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Seasonal phenotypic flexibility in body mass, basal thermogenesis, and tissue oxidative capacity in the male Silky Starling (*Sturnus sericeus*)

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Abstract

Background: Acclimatization to winter conditions is an essential prerequisite for the survival of small birds in the northern temperate zone. Changes in photoperiod, ambient temperature and food availability trigger seasonal physiological and behavioral acclimatization in many passerines. Seasonal trends in metabolic parameters are well known in avian populations from temperate environments; however, the physiological and biochemical mechanisms underlying these trends are incompletely understood. In this study, we used an integrative approach to measure variation in the thermogenic properties of the male Silky Starling (*Sturnus sericeus*) at different levels or organization, from the whole organism to the biochemical. We measured body mass (M_b), basal metabolic rate (BMR), energy budget, the mass of selected internal organs, state 4 respiration and cytochrome c oxidase (COX) activity in the heart, liver and muscle.

Methods: Oxygen consumption was measured using an open-circuit respirometry system. The energy intake of the birds were then determined using an oxygen bomb calorimeter. Mitochondrial state 4 respiration and COX activity in heart, liver and pectoral muscle were measured with a Clark electrode.

Results: The results suggest that acclimatization to winter conditions caused significant change in each of the measured variables, specifically, increases in M_b, organ mass, BMR, energy intake and cellular enzyme activity. Furthermore, BMR was positively correlated with body mass, energy intake, the mass of selected internal organs, state 4 respiration in the heart, liver and muscle, and COX activity in the heart and muscle.

Conclusions: These results suggest that the male Silky Starling's enhanced basal thermogenesis under winter conditions is achieved by making a suite of adjustments from the whole organism to the biochemical level, and provide further evidence to support the notion that small birds have high phenotypic plasticity with respect to seasonal changes.

Keywords: Basal metabolic rate (BMR), Cytochrome c oxidase (COX), Organ mass, Seasonal variation, *Sturnus sericeus*, State 4 respiration

Background

The thermogenic properties of animals are closely related to energy utilization (Webster and Weathers 2000; Vézina et al. 2006, 2007), energy distribution (Dawson and Marsh 1986), life-history strategy and evolutionary history (Diamond 1998; Wiersma et al. 2007; Swanson 2010). Furthermore, the thermogenesis of birds reflects their physiological capacity to adapt to environmental change (Swanson 1991a; McKechnie 2008; Zheng et al. 2013a, 2014a). Many investigators have studied the relationship between survival and the thermoregulation in birds

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and small mammals in tropical and temperate regions (Weathers 1979; Wikelski et al. 2003; Wiersma et al. 2007; Zheng et al. 2008a; Smit and McKechnie 2010; Swanson 2010; Nilsson and Nilsson 2016; Petit et al. 2017). In temperate regions, cold temperatures and reduced food availability affect the energy budgets of winter-active birds. In response, birds have evolved a range of morphological, physiological and behavioral mechanisms to enable them to adapt to the greater seasonal variation in climate experienced in temperate regions (Swanson 1990, 1991b; Zheng et al. 2008b, 2014b). Phenotypic flexibility refers to phenotypic changes that are reversible, temporary, and repeatable (Piersma and Drent 2003; Starck and Rahmaan 2003; Tieleman et al. 2003; McKechnie 2008). The study of phenotypic flexibility has become a central topic in evolutionary ecology and resident endothermic animals in temperate climates are ideal subjects for associated natural experiments (McKechnie et al. 2006; Liknes and Swanson 2011; Swanson et al. 2014). One example of phenotypic flexibility is the seasonal phenotypes developed by some small birds in response to seasonal climatic variation (Swanson 2010). Many small birds inhabiting seasonal environments increase body mass with winter acclimatization (McKechnie and Swanson 2010). The winter increase in body mass could result from increases in fat deposits and/or lean mass and thereby influence thermogenic demands (Cooper 2007; Zheng et al. 2014a). The winter phenotype of such birds is characterized by improved cold tolerance and enhanced capacity for thermogenesis, increases in both basal metabolic rate (BMR; minimum metabolic rate) and summit metabolic rate (M_{sum}; maximal thermoregulatory metabolic rate) (Swanson 1990, 1991a; McKechnie and Wolf 2004; Swanson and Garland 2009; McKechnie and Swanson 2010). BMR, a widely-accepted benchmark of metabolic expenditure in endotherms, is correlated with the energetic cost of thermoregulation and (McKechnie and Wolf 2004; McNab 2009). BMR is thought to increase in winter because of the higher metabolic cost of supporting thermogenic tissues (McKechnie 2008; Zheng et al. 2013a, 2014a; Swanson et al. 2017), whereas elevated M_{sum} is directly correlated with improved cold tolerance in winter birds (Swanson 2001; Swanson and Liknes 2006).

Thermogenesis, or thermoregulation, in small birds is an example of a flexible trait that can be seasonally modulated through adjustments at several levels of organization (McKechnie 2008; Swanson 2010; Petit et al. 2014). At the organismal level, winter acclimatized birds, often, but not always, have a higher BMR than summer acclimatized birds (Swanson 1990, 1991a; Zheng et al. 2008a, 2014a). At the organ level, alternation in the size, or mass, of metabolically active tissues and organs, such as the

liver, kidneys, digestive tract, or muscles, can influence BMR (Brand et al. 2003; Zheng et al. 2008b, 2014a; Liknes and Swanson 2011). At the biochemical level, changes in catabolic enzyme activity, and or, the capacity for metabolic substrate transport, can influence the mass-specific metabolic activity of organs, thereby affecting organismal metabolic rates (Swanson 1991b; Liu et al. 2008; Zheng et al. 2008b, 2014a; Liknes and Swanson 2011). Variation in cellular metabolic intensity is often measured by examining variation in state 4 respiration (reflecting oxidative phosphorylation capacity) or cytochrome c oxidase (COX) activity (a key regulatory enzyme of oxidative phosphorylation) (Zheng et al. 2008b, 2014a; Swanson 2010; Zhou et al. 2016; Hu et al. 2017). Seasonal changes in thermogenesis at the organismal level have been well documented in several species under natural conditions (Dawson and Carey 1976; Southwick 1980; Liknes and Swanson 1996; Smit and McKechnie 2010; Zheng et al. 2008b, 2014a; Wu et al. 2015). For instance, body mass and BMR in Chinese Bulbuls (*Pycnonotus sinensis*) were higher in spring and winter than in summer. The dry masses of several nutritional organs and mitochondrial protein content, state 4 respiration, and COX activity in liver and muscle were all heightened in winter relative to other seasons, and this enhanced performance has been linked to regulatory changes in nutritional and exercise organ masses and liver and muscle cellular aerobic capacity interact to promote seasonal metabolic flexibility in Chinese Bulbuls (Zheng et al. 2008b). Similarly, Eurasian Tree Sparrows (*Passer montanus*) living at high latitude exhibit greater thermogenic capacities compared with their low latitude counterparts. Consistently, state 4 respiration and COX activity in liver and muscle were remarkably higher with latitude. These results are consistent with a pronounced latitudinal phenotypic flexibility mediated through physiological and biochemical adjustments in Eurasian Tree Sparrows (Zheng et al. 2014b). Because changes in thermogenic regulation allow the organisms to rapidly and reversibly respond to environmental variation, the study in thermoregulation can provide insights into the mechanistic underpinnings of phenotypic flexibility and the adaptive modification of complex physiological traits, the hierarchical levels of physiological traits is well illustrated of physiological adjustment that enhance thermogenic performance (Swanson 2010; Swanson et al. 2017; Maria et al. 2015; Zhou et al. 2016).

The Silky Starling (*Sturnus sericeus*; Passeriformes, Sturnidae) is a resident species in vast areas of southern and southeastern China, South Korea, North Vietnam and the Philippines (MacKinnon and Phillipps 2000). Its preferred habitat is broadleaf, and conifer-broadleaf mixed forest, but it can also be found in orchards and farmland. The Silky Starling is omnivorous and primarily

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feeds on insects, fruits and seeds. Studies have found that male Silky Starlings have a larger body mass than females, and a higher body temperature, higher thermal conductance, and a lower BMR with a relatively wide thermal neutral zone (Zhang et al. 2006; Zhao et al. 2013). The Silky Starling also has high metabolic water production (MWP) and evaporative water loss (EWL) (Bao et al. 2014). However, understanding seasonal changes in thermogenesis at the organismal level in this species has been hampered by the lack of research on cellular responses to seasonal change. To resolve this problem we investigated a broad range of thermogenic responses to temperature in the Silky Starling, from the cellular to the organismal level. In addition, we measured M_b, BMR and the energy budget of individual birds, as well as changes in the mass of the liver, heart, pectoral muscle and digestive tract (gizzard, small intestine and rectum). We also measured cellular level state 4 respiration and COX activity in the heart, liver, and pectoral muscle. We hypothesized that the Silky Starling would acclimatize to winter conditions by increasing M_b, BMR, energy budget and internal organs mass. At the cellular level, we predicted that winter acclimatized birds would increase their BMR by activating state 4 respiration and increasing COX activity in the heart, liver, and pectoral muscle.

Methods

Study site

This study was carried out in Wenzhou City, Zhejiang Province (27°29′N, 120°51′E, 14 m in elevation), China. Eighteen male Silky Starlings (male Silky Starling has whitish head contrasting with female) were live-trapped in forest between July 2013 and January 2014. Nine birds were caught during summer (hereafter referred to as "summer birds") and nine in winter (hereafter "winter birds"). The climate of Wenzhou is warm-temperate with an average annual rainfall of 1500 mm spread across all months, and slightly more precipitation during spring and summer. Mean ambient temperature ranged from 31.3 ± 0.2 °C in summer (July 2013) to 8.6 ± 0.4 °C in winter (January 2014). Daily minimum and maximum ambient temperatures vary greatly, ranging from 25.4 to 38.6 °C in summer, and from 0.9 to 14.8 °C in winter (Wenzhou Bureau of Meteorology). M_b to the nearest 0.1 g was determined immediately upon capture with an electronic balance (Sartorius BT25S, Germany). Birds were then transported to the laboratory and kept outdoors for 1 or 2 days in 50 cm × 30 cm × 20 cm cages under natural photoperiod and temperature before physiological measurements were taken (Zhang et al. 2006). Food and water were supplied ad libitum. All experimental procedures were approved by the Animal Care and Use Committee of the Wenzhou University (Protocol 20130012).

Measurement of metabolic rate

Birds' metabolic rates were estimated by measuring their oxygen consumption in an open-circuit respirometry system (AEI technologies S-3A/I, USA). Metabolic chambers were 1.5 L in volume, made of plastic, and provided with a perch for the bird to stand on (Smit and McKechnie 2010). Chamber temperature was regulated by a temperature-controlled cabinet (Artificial climatic engine BIC-300, China) capable of regulating temperature to ± 0.5 °C. Water vapour and CO_2 were scrubbed from the air passing through the chamber in a silica gel/soda lime/silica column before passing through the oxygen analyzer. We measured the oxygen content of excurrent gas from metabolic chambers with an oxygen sensor (AEI technologies N-22M, USA). We used a flow control system (AEI technologies R-1, USA) to set the flow of excurrent gas. The pump was located downstream of the metabolic chamber and air pulled through the chamber at 300 mL/min by the pump during metabolic rate measurements (Zheng et al. 2014a; Wu et al. 2015). This maintained a fractional concentration of O_2 in the respirometry chamber of about 20%, calibrated to $\pm 1\%$ accuracy with a general purpose thermal mass flow-meter (TSI 4100 Series, USA) (McNab 2006). Oxygen consumption rates were measured at 25 ± 0.5 °C, which is within the thermal neutral zone of the Silky Starling (Bao et al. 2014). Baseline O_2 concentrations were obtained before and after each test (Li et al. 2010; Wu et al. 2015). All measurements of gas exchange were obtained during the resting-phase of birds' circadian cycles (between 20:00 and 24:00) in darkened chambers when individual birds could reasonably be expected to be post-absorptive. Food was removed 4 h before each measurement to minimize the heat increment associated with feeding (Zhang et al. 2006; Bao et al. 2014). We first ensured that birds were perching calmly in the chamber and started recording oxygen consumption at least 1 h later. Each animal was generally in the metabolic chamber for at least 2 h. The data obtained were used to calculate 5 min running means of instantaneous oxygen consumption over the entire test period using equation 2 of Hill (1972). The lowest 5 min mean recorded over the test period was considered the resting metabolic rate (Smit and McKechnie 2010). All values for oxygen consumption were expressed as mL O_2/h and corrected to STPD conditions (Christians 1999; Schmidt-Nielsen 1997). Body temperature (T_b) was measured during metabolic measurements with a lubricated thermocouple inserted into the cloaca, the output of which was digitized by an Oakton thermocouple meter (Eutech Instruments, Singapore). M_b was measured to the nearest 0.1 g before and after experiments; mean M_b was used in calculations. All measurements were made daily between 20:00 and 24:00.

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Energy budget

The energy intake of the birds caught in summer and winter was measured over 3 days according to previously established methods (Klaassen et al. 2004; Wu et al. 2014). During each test, subjects were housed individually in cages, where food was provided in excess of the animals' needs and water was provided ad libitum. The uneaten food and feces were collected after the 3-day period, separated manually and oven-dried at 65 °C for at least 72 h. The caloric contents of the dried food and feces were then determined using a C 2000 oxygen bomb calorimeter (IKA Instrument, Germany). Gross energy intake (GEI), fecal energy (FE), digestible energy intake (DEI) and digestibility of energy were calculated according to Li and Wang (2005) and Wu et al. (2014):

$$\text{GEI (kJ/day)} = \text{dry food intake (g/day)}$$
$$\times \text{caloric value of dry food (kJ/g)}$$
$$\text{FE (kJ/day)} = \text{dry mass of feces (g/day)}$$
$$\times \text{caloric value of dry feces (kJ/g)}$$
$$\text{DEI (kJ/day)} = \text{GEI (kJ/day)} - \text{FE (kJ/day)}$$
$$\text{Digestibility (\%)} = \text{DEI (kJ/day)/GEI (kJ/day)} \times 100\%.$$

Measurements of organ masses

Birds were euthanized by cervical dislocation at the end of the experiment and their heart, liver, pectoral muscle, gizzard, small intestine and rectum extracted and weighed to the nearest 0.1 mg. Part of the heart, liver and muscle was used to investigate state 4 respiration and COX activity (Zheng et al. 2008b, 2014a), but the other internal organs, including the remainder of the heart, liver and pectoral muscle, were dried to a constant mass over 2 days at 65 °C and weighed to the nearest 0.1 mg (Williams and Tieleman 2000; Liu and Li 2006).

Measurements of tissue state 4 respiration and cytochrome c oxidase (COX) activity

State 4 respiration in the heart, liver and pectoral muscle was measured at 30 °C in 1.96 mL of respiration medium (225 mM sucrose, 50 mM Tris/HCl, 5 mM $MgCl_2$, 1 mM EDTA and 5 mM KH_2PO_4, pH 7.2) with a Clark electrode (Hansatech Instruments LTD., England, DW-1), essentially as described by Estabrook (1967). State 4 respiration was measured over a 1 h period under substrate dependent conditions, with succinate as the substrate (Zheng et al. 2008b, 2010, 2013b). COX activity in the heart, liver and pectoral muscle were measured
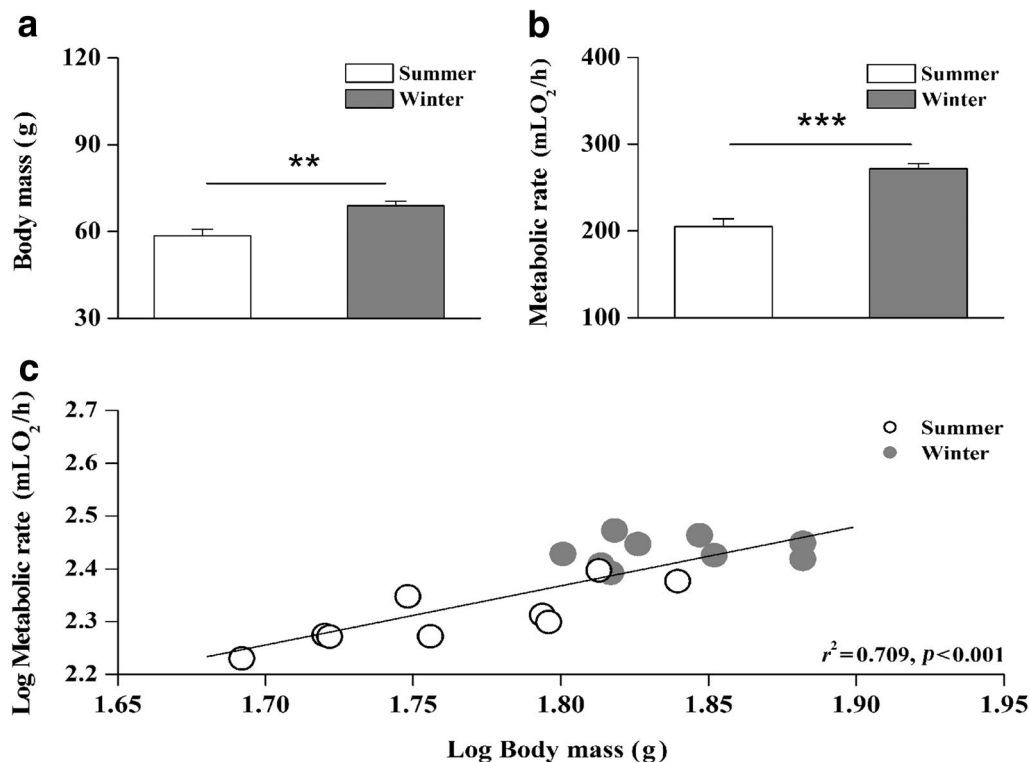
Fig. 1 Body mass (**a**), resting metabolic rate (**b**), and the correlation between body mass and basal metabolic rate (**c**), of Silky Starlings (*Sturnus sericeus*) captured in either winter or summer in Wenzhou City, China. Data are shown as mean ± SEM, **p < 0.01 and ***p < 0.001

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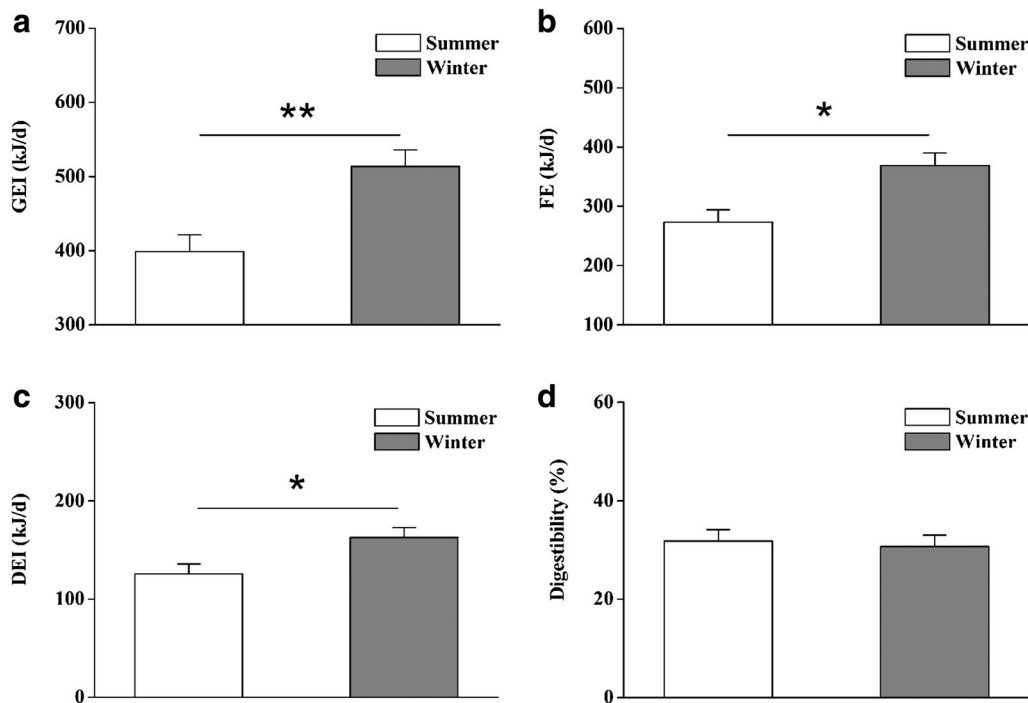
Fig. 2 Gross energy intake (**a**), fecal energy (**b**), digestible energy intake (**c**), and digestibility (**d**), of Silky Starlings (*Sturnus sericeus*) captured in either winter or summer in Wenzhou City, China. Data are shown as mean ± SEM, *$p < 0.05$ and **$p < 0.01$. *GEI* gross energy intake, *FE* fecal energy, *DEI* digestible energy intake

polarographically at 30 °C using a Clark electrode according to Sundin et al. (1987). We express state 4 respiration and COX activity measurements in terms of whole organ activity (μmol O_2/min per organ) (Wiesinger et al. 1989; Zheng et al. 2013b, 2014a; Zhou et al. 2016).

Statistics

Data were analyzed using SPSS (version 19.0). The normality of all variables was assessed using the Kolmogorov–Smirnov test and any non-normally distributed data were normalized by log-transformation prior to statistical analysis. Direct comparisons of the M_b and body temperature of starlings acclimatized to either winter or summer were made with Student t test. Because digestive efficiency consists of percentage data, these are not typically normally distributed, so we used arcsin-square root transformed prior to statistical analysis. Changes in metabolic rate, GEI, FE and DEI were analyzed by ANCOVA with tarsus length as a covariate. Differences in state 4 respiration and COX activity between birds caught in winter and summer were evaluated with Student t test where appropriate. Least-squares linear regression was used to test for allometric correlations between the log masses of different organs, log BMR and log M_b. For organ mass allometric regressions we used body mass minus wet organ mass for the organ in question to avoid
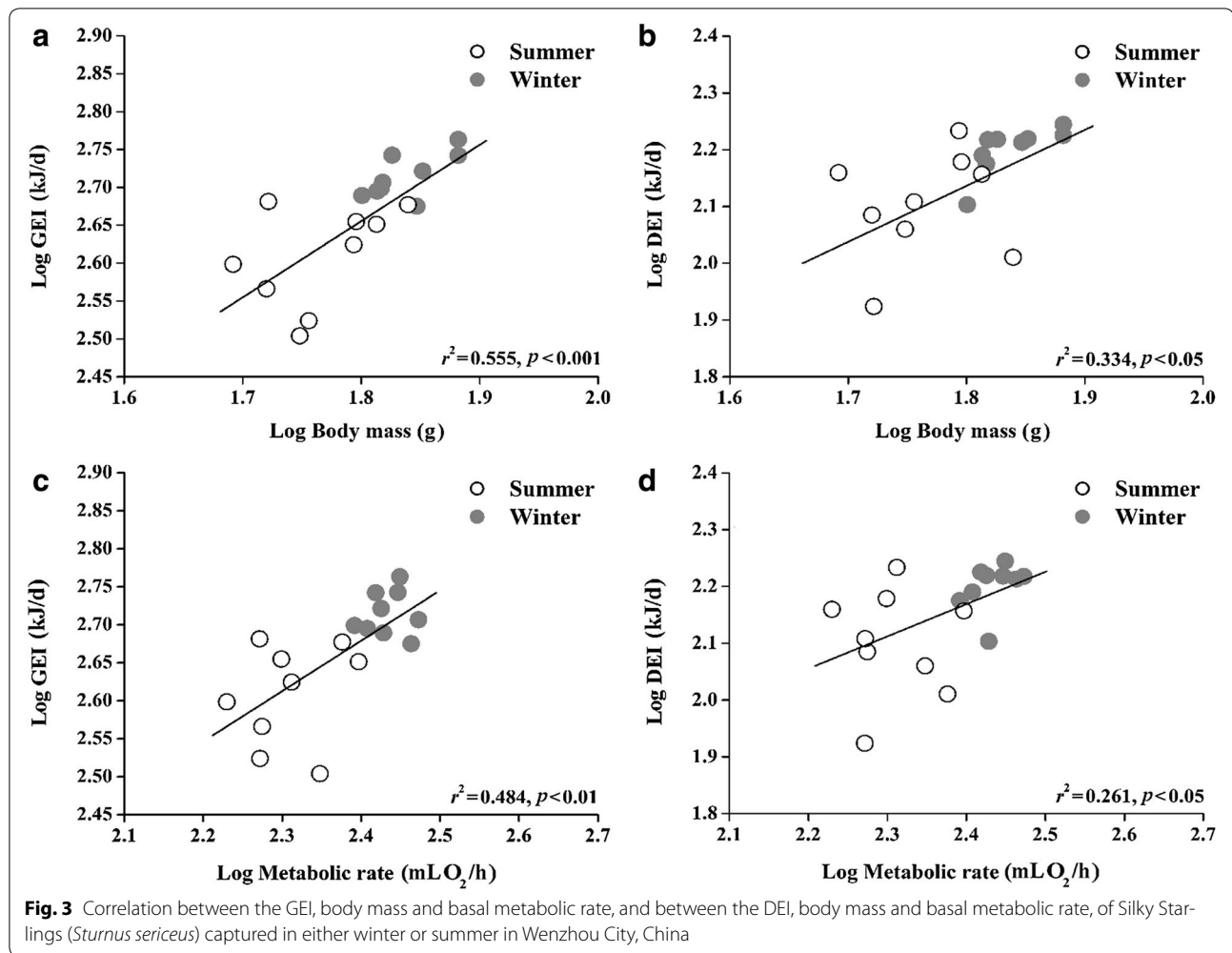
statistical problems with part-whole correlations (Christians 1999). Residuals were calculated from allometric equations and linear regression was used to determine if log organ mass residuals were significantly correlated with those of log BMR. Least-squares linear regression was also used to evaluate the relationship between log BMR and log M_b, and between log BMR, log state 4 respiration and log COX. All results were expressed as mean ± SEM; $p < 0.05$ was considered statistically significant.

Results

Body mass (M_b) and basal metabolic rate (BMR)

Winter birds had significantly higher M_b and BMR than summer birds (M_b, $t_{16} = 3.850$, $p < 0.01$, Fig. 1a; BMR, $t_{16} = 6.446$, $p < 0.001$, Fig. 1b); the BMR of winter birds exceeded that of their summer counterparts by 33%. After adjusting for body size by using tarsus length as a covariate, the BMR of winter birds averaged $262.2 ± 9.7$ O_2/h, still significantly higher than that of the summer birds ($214.8 ± 9.7$ O_2/h; $F_{1,15} = 8.185$, $p < 0.05$). There was a significant, positive correlation between log M_b and log BMR ($r^2 = 0.709$, $p < 0.001$; Fig. 1c). There was no significant difference in body temperature between winter and summer birds (winter, $42.2 ± 0.5$ °C; summer, $41.6 ± 0.5$ °C; $t_{16} = 0.833$, $p > 0.05$).

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Fig. 3 Correlation between the GEI, body mass and basal metabolic rate, and between the DEI, body mass and basal metabolic rate, of Silky Starlings (*Sturnus sericeus*) captured in either winter or summer in Wenzhou City, China

Energy budget

GEI, FE and DEI were all significantly higher in winter birds than in summer birds (GEI: ANCOVA, $F_{1,15} = 11.744$, $p < 0.01$, Fig. 2a: FE: ANCOVA, $F_{1,15} = 6.914$, $p < 0.05$, Fig. 2b; DEI: ANCOVA, $F_{1,15} = 4.681$, $p < 0.05$, Fig. 2c). GEI and DEI of winter birds were 27 and 24%, respectively, higher than those of summer birds. There was, however, no significant difference between winter and summer birds in digestibility ($t_{16} = 0.450$, $p = 0.659$; Fig. 2d). There were positive correlations between log GEI and log M_b (Fig. 3a), between log DEI and log M_b (Fig. 3b), between log GEI and log BMR (Fig. 3c), and between log DEI and log BMR (Fig. 3d).

Organ mass

Winter birds had a significantly heavier gizzard (ANCOVA, $F_{1,15} = 17.599$, $p < 0.01$), smaller intestine (ANCOVA, $F_{1,15} = 12.303$, $p < 0.01$), and total digestive tract (ANCOVA, $F_{1,15} = 17.567$, $p < 0.01$), than summer

birds (Fig. 4a). There were positive correlations between log gizzard mass and log GEI (Fig. 4b), and between log small intestine mass and log DEI (Fig. 4c). Winter birds also had a significantly heavier heart (ANCOVA, $F_{1,15} = 9.740$, $p < 0.01$), liver (ANCOVA, $F_{1,15} = 28.146$, $p < 0.001$), and pectoral muscle (ANCOVA, $F_{1,15} = 17.387$, $p < 0.01$), than summer birds (Fig. 5a). The slopes of allometric relationships between log organ mass and log M_b (minus wet mass of the organ) were positive for all organs; however, those for heart, pectoral muscle and gizzard were statistically significant (Table 1). The slopes of the statistically significant allometric regression lines were all > 1.0, indicating that the increase of organ mass with body size was greater than the increase in overall M_b (Table 1). There was a significant, positive correlation between log BMR residuals and log organ mass residuals for all organs except the heart and small intestine. However, the correlation between BMR and heart mass residuals was nearly significant (Table 1).

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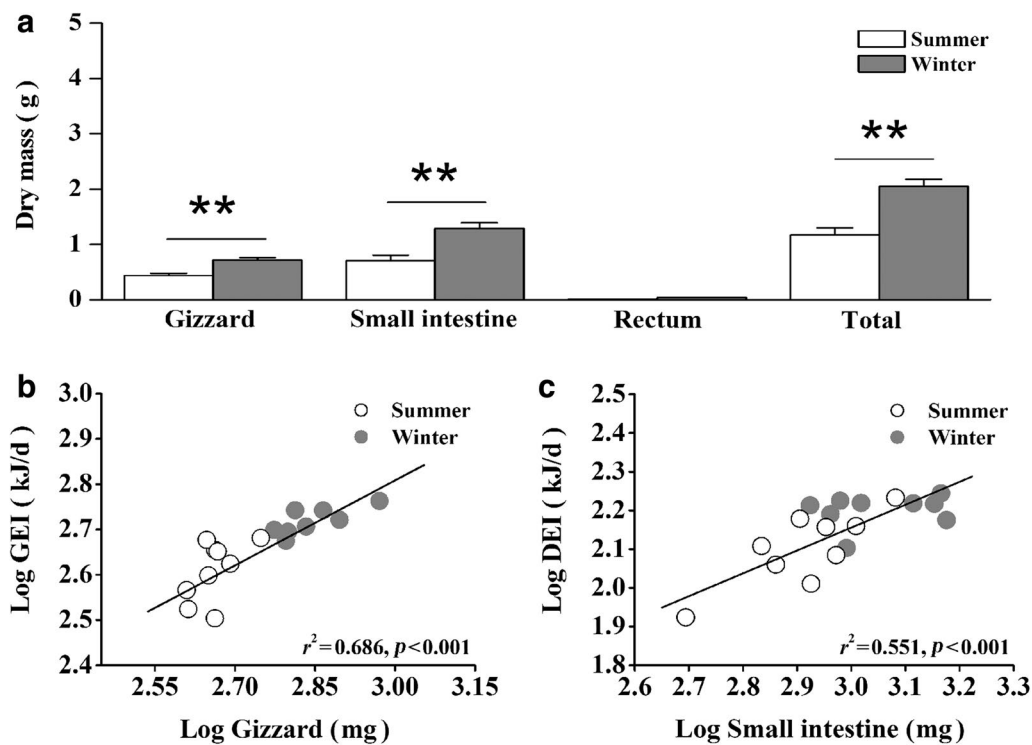
Fig. 4 Mass of the gizzard, small intestine, rectum and total gut (**a**), relationship between the GEI and gizzard mass (**b**), and between the DEI and small intestine mass (**c**), of Silky Starlings (*Sturnus sericeus*) captured in either winter or summer in Wenzhou City, China. Data are shown as mean ± SEM, **$p < 0.01$. *GEI* gross energy intake, *DEI* digestible energy intake

Tissue state 4 respiration and COX activity

Winter birds had significantly higher state 4 respiration in the heart (mass-specific, $t_{16} = 4.829$, $p < 0.001$; organ, $t_{16} = 4.266$, $p < 0.01$), liver (mass-specific, $t_{16} = 2.846$, $p < 0.05$; organ, $t_{16} = 3.489$, $p < 0.01$), and pectoral muscle (mass-specific, $t_{16} = 8.284$, $p < 0.001$; whole tissue, $t_{16} = 6.759$, $p < 0.001$) (Fig. 5b), compared to summer birds. Winter birds also had significantly higher COX activity in the heart (mass-specific, $t_{16} = 2.920$, $p < 0.05$; organ, $t_{16} = 2.591$, $p < 0.05$) and pectoral muscle (mass-specific, $t_{16} = 4.759$, $p < 0.001$; whole tissue, $t_{16} = 3.305$, $p < 0.01$), but not in the liver (mass-specific, $t_{16} = 1.265$, $p = 0.224$; organ, $t_{16} = 1.407$, $p = 0.178$) (Fig. 5c), relative to summer birds. There were significant, positive correlations between log BMR and log state 4 respiration in the heart, liver and pectoral muscle (Fig. 6a, c, e), and between log BMR and log COX activity in the heart and pectoral muscle (Fig. 6b, f). Although the correlation between log BMR and log COX activity in the liver was not statistically significant, it was nearly so ($p = 0.07$, Fig. 6d).
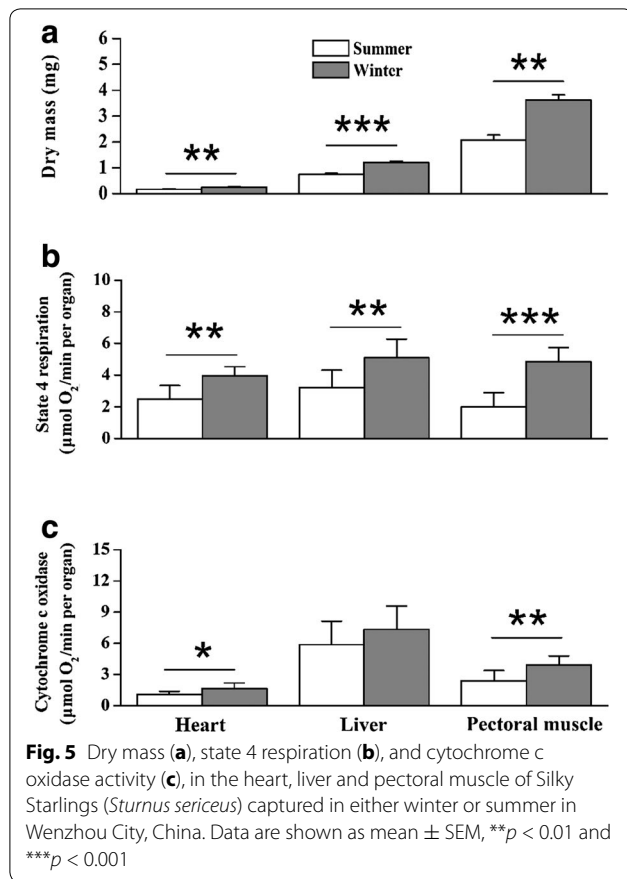
Discussion

The results of this study indicated that silky starlings captured in winter have significantly higher M_b, energy budget, organ mass, metabolic rate, and respiratory

enzyme activity, than those captured in summer. This suggests that the thermogenic response of Silky Starlings to winter conditions is achieved by a range of adjustments from the organismal to cellular level.

Seasonal variation in BMR, M_b, and energy budget

Many, but not all, small birds inhabiting temperate latitudes in the Northern hemisphere increase their BMR in winter relative to that in summer (McKechnie 2008; Smit and McKechnie 2010; Wu et al. 2015). For example, Cooper (2000) showed that the BMR of captive, winter-acclimatized Mountain Chickadees (*Poecile gambeli*) was higher than that measured in summer. Zheng et al. (2008b) also found that Eurasian Tree Sparrows captured in winter had higher BMR than those captured in summer. Similar results have also been observed in other free-living species (Rising and Hudson 1974; Southwick 1980; Liknes et al. 2002). Our data indicate that the BMR of the Silky Starling is 33% higher in winter than in summer. The functional significance of the winter increase in BMR is likely that it supports the maintenance costs of the thermogenic machinery. Seasonal variation in M_b, especially in small birds, is considered to be an adaptive strategy important for survival (Chamane and Downs 2009; Cooper 2000; Zhao et al. 2015). Our results suggest

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Fig. 5 Dry mass (**a**), state 4 respiration (**b**), and cytochrome c oxidase activity (**c**), in the heart, liver and pectoral muscle of Silky Starlings (*Sturnus sericeus*) captured in either winter or summer in Wenzhou City, China. Data are shown as mean ± SEM, **$p < 0.01$ and ***$p < 0.001$

that the Silky Starling is, on average, 18% heavier in winter than in summer. This increased winter M_b could reflect both increased thermogenic capacity and the accumulation of energy reserves (Dawson et al. 1983; Cooper 2007). An increase in M_b during winter is often due to increases in fat deposits, and or, the mass of metabolically active tissues (Williams and Tieleman 2000;

Zheng et al. 2008b). In addition to the seasonal variation in M_b, seasonal differences also occurred for some organ masses, so variation in these factors also likely contributes to the seasonal differences in BMR (Zheng et al. 2013a). An increased energy intake and budget can compensate for the increased energy expenditure associated with thermogenesis in cold conditions (Williams and Tieleman 2000). M_b is an important indicator of energy balance (Doucette and Geiser 2008). A positive balance results from an adaptive increase in energy intake that periodically exceeds energy output as birds replenish their fat reserves and metabolic organ mass (Swanson 1991a; Cooper 2007; Liu et al. 2008; Zheng et al. 2008b, 2014a). Many studies have found evidence of significant seasonal variation in daily energy intake within species (Kelly 1998; Webster and Weathers 2000; Guillemette and Butler 2012; Hegemann et al. 2012). In this study, winter birds had 27% higher GEI, and 29% higher DEI, compared to summer birds, and the strong positive correlation between M_b and BMR (see "Energy budget"), suggesting that enhancement of energy intake could be a general response to higher energy demands in small endotherms (Stokkan et al. 1986; Wu et al. 2014).

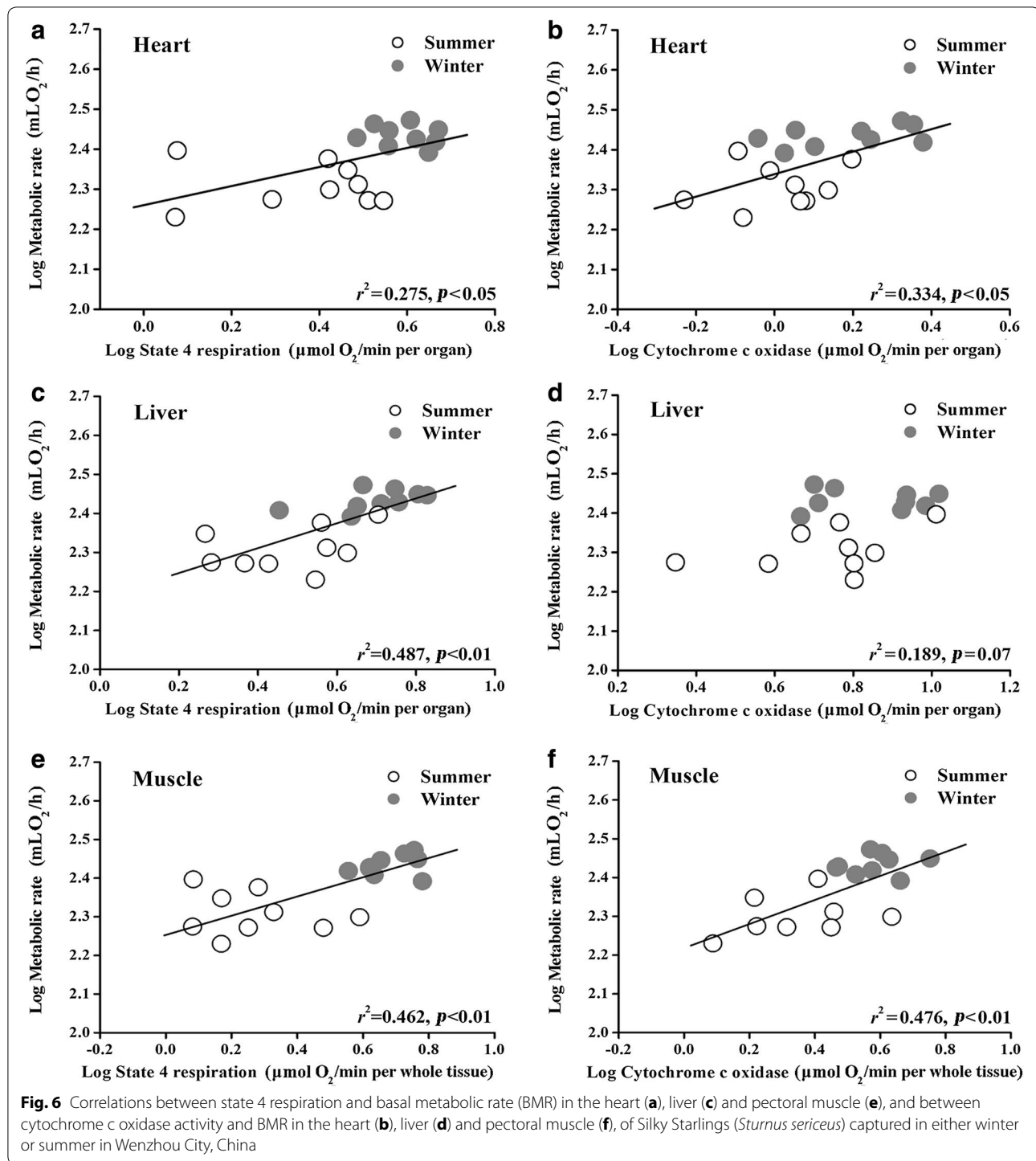### Seasonal variation in organ mass and tissue oxidative capacity

With respect to metabolic adjustments, the BMR of an animal is the sum of the metabolic rates of its organs and other metabolically active structures (Zheng et al. 2008a; Swanson 2010; Clapham 2012). The mechanisms underlying seasonal metabolic adjustments in birds may operate at multiple hierarchical levels. Several authors have suggested that much of the energy used in basal metabolism is consumed by visceral organs (Daan et al. 1990; Piersma et al. 1996; Burness et al. 1998), including the gizzard and small intestine (storage, digestion and

Table 1 Linear regression statistics for log organ mass versus log body mass (controlled for tarsus length) allometric equations (allometric correlations) and for log organ mass residuals versus log basal metabolic rate (BMR) residuals (residual correlations) in Silky Starlings (*Sturnus sericeus*) that had been acclimatized to different seasons from Wenzhou City, China

	Heart	Liver	Muscle	Gizzard	Small intestine	Rectum	Digestive tract
Allometric correlations							
R^2	0.493	0.086	0.627	0.309	0.001	0.073	0.100
p	*< 0.01*	0.238	*< 0.001*	*< 0.05*	0.908	0.275	0.199
Slope	1.812	0.533	2.391	1.095	0.068	1.106	0.660
Residual correlations							
R^2	0.152	0.204	0.461	0.258	0.052	0.249	0.248
p	0.062	*< 0.05*	*< 0.01*	*< 0.05*	0.183	*< 0.05*	*< 0.05*
Slope	0.931	0.863	1.541	0.946	0.832	2.294	1.159

Values in italics are statistically significant

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Fig. 6 Correlations between state 4 respiration and basal metabolic rate (BMR) in the heart (**a**), liver (**c**) and pectoral muscle (**e**), and between cytochrome c oxidase activity and BMR in the heart (**b**), liver (**d**) and pectoral muscle (**f**), of Silky Starlings (*Sturnus sericeus*) captured in either winter or summer in Wenzhou City, China

absorption), the heart and lungs (oxygen transport), the liver (catabolism), and the kidneys (elimination of waste) (Kersten and Piersma 1987; Daan et al. 1990; Hammond et al. 2001). Although the liver, kidneys, heart and digestive tract represent less than 10% of total M_b they contribute more than 60% of all energy expenditure while at

rest (Clapham 2012). The results of this study indicate that winter birds had a heavier gizzard, digestive tract, heart, liver, and pectoral muscles, than summer birds, and masses of these organs also co-varied with BMR at seasons in a manner consistent with a regulatory role for seasonal RMR variation in this species (Table 1). The data

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for Silky Starlings in this study are thus consistent with previous data from small birds and, collectively, these studies suggest that seasonal changes in organ masses are important general contributors to RMR variation in small birds. In addition, the mass of the gizzard and small intestine, where food storage, digestion and absorption occur, were strongly correlated with GEI and DEI (Fig. 4b, c), suggesting that winter birds had increased their gizzard and small intestine mass in response to increased food intake.

State 4 respiration is clearly connected with BMR whereas COX activity is correlated with maximal capacity for thermogenesis (the increase in COX activity may simply be a function of mass-specific values and/or larger organ mass). We found that state 4 respiration in the heart (mass-specific and organ), liver (mass-specific and organ) and pectoral muscle (mass-specific and whole tissue) of winter birds was significantly higher than in summer birds. In addition, COX activity in the heart (mass-specific and organ) and muscle (mass-specific and whole tissue) of winter birds was significantly higher than in summer birds. Moreover, BMR was significantly correlated with state 4 respiration in the heart, liver and pectoral muscle, and with COX activity in the heart and pectoral muscle (Fig. 6). Although the correlation between BMR and COX activity in the liver was not statistically significant, it was nearly so (Fig. 6d). Similar results have been reported in other small passerines from the same region, such as the Chinese Bulbul (Zheng et al. 2010, 2014a) and Eurasian Tree Sparrow (Liu et al. 2008; Zheng et al. 2008a, 2014b), and other small birds in northern hemisphere like Little Bunting (*Emberiza pusilla*) (Zheng et al. 2013a), Black-capped Chickadee (*Poecile atricapillus*), House Sparrow (*Passer domesticus*) (Liknes and Swanson 2011), and in small mammals such as the Plateau Pika (*Ochotona curzoniae*) (Wang et al. 2006a), Root Vole (*Microtus oeconomus*) (Wang et al. 2006b) and Mongolian Gerbil (*Meriones unguiculatus*) (Zhang and Wang 2007). These results suggest that modulation of cellular thermogenesis at the tissue and organ levels are important aspects of seasonal acclimatization in the Silky Starling. These studies collectively present an increasingly comprehensive picture of seasonal adjustments in thermoregulation from the cellular to the organismal level.

Conclusions

Organisms are thought to acclimatize to seasonal variation in the environment through a range of physiological and biochemical adjustments (Zheng et al. 2008b). The results of this study suggest that Silky Starlings primarily acclimatize to winter conditions by increasing their M_b, organ mass, BMR, energy intake, and tissue oxidative capacity. This suggests that a suite of adjustments, from the cellular to the organismal level, have the potential to contribute significantly to the thermogenic efforts of seasonal acclimatized birds. The observed seasonal changes in M_b, basal thermogenesis (BMR) and tissue oxidative capacity in Silky Starlings may all be important to the ability of this temperate passerine to survive winter conditions (Liknes and Swanson 2011; Zheng et al. 2008b, 2014a).

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