Phenotypic flexibility in body mass, basal metabolic rate and summit metabolism in southern red bishops (Euplectes orix): Responses to short term thermal acclimation

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A R T I C L E   I N F O

Article history:
Received 15 February 2013
Received in revised form 2 April 2013
Accepted 4 April 2013
Available online 8 April 2013

Keywords:
Body mass
Intraspecific variation
Metabolic expansibility
Phenotypic plasticity
Resting metabolic rate

A B S T R A C T

Avian basal metabolic rate (BMR) and summit metabolism (Msum) vary within individuals in response to seasonal acclimatization in free-ranging birds, and thermal acclimation under laboratory conditions. We examined relationships between acclimation air temperature (Tacc) and body mass (Mb), BMR and Msum in female southern red bishops (Euplectes orix) from a relatively mild coastal site and a seasonally colder, inland site. Following acclimation for 21 days to Tacc = 10, 22 or 35 °C, Mb, BMR and Msum were all significantly and negatively related to Tacc. The significant relationship between BMR and Tacc did not remain after Mb was included as a covariate, whereas that between Msum and Tacc did. A subsequent reverse acclimation protocol, where bishops were acclimated to a second Tacc value and then re-acclimated to the first, revealed that short-term changes in Mb and BMR were completely reversible, but changes in Msum were only partially reversible. Following the reverse-acclimation protocol, metabolic expansibility (Msum/BMR) varied significantly with air temperature, being greatest at Tacc = 35 °C. Our data suggest that the intraspecific variation previously reported for this species is at least partly driven by factors other than temperature.

1. Introduction

Avian resting metabolic rates generally show a high degree of phenotypic flexibility, with both basal metabolic rate (BMR; minimum normothermic resting metabolic rate) and summit metabolism (Msum; maximum normothermic resting metabolic rate) being adjusted in natural as well as artificial environments (reviewed by McKechnie, 2008; McKechnie and Swanson, 2010; Swanson, 2010). One key driver of phenotypic flexibility in avian BMR is short-term thermal acclimation, with BMR varying within individuals in response to exposure to various air temperatures (Ta) under laboratory conditions. In general, BMR is negatively correlated with Ta, with acclimation to low Ta resulting in birds increasing their BMR and vice versa (Tieleman et al., 2003; Klaassen et al., 2004; McKechnie et al., 2007; Cavieres and Sabat, 2008). This pattern of short-term variation within individual birds is qualitatively consistent with broad-scale interspecific patterns; species from cold climates generally have higher BMRs compared to species from warmer climates (Weathers, 1979; White et al., 2007; Jetz et al., 2008).

In contrast to BMR, almost nothing is known about phenotypic flexibility in Msum in response to short-term thermal acclimation. As the maximum rate of thermogenic heat production in a resting bird (Swanson and Garland, 2008), Msum is a key determinant of cold tolerance. Whereas numerous studies have examined seasonal variation in Msum in birds acclimatized to natural conditions (Dutenhoffer and Swanson, 1996; Swanson, 2001; Cooper, 2002; Liknes et al., 2002; Dawson and Olson, 2003; Wells and Schaeffer, 2012), to the best of our knowledge only one study has involved birds acclimated to a range of Ta under artificial conditions to examine Msum adjustments (Vezina et al., 2006). In the latter study, red knots (Calidris canutus) were acclimated to cold (4 °C), warm (25 °C) and variable (5.1-14.5 °C outdoor) environments for an acclimation period of 18 days. The Msum of birds in the cold and variable treatments was 13% higher than that of birds in the warm treatment, but showed no significant differences in mass-specific Msum.

A better understanding of the functional linkages between adjustments in BMR and Msum in response to short-term thermal acclimation is vital for elucidating the ecological significance of such phenotypic flexibility, and for relating patterns of phenotypic flexibility seen during short term acclimation in captive birds to those seen during seasonal acclimatization in wild birds. Smit and McKechnie (2010), for instance, argued that latitudinal variation in the direction and magnitude of seasonal BMR adjustments was driven by factors...
related to cold tolerance or energy conservation. This idea assumes that BMR and M_sum are indeed functionally linked. Several authors have reported significant correlations between BMR and M_sum in interspecific analyses (Dutenhoffer and Swanson, 1996; Rezende et al., 2002, but see also Wiersma et al., 2007), and BMR and M_sum are correlated in black-capped chickadees (Poecile atricapillus; Lewden et al., 2012). However, intraspecific links between BMR and M_sum remain largely unexplored, and thus laboratory studies examining adjustments in both these variables in acclimated birds are potentially informative. A second, related question that has received little attention is whether metabolic reaction norms vary within species, for instance between populations that differ in seasonal exposure to cold conditions.

To address the above questions, we examined BMR and M_sum adjustments in response to short-term thermal acclimation in two populations of southern red bishops (Euplectes orix), a small passerine widespread in southern Africa. One population inhabits a relatively mild habitat at sea-level, whereas the second inhabits a seasonally much colder habitat at higher altitude. We previously found that wild E. orix populations differ substantially in the magnitude and direction of changes in BMR and M_sum associated with seasonal acclimatisation (van de Ven et al., 2013). Bishops from a colder, inland site increased BMR (whole-animal and mass-specific) and mass-specific M_sum during winter, whereas birds from a milder, coastal site reduced mass-specific BMR (but not whole-animal BMR) and both whole-animal and mass-specific M_sum in winter (van de Ven et al., 2013). Here, we examine intraspecific variation in the magnitude and reversibility of BMR and M_sum phenotypic adjustments in artificial environments during short term thermal acclimation, again comparing bishops from a milder, coastal site and a colder, inland site. We predicted that thermal acclimation would result in negative relationships between air temperature and both BMR and M_sum, and that these responses would be reversible over time scales of weeks. We also examined the repeatability of BMR, M_sum, and the persistence of inter-individual differences under conditions causing rapid phenotypic adjustments has the potential to provide important insights into the ways in which these traits evolve (Swanson and King, in press).

2. Materials and methods

2.1. Animal capture and housing

We captured 30 southern red bishops (Euplectes orix) at each of two sites, namely a small wetland in the Kragga Kamma suburb of Port Elizabeth, Eastern Cape, South Africa (33° 59’ S 25° 30’ E, 175 m.a.s.l.) and a municipal dam surrounded by a wetland in the town of Barkly East, Eastern Cape, South Africa (30° 59’ S 27° 36’ E, 1795 m asl). These sites differ substantially in terms of seasonal temperature variation (Table 1), with Barkly East being similar in terms of winter temperatures and seasonal variation to Aliwal North (the inland site used by van de Ven et al. 2013). At both sites, adult female bishops were caught in mist nets in mid-winter, well outside of this species’ breeding season (Craig, 2005). As was the case in our previous study, we used only adult females to avoid potentially confounding effects arising from breeding season plumage ornamentation in males (van de Ven et al., 2013).

After capture, birds were individually marked with celluloid colour split rings and transported by road to the Department of Zoology at Nelson Mandela Metropolitan University (NMMU), where they were housed in cages (L:45 cm; W:30 cm; H:40 cm), with five birds per cage. Food (wild bird seed) and water were available ad libitum, and the photoperiod was 12 L:12D (photophase = 5:30 h to 17:30 h) throughout all experiments. Immediately upon arrival at NMMU, the cages containing the birds were placed in a constant environment room set to Ta ≈ 22 °C.

2.2. Metabolic rate measurements

Basal, resting and summit metabolic rates were measured indirectly as oxygen consumption (Vo2) using the same methods as van de Ven et al. (2013; PDF available from corresponding author), except that a 100-L constant-environment cabinet (Humidity Chamber, Labcon, South Africa) was used, allowing measurements in up to four birds simultaneously. The lower critical limit of thermoneutrality (Tlc) was established for each group of bishops in a subset of four individuals after each stage of acclimation or reverse-acclimation to ensure that BMR was measured at thermoneutrality. For all BMR measurements, the air temperature within the chamber was approximately 1 °C higher than the Tlc for that group, and ranged from 32 to 35 °C. For M_sum measurements, the chamber temperature was set to the helox cold limit (Tc) value previously established for that group, using the same approach as van de Ven et al. (2013), with the helox Tc varying between 4–8 °C. As was the case in our previous study (van de Ven et al., 2013), M_sum was measured in only one bird at a time, and body temperature (Tb) was measured with the use of a fine-gauge Cu-Crn thermocouple (TopTronic T235H) inserted approximately 5 mm into the cloaca until a slight withdrawal did not result in a change in the measured value. Body temperature following M_sum measurement was ≥2 °C lower than Tb before measurement in 95.3% of cases, and was ≥3 °C lower in 72.2% of cases, and we are thus confident that our protocol successfully elicited maximum resting metabolic heat production. Air temperature in the constant environment rooms and cabinet used for metabolic measurements was measured using Thermochron iButtons (DS1922L, Maxim, Sunnyvale, CA, USA, resolution = 0.0625 °C) calibrated in a circulating waterbath. The only other difference between our measurements and those of van de Ven et al. (2013) was that in the present study, BMR and M_sum were taken as the lowest and highest average values, respectively, over a 5-min period.

2.3. Experimental protocol

Our study consisted of three phases, namely initial acclimation to captivity, thermal acclimation and reverse thermal acclimation (Fig. 1). Sample sizes varied slightly from those indicated during the course of the experiments on account of occasional deaths, but in no treatment was n < 9.

In their study of metabolic responses to thermal acclimation in laughing doves, McKechnie et al. (2007) found that the BMR of doves decreased significantly following them being moved from outdoor aviaries into indoor cages, possibly on account of a reduction in exercise intensity. For this reason, we placed bishops in indoor cages immediately after capture, and began the experiments by acclimating all 60 individuals to Tc ≈ 22 °C in one room. In order to verify that birds had completely acclimated to these initial conditions, starting

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Table 1

<table>
<thead>
<tr>
<th>Site</th>
<th>Summer Minimum (°C)</th>
<th>Summer Maximum (°C)</th>
<th>Winter Minimum (°C)</th>
<th>Winter Maximum (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Port Elizabeth (175 m asl)</td>
<td>17.4 ± 0.2</td>
<td>25.5 ± 0.8</td>
<td>8.3 ± 0.6</td>
<td>20.5 ± 1.1</td>
</tr>
<tr>
<td>Barkly East (1795 m asl)</td>
<td>10.9 ± 0.9</td>
<td>26.3 ± 1.5</td>
<td>-0.8 ± 1.7</td>
<td>14.7 ± 1.5</td>
</tr>
<tr>
<td>Aliwal North (1300 m asl)</td>
<td>14.5 ± 0.8</td>
<td>30.4 ± 1.4</td>
<td>-2.8 ± 1.1</td>
<td>18.0 ± 1.3</td>
</tr>
</tbody>
</table>
in the second week of initial acclimation we first established the thermoneutral zone in four birds from each population by measuring RMR at 10, 15, 20, 25, 28, 30, 32, 35 and 38 °C in random order (three hours at each temperature), and then repeatedly measured BMR at Ta ≈ 32 °C in eight birds from each population, with five days between each individual’s successive BMR measurements. These measurements from a subset of birds were intended to ensure that they were fully acclimated to artificial conditions, and that subsequent experiments commenced from a state in which metabolic rate was stable. BMR was considered stable when there was no significant difference between the two most recent sets of measurements in the eight birds, which were compared using repeated-measures ANOVA. Typically, BMR stabilised after ~15 days.

The second phase of the study, thermal acclimation, commenced once we were certain that BMR was stable. Following the initial acclimation period, the birds from each population were divided into three groups of 10 each, and each group moved into one of three constant environment rooms set to approximately 10 °C, 22 °C or 35 °C. The actual temperatures of these rooms over the entire period of the experiments averaged 10.35 ± 0.25 °C, 21.96 ± 0.44 °C, and 34.87 ± 0.24 °C respectively. Birds were acclimated to these thermal conditions for 21 days, after which the TNZ was established for four birds per acclimation air temperature (Ta), and BMR and Msum was measured in each individual (i.e., 10 birds per Ta).

The third phase of the study, subsequent to the thermal acclimation described above, involved sequentially acclimating each bishop to a new Ta, and then reverse-acclimating it to the previous Ta (Fig. 1). Following the completion of the thermal acclimation phase described above, the birds were rotated between the three rooms (Fig. 1). For instance, following the ten bishops from the Barkly East population that were housed in the 10 °C room, five were transferred to the 22 °C room and five to the 35 °C room. Birds were then given 21 days to acclimate to the new thermal conditions, after which the TNZ, BMR and Msum were measured as described above. The birds were then returned to the same room they had been in during the thermal acclimation phase. For instance, the five birds that experienced Ta = 10 °C during Acclimation I and 22 °C during Acclimation II were now returned to the 10 °C room. After a further 21 days, the TNZ, BMR and Msum of each bird were measured for the third time (Fig. 1). The reverse thermal acclimation protocol described above was intended to investigate the reversibility of short-term thermal acclimation responses.

2.4. Data analyses

Once Mₙ and BMR stabilised following the initial period of acclimation to captivity, we first tested the dependence of BMR and Msum on Mn using linear regression models. Where no significant scaling was evident, we used t-tests to compare variables between coastal and inland birds, and paired t-tests to compare the Mn between capture and the end of the initial acclimation to captivity phase. All assumptions regarding normality and homoscedascity were verified used Shapiro-Wilk tests and Levene’s tests, respectively. Where necessary, data were log-transformed to meet the assumption of normality. In the case of Msum following initial acclimation, a significant relationship with Mn was evident, and we accordingly used analysis of covariance (ANCOVA) to compare coastal and inland birds.

For the thermal acclimation phase of the experiment (i.e., acclimation to one of three acclimation air temperatures following the initial acclimation to captivity), we analysed Mn, BMR and Msum using analyses of variance (ANOVA) with site (categorical) and Ta (continuous) as predictors. To analyse changes in mass-independent BMR and Msum, we followed the approach of Vézina et al. (2006), and added Mn as a covariate in models for BMR and Msum.

The analysis of the reverse-acclimation phase of our experiment was complicated by an unbalanced design: whereas for each site there were three experimental groups during the preceding thermal acclimation phase (corresponding with each of the three Ta values),
there were effectively six groups during the reverse-acclimation phase, on account of the various sequences of Tacc during this phase of the experiment. Therefore, we analysed these data with % change in Mb, BMR and Msum for each individual between the first and second Tacc experienced (Tacc 1 → Tacc 2), and between the second and third Tacc experienced (Tacc 2 → Tacc 1, since the birds were reverse-acclimated) as response variables. We fitted general linear mixed models (restricted maximum likelihood), with acclimation phase (Tacc 1 → Tacc 2 vs Tacc 2 → Tacc 1) and site (i.e., inland vs coastal) as fixed effects, the change in Tacc (ΔTacc) as a covariate, and individual as a random effect. This design allowed us to test whether acclimation responses were reversible, by comparing the magnitude of change in the response variables between successive rounds of acclimation in opposite directions, after taking into account the magnitude of the change in Tacc experienced by each bird. To analyse the reversibility of changes in mass-independent BMR and Msum, we included % change in Mb as a covariate. The denominator degrees of freedom for fixed effects were estimated using Satterthwaite’s method (Satterthwaite, 1946).

We estimated repeatability (r) values for each population from the variance components of repeated-measures ANOVA (Lessells and Boag, 1987), with acclimation phase (i.e., thermal acclimation, reverse acclimation 1 and reverse acclimation 2) and Tacc as fixed effects and individual as a random effect. We estimated the 95% confidence intervals for r using SE estimates calculated following Becker (1984), and considered r-values to be significant when the 95% confidence intervals did not include zero (Swanson and King, in press). Repeatabilities were estimated for both whole-animal and mass-independent (i.e., residuals from regressions against Mb) BMR and Msum. All statistical procedures in Mb, BMR and Msum, for each individual between the first and second Tacc experienced (Tacc 1 → Tacc 2), and between the second and third Tacc experienced (Tacc 2 → Tacc 1, since the birds were reverse-acclimated) as response variables. We fitted general linear mixed models (restricted maximum likelihood), with acclimation phase (Tacc 1 → Tacc 2 vs Tacc 2 → Tacc 1) and site (i.e., inland vs coastal) as fixed effects, the change in Tacc (ΔTacc) as a covariate, and individual as a random effect. This design allowed us to test whether acclimation responses were reversible, by comparing the magnitude of change in the response variables between successive rounds of acclimation in opposite directions, after taking into account the magnitude of the change in Tacc experienced by each bird. To analyse the reversibility of changes in mass-independent BMR and Msum, we included % change in Mb as a covariate. The denominator degrees of freedom for fixed effects were estimated using Satterthwaite’s method (Satterthwaite, 1946).

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were carried using SigmaPlot 12 or JMP 10 software. Values are presented as mean ± SD.

3. Results

3.1. Initial acclimation to captivity

Upon capture, inland birds (Mb = 23.89 ± 1.87 g, n = 30) were significantly heavier than coastal birds (Mb = 22.27 ± 2.71 g, n = 30; t1,58 = −2.684, P = 0.009). Following the initial acclimation period to Ta = 22 °C, the body mass of inland birds (23.58 ± 2.08 g, n = 30) remained significantly higher than that of birds from the coastal site (22.32 ± 2.64 g, n = 30; t1,58 = −1.266, P = 0.043). Body mass did not change significantly during the initial acclimation period in either the coastal (t1,29 = −0.0883, P = 0.930) or inland group (t1,29 = 0.620, P = 0.540).

Following the initial period of acclimation to Ta = 22 °C, neither BMR (t1,58 = −0.863, P = 0.392) nor mass-specific (M-S) BMR (t1,58 = 0.626, P = 0.534) differed significantly between birds from the inland and coastal sites, and mean BMR was 61.3 ± 9.2 mL O2 hr−1 (n = 60). Moreover, in neither the coastal nor the inland group was BMR following acclimation significantly related to Mb. In contrast, Msum was significantly related to Mb in both the coastal (F1,28 = 11.106, P = 0.002) and inland (F1,28 = 7.186, P = 0.012) groups, but did not differ significantly between the populations when Mb was accounted for (ANCOVA, F1,57 = 0.250, P = 0.619). The mean Msum following initial acclimation to captivity was 401.7 ± 34.6 mL O2 hr−1 (n = 60). The mean metabolic expansibility (i.e., Msum/BMR) was 6.69 ± 1.06, and did not differ between the two populations (t1,58 = 0.598, P = 0.552).

3.2. Thermal acclimation

Following acclimation to Ta = 10, 22 or 35 °C, Mb varied significantly with Tacc (F1,58 = 8.44, P = 0.005), but not with site (F1,58 = 0.907, P = 0.345) or site* Tacc (F1,58 = 2.632, P = 0.110) (Fig. 2). The change in acclimation air temperature (°C)

\[\begin{array}{c|c|c|c|c|c|c|c}
\text{Change in body mass} & \text{Change in BMR} & \text{Change in Msum} \\
\hline
\text{Coastal} & \text{Inland} & \text{Coastal} & \text{Inland} & \text{Coastal} & \text{Inland}
\end{array}\]

During reverse thermal acclimation of southern red bishops (Euplectes orix) from a coastal and an inland site, the percentage changes in body mass (A,B), basal metabolic rate (BMR; C,D) and summit metabolism (Msum; E,F) were significantly and approximately linearly related to the magnitude of change in acclimation air temperature (Tacc). Panels A,C and E show changes associated with bishops moved from a first Tacc to one of two other Tacc values, and panels B,D, and F show changes in the opposite direction, i.e., from the second Tacc experienced by each bird back to the first Tacc.
mean Mb of bishops acclimated to Ta = 10 °C was equivalent to 107.8% of that of bishops acclimated to Ta = 35 °C.

BMR was significantly related to Mb in coastal birds acclimated to 35 °C, but not in any of the other five combinations of site and Tacc, and so we analysed variation using ANOVA. BMR varied significantly with Tacc (F1,58 = 7.404, P = 0.009) but not with site (F1,58 = 1.291, P = 0.261) or site* Tacc (F1,58 = 2.267, P = 0.130) (Fig. 3). When Mb was added as a covariate, however, there was no significant effect of Tacc (F1,58 = 3.763, P = 0.058), nor site (F1,58 = 0.854, P = 0.360) or site* Tacc (F1,58 = 0.972, P = 0.329).

Msum was not significantly related to Mb in any of the six combinations of site and Tacc. Like Mb and BMR, Msum varied significantly with Tacc (F1,58 = 18.793, P < 0.001) but not with site (F1,58 = 0.220, P = 0.641) or site* Tacc (F1,58 = 0.149, P = 0.701) (Fig. 4). The mean Msum of bishops acclimated to Ta = 10 °C was equivalent to 119% of that of birds acclimated to Ta = 35 °C. When Mb was added as a covariate, the significant effect of Tacc on Msum remained (F1,58 = 8.678, P = 0.005), and site (F1,58 = 0.904, P = 0.346) and site* Tacc (F1,58 = 0.047, P = 0.830) remained non-significant. Metabolic expansibility did not vary with Tacc (F1,58 = 0.100, P = 0.753) or site (F1,58 = 0.001; Fig. 5) and unrelated to site (F1,63.9 = 0.0003, P = 0.978), and these patterns of variation remained qualitatively unchanged when % change in Mb was added as a covariate. Percentage change in Msum was also strongly related to M-I (log-transformed to meet the assumption of normality) following reverse acclimation (Table 2). An unexpected observation, which is linked to the observed effect of acclimation direction on changes in Msum concerned metabolic expansibility following the reverse-acclimation phase: when metabolic expansibility (Msum) / basal metabolic rate (BMR)) varied significantly with acclimation air temperature following reverse acclimation.

### 3.3. Reverse thermal acclimation

The % change in Mb was strongly dependent on ΔTacc (F1,57.12 = 55.637, P < 0.001; Fig. 5), and the slope of this relationship did not depend on the direction of acclimation (Tacc 1 → Tacc 2 vs Tacc 2 → Tacc 1; F1,57.12 = 0.221, P = 0.640). There was, however, a significant effect of site (F1,57.67 = 5.452, P = 0.023), with coastal birds having a higher least-squares mean (1.639 ± 1.069%) than inland birds (−1.878 ± 1.061%). Percentage change in BMR was also strongly related to ΔTacc (F1,54.659, P < 0.001; Fig. 5), and there was no significant effect of acclimation direction (F1,72.09 = 0.931, P = 0.338) or site (F1,65.46 = 0.417, P = 0.521), and these patterns of variation remained qualitatively unchanged when % change in Mb was added as a covariate. Percentage change in Msum was also strongly related to ΔTacc (F1,65.76 = 74.548, P < 0.001; Fig. 5) and unrelated to site (F1,65.03 = 0.0003, P = 0.986), but showed a significant effect of acclimation direction (F1,65.62 = 10.130, P = 0.002) which is reflected in the shallower slope of the regression model fitted to the % change in Msum as a function of ΔTacc during Tacc 2 → Tacc 1 (Fig. 5E) compared to during Tacc 1 → Tacc 2 (Fig. 5F). The significant effect of acclimation direction remained (F1,65.18 = 9.370, P = 0.003) when % change in Mb was included in the model.

In both the coastal and inland populations, Mb was highly and significantly repeatable (Table 2). In contrast, however, the repeatability values for BMR and Msum were not significantly different to zero in either population, neither when calculated for whole-animal nor mass-independent (i.e., residual) values (Table 2). An unexpected observation, which is linked to the observed effect of acclimation direction on changes in Msum concerned metabolic expansibility following the reverse-acclimation phase: when metabolic expansibility

### Table 2

<table>
<thead>
<tr>
<th></th>
<th>Coastal</th>
<th>Inland</th>
</tr>
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<tbody>
<tr>
<td>r</td>
<td>l. 95% CI</td>
<td>u. 95% CI</td>
</tr>
<tr>
<td>Mb</td>
<td>0.689*</td>
<td>0.226</td>
</tr>
<tr>
<td>BMR</td>
<td>0.035</td>
<td>−0.227</td>
</tr>
<tr>
<td>M-I BMR</td>
<td>0.001</td>
<td>−0.207</td>
</tr>
<tr>
<td>Msum</td>
<td>0.000</td>
<td>−0.207</td>
</tr>
<tr>
<td>M-I Msum</td>
<td>0.000</td>
<td>−0.207</td>
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*Significant r-values are indicated with asterisks.

### 4. Discussion

Southern red bishops showed considerable phenotypic flexibility in Mb, BMR and Msum in response to short-term thermal acclimation, with these responses being largely reversible over short time scales. Responses to acclimation were similar in the two populations, and we could detect no obvious differences between bishops from a warmer coastal site and cooler inland site during the thermal acclimation phase of the experiment, although inter-population differences in the relationship between Tacc and Mb became evident during the reverse-acclimation phase.

Our finding that Mb was negatively related to Tacc is consistent with the responses of some species to short-term thermal acclimation, but not others. Cold-acclimated red knots (Calidris canutus islandicus), garden warblers (Sylvia borin) and woodlarks (Lullula arborea) all showed higher Mb when compared with warm-acclimated counterparts (Tielman et al., 2003; Klaassen et al., 2004; Vézina et al., 2006; Barceló et al., 2009). In contrast, laughing doves (Streptopelia senegalensis), sky-larks (Alauda arvensis), Dunn’s larks (Eremalda dunni) and stripe-headed larks (Chersornames albofasciata) and rufous-collared sparrows (Zonotrichia capensis) all showed no significant differences in Mb when acclimated to differing Ta values (Tielman et al., 2003; McKechnie et al., 2007; Cavieres and Sabat, 2008). Collectively, these observations suggest that avian Mb responses to short thermal acclimation vary substantially among species. However, the response of Mb to thermal acclimation can also vary among studies of a single species; Williams and Tielman (2000) found that hoopoe larks (Alaemon alaides) acclimated to 15 °C for three weeks were significantly heavier than individuals acclimated to 36 °C, but in a later study individuals of the same species showed no significant difference in Mb when acclimated for three weeks to 15 °C or 35 °C (Tielman et al., 2003).

The relatively higher Mb of bishops acclimated to cold Ta is qualitatively consistent with the seasonal patterns of Mb changes reported for this species (van de Ven et al., 2013). In populations from a coastal site (Port Elizabeth, same area as in the present study) and an inland site (Alwal North, broadly similar but slightly colder conditions than Barkley East), Mb was significantly higher in winter compared to summer (van de Ven et al., 2013). However, the magnitude of the increase in Mb during the thermal acclimation phase of the present study (~8%...
higher at \( T_A = 10 ^\circ C \) vs 35 \( ^\circ C \) is smaller than that observed in wild, seasonally acclimatized birds (~13\% and 21\% higher in winter in coastal and inland birds, respectively (van de Ven et al., 2013). A possible explanation for this difference concerns the greater variability in \( T_A \) experienced by wild birds compared to captive individuals in our study; captive red knots experiencing naturally variable \( T_A \) (mean daily values ranging between 6 and 12 \( ^\circ C \)) showed similar changes in \( M_b \) to conspecifics acclimated to cold (\( T_{acc} \approx 4 \) \( ^\circ C \)) conditions (Vézina et al., 2006).

We did not investigate the processes underlying the adjustment of \( M_b \) during thermal acclimation. In wild birds, winter \( M_b \) increases are typically associated with winter fattening (Dawson and Marsh, 1986; Rogers et al., 1993) and/or changes in the mass of specific organs or muscles (Liu and Li, 2006; Vézina et al., 2011), although winter metabolic adjustments may also reflect changes in the metabolic intensity of organs mediated by upregulation of enzyme activity rather than their masses (e.g., Lïknes and Swanson, 2011). Several laboratory studies of acclimation have documented significant changes in the masses of several organs, including the intestine, kidneys, liver and stomach (Williams and Tieleman, 2000; Tieleman et al., 2003; Cavieres and Sabat, 2008), findings that support the energy demand hypothesis, which predicts that the masses of organs are adjusted to reflect food intake (Williams and Tieleman, 2000). In light of these results, it seems likely that the rapid adjustment of \( M_b \) in southern red bishops involved changes in the masses of specific organs, associated with greater food intake at lower \( T_A \), although we did not confirm this by measuring food intake. In addition, winter acclimatization is often associated with increases in the mass of flight muscles, which is thought to contribute to elevated shivering thermogenic capacity (reviewed by Swanson, 2010), and it may well be that a component of the \( M_b \) increases we observed in cold-acclimated birds were attributable to such changes, particularly in light of the concomitant increases in \( M_{sum} \) (discussed below).

There have been fewer studies examining the reversibility of acclimation-associated changes in \( M_b \). Our analysis of percentage change in \( M_b \) during reverse acclimation indicates that these adjustments are fully reversible, since the direction of change in \( T_{acc} \) had no significant effect on the magnitude of the response. These results are qualitatively consistent with those of Barceló et al. (2009), who found that rufous-collared sparrows decreased and then increased \( M_b \) when sequentially acclimated to warm (30 \( ^\circ C \)) and then cool (15 \( ^\circ C \)) conditions, and vice versa. Our results, like those of the latter authors, suggest that even in non-migratory passerine species, \( M_b \) is a highly flexible trait that is continually adjusted in response to changing environmental conditions. The high and significant repeatabilities for \( M_b \) in both populations of sparrows are qualitatively consistent with that observed in captive summer-acclimatized American goldfinches (Swanson and King, in press), although the overall repeatability for \( M_b \) in the latter study (0.348) was considerably lower than in the bishops.

The significant effect of site on percentage \( M_b \) change during the reverse acclimation phase of our experiments indicates that fractional changes were slightly but significantly more positive in bishops from the coastal site, and that the shape of the \( M_b \) reaction norm differs between the two populations. Cavieres and Sabat (2008) did not find significant changes in \( M_b \) associated with acclimation to either 15 \( ^\circ C \) or 30 \( ^\circ C \) in three rufous-collared sparrow populations from sites ranging in latitude from 27 to 40°S. However, the difference between \( T_{acc} \) in the latter study was only 15 \( ^\circ C \) (compared to 25 \( ^\circ C \) in our study), and may have been too small to fully investigate intraspecific variation in \( M_b \) reaction norms.

Southern red bishops increased BMR when acclimated to low \( T_A \) and vice versa, a result that is broadly consistent with virtually all species investigated to date (e.g., Tieleman et al., 2003; Klaassen et al., 2004; McKechnie et al., 2007; Cavieres and Sabat, 2008). The magnitude of the increase in BMR (~19% higher at \( T_A = 10 ^\circ C \) compared to \( T_A = 35 ^\circ C \)) is qualitatively similar to that observed in most species (reviewed by McKechnie, 2008). The negative relationship between BMR and \( T_{acc} \) was driven primarily by changes in \( M_{sum} \); when the latter variable was included as a covariate the significant effect of \( T_{acc} \) disappeared. These findings suggest that BMR in E. orix changes in concert with \( M_b \) rather than reflecting changes in mass-independent metabolic rates. The absence of significant changes in BMR independently of \( M_b \) contrasts with the findings of several other studies: for instance, in red knots the significant differences in BMR among thermal acclimation treatments remained significant when \( M_b \) was included as a covariate (Vézina et al., 2006), and in laughing doves BMR varied significantly with \( T_{acc} \) whereas \( M_b \) did not (McKechnie et al., 2007).

We found no evidence for differences between the two bishop populations in the shapes of the relationship between BMR and \( T_{acc} \). The similarity in relationships between BMR and environmental temperature in the two E. orix populations contrasts with the intraspecific variation in responses to thermal acclimation in rufous-collared sparrows documented by Cavieres and Sabat (2008). In their study, sparrows from a site with comparatively little seasonal variation (Copióapo, 27°S) did not respond to thermal acclimation with significant changes in BMR. In contrast, conspecifics from two sites with greater seasonal variation (Santiago, 33°S and Valdivia, 39°S) did show significant variation in BMR associated with \( T_{acc} \) (Cavieres and Sabat, 2008).

The similarity in responses of BMR to thermal acclimation in the two bishop populations was surprising given that inland and coastal populations of this species show divergent patterns of seasonal acclimatization. The BMR of wild birds from a coastal site (Port Elizabeth) did not vary between summer and winter, but mass-specific BMR was significantly lower in winter (van de Ven et al., 2013). Conspecifics from an inland site (Aliwal North), in contrast, showed large winter increases in both BMR (58\%) and mass-specific BMR (31\%; van de Ven et al., 2013). In the only other study comparing changes in BMR during acclimation vs acclimatization of which we are aware, a population of rufous-collared sparrows showed no significant seasonal difference in BMR, but significantly higher BMR (by ~19%) when acclimated to 15 °C compared to 30 °C (Maldonado et al., 2009).

As was the case for \( M_b \), changes in BMR in response to short-term thermal acclimation appeared to be fully reversible, with no significant effect of the direction of change in \( T_{acc} \) on the magnitude of the response. In a similar study of another passerine, the rufous-collared sparrow, Barceló et al. (2009) found that the thermal history of individuals affected the magnitude, but not direction, of changes in BMR. In their study, sparrows sequentially acclimated to warm (30 °C) and cool (15 °C) \( T_A \) increased BMR more rapidly in response to cool conditions than they decreased it in response to warm conditions, with the magnitude of BMR decreases during four weeks of warm-acclimation being smaller than BMR increases during cool-acclimation for the same period. In laughing doves, however, the magnitude of BMR changes during a 3-week acclimation period appeared to be independent of the direction (warm vs cold) of acclimation (McKechnie et al., 2007). Our finding that neither BMR nor mass-independent BMR were repeatable in southern red bishops is unexpected, since most studies examining the repeatability of avian BMR have reported significant repeatabilities (reviewed by Versteegh et al., 2008). Nevertheless, non-repeatable BMR has been reported in at least two other species, namely skylark (Alauda arvensis) and woodlark (Lullula arborea; Tieleman et al., 2003).

Like BMR, \( M_{sum} \) in southern red bishops was highly flexible, and was significantly related to \( T_{acc} \). Unlike the case for BMR during the thermal acclimation phase, the significant influence of \( T_{acc} \) remained when \( M_b \) was included as a covariate, indicating that mass-independent \( M_{sum} \) was up-regulated in bishops acclimated to lower temperatures. The latter finding contrasts with a previous study of red knots, in which \( M_{sum} \) differed significantly between individuals acclimated to cool (4 °C) and warm (25 °C) conditions, but did not differ when \( M_b \) was included as a covariate (Vézina et al., 2006). Whereas phenotypic flexibility in \( M_{sum} \) has been extensively studied in the context of seasonal acclimatization to cold winter temperatures (e.g., Swanson, 1990, 2001; Swanson and
Garland, 2009; Swanson and Olmstead, 1999; reviewed by McKechnie and Swanson, 2010), the only previous study to examine Msum responses to acclimation of which we are aware is that of Vézina et al. (2006).

The lack of a difference in relationship between Msum and Tb between the two bishop populations in the present study is at odds with the seasonal patterns evident in two wild populations. Bishops from Port Elizabeth significantly decreased both Msum and mass-specific Msum in winter, whereas birds from Aliwal North increased Msum by ~15% in winter, without any significant change in mass-specific values (van de Ven et al., 2013). Very little is known about seasonal variation in Msum in birds from tropical and sub-tropical latitudes, and the functional links between responses seen during thermal acclimation and seasonal acclimatization are virtually unexplored. Wells and Schaeffer (2012) examined seasonal acclimatization in seven tropical species (Mb range ~5 to 30 g) at a site in Panama and found that Msum was 12–35% higher in summer compared to winter, whereas a single temperate species investigated in the same study increased Msum by 45% during winter. A general pattern of winter Msum upregulation in temperate latitudes but downregulation in the tropics is qualitatively consistent with the pattern reported for BMR by Smit and McKechnie (2010), and supports the notion that seasonal adjustments in avian metabolic machinery vary in a consistent way with latitude. However, the intraspecific variation in seasonal changes in Msum in southern red bishops reported by van de Ven et al. (2013), together with the short-term changes associated with thermal acclimation we report here, suggest that temporal variation in Msum also reflects factors other than global latitudinal gradients in environmental temperature.

The reverse thermal acclimation phase of our study revealed that changes in Msum depend on the direction of changes in Tacc, unlike the case for Mb or BMR (Fig. 5E,F). Whereas there was a clear linear relationship between change in Tacc and percentage change in Msum when birds went from Tacc 1 to Tacc 2, the corresponding relationship during reverse-acclimation (i.e., Tacc 2 to Tacc 1) was less obvious (but nevertheless statistically significant). These patterns (which exist for both Msum and mass-independent Msum) could mean that 1) the magnitude of Msum changes was attenuated during the Tacc 2 to Tacc 1 phase compared to the Tacc 1 to Tacc 2 phase, and/or 2) that the rate of change in Msum during the former phase was slower than during the latter, and Msum had not stabilised at the end of the 3-week acclimation period. Whereas our data do not allow us to evaluate the second possibility, the significant variation in metabolic expensibility among groups following reverse-acclimation may provide a clue. Bishops first acclimated to 35 °C and then acclimated to the other two Tacc values before being reverse-acclimated to 35 °C showed substantially greater metabolic expensibilities than the other groups. This pattern is qualitatively consistent with the observation that whereas changes in Mb and BMR were fully reversible during the period over which the reverse-acclimation phase of our experiments were carried out, changes in Msum were not. In addition to changes in body composition (e.g., pectoral muscle mass), variation in metabolic expansibility could arise from changes in the activities of enzymes involved in mitochondrial respiration, such as cytochrome c oxidase (COX) and citrate synthase (CS) (Swanson, 2010). Both COX and CS activities in avian muscles have been reported as varying in response to acclimation and/or acclimatization in some species, but not in others (reviewed by Swanson, 2010). The mean metabolic expensibilities observed during initial acclimation (6.7), thermal acclimation (7.3) and reverse thermal acclimation to Tacc = 10 and 22 °C (7.8 and 6.8 respectively) are within the typical range for birds, but that of bishops reverse-acclimated to Tacc = 35 °C (9.5) is higher than any previously recorded avian value (Arens and Cooper, 2005).

There is no obvious explanation for the latter observation, since the capacity to increase resting heat production far above basal levels is generally thought to be related to very cold environments (Arens and Cooper, 2005) and possibly large supracoracoideus muscles associated with hovering flight (López-Calleja and Bozinovic, 1995; Swanson, 2010). We suspect that the high values we documented in one group of bishops reflects little more than an artefact of the rapid changes in both BMR and Msum elicited by our experimental protocol.

In conclusion, our data reveal that responses to short-term thermal acclimation in a non-migratory passerine from sub-tropical latitudes involve an interplay between changes in Mb and both minimum and maximum resting metabolic rates. These findings reiterate that the lower and upper limits of avian normothermic resting metabolic rates are highly flexible, and suggest that birds continuously vary their metabolic capacity in response to fluctuating conditions. The consistent negative relationships between air temperature and Mb, BMR and Msum under artificial conditions in individuals from two populations contrast with the seasonal patterns seen in free-ranging conspecifics, revealing that the seasonal changes that occur during acclimatization are driven by factors other than air temperature alone. Overall, however, our data for both seasonal acclimatization in wild bishops (van de Ven et al., 2013) and thermal acclimation in captive individuals (present study) may be interpreted as being broadly consistent with the predictions of the energy demand hypothesis (Williams and Tieleman, 2000). For instance, the seasonally stable BMR observed in wild coastal bishops (van de Ven et al., 2013) may reflect approximately constant food intake across seasons driven by higher activity levels when breeding in summer combined with only moderate increases in food intake associated with thermoregulatory demands during the comparatively mild winter. The observation that metabolic expensibility began to vary between experimental groups towards the end of the study highlights the potential for differential responses of BMR and Msum. Taken together with our previous results for wild bishops (van de Ven et al., 2013), the responses of E. orix to thermal acclimation highlight the need for future studies that address other potential determinants of metabolic adjustments, such as photoperiod and food availability. One useful approach may be to make regular measurements of metabolic rates in wild populations over the course of several years (Swanson and Olmstead, 1999; Broggi et al., 2007); such long-term physiological monitoring, combined with data on fluctuations in environmental variables, has the potential to shed considerable light on determinants of avian metabolic variation.

Acknowledgements

Sincere thanks go to A. Schultz and G. Grieve for their assistance with the bird capture. We are grateful to L. Minnie, T. Vink and the Mollentze family for their assistance in the field. Gratitude is expressed to R. Wasserman who provided useful comments on an earlier version of this article, and two anonymous reviewers whose comments improved the manuscript. All procedures in this study were approved by the Animal Ethics Committee of the Nelson Mandela Metropolitan University (A10-SCI-ZOO-003). Permits to capture study animals were granted by the Eastern Cape Department of Economic Development and Environmental Affairs (CRO 72/10CR, CRO 73/10CR, and CRO 70/11CR). This project was facilitated by funding from the National Research Foundation (NRF) to N.M. and A.E.M. Any opinion, findings, and conclusions or recommendations expressed in this material are those of the authors, and therefore the NRF does not accept any liability in regard thereto.

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