Interspecific variation of thermoregulation between small migratory and resident passerines in Wenzhou

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ABSTRACT

Physiological adaptation arises from several fundamental sources of phenotypic variation. Most analyses of metabolic adaptation in birds have focused on the basal metabolic rate (BMR), the lower limit of avian metabolic heat production. In this study, we investigated thermoregulation in three passerine species; the yellow-billed grosbeak *Eophona migratoria*, white-rumped munia *Lonchura striata* and black-throated bushtit *Aegithalos concinnus*, in Wenzhou, China. Metabolic rate was measured using the closed-circuit respirometer containing 3.5 L animal chambers. Body temperature (T_b) was measured during metabolic measurements using a lubricated thermocouple. The minimum thermal conductance of these species was calculated by measuring their T_b and metabolic rates. The yellow-billed grosbeak remained largely normothermic, and the white-rumped munia and black-throated bushtit exhibited variable T_b at ambient temperatures (T_a). Mean metabolic rates within thermal neutral zone were 2.48±0.09 O_2 (mL)/g/h for yellow-billed grosbeaks, 3.44±0.16 O_2 (mL)/g/h for white-rumped munias, and 3.55±0.20 O_2 (mL)/g/h for black-throated bushtits, respectively. Minimum thermal conductance of yellow-billed grosbeak, white-rumped munia and black-throated bushtit were 0.13±0.00, 0.36±0.01, and 0.37±0.01 O_2 (mL)/g/h/°C, respectively. The ecophysiological characteristics of these species were: (1) the yellowbilled grosbeak had relatively high T_b and BMR, a low lower critical temperature and thermal conductance, and a metabolic rate that was relatively insensitive to variation in T_a; all of which are typical of cold adapted species and explain its broader geographic distribution; (2) the white-rumped munia and black-throated bushtit had high thermal conductance, lower critical temperature, and relatively low BMR, all which are adapted to warm environments where there is little selection pressure for metabolic

thermogenesis. Taken together, these data illustrate small migratory and resident passerines that exhibit the different characteristics of thermoregulation.

Keywords: Basal metabolic rate; Body temperature; Thermal conductance; *Eophona migratoria*; *Lonchura striata*; *Aegithalos concinnus*

INTRODUCTION

Heat production in response to ambient temperature is commonly referred to as facultative, or adaptive, thermogenesis (Angilletta et al., 2010; Silva, 2006; Zhou et al., 2016). Endotherms primarily use mechanisms that equalize rates of heat production and loss to maintain a high and constant body temperature (Corp et al., 1997; Liu et al., 2004a). The necessity of maintaining an optimal body temperature is one of the major factors influencing the abundance and distribution of birds (Liu et al., 2005; Weathers, 1979), and birds have evolved many morphological and physiological adaptations to achieve this (Swanson & Merkord, 2013; Wiersma et al., 2007). Physiological adaptation arises from several fundamental sources of phenotypic variation. Most analyses of metabolic adaptation in birds have focused on the basal metabolic rate (BMR), the lower limit of avian metabolic heat production (McKechnie et al., 2006; McNab, 2009). BMR is a standardized baseline metabolic parameter that reflects a species' resting energy requirements in the absence of the increased metabolic demands associated with thermoregulation, digestion, activity or circadian rhythms (McKechnie, 2008; McNab, 2009; Zhou et al., 2016). BMR is a widely-accepted benchmark of metabolic expenditure for birds that is commonly used as a measure of

the energetic cost of thermoregulation. It has consequently been the focus of considerable research interest from environmental physiologists and comparative physiologists (e.g., Liu et al., 2005; Zheng et al., 2014a).

Ambient temperature (T_a) is considered one of the most important environmental factors affecting birds because it causes marked changes in their energy expenditure (Nzama et al., 2010) and has driven the evolution of a suite of morphological and physiological adaptations (Swanson et al., 2014). A considerable body of research has been conducted to examine the effects of temperature on animal adaptation, survival, and reproductive success (Sgueo et al., 2012; Zhou et al., 2016). In a comparative study of small birds with different habitats and habits, Rezende et al. (2002) recently emphasized the ecological significance of BMR. For example, tropical birds typically have a lower BMR than cold temperate birds, which is thought to be an adaptation to avoid heat stress and conserve water (Weathers, 1997; Wiersma et al., 2007). Conversely, the higher BMR of cold temperate and Arctic birds is thought to be an adaptation to colder temperatures and shorter breeding seasons (Klaassen, 1995; Zheng et al., 2014b). It has been suggested that the BMR of a number of long-distance migratory birds is lower in their tropical overwintering range than at their temperate breeding grounds (Lindström & Klaassen, 2003; Zheng et al., 2013). The higher metabolic capacities of high latitude species may involve a combination of genetic responses to climatic factors (Liknes & Swanson, 2011; Swanson, 2010; Wikelski et al., 2003). These findings indicate that environmental conditions are very important in shaping the thermoregulatory features of a species. There is now considerable evidence to show that the metabolic characteristics of birds are part of a network of physiological mechanisms that mediate major life-history trade-offs (Wikelski et al., 2003).

The yellow-billed grosbeak *Eophona migratoria* is a migratory bird that inhabits vast areas of northeast Asia. The white-rumped munia *Lonchura striata* is a common resident breeder in southern China and South Asia. The distribution of the black-throated bushtit *Aegithalos concinnus* ranges from the foothills of the Himalayas, across northern India through Nepal, to northern Vietnam and Taiwan (MacKinnon & Phillipps, 2000). Although all three species experience the same temperature and photoperiod in autumn and winter in Wenzhou, the yellow-billed grosbeak is a Palearctic bird that migrates to Wenzhou in winter whereas the white-rumped munia and black-throated bushtit are Indomalayan species that are resident in Wenzhou, and their thermoregulatory responses could differ (e.g., climatic differences across the ranges). To test the hypothesis that characteristics of thermoregulation in these three species are consistent with their respective biogeographic distributions, we compared body temperature, metabolic rate and thermal conductance among three small birds at different ambient temperatures. These results could make us better understand how these species adapt their environments. In addition, through the comparison with other small birds, it will help identify sources of variation in thermoregulatory characteristics in these small birds.

MATERIALS AND METHODS

Animals

Seven yellow-billed grosbeaks (six male, one female), ten white-rumped munias (six male, four female) and nine black-throated bushtits (seven male, two female) were captured by mist nets in Wenzhou city (N27°29', E120°51'), Zhejiang Province, China. The yellow-billed grosbeak *Eophona migratoria* is a granivorous and insectivorous migratory bird. The white-rumped munia *Lonchura striata* is a gregarious bird that feeds mainly on seeds. The black-throated bushtit *Aegithalos concinnus* mainly feeds on small insects and spiders, as well as small seeds, fruits and berries (particularly raspberries). The climate in Wenzhou is warm-temperate with the mean annual temperature is 18 ˚C. There are seven months of the year (March through September) in which the maximum temperature is above 37 ˚C (Zheng et al., 2008a; 2014a). All experiments were carried out from October to December 2012. Animals were kept in individual cages (50 cm×30 cm×20 cm) under natural photoperiod (14L:10D) with lights on at 0600h and temperature (25 ˚C). Food and water were supplied *ad libitum*. Yellow-billed grosbeaks and white-rumped munias were fed millet seeds, and black-throated bushtits were fed bird cake and mealworm *Tenebriomolitor* larvae. The mean body mass of yellow-billed grosbeaks, white-rumped munias and black-throated bushtits was 50.5±0.6 g (47.9-52.4 g), 12.6±0.3 g (11.1-14.5 g) and 6.8±0.1 g (6.4-7.4 g), respectively. All experimental procedures were approved by the Wenzhou City Animal Care and Use Committee, Zhejiang Province, China (Wu et al., 2015).

Measurement of metabolic rate

Oxygen consumption was measured using a closed-circuit respirometer according to the methods described by Górecki (1975) and Liu et al. (2004a; 2005). The volume of the metabolic chamber was 3.5 L and its temperature was controlled by a water bath in Artificial Climatic Engine (BIC-300, Shanghai) and maintained to ±0.5 ˚C. Every bird was tested only one Ta per day with at least two days between tests (Xia et al., 2013). Oxygen consumption rates were measured over a temperature range of 5 ˚C to 35 ˚C. Food was withheld four hours before animals were placed in the metabolic chamber to minimize the heat increment associated with feeding before each test. Birds were weighed to the nearest 0.1 g before being put in the chamber. Water and CO_2 were absorbed from the air in the chamber by silica gel and KOH. All measurements were made between 2000h and 2400h. Each trial lasted for one hour and commenced after animals had been inside the metabolic chamber for about one hour to acclimate. The reading interval for O_2 consumption was 10 min. Two or three consecutive, stable, minimum, recordings were used to calculate metabolic rates (Zheng et al., 2008b). Records of oxygen consumption when birds were active within the chamber were not used to compute the metabolic rate of each individual. Metabolic rates were expressed as O_2 (mL)/g/h, and corrected to standard

temperature and pressure conditions (Schmidt-Nielsen, 1997). Body mass was measured to the nearest 0.1 g before and after experiments. Mean body mass was used in calculations (Liu et al., 2005; Wu et al., 2015).

Measurement of body temperature

Body temperature (T_b) was measured during metabolic measurements using a lubricated thermocouple. This was inserted into the cloaca of each bird to a depth at which a slight withdrawal did not result in a change in the reading (1-2 cm). Thermocouple outputs were digitized using a thermocouple meter (Beijing Normal University Instruments Co., China) (Wu et al., 2015).

Calculation of thermal conductance

Total wet thermal conductance (C, O_2 (mL)/g/h/$^\circ$C) at any given T_a was calculated using the formula:

$$C=MR/(T_b-T_a) \quad (1)$$

Where MR is metabolic rate (O_2 (mL)/g/h/$^\circ$C), T_b the body temperature ($^\circ$C), and T_a the ambient temperature ($^\circ$C). This formula was suggested by Aschoff (1981) for calculating conductance at any given T_a.

Statistics

The data were analyzed using the SPSS statistical package (version 12.0 for windows). The effect of T_a on body temperature, metabolic rate and thermal conductance were analyzed using repeated measures ANOVA. Where appropriate, multiple *post hoc* comparisons were performed using the least significant difference method (LSD). The relationships between metabolic rate and T_b and T_a were modeled by fitting linear regression models. The relationships between thermal conductance and T_a were modeled by fitting exponential equation models, to the data, as appropriate. All results were expressed as mean±SE and $P<0.05$ was taken to be statistically significant.

RESULTS

Males and females of each species did not differ significantly in any measured variable ($P>0.05$ in all cases), so we pooled data for each species for subsequent analyses.

Yellow-billed grosbeak

The mean T_b of this species was 39.9±0.1 $^\circ$C. Although there was no significant difference in T_b over a range of T_a from 5 $^\circ$C to 32.5 $^\circ$C ($F_{7,41}$=1.059, $P>0.05$, Figure 1A), there was, however, significant differences in metabolic rate (MR) over this temperature range ($F_{7,41}$=21.231, $P<0.001$, Figure 1B). We were unsuccessful in identifying a join-point using a two-phase regression procedure (Nickerson et al., 1989), so we instead fit a linear regression model to data below 25 $^\circ$C. Below 25 $^\circ$C, MR increased with decreasing temperature as per the following equation:

MR (O_2 (mL)/g/h)= 5.07-0.11T_a ($P<0.001$, R^2=0.817, n=31) (2)

At 25 $^\circ$C, MR appeared independent of T_a, averaging 2.48±
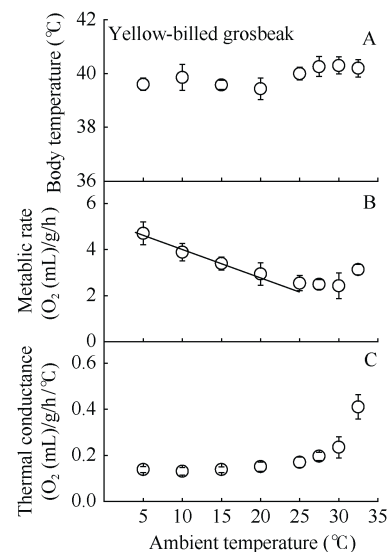

Figure 1 Mean body temperature (A), metabolic rate (B) and thermal conductance (C) of wild caught yellow-billed grosbeaks *Eophona migratoria* measured in an experimental facility in Wenzhou, China at ambient temperatures of approximately 5 $^\circ$C-32.5 $^\circ$C

0.09 O_2 (mL)/g/h. The line described by the above equation intersected MR at 23.5 $^\circ$C, the lower critical temperature. Thermal conductance increased from 15 $^\circ$C to 32.5°C ($F_{7,41}$=49.802, $P<0.001$, Figure 1C), as it was stable within the range of 5° C-15 $^\circ$C. Minimum thermal conductance was 0.13± 0.00 O_2 (mL)/g/h/$^\circ$C (Table 1). Thermal conductance increased exponentially from 25 $^\circ$C to 32.5 $^\circ$C as per the equation:

Log C (O_2 (mL)/g/h/$^\circ$C)=-2.00+0.05 T_a ($P<0.001$, R^2=0.746, n=22) (3)

Maximum thermal conductance was 0.41±0.02 O_2 (mL)/g/h/$^\circ$C at 32.5 $^\circ$C.

White-rumped munia

The T_b of the white-rumped munia fluctuated significantly over ambient temperatures between 5 $^\circ$C and 37.5 $^\circ$C ($F_{9,90}$= 11.190, $P<0.001$, Figure 2A). Mean T_b was 39.9±0.1 $^\circ$C (Table 1) and ranged from 39.1±0.2 $^\circ$C at 5 $^\circ$C to 40.6±0.2 $^\circ$C at 37.5 $^\circ$C. There was a positive, linear relationship between T_b and T_a over this temperature range as per the following equation:

T_b=38.96+0.04 T_a ($P<0.001$, R^2=0.511, n=100)   (4)

The MR of this species varied significantly between 5 $^\circ$C and 37.5 $^\circ$C ($F_{9,90}$=98.310, $P<0.001$, Figure 2B). Between 30 $^\circ$C and 35 $^\circ$C, MR appeared to be independent of Ta and averaged 3.44±0.16 O_2 (mL)/g/h (n=36). For Tas below 30 $^\circ$C,

MR (O_2 (mL)/g/h)=13.09-0.28 Ta ($P<0.001$, R^2=0.824, n=70) (5)

The line intersected MR at 34.5 $^\circ$C, so the lower critical temperature was 34.5 $^\circ$C. Thermal conductance varied significantly within a temperature range from 5 $^\circ$C to 37.5 $^\circ$C ($F_{9,90}$=51.434, $P<0.001$, Figure 2C), but was stable within a temperature range of 5 $^\circ$C to 20°C. Minimum thermal conductance was 0.36±0.01 O_2 (mL)/g/h/$^\circ$C. There was a

Table 1 Energetic parameters of wild-caught yellow-billed grosbeaks, white-rumped munias and black-throated bushtits measured in an experimental facility at Wenzhou, China

	Yellow-billed grosbeak	White-rumped munia	Black-throated bushtit
Body mass (g)	50.5±0.6	12.6±0.3	6.8±0.1
Body temperature (˚C)	39.9±0.1	39.9±0.1	38.9±0.1
BMR (O_2 (mL)/g/h)	2.48±0.09	3.44±0.16	3.55±0.20
Expectation ratio (% predicted)	172	124	99
a (O_2 (mL)/g/h)	5.05	12.59	14.20
b (O_2 (mL)/g/h/˚C)	-0.14	-0.24	-0.37
R^2	0.799	0.792	0.839
P	<0.001	<0.001	<0.001
T_{lc} (˚C)	23.5	34.5	28.8
Conductance (O_2 (mL)/g/h/˚C)	0.13±0.00	0.36±0.01	0.37±0.01
Expectation ratio (% predicted)	137	200	155

The equations are in the form of MR (O_2 (mL)/g/h)=a+b×T_a. Values are mean±SE. BMR is the basal metabolic rate, T_{lc} is the lower critical temperature. The expectationratio of BMR and conductance were predicted from the appropriate equation in Londoño et al. (2015) or Aschoff (1981), respectively. % predicted=(observed/predicted)×100.
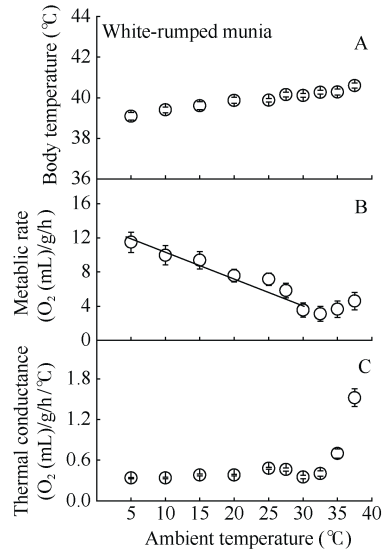

Figure 2 Mean body temperature (A), metabolic rate (B) and thermal conductance (C) of wild caught white-rumped munias *Lonchura striata* **measured in an experimental facility in Wenzhou, China at ambient temperatures of approximately 5 ˚C-37.5 ˚C**

significant, linear relationship between thermal conductance and T_a between 32.5 ˚C and 37.5˚C described by the equation:

Log C (O_2 (mL)/g/h/˚C)=-4.16+0.15 T_a (P<0.001, R^2=0.760, n=30)
(6)

Maximum thermal conductance attained 1.52±0.13 O_2 (mL)/g/h/˚C at 37.5 ˚C.

Black–throated bushtit

T_b varied significantly in this species between 5 ˚C and 34 ˚C ($F_{7,64}$=8.173, P<0.001, Figure3A). Mean T_b was 38.9±0.1 ˚C (Table 1) and tended to increase with T_a, ranging from 37.8±0.4 ˚C at 5 ˚C to 40.4±0.3 ˚C at 34 ˚C. The relationship between T_b and T_a over this temperature range can be described by the following equation:

T_b=37.36+0.08 T_a (P<0.001, R^2=0.362, n=72)   (7)

There were also significant differences in MR from 5 ˚C to 34˚C ($F_{7,64}$=58.547, P<0.001, Figure3B). At 28 ˚C, MR appeared to be independent of T_a and averaged 3.55±0.20 O_2 (mL)/g/h. For T_as below 28˚C,

MR (O_2 (mL)/g/h)=14.20-0.37 T_a (P<0.001, R^2=0.839, n=54) (8)

The line intersected MR at 28.8 ˚C, so the lower critical temperature was 28.8 ˚C. Thermal conductance increased significantly with T_a ($F_{7,64}$=20.001, P<0.001, Figure3C). Minimum thermal conductance was 0.37±0.00 O_2 (mL)/g/h/˚C from 5 ˚C to 28 ˚C. The relationship between thermal conductance and T_a can be described by the equation:

Log C (O_2 (mL)/g/h/˚C)=-2.40+0.07 T_a (P<0.001, R^2=0.643, n=27)
(9)

Maximum thermal conductance averaged 0.80±0.08 O_2 (mL)/g/h/˚C at 34 ˚C.

DISCUSSION

Interspecific variation in metabolic rate, thermal neutral zone (TNZ) and thermoregulation

Londoño et al. (2015) used allometric equations to calculate the expected the BMR of a range of bird species from their published body mass. According to Londoño et al. (2015), the

relationship between BMR and M_b for tropical species, BMR (watts)=0.449 $M_b^{0.589}$ (BMR=Watt, M_b=g); for temperate species, BMR (watts)=0.023 $M_b^{0.729}$. The BMRs of the yellow-billed grosbeak, white-rumped munia and black-throated bushtit were 172%, 124% and 99%, respectively, of the values predicted from body mass (Londoño et al., 2015). At lower T_a values, the BMRs of the yellow-billed grosbeak, white-rumped munia and black-throated bushtit generally decreased with increasing temperature, a pattern typical of endotherms (Schmidt-Nielsen, 1997; Willmer et al., 2005). Consequently we consider the minimum MR recorded in these species to be their true BMR. Many factors, such as body size, phylogeny, climate conditions, activity, and feeding habits, are thought to affect the metabolic levels of birds (McNab, 2000; 2009). Rezende et al. (2002) and McNab (2009) suggested that avian BMR is generally correlated with climate. A reduced level of endogenous heat production may thus be adaptive in low-latitude species (Wiersma et al., 2007; Wikelski et al., 2003), and conversely, higher metabolic rates may be adaptive in mid-latitude and high-latitude species (Swanson, 2010; Zheng et al., 2008b; 2014b). The low BMR of tropical species may arise directly from living in warm environments, with modest demands for metabolic thermogenesis and activity reflected in low rates of baseline energy expenditure (Jetz et al., 2008; White et al., 2007), and linked to their generally slow pace of life and lower investment in reproduction (Londoño et al., 2015; Ricklefs & Wikelski, 2002; Wiersma et al., 2007; Williams et al. 2010). Conversely, the higher BMR of temperate and arctic birds is thought to be an adaptation to colder temperatures and higher investment in reproduction (Klaassen, 1995; Londoño et al., 2015). Previously published data on the BMR of temperate and tropical birds, together with metabolic rates predicted from Londoño et al. (2015) equation are presented (Table 2). The BMR of temperate species is generally higher tropical species.

Table 2 Comparison of observed and predicted basal metabolic rates (BMR) of bird species from cold, temperate, and tropical regions

Species	Body mass (g)	BMR (Watt)	Expectation ratio (%)	Reference
Cold and temperate region				
Black-capped chickadee *Parus atricapillus*	13.8	0.35	223	1
Rufous-necked snowfinch *Montifringilla ruficollis*	22.8	0.47	211	2
Eurasian skylark *Alauda arvensi*	32.0	0.73	255	3
Woodlark *Lullula arborea*	25.6	0.58	237	3
Chestnut bunting *Emberiza rutila*	15.3	0.34	203	4
Little bunting *Emberiza pusilla*	11.3	0.26	196	4
Scarlet rosefinch *Carpodacus erythrinus*	24.2	0.57	242	5
Pallas's rosy finche *Carpodacus roseus*	22.5	0.54	242	6
Brambling *Fringilla montifringilla*	18.0	0.42	222	6
Common redpoll *Acanthis flammea*	11.6	0.29	210	6
Siberian accentor *Prunella montanella*	13.5	0.32	210	7
Chestnut-flanked white-eye *Zosterops erythropleura*	9.2	0.24	207	8
Yellow-browed bunting *Emberiza chrysophrys*	15.9	0.32	187	8
Black-faced bunting *Emberiza spodocephala*	15.1	0.40	239	9
Bohemian waxwing *Bombycilla garrulus*	64.9	0.84	175	9
Eurasian oystercatcher *Haematopus ostralegus*	554.0	2.91	127	10
Black-bellied plover *Pluvialis squatarola*	226.0	1.78	149	10
Ruddy turnstone *Arenaria interpres*	90.0	0.92	150	10
Little ringed plover *Charadrius dubius*	36.0	0.42	134	11
Common quail *Coturnix coturnix*	97.0	0.89	138	11
Eurasian sparrow hawk *Accipiter nisus*	135.0	0.95	116	11
King quail *Excalfactoria chinensis*	42.4	0.42	119	12
Barn owl *Tyto alba*	456.1	2.61	131	12
Western screech owl *Megascops kennicottii*	147.3	1.27	145	12
Rock parrot *Neophema petrophila*	48.4	0.63	162	13
Yellow-billed grosbeak *Eophona migratoria*	48.7	0.67	172	This Study

Species	Body mass (g)	BMR (Watt)	Expectation ratio (%)	Reference
Tropical				
Dusky munia *Lonchura fuscans*	9.5	0.10	60	14
Golden-collared manakin *Manacus vitellinus*	15.5	0.23	105	15
Red-capped manakin *Pipra mentalis*	12.3	0.19	101	15
Orange-cheeked waxbill *Estrilda melpoda*	7.5	0.13	90	16
Zebra finch *Poephila guttata*	12.1	0.18	96	16
Gouldian finch *Chloebia gouldiae*	15.5	0.22	98	16
Cut-throat finch *Amadina fasciata*	17.2	0.21	91	16
Java sparrow *Padda oryzivora*	25.4	0.31	104	16
Gouldian finch *Chloebia gouldiae*	17.1	0.23	99	17
Crested myna *Acridotheres cristatellus*	117.7	0.70	96	18
Puerto Rican tody *Todus mexicanus*	6.1	0.11	86	13
American pygmy kingfisher *Chloroceryle aenea*	11.8	0.17	90	11
Chestnut-backed antbird *Myrmeciza exsul*	28.3	0.29	92	11
Blue-crowned motmot *Momotus momota*	123.1	0.52	69	12
Andean cock-of-the-rock *Rupicola peruvianus*	246.5	1.08	96	12
White-tufted sunbeam *Aglaeactis castelnaudii*	6.9	0.10	73	12
Great sapphirewing *Pterophanes cyanopterus*	10.8	0.11	62	12
White-bellied woodstar *Chaetocercus mulsant*	3.6	0.07	75	12
Chestnut-capped puffbird *Bucco macrodactylus*	23.7	0.18	63	12
Semicollared puffbird *Malacoptila semicincta*	47.1	0.33	78	12
Black-fronted nunbird *Monasa nigrifrons*	86.0	0.46	76	12
White-fronted nunbird *Monasa morphoeus*	67.5	0.45	86	12
Rufous-breasted piculet *Picumnus rufiventris*	21.9	0.26	96	19
White-rumped munia *Lonchura striata*	12.6	0.24	124	This study
Black-throated bushtit *Aegithalos concinnus*	6.8	0.13	99	This study

Basal metabolic rates (BMR) was predicted by the appropriate equation in Londoño et al. (2015). % predicted=(observed/ predicted)×100. For each species, body mass, BMR and expectation ratio (%) are provided. References: 1 Chaplin, 1974; 2 Deng & Zhang, 1990; 3 Tieleman et al., 2002; 4 Liu et al., 2001a; 5 Liu et al., 2001b; 6 Liu et al., 2004a; 7 Liu et al., 2004b; 8 Liu et al., 2005; 9 Li et al., 2005; 10 McKechnie & Wolf, 2004; 11 Wiersma et al., 2007; 12 Londoño et al., 2015; 13 White et al., 2007; 14 Weathers, 1977; 15 Bartholomew et al., 1983; 16 Marschall & Prinzinger, 1991; 17 Burton & Weathers, 2003; 18 Lin et al., 2010.

Feeding habits are also an important factor affecting both the metabolic rates of animals and their geographic distribution. Birds feeding on seeds and fruits tend to have high metabolic rates that thought to be related to the consistency and abundance of food in their environment. In contrast, insectivorous birds tend to have lower metabolic rates (McNab, 1988). The yellow-billed grosbeak and white-rumped munia mainly feed on seeds with the addition of some insects and fruit in summer and autumn, whereas the black-throated bushtit is predominantly insectivorous. It is possible that the different dietary preferences of these species may affect their respective metabolic rates. Our data show that the BMR of the yellow-billed grosbeak and white-rumped munia were higher than the predicted BMR using allometric equations, whereas that of the black-throated bushtit was similar to predicted values, and that

this difference could be due to the grosbeak and munia being predominantly granivorous, whereas the bushtit is more insectivorous. These results are consistent with the previous studies (McNab, 1988).

TNZ is defined as the range of T_a at which temperature regulation is achieved only by control of sensible heat loss, without regulatory changes in metabolic heat production or evaporative heat loss (IUPS Thermal Commission, 1987; Willmer et al., 2005). A lower critical temperature and broader TNZ are typical of species that are adapted to cold (Schmidt-Nielsen, 1997; Willmer et al., 2005). For example, the arctic ptarmigan *Lagopus spp.* does not increase its metabolic rate unless the external temperature falls to -5 °C, and even then its metabolic rate is not greatly affected by ambient temperature (Mortensen & Blix, 1986). Conversely, a higher upper critical

temperature is typical of adaptation to hot climates, especially with regard to water conservation (Williams & Tieleman, 2000). For example, the Chinese hwamei (*Garrulaxcanorus*) may increase its metabolic rate if the ambient temperature drops to just 31 °C, and its metabolic rate is much more sensitive to change in ambient temperature (Wu et al., 2015; Xia et al., 2013). The lower critical temperature of the yellow-billed grosbeak was 23.5 °C, and the slope of the regression equation describing the relationship between metabolic rate and ambient temperature for this species was 0.14 (Table 1), results typical of a cold tolerant species. However, the white-rumped munia and black-throated bushtit increased their metabolic rates when the ambient temperature dropped to 30 °C and 28 °C, respectively, and the slopes of the regression equations describing the relationship between metabolic rate and temperature in these species were steeper; 0.24 O_2 (mL)/g/h/°C for the white-rumped munia and 0.37 O_2 (mL)/g/h/°C for black-throated bushtit (Table 1). These data indicate that these species are relatively intolerant to cold.
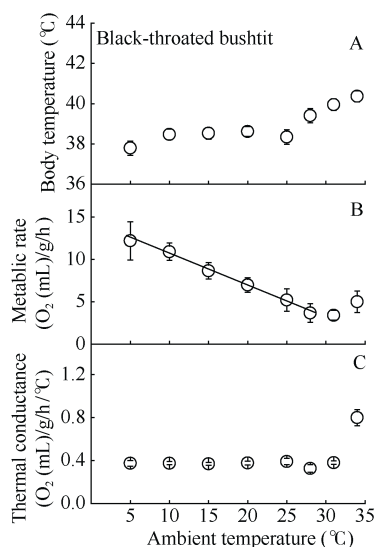

Figure 3 **Mean body temperature (A), metabolic rate (B) and thermal conductance (C) of wild-caught black-throated bushtits** *Aegithalos concinnus* **measured in an experimental facility in Wenzhou, China at ambient temperatures of approximately 5 °C-34 °C**

Interspecific variation in body temperature and thermal conductance

Considerable research has been devoted to the study of avian energetics, including body temperature (Clarke & Rothery, 2008; Prinzinger et al., 1991; Xia et al., 2013). The body temperature of birds depends upon their metabolic rate and heat loss. Small birds have higher body temperatures than larger ones because they have higher mass-specific rates of heat production. For example, the rest-phase T_b of most birds is 38.4 °C or less, but that of passerines is 39.0 °C (Prinzinger et al., 1991). We found that the yellow-billed grosbeak had the least variable T_b among the three species studied at lower T_a values. The high and constant T_b of the yellow-billed grosbeak would be

advantageous in boreal latitudes, whereas the lower T_b of the white-rumped munia and black-throated bushtit may be the result of an optimization process through which these species attempt to minimize their energy expenditure (McKechnie & Lovegrove, 2001).

According to Aschoff (1981) formula, minimal thermal conductance depends on body temperature, metabolic rate and body mass. Small birds have a relatively large surface to volume ratio, less insulation, and higher heat loss, resulting in higher thermal conductance (Schmidt-Nielsen, 1997). Our results show that the minimum thermal conductance of the yellow-billed grosbeak, white-rumped munia and black-throated bushtit were 137%, 200% and 155%, respectively, of the values predicted from Aschoff (1981) allometric equation, indicating that these species are poorly insulated for their body size. Birds that live at low latitudes generally have higher thermal conductance than expected based on their body mass, a feature that may facilitate heat loss (Schleucher & Withers, 2001). In Wenzhou, the mean temperature in October is about 23 °C, so birds inhabiting this region would be expected to have low metabolic heat production and high thermal conductance as a means of avoiding hyperthermia (Liu et al., 2006; Xia et al., 2013; Wu et al., 2015).

Metabolic properties and distribution

The metabolic properties of these species may also be an important factor affecting their distribution in China. In the present study, the high BMR, low lower critical temperature, and relatively stable metabolic rate, of the yellow-billed grosbeak are typical of a species adapted to cold. These features, in conjunction with its relatively lower thermal conductance and granivorous diet, may explain its broad geographic distribution. Conversely, the relatively low BMR, high thermal conductance, and temperature-sensitive metabolic rate, of the black-throated bushtit are typical of a tropical species. These features, together with its predominantly insectivorous diet, may explain why it is confined to relatively warm regions where insects and other invertebrates are more abundant. The white-rumped munia shares characteristics of both the yellow-billed grosbeak and black-throated bushtit. It has a relatively high BMR, thermal conductance, lower critical temperature, and its metabolic rate is relatively sensitive to changes in ambient temperature. These features, coupled with its food habit, may explain its relatively warm geographic distribution. In the present study, our data illustrate variation in the thermoregulatory characteristics of small passerine species that differ in their biogeographic distributions.

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