment at each T_a measured. T_b significantly varied among acclimation treatments but not among populations ($n \approx 10$ per population per T_{accl}). Inflection points were calculated using broken stick linear regressions and regression lines represent significant relationships from a linear mixed effects model (T_{accl} = 30 °C, $T_a > 41.966$ y = 0.265 x + 30.996; T_{accl} = 36 °C, $T_a < 43.384$ °C: y = 0.042 x – 40.433, $T_a > 43.384$ °C: y = 0.291 x – 29.542; T_{accl} = 42 °C, $T_a < 43.559$ °C: y = 0.062 x – 39.421, $T_a > 43.559$ °C: y = 0.246 x – 31.276).

4.4.4 Evaporative water loss

The EWL of sparrow-weavers significantly increased with increasing T_a, with a significant inflection at T_a = 43.0 °C ($P < 0.001$) above which EWL increased ($F_{1,195} = 93.110$, $P < 0.001$) at a rate ~ 4.5 × faster compared to lower T_a ($F_{1,161} = 14.398$, $P < 0.001$; figure 3). The top model explaining variation in EWL included other predictor variables and interactions terms along with T_a (T_{accl}, sex, population, T_{accl} × sex, T_{accl} × population, population × sex and T_{accl} × population × sex), but none of these had a significant effect on EWL ($P > 0.144$ for all interaction terms).

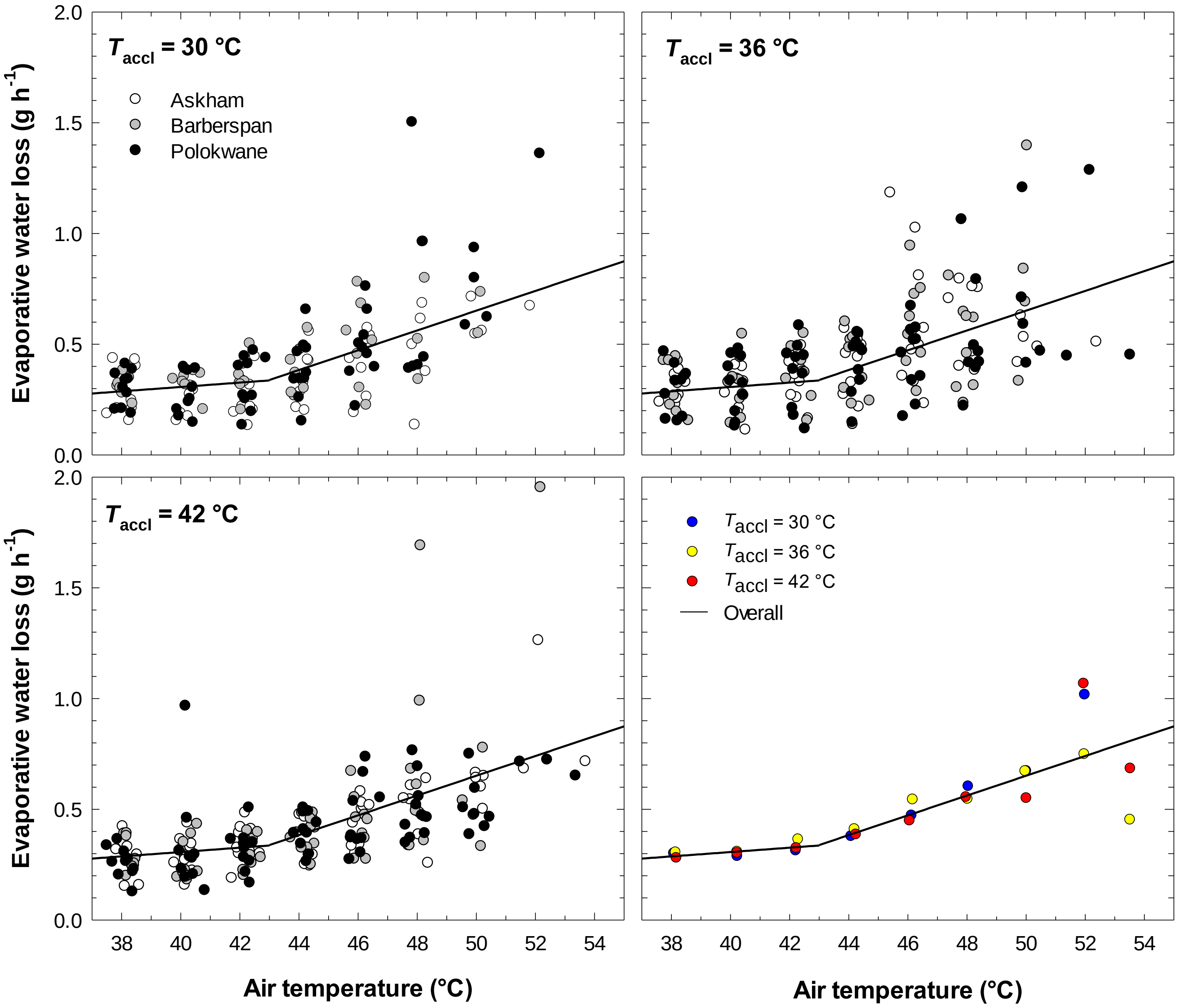

T_{accl} = 30 °C
T_{accl} = 36 °C
T_{accl} = 42 °C
Askham
Barberspan
Polokwane
T_{accl} = 30 °C
T_{accl} = 36 °C
T_{accl} = 42 °C
Overall
Evaporative water loss (g h^{-1})
Air temperature (°C)
0.0
0.5
1.0
1.5
2.0
38
40
42
44
46
48
50
52
54

Figure 3.

Figure 3. The evaporative water loss (EWL) of white-browed sparrow-weavers (*Plocepasser mahali*) was measured over an air temperature (T_a) range of ~ 38 to 54 °C. Sparrow-weavers were caught from three populations along a climatic gradient (Askham, Barberspan and Polokwane), and split among three acclimation temperature treatments (T_{accl} = 30 °C, 36 °C and 42 °C; first three panels). The bottom right panel is of mean EWL values of each acclimation group at each T_a measured. EWL did not significantly vary among acclimation treatments or populations ($n \approx 10$ per population per T_{accl}). Inflection points were calculated using broken stick linear regressions and regression lines represent significant relationships from a linear mixed effects model ($T_a <$ 43.005 °C: $y = 0.010 x – 0.087$; $T_a >$ 43.005 °C: $y = 0.045 x – 1.583$).

4.4.5 Resting metabolic rate

There was a significant inflection in RMR at T_a = 41.3 °C ($P < 0.001$; figure 4). At $T_a <$ 41.3 °C, the top model included only T_a as a predictor variable, with RMR decreasing with increasing T_a ($F_{1,161} = 186.837$, $P < 0.001$; figure 4). At $T_a >$ 41.3 °C, the top model describing variation in RMR was the null model (i.e., including no predictor variables), suggesting that none of the predictor variables included explained variation in RMR, and mean RMR was 0.2 ± 0.1 W in this T_a range (i.e., 41.3 °C < T_a < 54.0 °C; figure 4). Population and T_{accl} were not included as predictor variables in the top models explaining variation in RMR at any T_a. Repeating these analyses using CO_2 production instead of metabolic rate (W) as the response variable revealed the same patterns of variation and did not in any way affect my conclusions

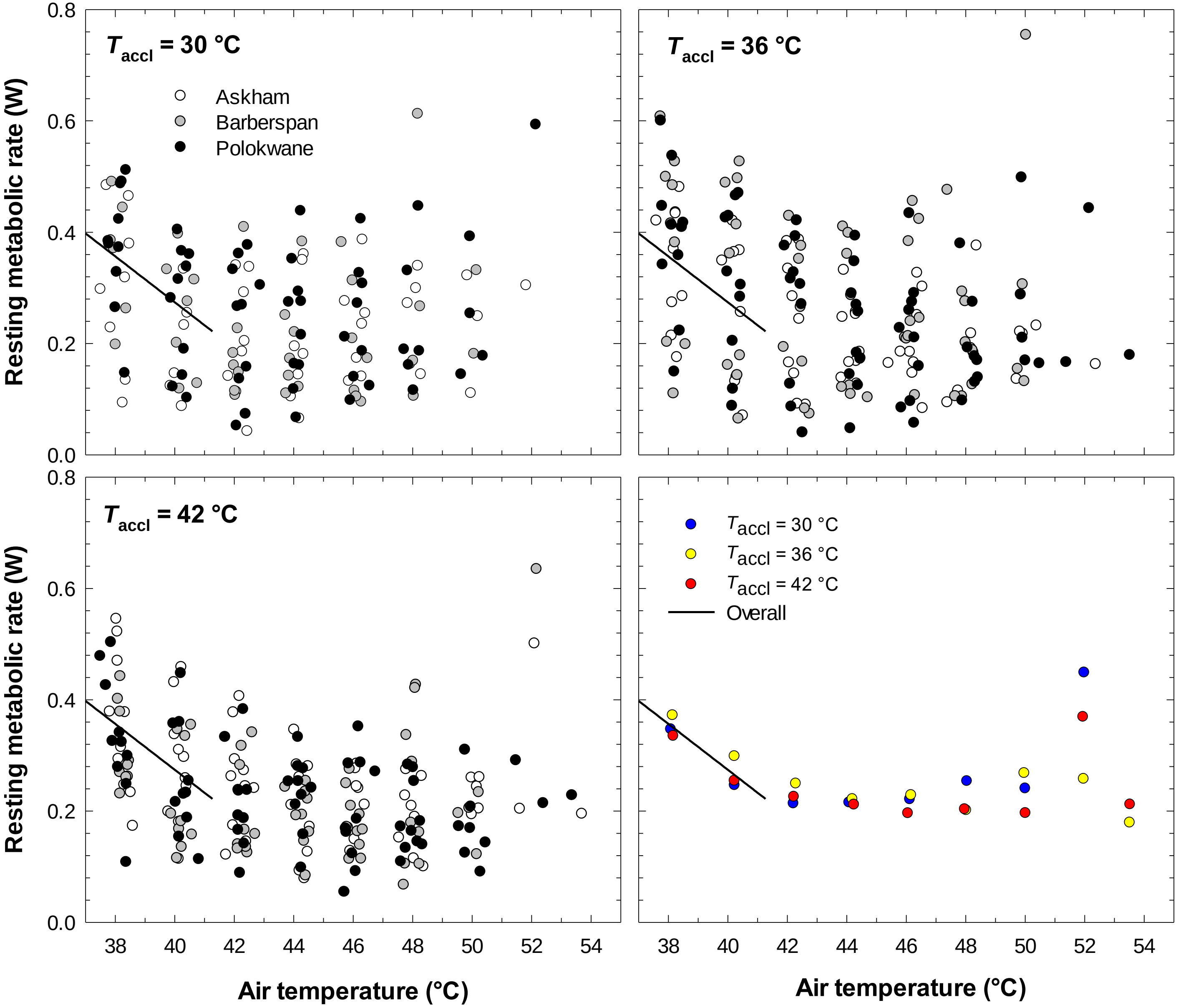
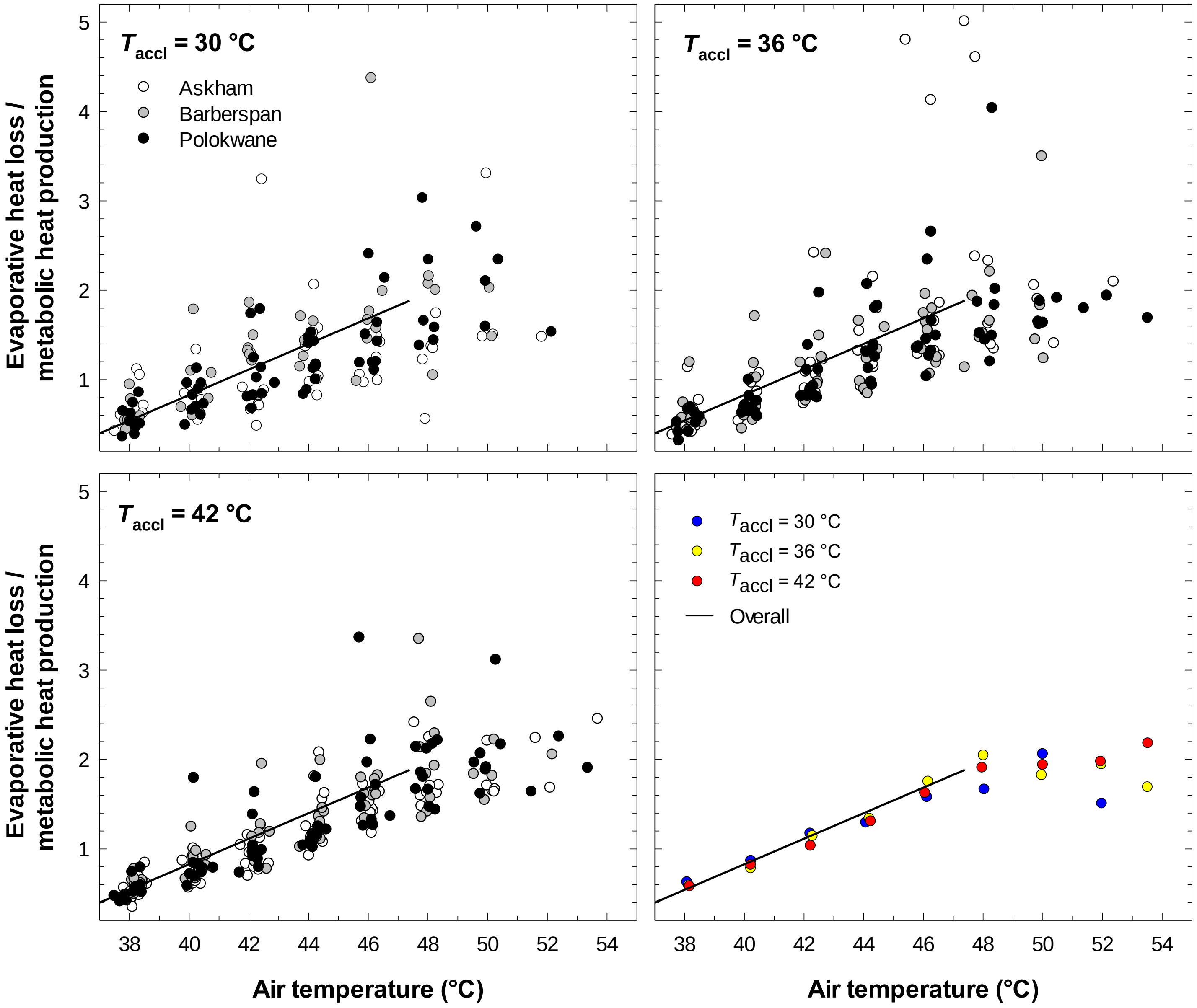

T_{accl} = 30 °C
○ Askham
● Barberspan
● Polokwane
T_{accl} = 36 °C
T_{accl} = 42 °C
● T_{accl} = 30 °C
● T_{accl} = 36 °C
● T_{accl} = 42 °C
Overall
Resting metabolic rate (W)
Resting metabolic rate (W)
0.0
0.2
0.4
0.6
0.8
38
40
42
44
46
48
50
52
54
Air temperature (°C)
Air temperature (°C)

Figure 4.

Figure 4. The resting metabolic rate (RMR) of white-browed sparrow-weavers (*Plocepasser mahali*) was measured over an air temperature (T_a) range of ~ 38 to 54 °C. Sparrow-weavers were caught from three populations along a climatic gradient (Askham, Barberspan and Polokwane), and split among three acclimation temperature treatments (T_{accl} = 30 °C, 36 °C and 42 °C; first three panels). The bottom right panel is of mean RMR values of each acclimation group at each T_a measured. RMR did not significantly vary among acclimation treatments or populations ($n \approx 10$ per population per T_{accl}). Inflection points were calculated using broken stick linear regressions and regression lines represent significant relationships from a linear mixed effects model ($T_a <$ 41.268 °C: $y = -0.041 x - 1.924$).

4.4.6 Evaporative cooling efficiency

There was a significant inflection point in EHL/MHP, although it was at a higher T_a than the inflection point for EWL and RMR (T_a = 47.4 °C; $P = 0.007$; figure 5). The top model explaining variation in EHL/MHP at $T_a <$ 47.4 °C included only T_a, and EHL/MHP significantly increased with increasing T_a ($F_{1,323} = 208.080$, $P < 0.001$; figure 5). At $T_a >$ 43.0 °C, the top model explaining variation in EHL/MHP was the null model, and the mean EHL/MHP over this range (i.e., 43.0 °C < T_a < 54.0 °C) was 1.9 ± 0.6 (figure 5). Population and T_{accl} were not included as predictor variables in the top models explaining variation in EHL/MHP at any T_a.



T_{accl} = 30 °C
○ Askham
● Barberspan
● Polokwane
T_{accl} = 36 °C
T_{accl} = 42 °C
● T_{accl} = 30 °C
● T_{accl} = 36 °C
● T_{accl} = 42 °C
— Overall
Evaporative heat loss / metabolic heat production
1
2
3
4
5
38
40
42
44
46
48
50
52
54
Air temperature (°C)

Figure 5.

Figure 5. The ratio of evaporative heat loss (EHL) to metabolic heat produced (EHL/MHP) of white-browed sparrow-weavers (*Plocepasser mahali*) was measured over an air temperature (T_a) range of ~ 38 to 54 °C. Sparrow-weavers were caught from three populations along a climatic gradient (Askham, Barberspan and Polokwane), and split among three acclimation temperature treatments (T_{accl} = 30 °C, 36 °C and 42 °C; first three panels). The bottom left panel is of mean EHL/MHP values of each acclimation group at each T_a measured. EHL/MHP did not significantly vary among acclimation treatments or populations ($n \approx 10$ per population per T_{accl}). Inflection points were calculated using broken stick linear regressions and regression lines represent significant relationships from a linear mixed effects model ($T_a < 47.388$ °C: $y = 0.143\ x – 4.889$).

4.5 Discussion

Sparrow-weavers showed phenotypic flexibility in M_b, T_b and their capacity to tolerate high T_a, with similar reactions norms in three populations. The strong effect of T_{accl}, but lack of any significant variation among populations, supports my hypothesis that previously documented interpopulation variation in heat tolerance and evaporative cooling capacity arises from phenotypic flexibility rather than localized genotypic adaptation or developmental plasticity (Noakes et al. 2016). As predicted, birds acclimated to the hottest daytime T_a were more heat tolerant compared to those from milder acclimation treatments. The data revealed the predicted patterns of variation in T_b, but not those for RMR or EWL; these findings differ from those of Noakes et al. (2016), who reported higher heat tolerance in desert sparrow-weavers compared to conspecifics from more mesic sites was associated with significantly lower EWL, RMR and $\mathrm{T_b}$ at higher T_a.

Noakes et al. (2016) found significant interpopulation variation in the M_b of free-ranging sparrow-weavers, whereas M_b following acclimation did not vary among populations but rather with T_{accl}. This suggests that interpopulation variation in M_b is not the result of hardwired genotypic differences among sparrow-weavers at different sites, but instead reflects a phenotypically plastic trait. Sparrow-weavers from the hottest acclimation treatment (T_{accl} = 42 °C) had significantly lower M_b than birds from the milder acclimation treatments, similar to the pattern of M_b variation observed in sparrow-weavers from desert *vs* mesic populations (Noakes et al. 2016). This could be the result of birds exposed to higher temperatures having poorer body condition on account of thermal stress and/or consuming less food. However, M_b was not a significant predictor for any response variable in the current study, suggesting that M_b differences are not responsible for thermoregulatory variation among acclimation treatments.

The current study is, to the best of my knowledge, the first to demonstrate plasticity in heat tolerance in response to short-term thermal acclimation in a passerine bird (figure 1). Interpopulation variation in the seasonal acclimatization of heat tolerance has previously been reported in sparrow-weavers, with a desert population having $T_{a,HT}$ values ~ 2.7 °C higher in summer compared to winter (Noakes et al. 2016). However, seasonal acclimatization of heat tolerance was associated with enhanced evaporative cooling capacities of desert sparrow-weavers during summer (reductions in EWL, RMR and T_b at $T_a \geq 40$ °C; Noakes et al. 2016), whereas in the present study the thermoregulation of sparrow-weavers acclimated to $T_a = 42$ °C varied from the milder acclimation treatments only by a reduction in T_b at $T_a \geq 38$ °C (figure 2). A possible explanation for the lack of variation in EWL in the current study is that sparrow-weavers had *ad libitum* access to water in all acclimation treatments, whereas wild, desert sparrow-weavers experience both higher T_a and scarce water supply during summer (Noakes et al. 2016). The magnitude of T_b variation among acclimation treatments (~ 0.6 °C) was similar to the magnitude reported for the seasonal acclimatization of T_b in desert sparrow-weavers (~ 0.7 °C; Noakes et al. 2016).

The two previous studies with data comparable to mine reported lower T_b in heat-acclimated individuals: *Zenaida asiatica mearnsii* (~ 1 °C lower at $T_a = 45$ °C; McKechnie and Wolf 2004) and *Columba livia* (~ 2 °C lower at $T_a > 35$ °C; Marder and Arieli 1988), associated with enhanced evaporative cooling efficiency via adjustments in the partitioning of

EWL into cutaneous and respiratory avenues. It is less clear how sparrow-weavers in the T_{accl} = 42 °C group maintained a lower T_b without increasing EWL or evaporative cooling efficiencies (EHL/MHP). One potential explanation could involve variation in thermal conductance as a result of post-acclimation M_b differences, although I consider this unlikely as M_b was not a predictor variable in the top models explaining T_b variation. Flexibility in T_b is an interesting response not frequently reported in the avian literature. The majority of acclimation studies focused on flexibility in RMR at thermoneutral or colder T_a (reviewed by McKechnie 2008; McKechnie and Swanson 2010), and so it is striking that three acclimation studies investigating flexibility of thermoregulation of wild birds at high T_a have all reported reduced T_b in heat-acclimated treatment groups (current study; Marder and Arieli 1988; McKechnie and Wolf 2004). The same pattern has been reported in heat-acclimated commercial chickens (Hutchinson and Sykes 1953; Sykes and Fataftah 1986; Abdelqader and Al-Fataftah 2014).

Maintaining lower T_b in hot environments could confer adaptive benefits by providing a greater capacity for heat storage (McNab and Morrison 1963; Tieleman et al. 2002). It has been argued that arid-zone birds should demonstrate a greater capacity for facultative hyperthermia compared to mesic species, but this has not been supported by studies directly comparing arid *vs* mesic birds (e.g., Tieleman and Williams 1999; Noakes et al. 2016). Smit et al. (2013) investigated T_b variation in free-ranging sparrow-weavers in the Kalahari Desert, and reported desert sparrow-weavers had a ~ 1 °C higher set-point T_b compared to semi-desert conspecifics, but not a greater capacity for hyperthermia (i.e., $T_b >$ modal T_b). Laboratory data for sparrow-weavers at high T_a reveal a contrasting pattern, with lower T_b in desert compared to mesic sparrow-weavers (Noakes et al. 2016), and also in sparrow-weavers acclimated to the hottest *vs* milder acclimation treatments (current study).

The EWL and RMR of sparrow-weavers were substantially lower in the present study compared to values previously reported for freshly-caught wild birds (Whitfield et al. 2015; Noakes et al. 2016). For example, EWL and RMR at T_a = 42 °C in acclimated sparrow-weavers were equivalent to just 20 % to 25 % of the values of wild conspecifics at the same T_a (Noakes et al. 2016). Previous studies have also documented lower metabolic rates in birds after periods of captivity (reductions from ~ 10 % to 40 %), with suggested mechanisms including lower stress levels after periods in captivity, changes in body composition and organ masses due to captive diets, and a decrease in pectoral muscle mass on account of reduced flight activity (Piersma et al. 1996; Al-Mansour 2005; McKechnie et al. 2007). Although the differences between my observed values and those reported by Noakes et al. (2016) are larger than documented elsewhere, the magnitude of these differences are nevertheless similar to the interspecific variation in BMR that occurs among similarly-sized species; for instance, the BMR of the ~ 24-g *Cacomantis variolosus* is equivalent to just 19 % of that of the 20-g *Phylidonyris novaehollandiae* (Hails 1983; Yuni and Rose 2005). I cannot rule out the possibility of these differences reflecting a methodological problem, but consider it unlikely as the same respirometry system and calibration procedures were used in all studies (Whitfield et al. 2015; Noakes et al. 2016).

The initial decrease in RMR with increasing T_a over the range of ~ 38 °C < T_a < 41.3 °C is puzzling (figure 4), as the upper critical temperature for this species is ~ 37 °C (Whitfield et al. 2015; Noakes et al. 2016). This pattern could potentially represent an artefact of my methods, such as birds calming down after being handled and placed in metabolic chambers, although an initial increase in RMR was not reported in previous studies on sparrow-weavers using a similar protocol (Whitfield et al. 2015; Noakes et al. 2016). The lack of a relationship between RMR and T_a > 41.3 °C is surprising as an increase in RMR with increasing T_a above normothermic T_b is expected in passerines as result of increasing respiratory EWL (e.g., McKechnie et al. 2017; Smith et al. 2017; Kemp and McKechnie 2019). Noakes et al. (2016) also reported that the relationship between T_a and RMR of sparrow-weavers at $T_a > 40$ °C was not as clear as those for other thermoregulatory variables, and van Dyk et al. (2019) found no relationship between T_a and RMR at low absolute humidities (~ 6 g m^{-3}) at 36 °C $\leq T_a \leq$ 44 °C, but increases in RMR with increasing T_a at higher humidities (up to 25 g m^{-3}).

The mean maximum EHL/MHP (2.1 ± 0.8) in the present study was similar to values previously reported for sparrow-weavers (1.79 ± 0.30: Whitfield et al. 2015; ~ 1.0 to 2.3: Noakes et al. 2016). There were a few outliers, with one individual exhibiting EHL/MHP = 5.0 at T_a = 47.4 °C (figure 5), a value close to the highest EHL/MHP ever recorded in a bird (EHL/MHP = 5.2 at T_a = 56 °C in a caprimulgid; O’Connor et al. 2017). Such high EHL/MHP values are surprising in a species that uses panting to dissipate heat at $T_a > T_b$ (Wolf and Walsberg 1996; Tieleman and Williams 2002), but as the associated EWL and RMR values appeared valid I did not exclude these data. EHL/MHP increased with increasing $T_a <$ 47.4 °C but plateaued at higher T_a (figure 5), suggesting that maximum evaporative cooling efficiencies of sparrow-weavers had been reached.

The variation among acclimation treatments I observed adds to the growing number of studies demonstrating considerable plasticity in the thermal physiology of sparrow-weavers (Smit and McKechnie 2010, 2015; Smit et al. 2013; Noakes et al. 2016, 2017). Little is known about the shape of avian thermoregulatory reaction norms [i.e., the range of phenotypic trait values a single genotype can produce in response to environmental conditions (Schlichting and Pigliucci 1998)]. The majority of studies investigating flexibility in avian thermoregulation have focused on metabolic responses at thermoneutral or colder T_a (e.g., basal metabolic rate [BMR] and summit metabolism [M_{sum}]; McKechnie 2008; McKechnie and Swanson 2010). The reaction norm for avian BMR has been demonstrated as approximately linear for *Streptopelia senegalensis* over the range 10 °C ≤ T_{accl} ≤ 35 °C McKechnie et al. 2007), and for *Poecile atricapillus* over minimum T_{a} ranging from -20 ˚C to 30 ˚C (Petit and Vézina 2014). The latter study also quantified variation in M_{sum} and reported the reaction norm to be approximately linear between upper and lower limits (Petit and Vézina 2014). Moreover, flexibility in BMR in response to thermal acclimation in a mammal, *Phodopus sungorus*, varies among and within individuals between seasons (i.e., summer *vs* winter; Boratyński et al. 2016, 2017), demonstrating that the scope of thermoregulatory reaction norms are not necessarily fixed in individuals.

The lack of variation of any thermoregulatory variables (EWL, RMR, EHL/MHP or T_{b}) among sparrow-weaver populations following acclimation suggest the shapes of reaction norms for heat tolerance and evaporative cooling capacities are similar across this species' range. Previous studies have reported lower EWL and RMR at thermoneutrality in arid-zone species compared to mesic counterparts (Williams 1996; Tieleman et al. 2002, 2003b), and some studies suggest these patterns also exist at the intraspecific level (MacMillen and Hinds 1998; Sabat et al. 2006; Noakes et al. 2016). The absence of interpopulation variation in RMR and EWL of sparrow-weavers after acclimation highlights that these patterns can arise from phenotypic flexibility, although common-garden experiments have demonstrated a genotypic basis for metabolic differences among populations of *Saxicola torquata* (Wikelski et al. 2003) and great tits *Parus major* (Broggi et al. 2005). Moreover, variation in EWL between populations of a kangaroo rat, *Dipodomys merriami*, has been argued to arise from a combination of genotypic adaptation, developmental plasticity and acclimatization (Tracy and Walsberg 2000, 2001), and interpopulation variation in the T_{b} of another rodent, *Rattus fuscipes,* is determined by an interaction between phylogenetic relatedness and climate (Glanville et al. 2012). Collectively, these studies reveal that patterns of thermoregulatory variation in endotherms can be the result of either phenotypic plasticity, genotypic adaptation, or a combination thereof.

It has been hypothesized that birds from more temporally heterogenous and unpredictable environments have greater flexibility in their thermal physiology, and that this pattern could also exist at the intraspecific level (Schlichting and Pigliucci 1998; Tieleman et al. 2003a; Cavieres and Sabat 2008). The capture site near Askham in the Kalahari Desert experiences the greatest annual variation in climatic variables compared to the other capture sites in the current study (e.g., in terms of rainfall and seasonal T_a extremes; table 1), but I found no variation in the shape of thermoregulatory reaction norms among populations in the current study. It is possible that the reaction norms of populations could vary in their upper limit or in the rate at which birds adjust their physiology to different T_a (McKechnie 2008). Annual rainfall at Askham is unpredictable with some years having little to no rain, and thus sparrow-weavers from this population would benefit from being able to rapidly adjust their physiology to conserve water during particularly hot, dry summers.

In conclusion, my results reiterate the importance of considering adaptive thermoregulation when predicting the response of birds to anthropogenic climate change (Williams et al. 2008; Angilletta et al. 2010; Boyles et al. 2011). I demonstrated phenotypic flexibility in the ability of a passerine bird to handle high T_a, suggesting that individuals (at least those from cooler sites) will be able to acclimatize to rising T_a during their lifetime (Boyles et al. 2011). However, the capacity for physiological adjustments will ultimately be constrained by biophysical parameters and physiological limits, meaning that there are likely upper limits to birds' capacity to increase their heat tolerance and evaporative cooling capacity (Boyles et al. 2011; Oswald and Arnold 2012). It may be that birds living in hot, desert environments are already close to these upper physiological limits. I found no fixed interpopulation differences in the heat tolerance of sparrow-weavers from sites along a climatic gradient, supporting the idea that climate change is likely to occur at a pace too rapid for species to undergo genotypic adaptation to cope with higher T_a (Boyles et al. 2011).

4.6 Literature cited

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CHAPTER 5:

General discussion and conclusions

This thesis adds to the growing evidence for considerable variation in the avian thermal physiology of a typical Afrotropical passerine bird, the white-browed sparrow-weaver (*Plocepasser mahali*; Smit and McKechnie 2010, 2015; Smit et al. 2013; Noakes et al. 2016, 2017). The magnitude and direction of seasonal acclimatization of basal metabolic rate (BMR) and summit metabolism (M_{sum}; Noakes et al. 2017), as well as heat tolerance and evaporative cooling capacity (Noakes et al. 2016), has been reported to vary among sparrow-weaver populations across climatic gradients of ~ 8 °C and 11 °C in mean daily winter minimum and summer maximum air temperatures (T_{a}) respectively. However, these studies gave no information on the origin of these interpopulation differences, particularly whether they result from phenotypic flexibility (reversible changes within individuals, e.g., acclimatization), development plasticity (ontogenic variation that becomes fixed on reaching maturity) or genotypic adaptation (hard-wired differences in response to different selection pressures; Piersma and Drent 2003; Angilletta et al. 2010).

I found considerable variation in the magnitude and direction of seasonal acclimatization of BMR and M_{sum} over a four-year period within arid-zone populations of sparrow-weavers and scaly-feathered weavers (*Sporopipes squamifrons*; Noakes and McKechnie in press, chapter 1). I also quantified seasonal changes in the body composition of sparrow-weavers in relation to metabolic variation, and found a winter increase in the mass of the pectoral muscle and heart was unexpectedly associated with a winter decrease in maximum thermogenic capacity (i.e., M_{sum}; chapter 2). I used short-term thermal acclimation experiments to investigate whether flexibility in BMR and M_{sum}, as well as heat tolerance and evaporative cooling capacity, varies among sparrow-weaver populations (chapter 3, 4). The reaction norms for BMR and the thermoregulatory responses of birds at high T_a did not vary among populations, and M_{sum} did not vary among acclimation temperature (T_{accl}) treatments. The data reveal considerable flexibility in the thermal physiology of Afrotropical birds, and suggest previously documented interpopulation differences in sparrow-weaver thermoregulation is primarily the result of acclimatization to local conditions.

5.1 Phenotypic flexibility within populations

I found considerable interannual variation in the magnitude and direction of seasonal acclimatization of BMR and M_{sum} in two Afrotropical passerine species (Noakes and McKechnie in press, chapter 1). In particular, there was considerable flexibility in the BMR of sparrow-weavers (~ 20 % lower to 68 % higher in winter compared to summer; Noakes and McKechnie in press, chapter 1), comparable to the range of variability in seasonal patterns across all subtropical species for which data is available (35 % lower to 60 % higher in winter; McKechnie et al. 2015). Moreover, the winter BMR increase of 68 % relative to summer values exceeds by a small margin the greatest seasonal metabolic change previously reported in a field-acclimatized bird, 64 % higher BMR during winter in temperate-zone *Passer domesticus* (Wisconsin USA; Arens and Cooper 2005). Such metabolic flexibility in a subtropical bird supports the idea that the milder winters of lower latitudes allow for greater variation in patterns of seasonal acclimatization (Noakes et al. 2017).

In contrast to the pattern characteristic of higher-latitude birds (Swanson 2010; McKechnie et al. 2015), M_{sum} was never significantly higher in winter compared to summer in sparrow-weavers (no seasonal change to 38 % lower in winter) nor scaly-feathered weavers (no significant seasonal variation; Noakes and McKechnie in press, chapter 1). Higher M_{sum} in north-temperate birds has been related to enhanced cold tolerance (Swanson 2001; Cooper 2002; Swanson and Liknes 2006), and variation in BMR and M_{sum} has been positively correlated with short-term (days to weeks) fluctuations in minimum T_a (Swanson and Olmstead 1999; Broggi et al. 2007; Petit et al. 2013; Petit and Vézina 2014). In contrast, changes in M_{sum} were never associated with adjustments in cold limit temperature (T_{CL}; helox temperature that M_{sum} was reached) in sparrow-weavers and scaly-feathered weavers, and metabolic rates never varied with minimum T_a in a manner reflecting changes in thermogenic capacity (Noakes and McKechnie in press, chapter 1). However, the BMR and M_{sum} of both species were lower in seasons with lower food abundance, which could reflect a metabolic downregulation to conserve energy (chapter 1, Noakes and McKechnie in press).

There was no significant seasonal change in the dry mass of the digestive and excretory organs of sparrow-weavers (gizzard, intestines and liver; chapter 2), and during the same year there was also no seasonal change in BMR (although there was significant BMR variation in other years; Noakes and McKechnie in press, chapter 1), which is consistent with previous studies suggesting these traits are functionally linked (Swanson 2010; Petit et al. 2014; Barceló et al. 2017). In contrast to studies on temperate-zone species (Swanson 2010; Swanson and Vézina 2015), significantly higher pectoral muscle and heart mass (~ 9 % and 18 % higher dry mass respectively) during winter was not reflected as an increase in M_{sum}, but rather a ~ 26 % decrease (chapter 2). To the best of my knowledge, this is the first study to report an increase in pectoral muscle mass with no associated increase in thermogenic capacity, a surprising observation as larger pectoral muscles should provide greater heat production (Hohtola 1982; Marsh and Dawson 1989; Milbergue et al. 2018). It has been suggested that changes in the cellular metabolic intensity of muscles are required to increase thermogenic capacity (Barceló et al. 2017), and it is possible that prerequisites at the cellular level were not met to allow a winter M_{sum} increase in sparrow-weavers. Larger pectoral muscles without a concomitant increase in thermogenic capacity further supports the idea that factors other than enhancing cold tolerance may drive patterns of metabolic variation in subtropical birds. Moreover, this supports the hypothesis that variation in avian M_{sum} can be uncoupled from variation in pectoral muscle mass (Barceló et al. 2017).

5.2 Phenotypic flexibility among populations

I found flexibility in the BMR and thermoneutral EWL of sparrow-weavers, as well as heat tolerance and evaporative cooling capacity, in response to thermal acclimation (chapters 3, 4). There were similar reaction norms among sparrow-weaver populations for BMR and thermoregulatory responses at high T_a (38 °C $\leq T_a \leq$ 54 °C), suggesting that previously documented interpopulation differences in these traits are the result of phenotypic flexibility (i.e., acclimatization to local climates; Noakes et al. 2016, 2017). The only interpopulation difference that persisted after acclimation, was higher M_{sum} in sparrow-weavers from a desert site with cool winters (Askham), however, the functional significance of this pattern is unclear as it was not associated with lower T_{CL} in Askham birds (chapter 3). Moreover, this interpopulation difference could be the result of genotypic adaptation, developmental plasticity or an epigenetic effect.

As reported in previous acclimation studies on lower-latitude birds (Williams and Tieleman 2000; Tieleman et al. 2003; Cavieres and Sabat 2008), BMR and EWL were lower in birds from the hottest acclimation treatment (T_{accl} = 35 °C) compared to cooler treatments (~ 12 % and 25 % lower respectively; chapter 3). In contrast to temperate-zone species (e.g., Barceló et al. 2017; Vézina et al. 2017; Milbergue et al. 2018), M_{sum} did not vary with T_{accl}. Moreover, the greater metabolic flexibility in field-acclimatized birds among seasons (Noakes et al. 2017; Noakes and McKechnie in press, chapter 1) in comparison to flexibility in response to thermal acclimation (chapter 3), highlights the potential importance of environmental factors other than minimum T_a in driving metabolic variation in this subtropical species (e.g., food availability). To the best of my knowledge, no previous acclimation studies have investigated flexibility in M_{sum}, nor metabolic values in response to $T_{\text{accl}} < 10$ °C, in lower-latitude birds.

Most previous acclimation studies have only included two T_{accl} treatments, giving little information on the shape of avian metabolic reaction norms (McKechnie 2008). The reaction norm for BMR has been reported as approximately linear in *Streptopelia senegalensis* (across $T_{\text{accl}} = 10$ °C, 22 °C and 35 °C; McKechnie et al. 2007), as well as in field-acclimatized *Poecile atricapillus* (minimum T_a ranging from -20 ˚C to 30 ˚C; Petit and Vézina 2014). The reaction norm for BMR in sparrow-weavers does not appear to be exactly linear, as BMR was similar in birds from $T_{\text{accl}} = 5$ ˚C and 20 ˚C, but lower in individuals from $T_{\text{accl}} = 35$ ˚C (chapter 3). The reaction norm for M_{sum} in *Po. atricapillus* was approximately linear between lower and upper limits (i.e., -10 °C and 24 °C respectively; Petit and Vézina 2014), whereas the M_{sum} of sparrow-weavers did not vary with T_{accl} between 5 ˚C and 35 ˚C (chapter 3).

The acclimation experiment investigating flexibility in thermoregulatory responses at high T_a, found that sparrow-weaver from the hottest treatment ($T_{\text{accl}} = 42$ °C) were more heat tolerant than those from milder treatments ($T_{\text{accl}} = 30$ °C and 36 °C), reaching ~ 2 °C higher T_a before the onset of severe hyperthermia and maintaining significantly lower T_b at $T_a \geq 38$ °C (Noakes and McKechnie 2019, chapter 4). However, there was no significant variation in RMR or EWL among T_{accl} treatments, which contrasts with seasonal acclimatization in a desert population where sparrow-weavers had higher heat tolerance, and lower T_b, EWL and RMR at $T_a \geq 40$ °C, during summer compared to winter (Noakes et al. 2016). It is not clear how sparrow-weavers at $T_{\text{accl}} = 42$ °C maintained lower T_b without adjusting EWL or RMR. Lower T_b in heat-acclimated individuals has been reported in two columbid species, however, this was associated with enhanced evaporative cooling efficiency via adjustments in the partitioning of EWL into cutaneous and respiratory avenues (*Columba livia* at $T_a > 35$ °C:

Marder and Arieli 1988; *Zenaida asiatica mearnsii* at T_a = 45 °C: McKechnie and Wolf 2004). To the best of my knowledge, this is the first study to demonstrate plasticity in heat tolerance in response to short-term thermal acclimation in a passerine bird.

5.3 Global variation in avian thermal physiology

The data I have presented here add to the growing evidence of considerable flexibility in avian thermal physiology, and supports the idea of considerable variation in seasonal metabolic acclimatization in birds at lower latitudes (McKechnie et al. 2015). This does not necessarily imply lower-latitude birds have an inherently greater physiological flexibility than their higher-latitude counterparts, but rather that extreme winter T_a at higher latitudes overwhelm other environmental factors that could drive patterns of seasonal metabolic variation (Noakes et al. 2017). It has been proposed that the magnitude and direction of seasonal metabolic adjustments is determined by a continuum between enhanced cold tolerance and energy conservation, associated with fluctuations in T_a and food availability respectively (Smit and McKechnie 2010). My results for sparrow-weavers and scaly-feathered weavers partly support this idea, as BMR and M_{sum} were consistently lower during seasons of lower food abundance, however, metabolic variation was never related to minimum T_a in a manner reflecting enhanced cold tolerance (Noakes and McKechnie in press, chapter 1). There was substantial variation in food abundance over the study period but less variation in daily minimum T_a among winters (~ 1.4 °C range in mean values), and thus food availability may be a more limiting factor during the milder winters of the Kalahari Desert.

It has been hypothesized that birds from environments with higher variability and/or unpredictability in climates should have greater physiological flexibility (Schlichting and Pigliucci 1998; Tieleman et al. 2003; Cavieres and Sabat 2008). I therefore expected greater flexibility in Askham sparrow-weavers in response to thermal acclimation on account of considerable seasonality in T_a and less predictable rainfall at this desert site, but found similar thermoregulatory reaction norms among populations across the range of T_{accl} measured (chapter 3, 4). It is possible that reaction norms vary in a manner outside the scope of my experiments, such as in the upper/lower limits or the rate at which individuals adjust their physiology (McKechnie 2008). Moreover, reactions norms are not necessarily fixed in individuals, for example, flexibility in BMR varies among and within individuals between seasons in a mammal, *Phodopus sungorus* (Boratyński et al. 2016, 2017).

The absence of interpopulation variation in the thermoregulatory responses of sparrow-weavers after acclimation (except for the higher M_{sum} in Askham birds; Chapter 3, 4), suggest previously reported interpopulation differences in seasonal acclimatization are largely the result of phenotypic flexibility (Noakes et al. 2016, 2017). This highlights the importance of considering phenotypic flexibility in studies comparing physiological differences among birds, although previous studies have demonstrated a genotypic basis for metabolic differences among populations of *Saxicola torquata* and *Parus major* (Wikelski et al. 2003; Broggi et al. 2005). Moreover, interpopulation variation in two rodent species has been argued to arise from a combination of factors, with EWL differences in *Dipodomys merriami* arising from genotypic adaptation, developmental plasticity and acclimatization (Tracy and Walsberg 2000, 2001), and T_b differences of *Rattus fuscipes* from both phylogenetic relatedness and climate (Glanville et al. 2012). Collectively, these studies reveal that patterns of thermoregulatory variation in endotherms can be the result of phenotypic plasticity, genotypic adaptation, or a combination thereof.

The increasing evidence for adaptive thermoregulation suggest that endotherms may be able to adjust their physiology to cope with changing climates (Williams et al. 2008; Angilletta et al. 2010; Boyles et al. 2011; Smit et al. 2013). This challenges the assumptions of the majority of climate envelope and correlative models predicting species' responses to anthropogenic climate change, which typically assume endotherms occur in a fixed climate space (Boyles et al. 2011; Pearson and Dawson 2013; Milne et al. 2015). I found considerable phenotypic flexibility in the thermal physiology of sparrow-weavers, suggesting individuals will be able to acclimatize to changing climates during their lifetime (Boyles et al. 2011). However, these adjustments will ultimately be constrained by biophysical parameters and physiological limits (Boyles et al. 2011; Oswald and Arnold 2012), and it is possible that birds living in hot, desert environments may already be close to the upper limits of their ability to increase heat tolerance and evaporative cooling capacity.

In conclusion, I found considerable phenotypic flexibility in the thermal physiology of Afrotropical passerine birds, focusing on sparrow-weavers as a model species. Interannual variation in seasonal metabolic acclimatization supports the idea of greater metabolic variability in birds inhabiting lower latitudes (Noakes and McKechnie in press, chapter 1; McKechnie et al. 2015). In contrast to temperate-zone species, metabolic variation was not related to minimum T_a or enhanced cold tolerance, but food abundance seems to be an important driver of metabolic adjustments in subtropical birds (Noakes and McKechnie in press, chapter 1). I found similar thermoregulatory reaction norms among populations of sparrow-weavers across a climatic gradient (chapter 3, 4), suggesting previously reported interpopulation differences in this species are primarily the result of phenotypic flexibility rather than genotypic adaptation or developmental plasticity (Noakes et al. 2016, 2017). More studies are required to better understand how physiological flexibility varies with latitude in birds, and what these patterns mean for endotherms faced with changing climates.

5.4 Literature cited

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