Zoological Studies

Physiological and Biochemical Thermoregulatory Responses in Male Chinese Hwameis to Seasonal Acclimatization: Phenotypic Flexibility in a Small Passerine

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Many small birds living in regions with seasonal fluctuations and ambient temperatures typically respond to cold by increasing metabolic thermogenesis, internal organ mass and the oxidative capacity of certain tissues. In this study, we investigated seasonal adjustments in body mass, resting metabolic rate (RMR), evaporative water loss (EWL), the mass of selected internal organs, and two indicators of cellular aerobic respiration (mitochondrial state-4 respiration and cytochrome *c* oxidase activity) in Chinese hwamei (*Garrulax canorus*) that had been captured in summer or winter from Wenzhou, China. RMR and EWL were higher in winter than in summer. State-4 respiration in the heart, liver, kidneys and pectoral muscle, as well as cytochrome *c* oxidase activity in the liver, kidneys and pectoral muscle were also higher in winter than summer. In addition, there was a positive correlation between RMR and EWL, and between RMR and indicators of cellular metabolic activity in the heart, liver, kidneys and pectoral muscle. This phenotypic flexibility in physiological and biochemical thermoregulatory responses may be important to the hwamei's ability to survive the unpredictable, periodic, cold temperatures commonly experienced in Wenzhou in winter.

Key words: Chinese hwamei (*Garrulax canorus*), Cytochrome *c* oxidase, Evaporative water loss, Organ mass, Resting metabolic rate, State-4 respiration.

BACKGROUND

Phenotypic flexibility can be defined as reversible, temporary, and repeatable changes in the traits of organisms in response to changes in internal or external environmental conditions (Piersma and Drent 2003; Starck and Rahmaan 2003; Tieleman et al. 2003a) and has become an important topic in evolutionary ecology and a subject of considerable interest to environmental and comparative physiologists (Vézina et al. 2006; McKechnie et al. 2007; Swanson 2010).

Birds resident in temperate regions provide the opportunity to conduct natural experiments on phenotypic flexibility in response to seasonal climates (Liknes and Swanson 2011; Zheng et al. 2014a). Winter in temperate regions is typically a period of energetic stringency for small birds because colder ambient temperatures, decreased food abundance, and longer nights reduce the daylight available to forage for food (Swanson 1990 1991; Liknes and Swanson 2011; Zheng et al. 2014b). Temperate bird species often have morphological, physiological, and behavioral

adaptations that helps them to cope with different, seasonal energy requirements and enhance their reproductive success (Yuni and Rose 2005).

Basal metabolic rate (minimum maintenance metabolic rate, BMR) in small birds 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 summit metabolic rate is directly correlated with improved cold tolerance in winter birds (Swanson 2001; Swanson and Liknes 2006). BMR, a widely-accepted benchmark of metabolic expenditure in endotherms, is one of the fundamental physiological standards for assessing the energy cost of thermoregulation (McKechnie and Wolf 2004; Swanson et al. 2017). Many small birds, such as the Eurasian tree sparrow (*Passer montanus*) (Zheng et al. 2008b 2014b), Chinese bulbuls (*Pycnonotus sinensis*) (Zheng et al. 2008a 2010 2014a), Chinese hwamei (*Garrulax canorus*) (Wu et al. 2015) and silky starling (*Sturnus sericeus*) (Li et al. 2017) have a higher BMR in winter than summer. The better cold resistance and higher winter BMR may indicate a functional link between the maximum sustained level of energy expenditure, *i.e.*, metabolic or working capacity, and BMR (Klaassen et al. 2004; Swanson 2010; Zheng et al. 2008b 2014a). BMR in small birds is an example of a flexible trait that can be seasonally modulated through adjustments at several levels (McKechnie 2008; Swanson 2010; Zheng et al. 2014a). At the organ level, changes in the mass of metabolically active organs could influence BMR (Daan et al. 1990; Hammond et al. 2001) and the relative sizes of metabolically expensive internal organs, such as the heart, intestines, liver, and kidneys, are thought to be important causes of variation in avian BMR, both among individuals and between seasons (Kersten and Piersma 1987; Daan et al. 1990; Piersma et al. 1996; Zheng et al. 2014a). At the cellular or tissue level, other adjustments, such as cellular hypertrophy, changes in capillary density, and cellular proliferation and extrusion in the intestine, could complement organ level adjustments to enhance metabolic and nutritional capacity (Starck and Rahmaan 2003; Swanson 2010). At the biochemical level, changes in catabolic enzyme activity and/or capacities for metabolic substrate transport could influence the mass-specific metabolic intensity of organs and thereby affect BMR (Liknes and Swanson 2011; Zheng et al. 2008b 2014a). Variation in cellular metabolic activity is often measured by examining variation in mitochondrial state-4 respiration (reflects oxidative phosphorylation capacity) or the activity of cytochrome *c* oxidase (COX) (a key regulatory enzyme involved in oxidative phosphorylation) (Zheng et al. 2008b 2014a 2014b).

Water balance may be under similar selective pressures as energy expenditure, especially in birds exposed to high ambient temperatures or where there is a scarcity of drinking water (Versteegh et al. 2008). Evaporative water loss (EWL) is the major avenue of water efflux in birds, especially for small species in which EWL is five times greater than urinary and fecal water loss (Dawson 1982; Williams and Tieleman 2005). EWL is widely used as a measure of water relations in inter- or intraspecific studies of birds in different environments (Clement et al. 2012; Xia et al. 2013). Several factors, such as temperature, food quantity and quality, and physiological status, have been implicated in the regulation of variation in EWL (Williams and Tieleman 2005; Xia et al. 2013). Birds living in deserts often rely on the water content of their food and metabolic water to supply their needs (Williams 1999; Tieleman et al. 2003a b). However, less effort has been devoted to understanding the ecological and evolutionary significance of variation in EWL than has been applied to questions about variation in BMR (Williams and Tieleman 2000). Thermal conductance, the reciprocal of insulation, has traditionally been calculated as the slope of the line relating oxygen consumption to ambient temperature (Weathers 1997). Animal thermal conductance mainly depends on the ratio of their surface area to volume and is also affected by ambient temperature. In general, birds that live at low latitudes have higher conductance than expected based on their body mass (Weathers 1997). Small birds have a relatively large surface area and poor thermal insulation, resulting in relatively high thermal conductance (Wu et al. 2015).

The Chinese hwamei (*Garrulax canorus*) (Passeriformes, Leiothrichidae) is an endemic Asian species found in central and southern China and northern and central Vietnam (Li et al. 2006; MacKinnon and Phillipps 2000). Within its natural range, the hwamei preferentially inhabits scrubland, open woodland, secondary forest, parks and gardens up to 1800 meters above sea level (MacKinnon and Phillipps 2000). The Chinese hwamei is omnivorous, feeding mainly on arthropods (insects and spiders) and mollusks (snails and slugs) which it finds on the ground among leaf litter in the breeding season, but also eats fruits and seeds in autumn and winter (Zheng and Zhang 2002). The reported eco-physiological properties of the hwamei are a relatively lower BMR than predicted for their body mass from allometric equations (McKechnie and Wolf 2004; McKechnie and Swanson 2010), high body temperature (Wu et al. 2015), narrow thermal neutral zone (TNZ) and high metabolic water production and evaporative water loss (MWP/EWL) (Xia et al. 2013). It also has similar capacity for daily and seasonal

metabolic acclimatization observed in other temperate passerines, which has been associated with a significant increase in oxygen consumption during day-light hours and winter relative to that at night and summer (Zhao et al. 2015). Because it feeds mainly on insects, these physiological traits and feeding habits could limit its distribution to a narrow habitat zone where the climate is relatively warm and food resources abundant (Xia et al. 2013). Although there is a considerable amount of information on physiological changes in the hwamei in response to temperature (Zhou et al. 2016), there is no information on cellular metabolic changes in this species in response to different seasonal conditions, such as variation in food abundance, temperature, or photoperiod.

Our data illustrate species-specific adaptations by the hwamei to seasonal adjustments. This information improves our understanding of the morphological, physiological, and biochemical adaptations by which small bird species make thermogenic adjustments to seasonal changes. We selected the hwamei as a study species because (i) it is resident in the warm, mesic climate of Zhejiang Province and (ii) because relatively few data on the physiology of the Timaliidae are currently available (Xia et al. 2013; Zhou et al. 2016). We used an integrated approach to measure and compare the thermogenic properties, including BMR, EWL, thermal conductance, internal organ mass, and two indicators of the level of cellular aerobic respiration, mitochondrial state-4 respiration and cytochrome *c* oxidase (COX) activity, in the heart, liver, kidneys and pectoral muscle of hwameis captured in summer and winter. We hypothesized that, like other small birds, the Chinese hwamei display seasonal physiological and biochemical adjustments in BMR, EWL, internal organ mass, and tissue oxidative capacity in response to changing seasonal energy demands. As energy demand increases because of lower values of ambient temperature (winter), hwameis ingest more food, with the result that key organs involved in catabolism (stomach, intestine and liver), oxygen transport to the tissues (heart) and the elimination of waste (kidneys) are stimulated to hypertrophy. Because these organs have a high metabolic intensity, total oxygen increases as cellular enzyme activity associated with bodily metabolic functions enhance. When oxygen requirements are elevated, ventilation rate increases, with a concomitant increase in EWL.

MATERIALS AND METHODS

Ambient temperature

This study was carried out in Wenzhou City, Zhejiang Province (27°29'N, 120°51'E, 14 m in elevation), China. The climate in Wenzhou is warm-temperate with an average annual rainfall of 1500 mm spread across all months with slightly more precipitation during spring and summer. Mean ambient temperature ranged from 31.3 ± 0.2°C in summer (July to August 2013) to 8.6 ± 0.4°C in winter (January to February 2014) (Wu et al. 2015; Zhou et al. 2016). Ambient temperature ranged from 26.4 ± 0.1°C to 34.6 ± 0.2°C in summer, and from 5.9 ± 0.5°C to 14.8 ± 0.6°C in winter (Fig. 1).

Study animals

A total of fifteen male hwameis were live-trapped in the forest between July 2013 and January 2014. Seven birds were caught in August (hereafter referred to as "summer birds") and eight in February (hereafter "winter birds"). Body mass was measured to the nearest 0.1 g 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 $50 \times 30 \times 20$ cm^3 cages under natural photoperiod and temperature before physiological measurements were taken (Wu et al. 2015). Birds were maintained on commercial hwamei pellets (20% crude protein, 6% crude fat, 4% crude fibre, 1% calcium, 0.5% lysine, and 0.5% methionine+cystine; Xietong Bioengineering Co. Ltd, Jiangsu, China) (Zhou et al. 2016). Food and water were provided *ad libitum* (Xia et al. 2013). All experimental procedures were approved by the Wenzhou City Animal Care and Use Committee (Protocol 20130014).

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), as described in previous publications (Wu et al. 2015; Zhou et al. 2016). Resting metabolic rate (RMR) is the energy required to perform vital body functions while the body is at rest. It is doubtful whether BMRs can ever be achieved in the laboratory, so the term RMR is often used to refer to such measurements, even when the standard conditions for BMR have been met (Zheng et al. 2014b). Metabolic chambers were 1.5 L in volume, made of plastic, and contained a perch for the bird to stand on (Smit and McKechnie 2010). Water vapour and

CO_2 were scrubbed from the air by passing it through a silica gel/soda lime/silica column before it passed 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 was pulled through the chamber at 300 ml min-1 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 ± 1% accuracy with a general purpose thermal mass flow-meter (TSI 4100 Series, USA). Oxygen consumption rates were measured at 30 ± 0.5°C, which is within the hwamei's thermal neutral zone (Wu et al. 2015). Chamber temperature was regulated by a temperature-controlled cabinet (Artificial climatic engine BIC–300, China) capable of regulating temperature to ± 0.5°C. Baseline O_2 concentrations were obtained before and after each test (Li et al. 2010). 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 (Liu et al. 2005; Xia et al. 2013). We first ensured that birds were perching calmly in the chamber and began recording oxygen consumption at least 1 h afterwards. Each animal was in the metabolic chamber for at least 2 h. The data obtained were used to calculate 5-minute running means of instantaneous oxygen consumption over the entire test period using equation 2 from 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^{-1} and corrected to STPD conditions (Schmidt-Nielsen 1997). Body temperature (T_b) was measured during metabolic measurements using a lubricated thermocouple inserted into the cloaca, and the output was digitized using an Oakton thermocouple meter (Eutech Instruments, Singapore). Body mass was measured to the nearest 0.1 g before, and after, experiments, and mean body mass was used in calculations.
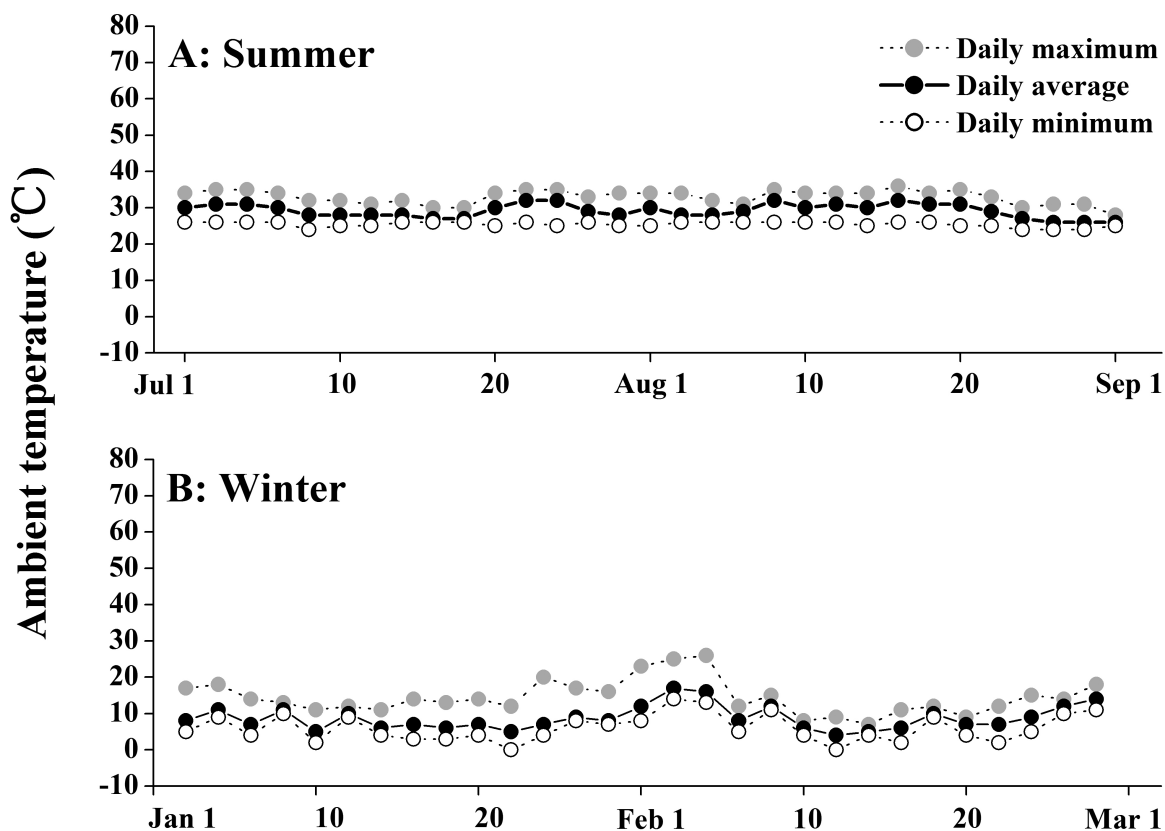
Fig. 1. Minimum, maximum and mean ambient daily summer (July to August 2013) and winter (January to February 2014) temperatures in Wenzhou, China. Mean ambient temperature ranged from 31.3 ± 0.2°C in summer to 8.6 ± 0.4°C in winter.

Measurement of evaporative water loss (EWL)

The water content of the air was determined before the experiment. A 'U' tube (containing silica gel) was connected in series behind the respiratory chamber and weighed (± 0.1 mg). The amount of evaporative water lost (EWL) by each bird was absorbed by the silica gel and could therefore be measured by reweighing the U tube at the end of the experiment. Paraffin was placed at the bottom of the respiratory chamber to prevent feces from influencing the EWL metrical value. The duration of the experiment was 1 h. EWL was calculated according to the formula in Ganey et al. (1993) and Xia et al. (2013): EWL (mg h^{-1}) = (tube weight after the experiment–tube weight before the experiment)/time

Thermal conductance

Total wet thermal conductance (*C*, ml O$_2$ g^{-1} h^{-1} °C^{-1}) at given T_a was calculated from the formula: C = MR / (T_b - T_a), where MR is metabolic rate (ml O$_2$ g^{-1} h^{-1}), T_b is body temperature (°C), and T_a is ambient temperature (°C). This formula was suggested by Aschoff (1981) for calculating conductance at given T_a.

Measurements of organ mass

Birds were euthanized by cervical dislocation at the end of the experiment and their heart, liver, kidneys and pectoral muscle extracted and weighed to the nearest 0.1 mg. A sample of each of these tissues was used to investigate state-4 respiration and COX activity (Zheng et al. 2008b 2014a) and the remainder was dried to a constant mass over 2 d at 65°C and weighed to the nearest 0.1 mg (Williams and Tieleman 2000; Liu and Li 2006).

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

State-4 respiration in the heart, liver, kidneys 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$_2$PO$_4$, 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 2013a). COX activity was measured polarographically at 30°C using a Clark electrode according to Sundin et al. (1987). State-4 respiration and COX activity measurements are expressed as mean mass-specific (μmol O$_2$ min^{-1} organ^{-1}) (Wiesinger et al. 1989; Zheng et al. 2013b 2014a).

Statistics

Data were analyzed using SPSS (version 19.0). The normality of all variables was examined using the Kolmogorov-Smirnov test and non-normally distributed data were log$_{10}$-transformed prior to analyses. Direct comparisons of the mean body mass, body temperature, RMR, EWL, and thermal conductance of birds caught in summer or winter were made using the Student Newman Keul's (SNK) *post hoc* test. Differences in organ dry mass, state-4 respiration, and COX between birds caught in summer and winter were also evaluated with the Student Newman Keul's (SNK) *post hoc* test where appropriate. Least squares linear regression was used to test for allometric correlations between the log masses of different organs. 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 RMR. Least squares linear regression was also used to evaluate the relationship between log RMR, log body mass, log EWL and log thermal conductance, between log EWL and log body mass, and between log RMR, log state-4 respiration and log COX. All results are expressed as mean ± *SEM*; *p* < 0.05 was considered statistically significant.

RESULTS

Body mass, body temperature, resting metabolic rate (RMR), evaporative water loss (EWL) and thermal conductance

The mean body weights of hwamei caught in summer and winter were 51.3 ± 1.6 g and 54.6 ± 1.9 g, respectively, which are not significantly different ($F_{1,13}$ = 1.788, *p* = 0.205; Fig. 2A). The body temperatures of birds caught in summer and winter were also not significantly different ($F_{1,13}$ = 0.959, *p* = 0.345); overall mean body temperature was 42.5°C. The mass-specific RMR (ml O$_2$ g^{-1} h^{-1}) of hwamei caught in winter was significantly higher ($F_{1,13}$ = 17.989, *p* = 0.001) than those caught in summer (32%), and winter mean whole-organism RMR (ml O$_2$ h^{-1}) was 42% higher than summer RMR ($F_{1,13}$ = 19.047, *p* = 0.001; Fig. 2B). Birds caught in winter had significantly higher EWL ($F_{1,13}$ = 14.402, *p* = 0.002; Fig. 2C) and thermal conductance ($F_{1,13}$ = 8.419, *p* = 0.012; Fig. 2D) than those caught in

summer. There was a significant, positive correlation between body mass and RMR (r^2 = 0.301, p = 0.034; Fig. 3A), body mass and EWL (r^2 = 0.281, p = 0.042; Fig. 3B), RMR and EWL (r^2 = 0.372, p = 0.017; Fig. 3C), and RMR and thermal conductance (r^2 = 0.517, P = 0.003; Fig. 3D).

Organ mass

With the exception of the heart, which was significantly heavier in birds caught in winter than those caught in summer ($F_{1,13}$ = 5.753, p = 0.032), there were no significant differences in the weights of the organs examined (Fig. 4A). There were significant positive, partial correlations between RMR and the dry mass of the heart and kidney (Table 1). No significant, positive residual correlations were found between RMR and the dry mass of any other organs (Table 1).

State-4 respiration and COX activity in selected tissues

Birds caught in winter had significantly higher state-4 respiration in the pectoral muscle (mass-specific, $F_{1,13}$ = 15.375, p = 0.002; whole tissue, $F_{1,13}$ = 8.087, p = 0.014), heart (mass-specific, $F_{1,13}$ = 47.588, p = 0.000; organ, $F_{1,13}$ = 43.265, p = 0.000), liver (mass-specific, $F_{1,13}$ = 37.881, p = 0.000; organ, $F_{1,13}$ = 223.431, p = 0.000) and kidney (mass-specific, $F_{1,13}$ = 19.236, p = 0.001; organ, $F_{1,13}$ = 25.160, p = 0.000) compared to those caught in summer (Fig. 4B). Birds caught in winter also had higher COX activity in the pectoral muscle (mass-specific, $F_{1,13}$ = 18.179, p = 0.001; whole tissue, $F_{1,13}$ = 14.368, p = 0.002), liver (mass-specific, $F_{1,13}$ = 19.288, p = 0.001; organ, $F_{1,13}$ = 10.800, p = 0.006) and kidneys (mass-specific, $F_{1,13}$ = 6.854, p = 0.021; organ, $F_{1,13}$ = 17.150, p = 0.001), but not in the heart (mass-specific, $F_{1,13}$ = 0.001, p = 0.998; organ, $F_{1,13}$ = 1.230, p = 0.289), than those caught in summer (Fig. 4C). There were significant, positive correlations between RMR and state-4 respiration in the pectoral muscle, heart liver and kidney (Figs. 5A, C, E, G) and between RMR and COX activity the pectoral muscle, liver and kidneys (Figs. 5B, F, H).

DISCUSSION

Seasonal changes in environmental factors such as temperature photoperiod, and food quality and availability, are important to temperate zone birds (Bush et al. 2008; Swanson and Merkord 2013). Small, temperate zone birds cope with such seasonal changes through a wide array of strategies. These include, but are not limited to, adjustments in body mass (Polo and Carrascal 2008; Vézina et al. 2007), metabolic rates (McKechnie and Swanson 2010; Smit and McKechnie 2010), organ mass, and tissue oxidative
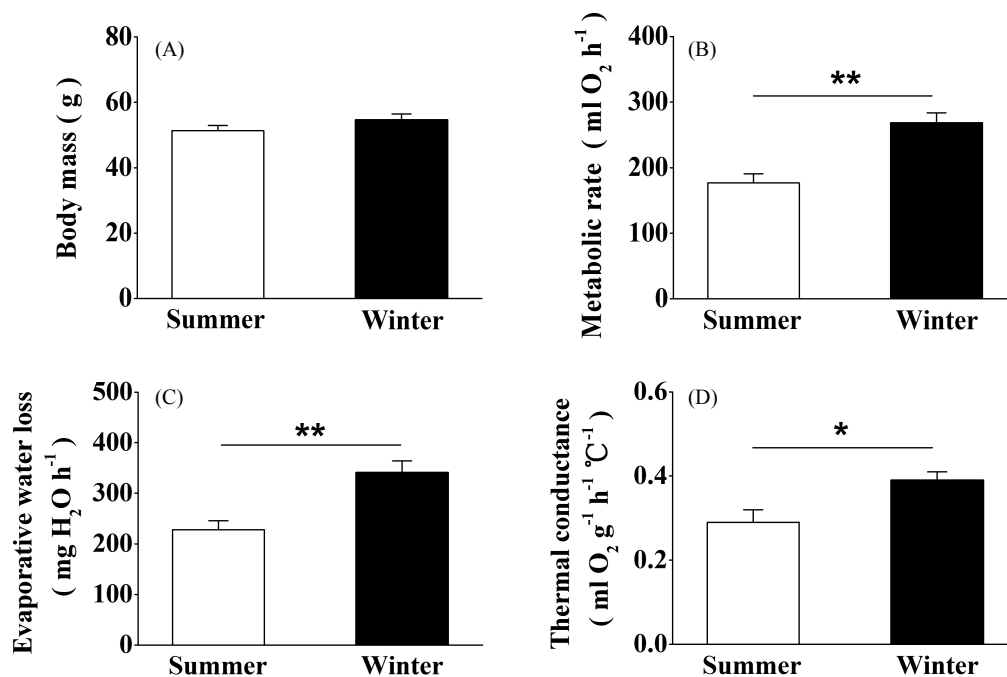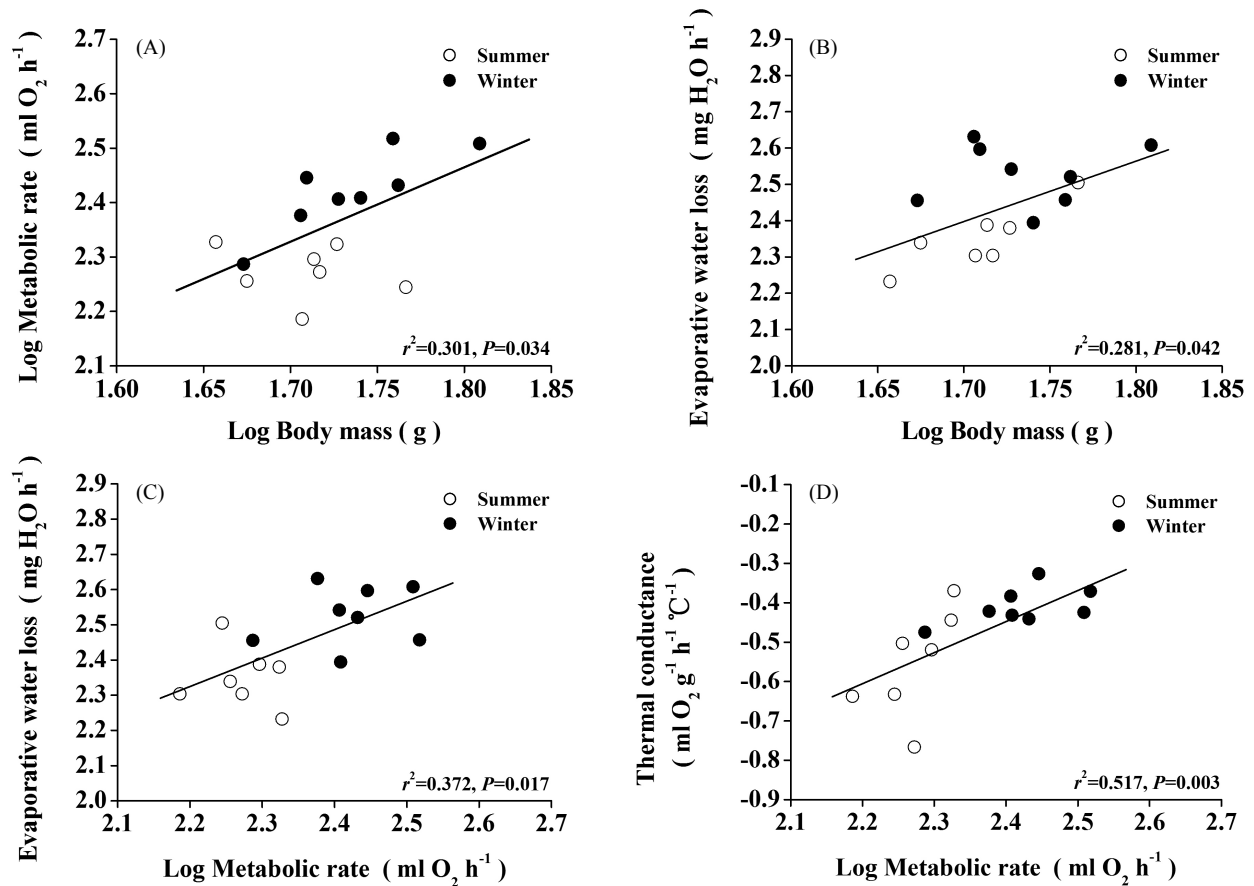
Fig. 2. Seasonal variation in body mass (A), resting metabolic rate (B), evaporative water loss (C) and thermal conductance (D) in Chinese hwamei (*Garrulax canorus*) captured in either summer or winter in Wenzhou, China. Data are shown as mean ± *SEM*, **p < 0.01.

capacity (Lindsay et al. 2009; Zheng et al. 2008a 2014a). We found that the Chinese hwamei displays significant seasonal variation in RMR, EWL and thermal conductance–they were higher in winter than in summer. Hwamei caught in winter also displayed higher respiratory enzyme activity than those caught in summer.

Seasonal variation in body mass, RMR, EWL and thermal conductance

Adjustments in body mass and RMR are major components of seasonal adaptation in animals (Zheng et al. 2008b; Wu et al. 2015; Zhao et al. 2015). Changes in body mass are an important adaptive strategy for many small birds and those inhabiting seasonal environments


Fig. 3. Correlations between body mass and resting metabolic rate (RMR) (A), between body mass and EWL (B), between RMR and EWL (C), and between RMR and thermal conductance (D) in Chinese hwameis (*Garrulax canorus*) captured in either summer or winter in Wenzhou, China.

Table 1. Linear regression statistics for log organ mass versus log body mass allometric equations (allometric correlations) and for log organ mass residuals versus log resting metabolic rate (RMR) residuals (residual correlations) in Chinese hwamei (*Garrulax canorus*) that had been acclimatized to different seasons. Bold indicateds statistical differences

	Pectoral muscle	Heart	Liver	Kidney
Allometric correlations				
r	0.144	0.798	0.244	0.597
P	0.607	**0.001**	0.379	**0.019**
Residual correlations				
r	0.214	0.434	0.096	0.047
P	0.443	0.106	0.734	0.868

either maintain a stable body mass, or increase it, when exposed to winter conditions (Zheng et al. 2008b 2014a; Swanson and Merkord 2013). In addition, the capacity to increase RMR through enhanced thermogenic properties is also an important factor for birds in cold environments (Arens and Cooper 2005; Nzama et al. 2010). Many small, winter-active birds inhabiting warm, temperate regions in the northern hemisphere increase their RMR in winter relative to that in summer (Pohl and West 1973; Southwick 1980; Smit and McKechnie 2010). Previous studies indicate that the hwamei undergoes significant seasonal changes in body mass, and is, on average, 10% or 7% heavier in winter than that in summer (Wu et al. 2015; Zhao et al. 2015). Zhou et al (2016) also found that cold-acclimated hwameis had a higher body mass than control birds. The results of this study indicate that seasonal variation in body mass in the Chinese hwamei is relatively small; birds caught in winter were 6% heavier than those caught in summer, but this difference was not statistically significant. However, we found that RMR in the hwamei was 42% higher in birds caught in winter compared to

those caught in summer. These data are consistent with those of previous studies (Wu et al. 2015; Zhao et al. 2015; Zhou et al. 2016), indicating that elevated RMR is an important component of winter acclimatization for the Chinese hwamei. In this study, we also found that seasonal variation in EWL in the Chinese hwamei was 50% higher in birds caught in winter than those caught in summer, and positive correlation was found between RMR and EWL. However, the nature of the relationship between RMR and EWL is unclear. One might predict that higher metabolic rates mandate increased ventilation, accomplished by increasing breathing frequency, resulting in an elevated EWL. For example, Williams and Tieleman (2000) found that hoopoe larks (*Alaemon alaudipes*) in the cold-acclimated group (15°C) had a significantly higher EWL than in the warm-acclimated group (36°C). Thermal conductance depends on body mass. The major reasons for this is size-dependent changes in the surface to volume ratio, the relationship between plumage thickness and size, and the fact that the thickness of the boundary layer depends on the radius of curvature,
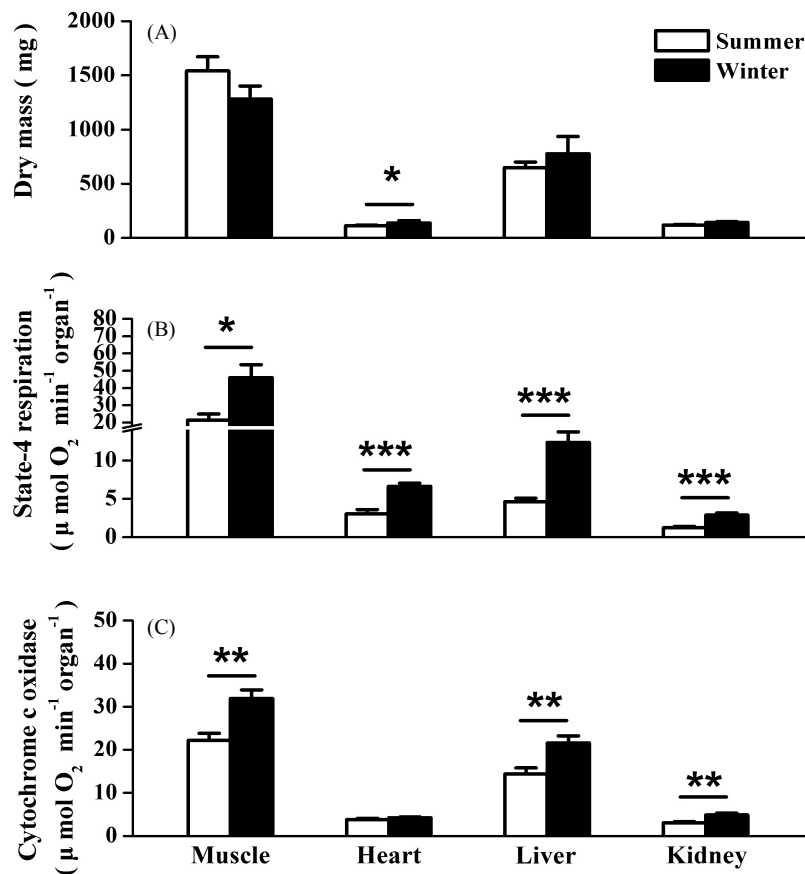
Fig. 4. Seasonal variation in dry mass (A), state-4respiration (B), and cytochrome c oxidase (C) in the pectoral muscle, heart, liver and kidneys of hwameis (*Garrulaxcanorus*) captured in either summer or winter in Wenzhou, China. Data are shown as mean ± *SEM*, *p < 0.05, **p < 0.01, ***p < 0.001.

which in turn changes with size (Aschoff 1981). We found that the hwamei has higher thermal conductance in winter than summer, which is consistent with data previously reported on Chinese bulbuls (Zheng et al. 2008a). Marschall and Prinzinger (1991) noted a positive correlation between thermal conductance and BMR, species with a lower than expected BMR having a lower than expected conductance and *vice versa*.
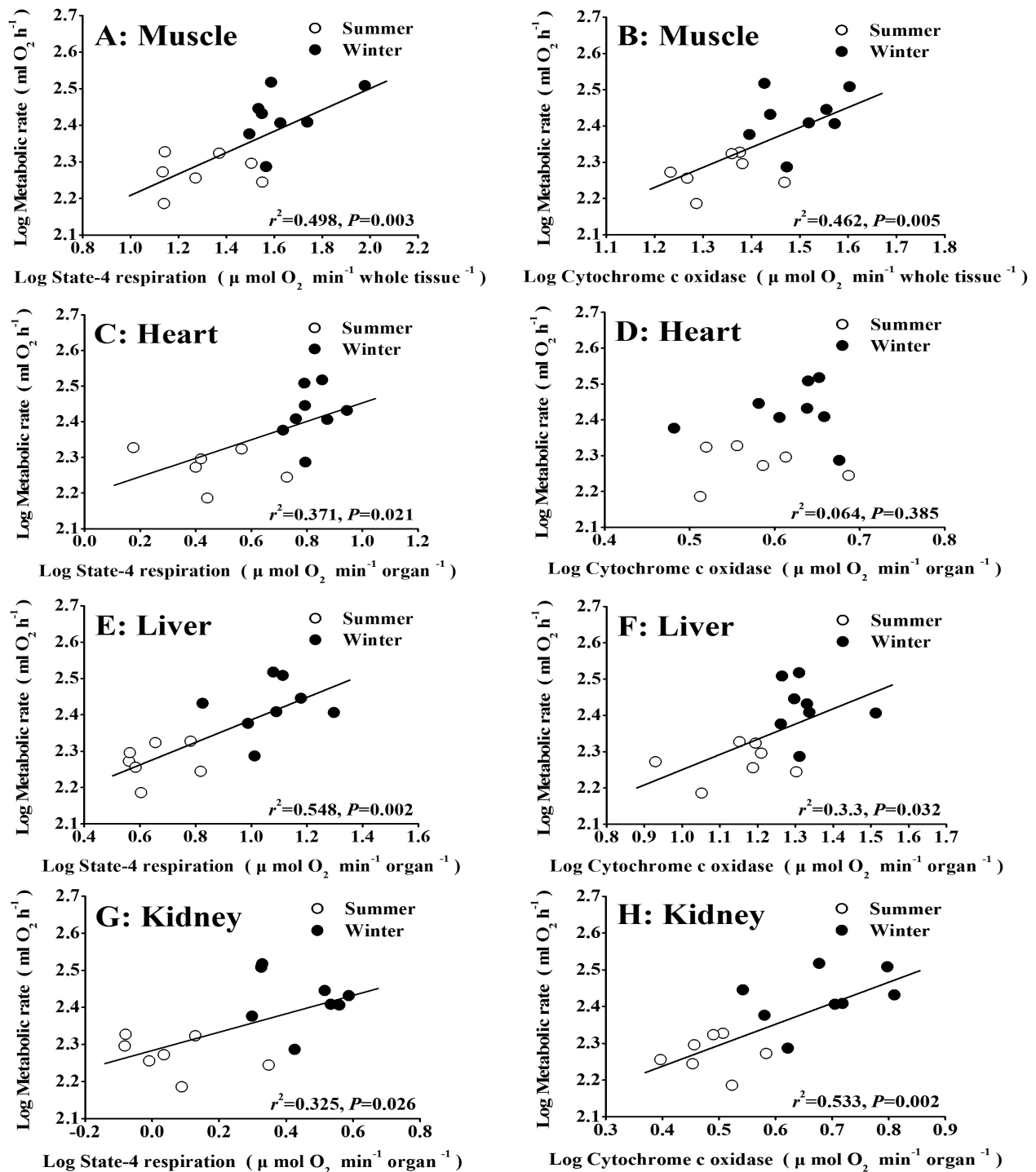
Fig. 5. Correlations between resting metabolic rate (RMR) and state-4 respiration in the pectoral muscle (A), heart (C), liver (E) and kidneys (G), and between RMR and cytochrome *c* oxidase activity in the pectoral muscle (B), heart (D), liver (F) and kidneys (H), in Chinese hwameis (*Garrulax canorus*) captured in either summer or winter in Wenzhou, China.

A positive relationship between thermal conductance and BMR appears to hold for species like the orange-cheeked waxbill *Estrilda melpoda* and the cut-throat finche *Amadina fasciata* (Marschall and Prinzinger 1991), the Chinese bulbul (Zheng et al. 2008a) and the Chinese hwamei (Wu et al. 2015). From the perspective of thermoregulation, high thermal conductances are unfavorable to birds in winter; however, in small birds, increasing metabolic heat production is the main way to compensate for heat loss in winter. This shows that small birds rely on metabolic capacity rather than insulation to maintain their body temperature in cold weathers and the physiological thermoregulatory responses of small bird species in warm, temperate climates has high phenotypic plasticity (Zhou et al. 2016).

Seasonal variation in organ mass and cellular thermogenic capacity

Although the heart, liver, kidneys and digestive tract represent < 10% of total body mass, they account for 50–70% of basal energy consumption (Daan et al. 1990; Clapham 2012; Vézina and Williams 2005). Therefore, adjusting organ mass could also be a means by which the hwamei increases its RMR in winter. However, with the exception of the heart, which was significantly heavier in winter-caught birds, we found no significant seasonal differences in the dry mass of the liver, kidneys or pectoral muscle. This suggests that seasonal variation in organ mass is not always associated with seasonal variation in RMR. State-4 respiration is clearly connected with RMR 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) (Li et al. 2017). In this study, our data indicate that winter-caught birds displayed higher levels of state-4 respiration in all four organs measured compared with those caught in summer. In addition, winter-caught birds showed higher levels of COX in the liver, kidneys, and pectoral muscles than in the summer-caught birds. These results are consistent with those of previous studies. For example, Chinese hwameis acclimated to 15°C displayed higher levels of state-4 respiration and COX activity in the liver, heart and kidneys than those kept at 35°C, suggesting that high aerobic capacity in the active organs could play an important role in the thermogenesis of cold-exposed animals (Zhou et al. 2016). High levels of state-4 respiration and COX activity are related to elevated RMR (Zheng et al. 2013a), a finding that is supported by the significant correlations between state-4 respiration, COX, and RMR in this study (Fig. 5). These results are also consistent with those obtained from other avian species, including the Chinese bulbuls (Zheng et al. 2010 2013a 2014a), Eurasian tree sparrow (Liu et al. 2008; Zheng et al. 2008b 2014b), silky starling (Li et al. 2017), and several small mammals, including plateau pikas (*Ochotona curzoniae*) (Wang et al. 2006a) and root voles (*Microtus oeconomus*) (Wang et al. 2006b). These results suggest that modulation of cellular thermogenesis at the tissue and organ levels are important aspects of seasonal acclimatization in the Chinese hwamei.

In conclusion, phenotypic plasticity is often hypothesized as the means by which organisms adjust to changing environmental conditions (Bozinovic et al. 2003; Zheng et al. 2014a; McKechnie et al. 2006). Our study demonstrates that the Chinese hwamei displays elevated and whole-body response to seasonal acclimatization, including enhanced RMR, EWL, and increased enzymatic activity in specific organs. These results show that the Chinese hwamei exhibits a pronounced seasonal phenotypic flexibility with physiological and biochemical adjustments. Such high phenotypic flexibility may be important to enable the hwamei to survive the in winter cold.

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