Effects of temperature acclimation on body mass and energy budget in the Chinese bulbul *Pycnonotus sinensis*

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**Abstract:** Chinese bulbuls (*Pycnonotus sinensis*) are small passerine birds that inhabit areas of central, southern and eastern China. Previous observations suggest that free–living individuals of this species may change their food intake in response to seasonal changes in ambient temperature. In the present study, we randomly assigned Chinese bulbuls to either a 30 °C or 10 °C group, and measured their body mass (BM), body temperature, gross energy intake (GEI), digestible energy intake (DEI), and the length and mass of their digestive tracts over 28 days of acclimation at these temperatures. As predicted, birds in the 30 °C group had lower body mass, GEI and DEI relative to those in the 10 °C group. The length and mass of the digestive tract was also lower in the 30 °C group and trends in these parameters were positively correlated with BM, GEI and DEI. These results suggest that Chinese bulbuls reduced their absolute energy demands at relatively high temperatures by decreasing their body mass, GEI and DEI, and digestive tract size.

**Keywords:** Body mass; Energy budget; *Pycnonotus sinensis*; Temperature acclimation

Thermogenesis and adjustment of energy intake are important for the survival of winter—particularly active birds in their natural environment (O’Connor, 1995, Yuni & Rose, 2005). The daily energy expenditure of many birds is usually higher in winter (Bryant et al, 1985; O’Connor, 1995; Stokkan et al, 1986) and this change may be triggered by environmental factors such as photoperiod, ambient temperature, and diet quality and/or quantity (Starck, 1996; 1999; Boon et al, 2000; Klaassen et al, 2004). Temperature is an important environmental zeitgeber for seasonal acclimatization in birds (McKechnie et al, 2007; Swanson, 2001; Tieleman et al, 2003; Vézina et al, 2006). Low-temperature stress during winter in temperate zones may induce an increase in energy expenditure during a season of generally decreased food availability (Krams et al, 2010). Many birds have a variety of strategies to cope with low temperature stress, such as increasing body mass, food intake and thermogenesis (Bednekoff et al, 1994; Goymann et al, 2006; Krams et al, 2010; Webster & Weathers, 2000; Williams & Tieleman, 2000; Swanson, 2010). The body mass of a bird is the sum of its energy intake and energy expenditure. In a bird maintaining constant body mass, time–averaged energy intake equals time–averaged energy use (Hammond & Diamond, 1997). This balance depends on the interplay between the intake and digestive processing of matter and energy, and their allocation among diverse functions, including thermoregulation, growth and reproduction (Caviedes-Vidal et al, 2007). Accordingly, morphological changes in the digestive tract provide a useful indication of energy expenditure (Williams & Tieleman, 2000; Karasov et al, 2011).

Phenotypic flexibility is a response to environmental conditions varying predictably, or of more stochastic fluctuations in the environment (Piersma & Drent, 2003; Starck & Rahmaan, 2003). Such responses to variable conditions ought to be reversible and repeatable.
digestive tract will decrease accordingly. Furthermore, when individuals have low energy intake in response to warm ambient temperatures similar to those typically experienced in summer.

We hypothesized that phenotypic variation in body mass, energy budget and digestive organs may depend not only on resorption and assimilation processes, but also on fine adjustment of its morphology, such as epithelial resorptive surface dimensions, volume and transport efficiency (DeGolier et al, 1999; Goymann et al, 2006; Karasov et al, 2004; Lavin et al, 2008; Starck & Rahmaan, 2003; Webster & Weathers, 2000).

Chinese bulbuls (Pycnonotus sinensis) are small passerines that are distributed over vast areas of central, southern and eastern China (MacKinnon & Phillipps, 2000). They have been reported to have high body temperature ($T_b$), high upper critical temperature ($T_{uc}$), low BMR, a relatively wide thermal neutral zone (TNZ) (Zhang et al, 2006), and they increase their body mass and BMR in response to colder temperatures (Zheng et al, 2008b). There is evidence that this species enhances its thermogenic capacity by increasing organ mass and respiratory enzyme activity (Zhang et al, 2008; Zheng et al, 2010). For this study, we selected Chinese bulbuls as a study species because they are resident in Zhejiang Province where we are based, and global warming appears to have allowed this species to colonize northeastern and northwestern China (Li et al, 2006; Song, 2006), and previous studies (Ni et al, 2010; Peng et al, 2010; Zhang et al, 2006, 2008; Zheng et al, 2008a, 2010; Zhou et al, 2010) provide critical background information required for our research. In this report, we investigated changes in body mass, energy budget and digestive tract morphology under two different metabolic loads, or energy demands, elicited by ambient temperature. We hypothesized that phenotypic variation in body mass, energy budget and digestive organs may play important roles in the adaptation of Chinese bulbuls to changing environmental conditions. We predicted that Chinese bulbuls will decrease their body mass and energy intake in response to warm ambient temperatures similar to those typically experienced in summer. Furthermore, when individuals have low energy requirements we predict that the mass and length of their digestive tract will decrease accordingly.

**MATERIALS AND METHODS**

In Wenzhou, the climate is warm-temperate with an average annual rainfall of 1 700 mm spread across all months, with slightly more precipitation during winter and spring. The mean annual temperature is 18 °C. Mean maximum daily temperature ranges from 39 °C in July to 8 °C in January, and mean minimum daily temperature from 28 °C in July to 3 °C in the same month. Mean winter and summer temperatures are 6 °C and 31 °C, respectively (Zheng et al, 2008b).

**Animals**

Chinese bulbuls were captured in mist nets in Wenzhou city during March 2011. Body mass to the nearest 0.1 g was determined immediately upon capture with a Sartorius balance (model BT25S). Birds were transported to the laboratory on the day of capture and caged outdoors for 1 or 2 d in 50×30×20 cm enclosures under natural photoperiods and temperatures of 13±1 °C before temperature acclimatization and measurements commenced. Food and water were supplied *ad libitum*. Birds were moved into individual cages for at least two weeks, after which 16 birds were randomly assigned to either the 30 °C or 10 °C group (eight birds in each group). Both groups were acclimated to these temperatures for 28 days. Body temperature was measured at 21:00 and 23:00 using a digital thermometer (Beijing Normal University Instruments Co.). The probe of the thermometer was inserted 3 cm into each bird’s cloaca and a reading taken within 30 s.

**Energy budget**

We regarded digestive energy intake as an index of total daily energy expenditure. During the 28 day experimental period, food was provided quantitatively and water was provided *ad libitum*. Food residues and feces were collected during the two days before temperature acclimation began (week 0) and once a week (every seventh day) thereafter throughout the four week experimental period. These residues were separated manually, then oven-dried at 60 °C until a constant mass was obtained. The caloric content of the residual food and feces were determined using a C200 oxygen bomb calorimeter (IKA Instrument, Germany). Gross energy intake (GEI), feces energy (FE), digestible energy intake (DEI), and digestibility of energy were calculated according to Grodzinski & Wunder (1975) and Ni et al (2010):

\[
\text{GEI (kJ /day)}=\text{dry food intake (g/day)}\times\text{caloric value of dry food (kJ/g)}
\]

\[
\text{FE (kJ/day)}=\text{dry mass of feces (g/day)}\times\text{caloric value of dry feces (kJ/g)}
\]

\[
\text{DEI (kJ/day)}=\text{GEI (kJ/day)}–\text{FE (kJ/day)}
\]

\[
\text{Digestibility (％)}=\text{DEI (kJ/day)/GEI (kJ/day)}\times100%
\]

**Measurements of organ masses**

Following the observation period, all birds were killed humanely and their digestive tracts (gizzard, small intestine and rectum) were removed, measured (±1 mm) and weighed (±0.1 mg) at the end of the 28 day acclimation period. The gizzard, small intestine and
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Rectum were then rinsed with saline to remove all gut contents before being dried and reweighed. These organs were then dried to a constant mass over 2 d at 75 °C and reweighed to the nearest 0.1 mg (Liu & Li, 2006; Williams & Tieleman, 2000).

Statistics

Data were analyzed using SPSS 12.0. All variables were tested for normality using the Kolmogorov-Smirnov test. Non-normally distributed data were transformed to natural logarithms. Repeated-measures analysis of variance (RM-ANOVA) was used to determine the significance of changes in body mass, body temperature, GEI, FE, DEI and digestibility over time. Least significant difference (LSD) post hoc tests were used to detect significant differences in the above parameters within the same group of birds between different days of temperature acclimation. The independent sample t-test was used to assess the significance of any differences in the body mass between 10 °C and 30 °C birds measured on the same day. Differences in the above variables between 10 °C and 30 °C birds, except for those in body mass, were evaluated using ANOVA or ANCOVA with body mass as a covariate where appropriate. In the case of digestibility, which was a percentage value, the arcsine-square-root transformation was performed prior to analysis to normalize the data. All results are expressed as mean±SEM, with P<0.05 being considered statistically significant.

RESULTS

There were no significant differences in body mass, body temperature, GEI, FE, DEI or digestibility between the 10 °C and 30 °C birds prior to the experiment.

Body mass and body temperature

The body mass of the 30 °C birds decreased significantly throughout the course of the experiment (RM-ANOVA, F_{4,35}=2.718, P<0.05, Figure 1A). Although this decrease in body mass was not significant after day 7 or day 14 (post hoc, P>0.05) and by day 28 the body mass of the 30 °C group had decreased by 11.2% relative to that measured on day 0 (post hoc, P<0.05). In contrast, the 10 °C birds maintained relatively constant body masses over the duration of the experiment (RM-ANOVA, F_{4,35}=0.420, P>0.05).

There was no significant difference in body mass between the 30 °C and 10 °C birds after day 7 (t_{14}=0.550, P=0.595), day 14 (t_{14}=0.196, P>0.05) and day 21 (t_{14}=0.212, P=0.595), but there was after day 28 (t_{14}=2.371, P<0.05). The end of experiment, the body mass of the 30 °C group was 7.5% lower than that of the 10 °C group (Figure 1A).

Neither the 10 °C nor the 30 °C group underwent significant changes in body temperature during the course of the experiment (RM-ANOVA, 10 °C, F_{4,35}=1.861, P>0.05; 30 °C, F_{4,35}=1.349, P>0.05, Figure 1B), nor were any significant between-group differences in body temperature detected (day 7, F_{1,14}=2.679, P>0.05; day 14, F_{1,14}=0.009, P>0.05; day 21, F_{1,14}=0.260, P>0.05; day 28, F_{1,14}=0.531, P>0.05).

Energy intake and digestibility

Birds in the 10 °C group did not undergo significant changes in FE (RM-ANOVA, F_{4,34}=2.239, P>0.05, Figure 2B) or digestibility (RM-ANOVA, F_{4,34}=1.283, P>0.05, Fig. 2D), but did undergo significant changes in GEI (RM-ANOVA, F_{4,34}=24.508, P<0.05; Figure 2A) and DEI (RM-ANOVA, F_{4,34}=6.494, P<0.01, Figure 2A) and DEI (RM-ANOVA, F_{4,34}=6.494, P<0.01, Figure 2C). Although no significant change in these two parameters occurred during the first three weeks of the experiment, both had decreased significantly by day 28 (post hoc, P<0.05).

Birds in the 30 °C group underwent significant changes in GEI, FE, DEI and digestibility (RM-ANOVA, GEI, F_{4,34}=24.508, P<0.001, Fig. 2A; FE, F_{4,34}=15.741, P<0.001 Figure 2B; DEI F_{4,34}=22.236, P<0.001 Figure 2C; digestibility, F_{4,34}=3.438, P<0.05; Figure 2D). Significant changes in GEI, FE and DEI were evident by Figure 1 Trend in body mass and body temperature in Chinese bulbuls acclimated to either 30 °C or 10 °C

Data are presented as mean±SE. Different letters (a or b) indicate significant differences (P<0.05) between measurements made on birds acclimated to 30 °C on different days; *. P<0.05.
Figure 2  Trends in GEI, FE, DEI and digestibility in Chinese bulbuls acclimated to either 30 °C or 10 °C

Data are presented as mean±SEM; Different letters (a, b, c, or d for the 30 °C group; and A, B or C for the 10 °C group) indicate significant differences (P<0.05) in measurements made on birds in either the 30 °C or 10 °C group on different days; **: P<0.01; ***: P<0.001 indicate significant differences between the group of birds acclimated to 30°C and those acclimated to 10°C.

day 7 (post hoc, GEI, P<0.05; FE, P<0.05; DEI, P<0.05) and continued over the subsequent three weeks. The GEI, FE and DEI of the 30 °C group were significantly lower than those of the 10 °C group on days 7, 14, 21 and 28 (ANOVA, day 7, GEI, F1,13=24.271, P<0.001; FE, F1,13=14.812, P<0.01; DEI F1,13=14.395, P<0.01; day 28, GEI, F1,13=46.130, P<0.001; FE, F1,13=18.252, P<0.01; DEI F1,13=96.832, P<0.001). No significant difference in digestibility between the 10 °C and 30 °C groups was observed (ANOVA, day 28, F1,13=0.305, P>0.05). Log GEI and DEI were positively correlated with log body mass at day 28 (GEI: \( r^2 = 0.372, P<0.01; \) DEI: \( r^2 = 0.458, P<0.01 \), Figure 3).

Digestive tract morphology

Although no significant changes in gizzard and rectum length (ANOVA, gizzard, F1,13=0.027, P>0.05; rectum, F1,13=1.108, P>0.05, Figure 4) were detected, the length of the total digestive tract and small intestine varied significantly over the course of the experiment (ANOVA, total digestive tract, F1,13=5.325, P<0.05;...
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small intestine, $F_{1,13}=5.626, P<0.05$, Figure 4), becoming shorter in 30 °C birds than in 10 °C birds. The log of total digestive tract length was positively correlated with log body mass, GEI and DEI at day 28 (body mass: $r^2=0.267, P<0.05$, Figure 6; GEI: $r^2=0.237, P<0.05$, Figure 7; DEI: $r^2=0.399, P<0.01$, Figure 8).

There was no significant difference in gizzard mass between the 10 °C and 30 °C birds at the end of the experiment (ANCOVA, wet mass with content, $F_{1,13}=0.706, P>0.05$; wet mass without contents, $F_{1,13}=0.396, P>0.05$, dry mass, $F_{1,13}=0.434, P>0.05$, Figure 5).

However, the mass of the small intestine changed significantly (ANCOVA, wet mass with contents, $F_{1,13}=7.158, P<0.05$; wet mass without contents, $F_{1,13}=13.491, P<0.01$; dry mass, $F_{1,13}=15.569, P<0.01$, Figure 5). Small intestine wet mass with contents, wet mass without contents and dry mass were 30.1%, 35.0% and 36.3% lower, respectively, in the 30 °C group than in their 10 °C counterparts.

No significant difference in rectal mass between 10 °C and 30 °C birds was evident at day 28 (ANCOVA, wet mass with contents, $F_{1,13}=1.832, P>0.05$; wet mass without contents, $F_{1,13}=1.311, P>0.05$, dry mass, $F_{1,13}=0.484, P>0.05$, Figure 5).

However, bulbuls in the 30 °C group had a lighter digestive tract overall than 10 °C birds (ANCOVA, wet mass with contents, $F_{1,13}=7.580, P<0.05$; wet mass without contents, $F_{1,13}=15.858, P<0.01$; dry mass, $F_{1,13}=16.275, P<0.01$, Figure 5). The log of wet and dry mass of the complete digestive tract was positively correlated with log body mass, GEI and DEI at day 28 (body mass, wet mass: $r^2=0.462, P<0.01$; dry mass: $r^2=0.626, P<0.001$, Figure 6; GEI, wet mass: $r^2=0.635, P<0.001$; dry mass: $r^2=0.726, P<0.001$, Figure 7; DEI, wet mass: $r^2=0.615, P<0.001$; dry mass: $r^2=0.712, P<0.001$, Figure 8).

**DISCUSSION**

Our results suggest that ambient temperature had significant effects on the body mass, GEI, FE, and DEI of...
Chinese bulbuls, all of which decreased significantly in birds acclimatized to 30 °C. These birds also underwent a significant decrease in the length and mass of the digestive tract.

Ambient temperature is known to have significant effects on many morphological physiological and biochemical parameters of birds, including body mass (Tieleman et al., 2003; Cooper, 2007; McKechnie et al., 2007; Zheng et al., 2008a). Our body mass results were consistent with those of previous reports on the response of Chinese bulbuls to seasonal changes (Zhang et al., 2008; Zheng et al., 2008b, 2010). We found that bulbuls acclimatized to 10 °C maintained a relatively constant body mass but that those acclimatized to 30 °C decreased in body mass over the four week experimental period, and by the end of the experiment the body mass of these birds was 7.5% lower than that of those kept at 10 °C (Figure 1A). The decrease in the body mass of bulbuls acclimatized to 30 °C reflected an imbalance between energy intake and expenditure, a hypothesis supported by changes in other parameters, such as GEI, DEI, and the length and mass of the digestive tract.

Environmental temperature can alter the energy intake of birds. Cold winter temperatures will increase energy consumption because of the increased need for energy to maintain body temperature. Warmer temperatures on the other hand decrease energy requirements because less heat needs to be produced to maintain body temperature (Cain, 1973; Syafwan et al., 2012). There is marked variation across avian species in the reported energy budgets associated with acclimatization or acclimation to changing ambient temperatures (Salvante et al., 2010; Syafwan et al., 2012). For example, the GEI of warm (38 °C) acclimated Muscovy ducks *Cairina moschata* decreased by 49% relative to that of control (20 °C) birds, and that of cold (−10 °C) acclimated ducks increased by 62% relative to that of control birds in an 11 hour photoperiod (Cain, 1973). Similarly, the GEI of summer acclimatized Elliot’s pheasants *Syrmaticus ellioti* was 29% and 34% lower than that of autumn and winter acclimatized birds, respectively (Lou et al., 2003). This suggests that changes in GEI and DEI are a common response to variation in ambient temperature for small birds and mammals in both warm and temperate habitats (Stokkan et al., 1986). We found that Chinese bulbuls acclimatized to 30 °C underwent a 47% decrease in GEI, and a 45% decrease in DEI, relative to those kept at 10 °C. These changes in GEI and DEI were positively correlated with body mass (Figure 3). These results suggest that bulbuls acclimatized to 30 °C experienced an obvious decrease in energy requirements. One could predict that, if different physiological systems have to compete for energy, the combination of these energetically challenging processes would affect heat production, as we found. Chinese bulbuls acclimatized to 30 °C for four weeks decreased their basal metabolic rate (BMR), liver, kidney and small intestine mass, and mitochondrial state–4
respiration and cytochrome c oxidase (COX) activity in liver and muscle, compared to those kept at 10 °C (Zheng et al., 2013). In view of the mass–specific energy metabolism of these organs and/or tissues, the observed decreases in body mass, GEI and DEI were not surprising.

Environmental temperature also influences morphological and physiological functions of the digestive tract in birds (Williams & Tieleman, 2000; Starck & Rahmaan, 2003; Karasov, 2011; Karasov et al., 2011). During cold conditions, when energy demands increase, small birds may increase their energy intake, which simultaneously compromises their digestive-efficiency unless they also undergo associated changes in gut size, enzyme activity, nutrient uptake and/or food transit time (McKinney & McWilliams, 2005; Karasov et al., 2011). For example, warm (35 °C) acclimated woodlarks, skylarks, spike–heeled larks Chersomanes albofasciata and Dunn’s lark underwent a respective 21%, 22%, 9%, and 24% decrease in stomach mass, and a 21%, 23%, 23%, and 21% decrease in intestinal mass, relative to that of cold (15 °C) acclimated conspecifics (Tieleman et al, 2003). Similarly, summer acclimatized spruce grouse Canachites canadensis had a 44% lower ventricular mass and a 21% shorter small intestine than winter acclimatized birds (Pendergast & Boag, 1973). According to Sibly’s (1981) model of optimal digestion, one of the advantages of increasing digestive tract size is that it allows an increase in the mean retention time of digesta, thereby increasing digestibility if the ingestion rate is constant. Alternately, it allows a constant mean retention time of digesta, which maintains digestive efficiency if the ingestion rate increases. Our results showed that bulbuls acclimatized to 30 °C decreased the size of their gastrointestinal tracts relative to those bulbuls kept at 10 °C. The significant, positive relationship between log body mass, GEI and DEI (Figure 6, Figure 7 and Figure 8), suggests that this was an adaptive response to decreased body mass and energy intake.

In conclusion, Chinese bulbuls in the 30 °C group had lower body mass, GEI and DEI relative to those in the 10 °C group. The length and wet and dry mass of the digestive tract of bulbuls were also lower in the 30 °C group and had significant positive relationships with body masses, GEI and DEI. Chinese bulbuls reduced their energy demands at high temperatures by decreasing their GEI and DEI, and digestive tract size.

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