



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

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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. We 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*). We also measured pectoral muscle thickness using a portable ultrasound system to determine whether we could non-lethally estimate muscle size. A concurrent study measured seasonal changes in BMR and M_{sum} in the same population of sparrow-weavers, but different individuals. 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. We 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 our knowledge, this is the first study to report an increase in avian pectoral muscle mass but a concomitant decrease in thermogenic capacity.

Keywords Phenotypic flexibility · Pectoral muscle · Organs · Acclimatization · Summit metabolism · Basal metabolic rate · Subtropical birds

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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 a non-reproductive, non-growing individual, and primarily 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/or liver; Williams and Tieleman 2000; Swanson 2010; Zheng et al. 2014b), 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 pectoral muscle mass and increased support costs of the skeletal muscles involved in shivering thermogenesis (Chappell et al. 1999; Vézina et al. 2017; Milbergue et al. 2018).

Avian M_{sum} represents the ceiling of an individual's thermogenic capacity, measured as the maximum resting metabolic rate during acute 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, b; 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 hot 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 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 in the mass of digestive and excretory organs has been reported for two subtropical passerines in China (Zheng et al. 2014a, b). To the best of our 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 muscle 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). As far as we are aware, 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). In contrast, this method did not discriminate between the larger pectoral muscles of 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, we 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). We 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 (i.e., different individuals to those in the present study; Noakes and McKechnie 2020), allowing us to compare patterns of metabolic variation with body composition changes at the population, but not individual, level. We predicted that seasonal variation in BMR and M_{sum} is associated with parallel changes in organ and muscle masses as commonly observed in temperate species. Specifically, we expected that higher BMR is 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} is associated with increases in the mass of pectoral muscles and potentially the heart (Swanson 2010; Swanson and Vézina 2015).

Materials and methods

Study species and site

We 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, $n = 10$ per season; Table 1; Noakes and McKechnie 2020). We 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 m³), and all body composition data were collected during the following day (< 24 h after capture). The natural diet of sparrow-weavers includes insects and grass seeds (~30% insects, 70% seeds; Ferguson 1988; du Plessis 2005), and we provided captive birds with water, mealworms and a wild bird seed mix. To avoid trapping reproductive individuals, we did not catch sparrow-weavers from breeding nests or over the peak egg-laying period (November–December; du Plessis 2005). Individual sparrow-weavers were sexed according to the colour of their beaks (total $n = 63$, 31 males and 32 females; du Plessis 2005).

Ultrasound measurements of pectoral muscle thickness

We validated the use of portable ultrasonography to estimate the mass of pectoral muscles of sparrow-weavers. The M_p 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). We 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, we took measurements of only the left pectoral muscle to reduce handling time. During the first season (summer), two observers worked together to take measurements from each individual, 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. We parted the breast feathers and applied ultrasound transmission gel (NCCB-0234, Ningbo Medelast Co, Ltd, Ningbo, China) over the left pectoral muscle. We 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. We 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.

Table 1 Seasonal variation in the basal metabolic rate, summit metabolism and cold limit temperature (mean \pm standard deviation) of a southern African arid-zone passerine bird, the white-browed sparrow-weaver (*Plocepasser mahali*; $n = 10$ per summer and winter 2016, different individuals from the same population as the present study)

Category	Variable	Summer	Winter
<i>Plocepasser mahali</i>	Basal metabolic rate (W)	0.60 \pm 0.09	0.60 \pm 0.07
	Summit metabolism (W)	4.55 \pm 0.70*	3.35 \pm 0.42*
	Cold limit temperature (°C)	- 2.52 \pm 3.82	- 3.74 \pm 5.21
Climatic variables	Minimum temperature (°C)	21.4 \pm 3.3	1.4 \pm 4.0
	Maximum temperature (°C)	39.0 \pm 3.9	24.5 \pm 3.7
	Total rainfall (mm)	70	0
	Total insect abundance	6509	13,101
Food availability	Grass cover (%)	89.8	75.4

Statistical results are from linear models fitted to data, and asterisks denote significant seasonal variation ($p < 0.05$). There was also seasonal variation in weather variables at the study site: mean \pm standard deviation values of the daily minimum and maximum temperatures, and total rainfall, and fluctuations in food availability: total insect abundance and grass seed abundance (estimated using percentage grass cover). All data obtained for 2016 from Noakes and McKechnie (2020), with cold limit temperature as defined by those authors

1999; Swanson et al. 2013; Swanson and Merkord 2013). We obtained three thickness measurements per individual, and used the average value for further analyses.

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, we euthanized birds via cervical dislocation and measured the total M_b of the carcass. We then excised the liver, intestines (from gizzard to vent, i.e., gut tube), gizzard, heart and both pectoral muscles still attached to the keel (hereafter, pectoral muscles-keel complex), removing all attached mesenteries and fat deposits, and rinsed and temporarily stored samples in saline solution, while dissection continued. We removed the intestines with the pancreas still attached, and thus mass values includes the pancreas. We also measured the length of the intestines (to the nearest 0.01 mm), but have these measurements for only a few individuals in the first season of data collection (summer: $n = 5$, winter: $n = 15$). We 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, we measured the wet mass of the organs and pectoral muscles-keel complex after blotting each dry with absorbent paper. After completion of measurements, we wrapped all tissues in tin foil, stored samples in resealable plastic bags along with the remaining carcass, and froze samples at $-18\text{ }^\circ\text{C}$ until dry mass measurements were performed. Prior to dry mass measurements, we defrosted the pectoral muscles-keel complex and dissected the left pectoral muscle along with the attached supracoracoideus muscle, and the reported wet and dry pectoral mass includes both muscles.

To measure the dry mass of organs and the left pectoral muscle of sparrow-weavers, we 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 $55\text{ }^\circ\text{C}$. We removed samples from the oven once a day to measure mass, and continued measurements until we obtained the same mass for three consecutive days. The final mass measured was considered the dry mass of each organ and pectoral muscle.

Data analyses

All values are presented as mean \pm standard deviation. We used the “lm” base function in R 3.5.2 (R Core Team 2018) to fit linear models to our 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. We 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 our response variables, by selecting models with the lowest second-order Akaike Information Criterion (AICc) values. If multiple top models had AICc values that differed by < 2 , we chose the most parsimonious model.

We 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. We 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. We 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). We 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). We estimated the repeatability of ultrasound measurements (using the three measurements per individual) by calculating intraclass correlation coefficients using the “ICCest” function from the ICC package (Wolak et al. 2012).

Results

The top model explaining variation in the M_b of sparrow-weavers included only sex, with M_b being significantly higher in males ($41.9 \pm 3.0\text{ g}$) compared to females ($38.6 \pm 1.9\text{ g}$; $F_{1,26} = 10.928$, $p = 0.003$). 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). There was no seasonal variation in M_b , suggesting that adjustments in M_b were not driving seasonal changes in organ and muscles masses (Table 3).

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).

Table 2 Statistical results (F value, p value, degrees of freedom [df]) are from the top linear models fitted to data of seasonal variation (summer vs winter) in the dry and wet mass of the gizzard, intestines (including pancreas), liver, heart, and left pectoral muscle (including supracoracoideus muscle) of a southern African arid-zone passerine bird, the white-browed sparrow-weaver (*Plocepasser mahali*; $n = 15$ per season)

Category	Response variable	Predictor variables					
		Body mass			Season		
		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			

Predictor variables included body mass and/or season (no statistical results indicates a predictor variable was not included in the top model). 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 Mean \pm standard deviation values of seasonal variation (summer vs winter) in the body mass, and wet and dry masses of the gizzard, intestines (including pancreas), liver, heart, and left pectoral

muscle (including supracoracoideus muscle) of a southern African arid-zone passerine bird, the white-browed sparrow-weaver (*Plocepasser mahali*; $n = 15$ per season)

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

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 ($p < 0.05$)

However, contrasting patterns of significant seasonal variation were apparent for wet masses of the digestive organs, with $\sim 17\%$ higher gizzard mass and $\sim 12\%$ lower intestines mass in winter compared to summer (Tables 2, 3). We 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 ($\sim 7\%$ and 9% higher, respectively) and heart ($\sim 8\%$ and 18% higher, respectively) were significantly heavier (both wet and dry mass) during winter compared to summer (Tables 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 (Fig. 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

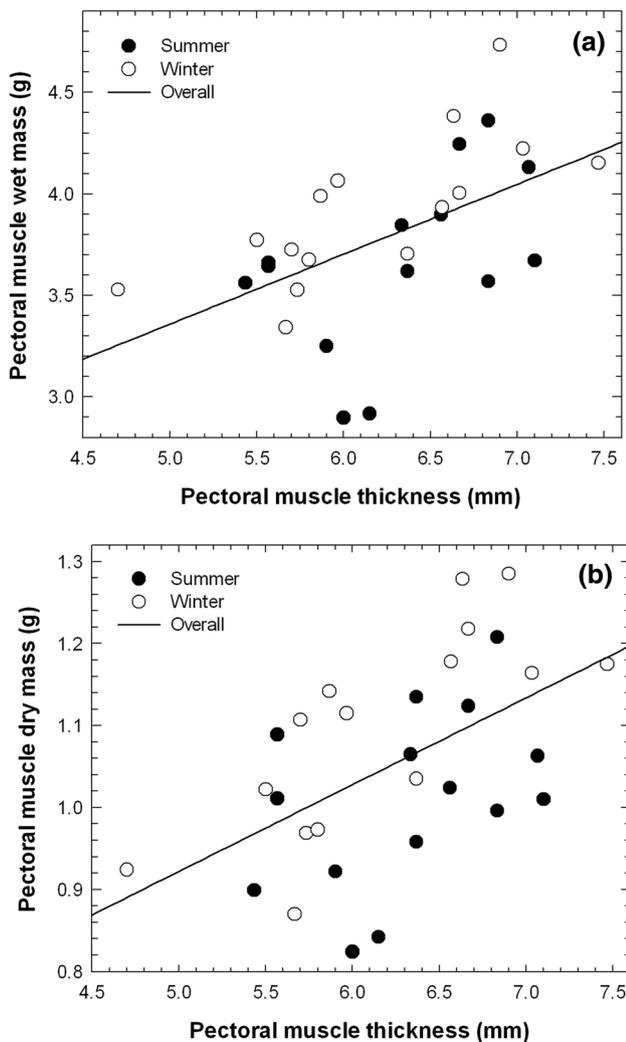


Fig. 1 Pectoral muscle thickness measured using ultrasonography emerged as a weak predictor of the pectoral muscle mass of white-browed sparrow-weavers (*Plocepasser mahali*). Data were collected during both summer and winter, and data from both seasons are presented together ($n=15$ per season). Solid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

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 (Fig. 1; Tables 2, 3). The repeatability of pectoral muscle thickness, calculated for the three ultrasound measurements per individual, was relatively high for both seasons combined (0.74), but lower in summer (0.68) compared to winter (0.80).

Discussion

Our 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 in a concurrent study: Noakes and McKechnie 2020). 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). We 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 of birds from the same population (i.e., M_{sum} ; Table 1; Noakes and McKechnie 2020), 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 our 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 our predictions and the results of previous studies reporting correlations between these traits in birds (Williams and Tieleman 2000; Swanson 2010). The direction and magnitude of seasonal acclimatization of BMR vary interannually in the same Kalahari Desert population of sparrow-weavers (from $\sim 20\%$ lower to 68% higher in winter over a 4-year period; Noakes and McKechnie 2020), and presumably variation in organ mass would mirror patterns of BMR variation as documented in other subtropical species (e.g., Chappell et al. 1999; Cavieres and Sabat 2008; Zheng et al. 2014b). 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 ($\sim 43\%$, 40% , 43% and 37% higher in winter, respectively; Williams and Tieleman 2000).

We found contrasting patterns of seasonal variation in the wet masses of the intestines and gizzard ($\sim 12\%$ higher and 16% lower in winter, respectively; Table 3), and contrasting patterns in the small intestine and gizzard mass were also reported in *P. sinensis* from Wenzhou, China ($\sim 43\%$ 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

Table 4 Statistical results from models fitted to investigate the relationship between pectoral muscle thickness (measured using ultrasonography), and the actual pectoral muscle mass (wet and dry) in a southern African arid-zone passerine bird, the white-browed sparrow-weaver (*Plocepasser mahali*; $n = 15$ per season)

Response variable	Season	Predictor variable: pectoral muscle thickness					
		<i>F</i> value	<i>df</i>	<i>p</i> value	r^2	<i>m</i>	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

Data were collected during the summer and winter, and linear regression models were fitted to data for both seasons together, and then for each season 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

is likely more relevant than changes in wet masses in the context of BMR variation (Table 1; Noakes and McKechnie 2020).

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 thermoregulatory 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 et al. 2017). This notion is supported by data for sparrow-weavers: summer and winter BMR decreased with decreasing food abundance over a 4-year period (Noakes and McKechnie 2020), 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). However, this implies that changes in the rate of food consumption of sparrow-weavers is driven by energy supply (i.e., food availability), whereas changes in temperate-zone birds are driven by energy demand (i.e., thermogenic costs).

Our data reveal significantly higher pectoral muscle and heart mass in winter compared to summer, but in contrast to our predictions this was not reflected in a winter increase in M_{sum} of sparrow-weavers, but rather a ~26% decrease (Noakes and McKechnie 2020). As far as we are aware, 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 (~12–18% higher; e.g., Cooper 2002; Liknes and

Swanson 2011; Vézina et al. 2017). This pattern is surprising considering that larger pectoral muscles are presumed to provide higher shivering capacity and thus 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 significant winter increase in M_{sum} over a 4-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 2020). Winters in the Kalahari Desert are relatively mild compared to higher latitudes (mean winter minimum $T_a > 0$ °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 2020). 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 2020). 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 (Fig. 1), but correlation coefficients for these relationships were low (Table 4) compared to most previous studies (typically: $r^2 = 0.66$ – 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; Fig. 1; Tables 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 *P. domesticus*. 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 our sample sizes (summer: $n = 32$, winter: $n = 31$) were greater than those of Swanson and Merkord (2013; summer: $n = 13$, winter: $n = 17$). The repeatability value of our ultrasound measurements of pectoral muscle thickness (0.74 for both seasons combined) were similar to/higher than those reported in previous studies (range 0.29–0.96), supporting the notion that high repeatability does not guarantee strong predictive equations for muscle mass (reviewed by Royer-Boutin et al. 2015).

Before modern advances 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, $M_b \approx 120$ – $10,000$ g, $r^2 = 0.70$ – 0.92 ; Sears 1988; Dietz et al. 1999; Battley et al. 2004). Our 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 *P. 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 ($r^2 = 0.02$ – 0.76) from measurements on *P. atricapillus* ($M_b \approx 11$ g, 12 MHz frequency), varying with the life-state of birds (alive or dead) and the observer conducting measurements. Our 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, we found no seasonal variation in the dry mass of digestive and excretory organs or the BMR of sparrow-weavers (Noakes and McKechnie 2020), 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 2020), 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 our 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 we are 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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