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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Abstract

Avian basal metabolic rate (BMR) and summit metabolism (M_{sum}) 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 (T_{acc}) and body mass (M_b), BMR and M_{sum} 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 $T_{acc} \approx 10$, 22 or 35 °C, M_b, BMR and M_{sum} were all significantly and negatively related to T_{acc}. The significant relationship between BMR and T_{acc} did not remain after M_b was included as a covariate, whereas that between M_{sum} and T_{acc} did. A subsequent reverse acclimation protocol, where bishops were acclimated to a second T_{acc} value and then re-acclimated to the first, revealed that short-term changes in M_b and BMR were completely reversible, but changes in M_{sum} were only partially reversible. Following the reverse-acclimation protocol, metabolic expansibility (M_{sum}/BMR) varied significantly with air temperature, being greatest at T_{acc} = 35 °C. Our data suggest that the intraspecific variation in seasonal metabolic variation previously reported for this species is at least partly driven by factors other than temperature.

Keywords

body mass intraspecific variation metabolic expansibility phenotypic plasticity resting metabolic rate

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 (M_{sum}; 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 (T_a) under laboratory conditions. In general, BMR is negatively correlated with T_a, with acclimation to low T_a resulting in birds increasing their BMR and *vice versa* (Cavieres and Sabat, 2008; Klaassen et al., 2004; McKechnie et al., 2007; Tieleman et al., 2003). 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 (Jetz et al., 2008; Weathers, 1979; White et al., 2007).

In contrast to BMR, almost nothing is known about phenotypic flexibility in M_{sum} in response to short-term thermal acclimation. As the maximum rate of thermogenic heat production in a resting bird (Swanson and Garland, 2009), M_{sum} is a key determinant of cold tolerance. Whereas numerous studies have examined seasonal variation in M_{sum} in birds acclimatized to natural conditions (Cooper, 2002; Dawson and Olson, 2003; Dutenhoffer and Swanson, 1996; Liknes et al., 2002; Swanson, 2001; Wells and Schaeffer, 2012), to the best of our knowledge only one study has involved birds acclimated to a range of T_a under artificial conditions to examine M_{sum} adjustments (Vézina 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 M_{sum} 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 M_{sum}.

A better understanding of the functional linkages between adjustments in BMR and M_{sum} 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 M_b, BMR and M_{sum}; the degree to which physiological traits are repeatable is a key variable determining how natural selection operates on them (Falconer and Mackay, 1996), 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, 2013).

2. Materials and methods

2.1. Animal capture and housing

We captured 30 southern red bishops at each of two sites, namely a small wetland in the 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).

Table 1. Seasonal air temperature variation at two sites (Port Elizabeth and Barkly East) in the Eastern Cape province of South Africa where southern red bishops (*Euplectes orix*) were captured for thermal acclimation experiments. Data from a third site (Aliwal North) are also included; although no bishops from this site were involved in the present study, we previously measured seasonal metabolic responses in this species here (van de Ven et al., 2013). Data (mean ± SD) are mean monthly values for the period 2005 – 2011, obtained from the South African Weather Service.

Site	**Summer**		**Winter**	
	Minimum (°C)	**Maximum (°C)**	**Minimum (°C)**	**Maximum (°C)**
Port Elizabeth (175 m asl)	17.4 ± 0.2	25.5 ± 0.8	8.3 ± 0.6	20.5 ± 1.1
Barkly East (1795 m asl)	10.9 ± 0.9	26.3 ± 1.5	-0.8 ± 1.7	14.7 ± 1.5
Aliwal North (1300 m asl)	14.5 ± 0.8	30.4 ± 1.4	-2.8 ± 1.1	18.0 ± 1.3

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 12L:12D (photophase = 5h30 to 17h30) throughout all experiments. Immediately upon arrival at NMMU, the cages containing the birds were placed in a constant environment room set to $T_a \approx 22$ °C.

2.2. Metabolic rate measurements

Basal, resting and summit metabolic rates were measured indirectly as oxygen consumption ($\dot{V}_{O_2}$) 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 (T_{lc}) 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 T_{lc} for that group, and ranged from 32 to 35°C. For M_{sum} measurements, the chamber temperature was set to the helox cold limit (T_{cl}) value previously established for that group, using the same approach as van de Ven et al. (2013), with the helox T_{cl} 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 (T_b) was measured with the use of a fine-gauge Cu-Cn 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

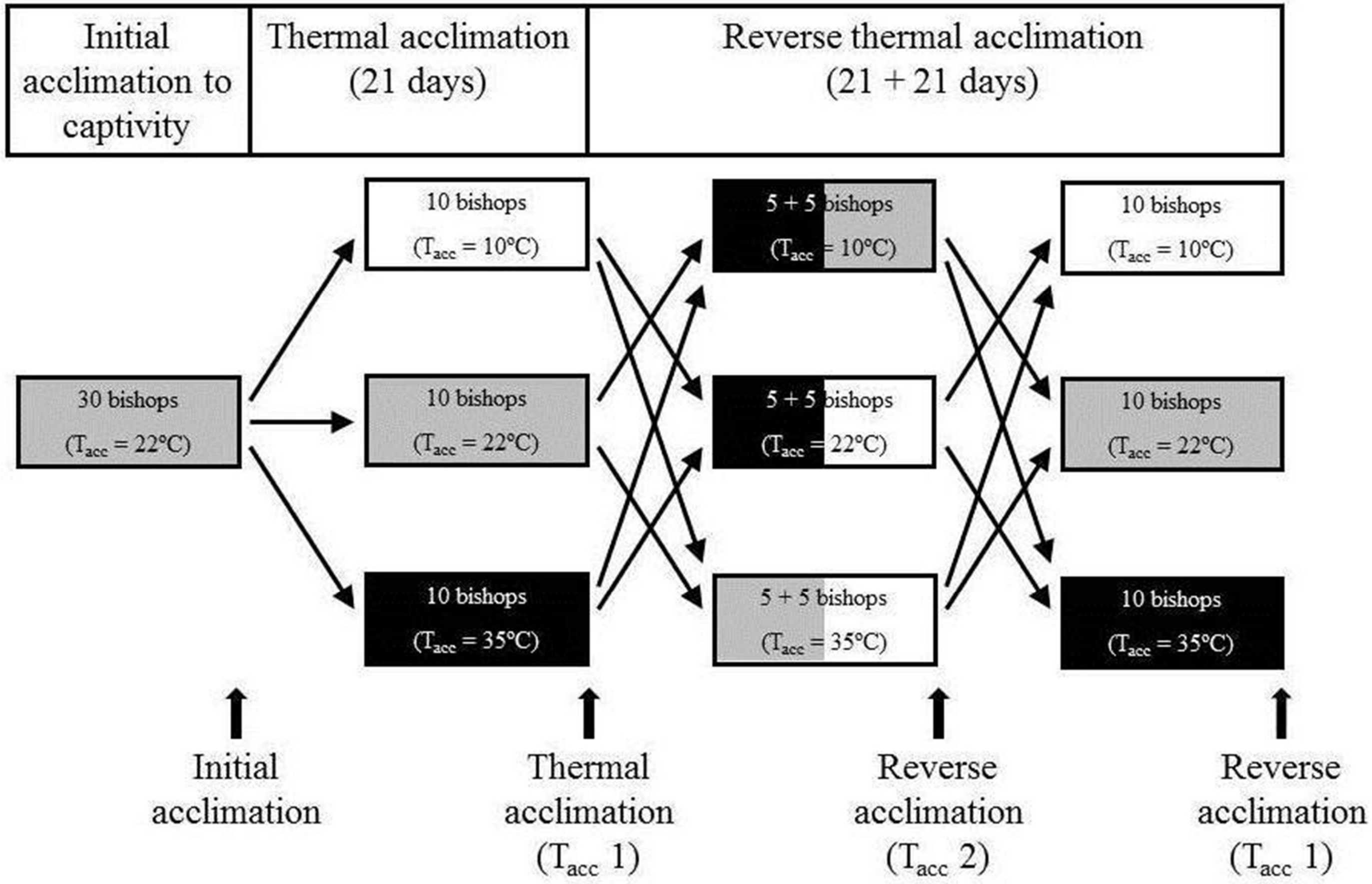
ºC lower than T_b before measurement in 95.3 % of cases, was ≥ 3 ºC lower in 72.2 % of cases, and we are thus confident that our protocol successfully elicited maximum resting metabolic heat production. Air temperature in the constant environment rooms and cabinet used for metabolic measurements was measured using Thermochron iButtons (DS1922L, Maxim, Sunnyvale, CA, U.S.A., 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 (Figure 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 $T_a \approx 22$ °C in one room. In order to verify that birds had completely acclimated to these initial conditions, starting 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 $T_a \approx 32$ °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

Figure 1. Diagrammatic summary of experimental design for thermal acclimation and reverse thermal acclimation experiments with southern red bishops (*Euplectes orix*) from two sites, namely Port Elizabeth (coastal) and Barkly East (inland). The experimental sequence shown here applies to 30 birds from each site. Shading is used to indicate the pathway along which individual birds experienced various acclimation air temperature (T_{acc}) values. For instance, the 10 bishops first acclimated to T_{acc} = 10 °C (no shading) were then exposed to T_{acc} = 22 °C (5 birds) and T_{acc} = 35 °C (5 birds), before being reverse-acclimated to T_{acc} = 10 °C. Arrows indicate the points at which body mass, basal metabolic rate and summit metabolism where measured.



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 (T_{acc}), and BMR and M_{sum} was measured in each individual (i.e., 10 birds per T_{acc}).

The third phase of the study, subsequent to the thermal acclimation described above, involved sequentially acclimating each bishop to a new T_{acc} value, and then reverse-acclimating it to the previous T_{acc} (Figure 1). Following the completion of the thermal acclimation phase described above, the birds were rotated between the three rooms (Figure 1). For instance, of 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 M_{sum} 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 T_a = 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 M_{sum} of each bird were measured for the third time (Figure 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 M_b and BMR stabilised following the initial period of acclimation captivity, we first tested the dependence of BMR and M_{sum} on M_b 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 M_b 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 M_{sum} following initial acclimation, a significant relationship with M_b 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 M_b, BMR and M_{sum} using analyses of variance (ANOVA) with site (categorical) and T_{acc} (continuous) as predictors. To analyse changes in mass-independent BMR and M_{sum}, we followed the approach of Vézina et al. (2006), and added M_b as a covariate in models for BMR and M_{sum}.

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 T_{acc} values), there were effectively six groups during the reverse-acclimation phase, on account of the various sequences of T_{acc} during this phase of the experiment. Therefore, we analysed these data with

% change in M_b, BMR and M_{sum} for each individual between the first and second T_{acc} experienced (T_{acc} 1 → T_{acc} 2), and between the second and third T_{acc} experienced (T_{acc} 2 → T_{acc} 1, since the birds were reverse-acclimated) as response variables. We fitted general linear mixed models (restricted maximum likelihood), with acclimation phase (T_{acc} 1 → T_{acc} 2 *vs* T_{acc} 2 → T_{acc} 1) and site (i.e., inland vs coastal) as fixed effects, the change in T_{acc} (ΔT_{acc}) 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 T_{acc} experienced by each bird. To analyse the reversibility of changes in mass-independent BMR and M_{sum}, we included % change in M_b 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 T_{acc} 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, 2013). Repeatabilities were estimated for both whole-animal and mass-independent (i.e., residuals from regressions against M_b) for BMR and M_{sum}. All statistical procedures were carried using 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 (M_b = 23.89 ± 1.87 g, n = 30) were significantly heavier than coastal birds (M_b = 22.27 ± 2.71 g, n = 30; $t_{1,58}$ = -2.684, P = 0.009). Following the initial acclimation period to T_a = 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, $t_{1,58}$ = -1.266, P = 0.043). Body mass did not change significantly during the initial acclimation period in either the coastal ($t_{1,29}$ = -0.0883, P = 0.930) or inland group ($t_{1,29}$ = 0.620, P = 0.540).

Following the initial period of acclimation to T_a = 22 °C, neither BMR ($t_{1,58}$ = -0.863, P = 0.392) nor mass-specific (M-S) BMR ($t_{1,58}$ = 0.626, P = 0.534) differed significantly between birds from the inland and coastal sites, and mean BMR was 61.3 ± 9.2 mL O_2 hr^{-1} (n = 60). Moreover, in neither the coastal nor the inland group was BMR following acclimation significantly related to M_b. In contrast, M_{sum} was significantly related to M_b in both the coastal ($F_{1,28}$ = 11.106, P = 0.002) and inland ($F_{1,28}$ = 7.186, P = 0.012) groups, but did not differ significantly between the populations when M_b was accounted for (ANCOVA, $F_{1,57}$ = 0.250, P = 0.619). The mean M_{sum} following initial acclimation to captivity was 401.7 ± 34.6 mL O_2 hr^{-1} (n = 60). The mean metabolic expansibility (i.e., M_{sum}/BMR) was 6.69 ± 1.061, and did not differ between the two populations ($t_{1,58}$ = 0.598, P = 0.552).

3.2. Thermal acclimation

Following acclimation to T_a = 10, 22 or 35 °C, M_b varied significantly with T_{acc} ($F_{1,58}$ = 8.44., P = 0.005), but not with site ($F_{1,58}$ = 0.907, P = 0.345) or site* T_{acc} ($F_{1,58}$ = 2.632, P = 0.110)

(Figure 2). The mean M_b of bishops acclimated to $T_a = 10$ °C was equivalent to 107.8 % of that of bishops acclimated to $T_a = 35$ °C.

BMR was significantly related to M_b in coastal birds acclimated to 35 °C, but not in any of the other five combinations of site and T_{acc}, and so we analysed variation using ANOVA. BMR varied significantly with T_{acc} ($F_{1,58} = 7.404$, $P = 0.009$) but not with site ($F_{1,58} = 1.291$, $P = 0.261$) or site* T_{acc} ($F_{1,58} = 2.367$, $P = 0.130$) (Figure 3). When M_b was added as a covariate, however, there was no significant effect of T_{acc} ($F_{1,58} = 3.763$, $P = 0.058$), nor site ($F_{1,58} = 0.854$, $P = 0.360$) or site* T_{acc} ($F_{1,58} = 0.972$, $P = 0.329$).

M_{sum} was not significantly related to M_b in any of the six combinations of site and T_{acc}. Like M_b and BMR, M_{sum} varied significantly with T_{acc} ($F_{1,58} = 18.793$, $P < 0.001$) but not with site ($F_{1,58} = 0.220$, $P = 0.641$) or site* T_{acc} ($F_{1,58} = 0.149$, $P = 0.701$) (Figure 4). The mean M_{sum} of bishops acclimated to $T_a = 10$ °C was equivalent to 119 % of that of birds acclimated to $T_a = 35$ °C. When M_b was added as a covariate, the significant effect of T_{acc} on M_{sum} remained ($F_{1,58} = 8.678$, $P = 0.005$), and site ($F_{1,58} = 0.904$, $P = 0.346$) and site* T_{acc} ($F_{1,58} = 0.047$, $P = 0.830$) remained non-significant. Metabolic expansibility did not vary with T_{acc} ($F_{1,58} = 0.100$, $P = 0.753$) or site ($F_{1,53} = 2.291$, $P = 0.136$), and averaged 7.29 ± 1.65.

3.3. Reverse thermal acclimation

The % change in M_b was strongly dependent on ΔT_{acc} ($F_{1,57.12} = 55.637$, $p < 0.001$; Figure 5), and the slope of this relationship did not depend on the direction of acclimation ($T_{acc}\ 1 \rightarrow T_{acc}\ 2$ *vs* $T_{acc}\ 2 \rightarrow T_{acc}\ 1$; $F_{1,57.12} = 0.221$, $p = 0.640$). There was, however, a significant effect of site ($F_{1,57.67} = 5.452$, $p = 0.023$), 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 ΔT_{acc} ($F_{1,74.9} = 54.659$, $p < 0.001$; Figure 5), and there was no significant effect of acclimation direction ($F_{1,72.69} = 0.931$, $p = 0.338$) or site ($F_{1,65.46} = 0.417$, $p = 0.521$), and these patterns of variation remained qualitatively unchanged when % change in M_b was

Figure 2. The body mass of southern red bishops (*Euplectes orix*) from a coastal and an inland site varied significantly with the air temperature experienced during short-term (21 days) thermal acclimation. The mean body masses of wild bishops recorded in a previous study (van de Ven et al., 2013) are indicated by horizontal lines: solid lines = coastal birds (Port Elizabeth), dashed lines = inland birds (Aliwal North), red lines = summer values, and blue lines = winter values.

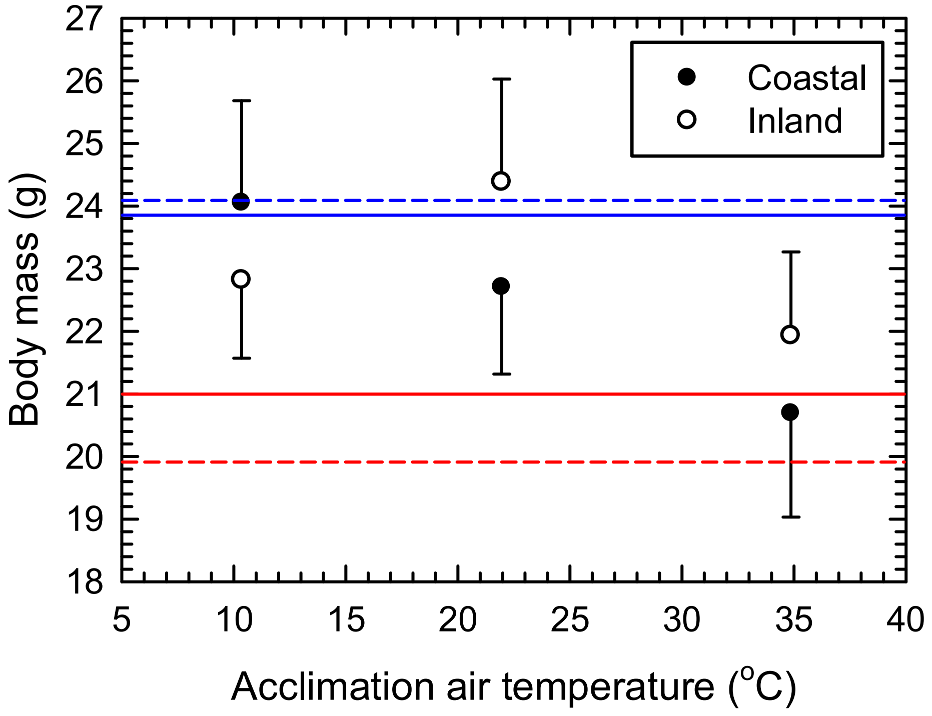


Figure 3. The basal metabolic rate (BMR; upper panel) and mass-specific basal metabolic rate (M-S BMR; lower panel) of southern red bishops (*Euplectes orix*) from a coastal and an inland site varied as a function of the air temperature experienced during short-term (21 days) thermal acclimation. The mean BMR and M-S BMR of wild bishops recorded in a previous study (van de Ven et al., 2013) are indicated by horizontal lines: solid lines = coastal birds (Port Elizabeth), dashed lines = inland birds (Aliwal North), red lines = summer values, and blue lines = winter values

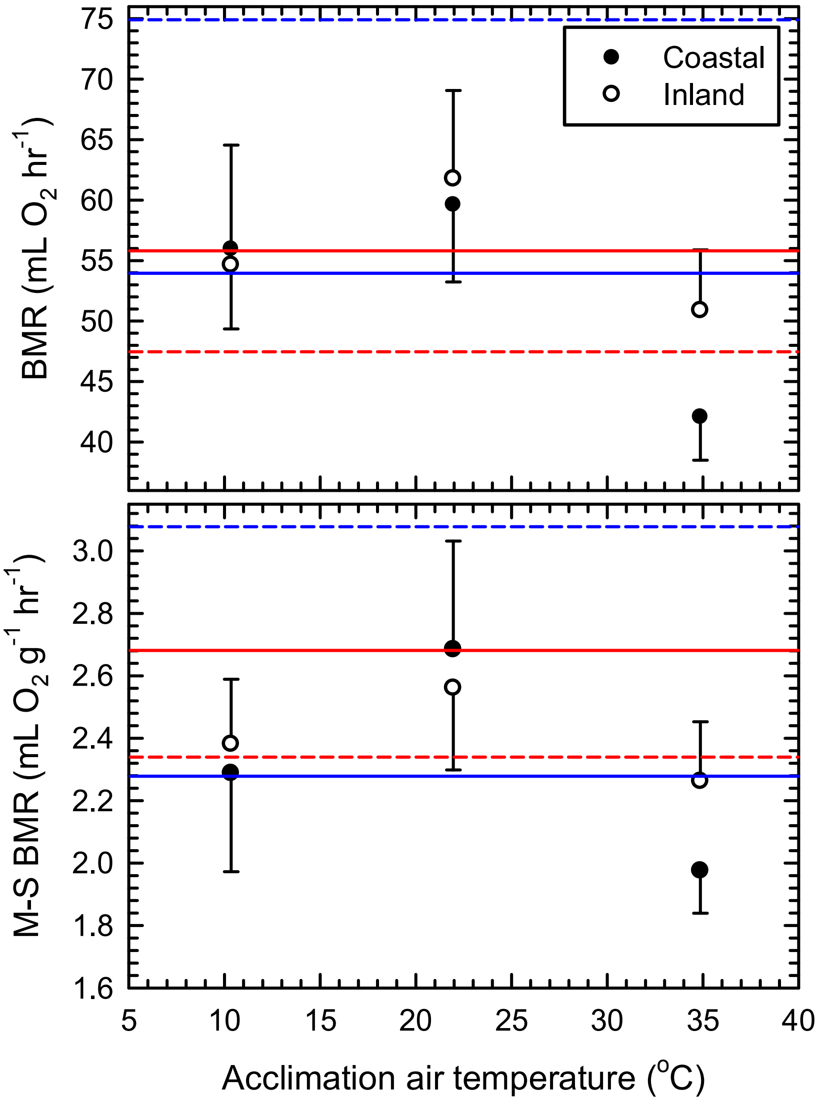


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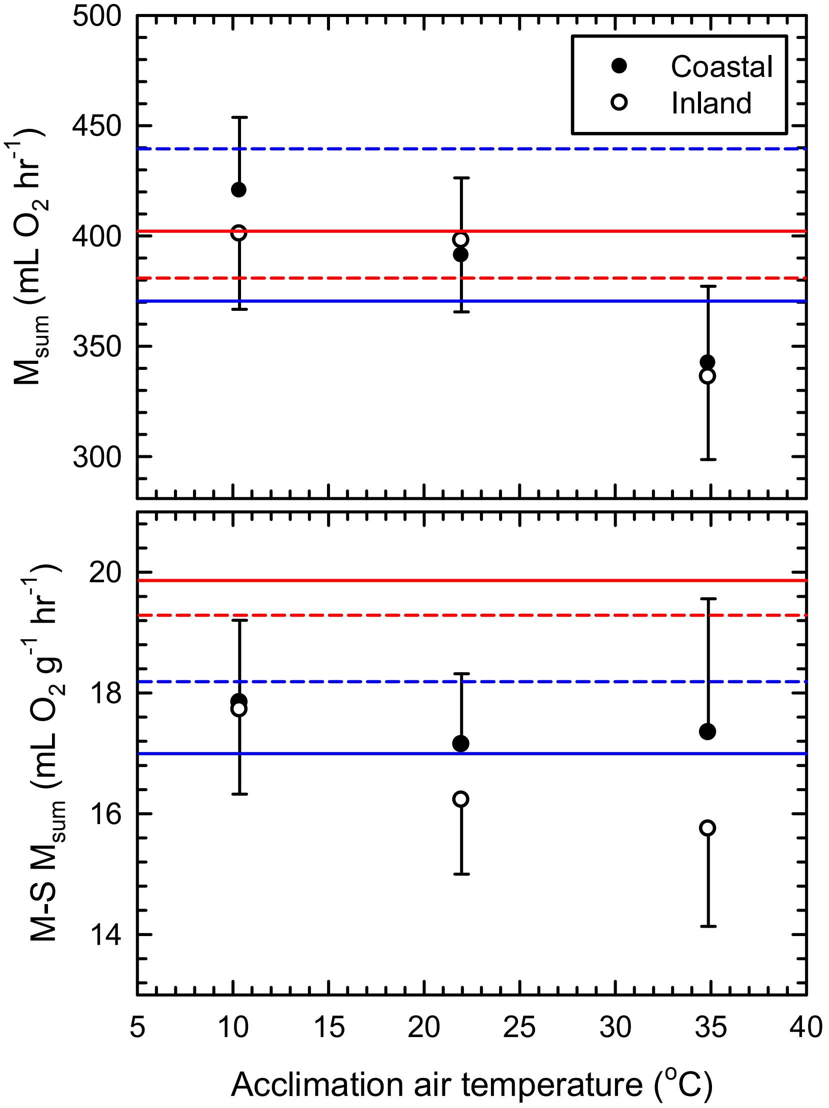
Figure 4. The summit metabolism (M_{sum}; upper panel) and mass-specific summit metabolism (M-S M_{sum}; lower panel) of southern red bishops (*Euplectes orix*) from a coastal and an inland site varied as a function of the air temperature experienced during short-term (21 days) thermal acclimation. The mean M_{sum} and M-S M_{sum} of wild bishops recorded in a previous study (van de Ven et al., 2013) are indicated by horizontal lines: solid lines = coastal birds (Port Elizabeth), dashed lines = inland birds (Aliwal North), red lines = summer values, and blue lines = winter values.



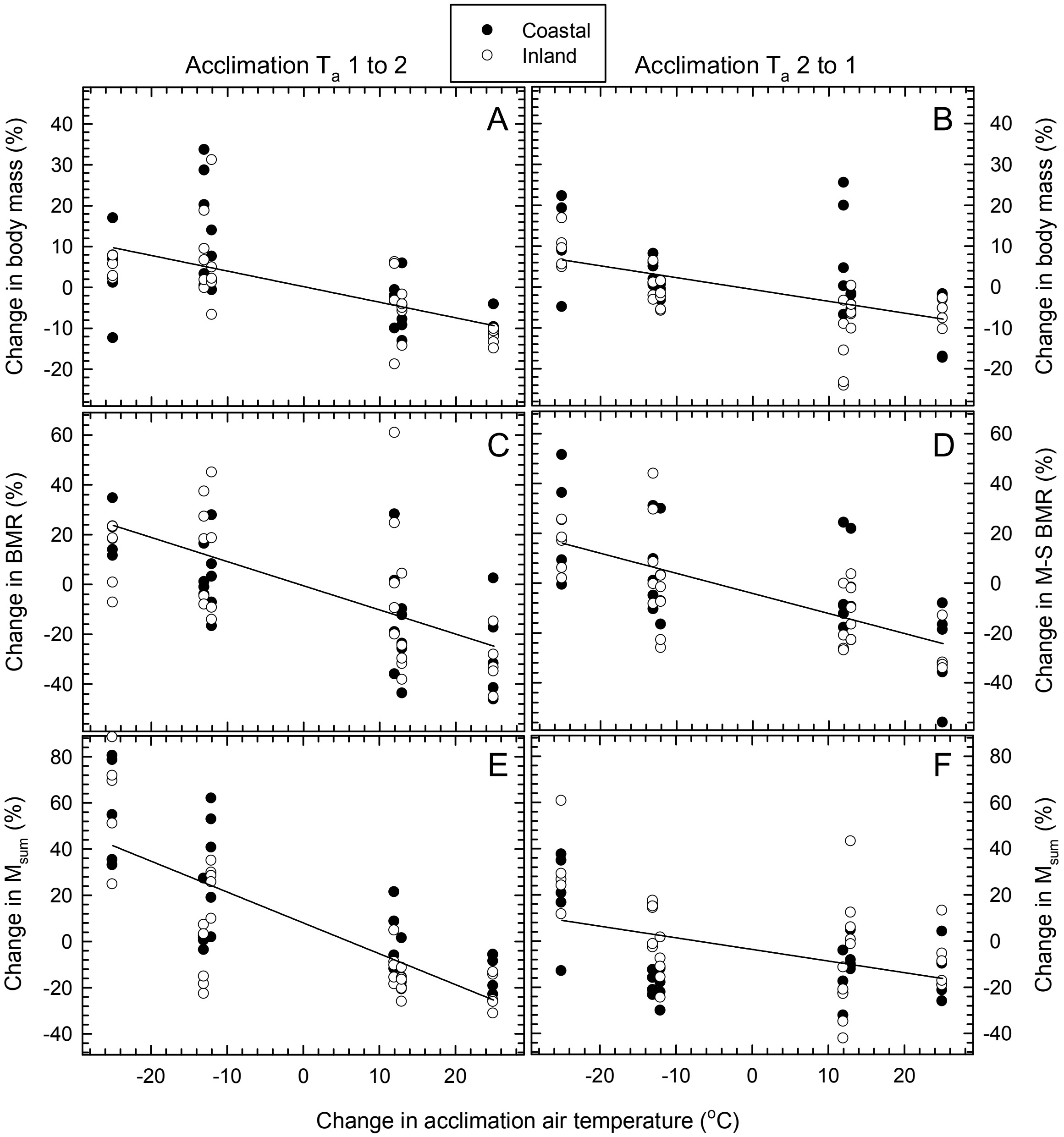
added as a covariate. Percentage change in M_{sum} was also strongly related to ΔT_{acc} ($F_{1,65.76}$ = 74.548, $p < 0.001$; Figure 5) and unrelated to site ($F_{1,63.9}$ = 0.0003, p = 0.986), but showed a significant effect of acclimation direction ($F_{1,65.62}$ = 10.130, p = 0.002) which is reflected in the shallower slope of the regression model fitted to the % change in M_{sum} as a function of ΔT_{acc} during T_{acc} 2 → T_{acc} 1 (Figure 5E) compared to during T_{acc} 1 → T_{acc} 2 (Figure 5F). The significant effect of acclimation direction remained ($F_{1,66.18}$ = 9.370, P = 0.003) when % change in M_b was included in the model.

Table 2. Repeatabilities (*r*) for body mass (M_b), basal metabolic rate (BMR) and summit metabolism (M_{sum}) in two populations of southern red bishop (*Euplectes orix*) during thermal acclimation and subsequent reverse thermal acclimation, with lower and upper 95 % confidence intervals (l. 95 % CI and u. 95 % CI, respectively). Mass-independent analyses (i.e., based on residuals) are indicated as "M-I". Significant *r*-values are indicated with asterices.

	Coastal			**Inland**		
	r	**l. 95 % CI**	**u. 95 % CI**	***r***	**l. 95 % CI**	**u. 95 % CI**
M_b	0.689*	0.226	1.152	0.768*	0.388	1.147
BMR	0.035	-0.227	0.296	0.042	-0.231	0.315
M-I BMR	0.035	-0.227	0.297	0.032	-0.225	0.289
M_{sum}	0.001	-0.207	0.208	0.002	-0.208	0.211
M-I M_{sum}	0.000	-0.207	0.208	0.001	-0.207	0.208

In both the coastal and inland populations, M_b was highly and significantly repeatable (Table 2). In contrast, however, the repeatability values for BMR and M_{sum} 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 likely linked to the observed effect of acclimation direction on changes in M_{sum}, concerned metabolic expansibility following the reverse-acclimation phase: when metabolic

Figure 5. 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 (M_{sum}; E,F) were significantly and approximately linearly related to the magnitude of change in acclimation air temperature (T_{acc}). Panels A,C and E show changes associated with bishops moved from a first T_{acc} to one of two other T_{acc} values, and panels B,D, and F show changes in the opposite direction, i.e., from the second T_{acc} experienced by each bird back to the first T_{acc}.



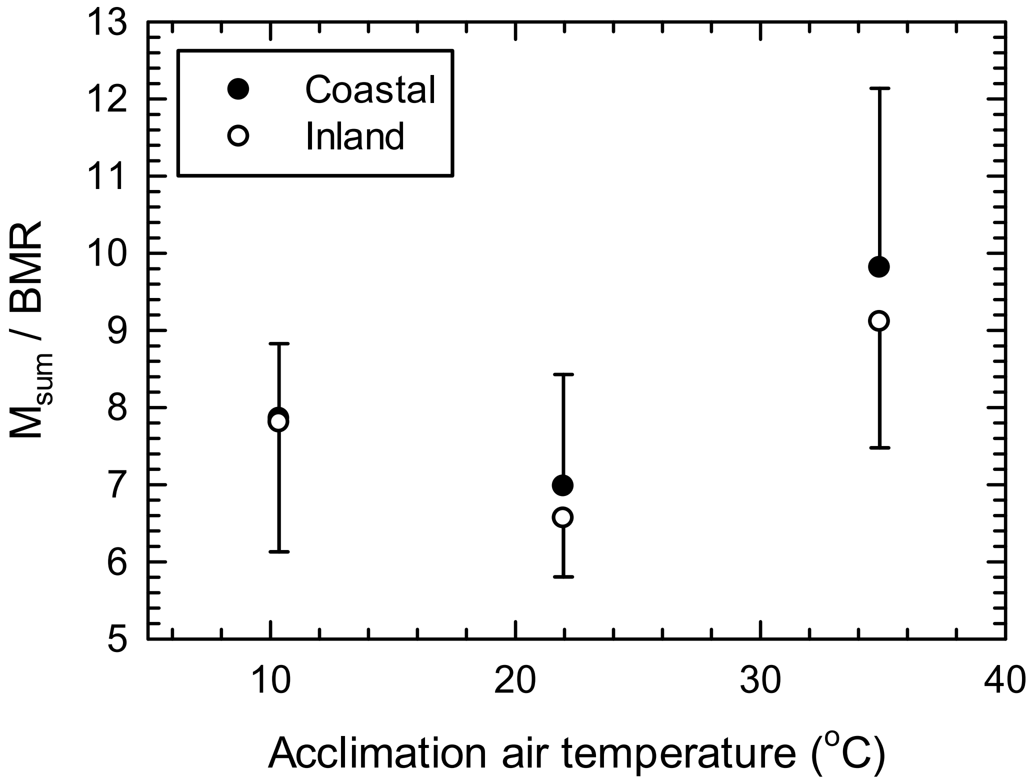
expansibility (log-transformed to meet the assumption of normality) following reverse acclimation was analysed by ANOVA, it was significantly and positively related to T_{acc} ($F_{1,58}$ = 4.432, P = 0.034). The metabolic expansibility of bishops reverse-acclimated to 10 °C was 7.83 ± 1.33 (n =20), whereas that of bishops acclimated to 35 °C was 9.46 ± 1.99 (n = 20) (Figure 6).

4. Discussion

Southern red bishops showed considerable phenotypic flexibility in M_b, BMR and M_{sum} 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 T_{acc} and M_b became evident during the reverse-acclimation phase.

Our finding that M_b was negatively related to T_{acc} is consistent with the responses of some species to short-term thermal acclimation, but not others. Cold-acclimated red knots (*Calidris canutus islandica*), garden warblers (*Sylvia borin*) and woodlarks (*Lullula arborea*) all showed higher M_b when compared with warm-acclimated counterparts (Barceló et al., 2009; Klaassen et al., 2004; Tieleman et al., 2003; Vézina et al., 2006). In contrast, laughing doves (*Streptopelia senegalensis*), skylarks (*Alauda arvensis*), Dunn's larks (*Eremalauda dunni*), and spike-heeled larks (*Chersomanes albofasciata*) and rufous-collared sparrows (*Zonotrichia capensis*) all showed no significant differences in M_b when acclimated to differing T_a values (Cavieres and Sabat, 2008; McKechnie et al., 2007; Tieleman et al., 2003). Collectively, these observations suggest that avian M_b responses to short thermal acclimation vary substantially among species. However, the response of M_b to thermal

Figure 6. In southern red bishops (*Euplectes orix*) from a coastal and an inland site, metabolic expansibility [i.e., summit metabolism (M_{sum}) / basal metabolic rate (BMR)] varied significantly with acclimation air temperature.



acclimation can also vary among studies of a single species; Williams and Tieleman (2000) found that hoopoe larks (*Alaemon alaudipes*) 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 M_b when acclimated for three weeks to 15 °C or 35 °C (Tieleman et al., 2003).

The relatively higher M_b of bishops acclimated to cold T_a is qualitatively consistent with the seasonal patterns of M_b 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 (Aliwal North, broadly similar but slightly colder conditions than Barkley East), M_b was significantly higher in winter compared to summer (van de Ven et al., 2013). However, the magnitude of the increase in M_b during the thermal acclimation phase of the present study (~ 8 % higher at T_a = 10 °C vs 35 °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 bishops compared to captive individuals in our study; captive red knots experiencing naturally variable T_a (mean daily values ranging between 6 and 12 ºC) showed similar changes in M_b to conspecifics acclimated to cold ($T_{acc} \approx$ 4 ºC) 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., Liknes 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 (Cavieres and Sabat, 2008; Tieleman et al., 2003; Williams and Tieleman, 2000), 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 far 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 °C) and then cool (15 °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 bishops are qualitatively consistent with that observed in captive summer-acclimatized American goldfinches (Swanson and King, 2013), 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 °C or 30 °C in three rufous-collared sparrow populations from sites ranging in latitude from 27 to 40°S. However, the difference between T_{acc}s in the latter study was only 15 °C (compared to 25 °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 response that is broadly consistent with virtually all species investigated to date (e.g., Cavieres and Sabat, 2008; Klaassen et al., 2004; McKechnie et al., 2007; Tieleman et al., 2003). The magnitude of the increase in BMR (~ 19 % higher at T_a = 10 °C compared to T_a = 35 °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_b; 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 (Copiapo, 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 bishops 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 M_{sum} responses to acclimation of which we are aware is that of Vézina et al. (2006).

The lack of a difference in relationship between M_{sum} and T_a 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 M_{sum} and mass-specific M_{sum} in winter, whereas birds from Aliwal North increased M_{sum} 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 M_{sum} 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 (M_b range ≈ 5 to 30 g) at a site in Panama and found that M_{sum} was 12 – 35 % higher in summer compared to winter, whereas a single temperate species investigated in the same study increased M_{sum} by 45 % during winter. A general pattern of winter M_{sum} 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 M_{sum} 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 M_{sum} also reflects factors other than global latitudinal gradients in environmental temperature.

The reverse thermal acclimation phase of our study revealed that changes in M_{sum} depend on the direction of changes in T_{acc}, unlike the case for M_b or BMR (Figure 5E,F). Whereas there was a clear linear relationship between change in T_{acc} and percentage change in M_{sum} when birds went from T_{acc} 1 to T_{acc} 2, the corresponding relationship during reverse-acclimation (i.e., T_{acc} 2 to T_{acc} 1) was less obvious (but nevertheless statistically significant). These patterns (which exist for both M_{sum} and mass-independent M_{sum}) could mean that a) the magnitude of M_{sum} changes was attenuated during the T_{acc} II to T_{acc} I phase compared to the T_{acc} I to T_{acc} II phase, and/or b) that the rate of change in M_{sum} during the former phase was slower than during the latter, and M_{sum} 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 expansibility among groups following reverse-acclimation may provide a clue. Bishops first acclimated to 35 °C and then acclimated to the other two T_{acc} values before being reverse-acclimated to 35 °C showed substantially greater metabolic expansibilities than the other groups. This pattern is qualitatively consistent with the observation that whereas changes in M_b and BMR were fully reversible during the period over which the reverse-acclimation phase of our experiments were carried out, changes in M_{sum} 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 expansibilities observed during initial acclimation (6.7), thermal acclimation (7.3) and reverse thermal acclimation to T_{acc} = 10 and 22 ºC (7.8 and 6.8 respectively) are within the typical range for birds, but that of bishops reverse-acclimated to T_{acc} = 35 ºC (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 M_{sum} 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 M_b 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 M_b, BMR and

M_{sum} 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 expansibility began to vary between experimental groups towards the end of the study highlights the potential for differential responses of BMR and M_{sum}. 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 (Broggi et al., 2007; Swanson and Olmstead, 1999); 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.

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